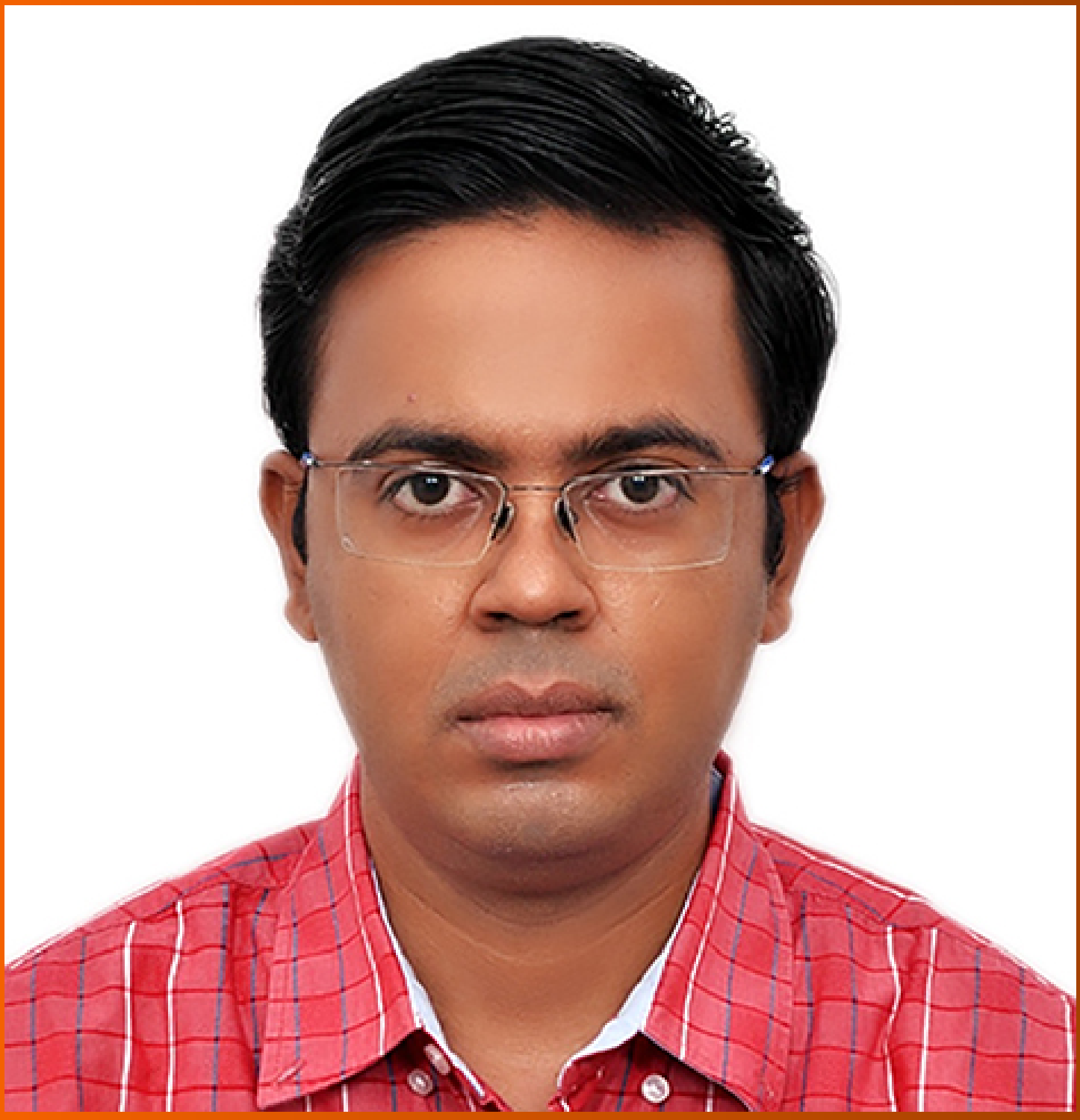
ISSN 2220-3249 (online)

World Journal of *Virology*

Quarterly Volume 14 Number 1 March 25, 2025



Published by Baishideng Publishing Group Inc

World Journal of Virology

Contents

Quarterly Volume 14 Number 1 March 25, 2025

EDITORIAL

Shamim L, Musharaf I, Nashwan AJ. Dexamethasone in coronavirus disease 2019 care: Dosage and utilization insights. *World J Virol* 2025; 14(1): 98765 [DOI: 10.5501/wjv.v14.i1.98765]

Nagoba BS, Dhotre SV, Gavkare AM, Mumbre SS, Dhotre PS. Convergence of COVID-19 and recurrent stroke: In-hospital mortality risks explored. *World J Virol* 2025; 14(1): 99904 [DOI: 10.5501/wjv.v14.i1.99904]

REVIEW

Cenci Dietrich V, Costa JMC, Oliveira MMGL, Aguiar CEO, Silva LGO, Luz MS, Lemos FFB, de Melo FF. Pathogenesis and clinical management of arboviral diseases. *World J Virol* 2025; 14(1): 100489 [DOI: 10.5501/wjv.v14.i1.100489]

MINIREVIEWS

Moliya P, Singh A, Singh N, Kumar V, Sohal A. Insights into gastrointestinal manifestation of human immunodeficiency virus: A narrative review. *World J Virol* 2025; 14(1): 99249 [DOI: 10.5501/wjv.v14.i1.99249]

Karanam SK, Nagvishnu K, Uppala PK, Edhi S, Varri SR. Crimean-Congo hemorrhagic fever: Pathogenesis, transmission and public health challenges. *World J Virol* 2025; 14(1): 100003 [DOI: 10.5501/wjv.v14.i1.100003]

ORIGINAL ARTICLE

Retrospective Study

Shahid Y, Butt AS, Jamali I, Ismail FW. Rising incidence of acute hepatitis A among adults and clinical characteristics in a tertiary care center of Pakistan. *World J Virol* 2025; 14(1): 97482 [DOI: 10.5501/wjv.v14.i1.97482]

Saeed NK, Almusawi SK, Albalooshi NA, Al-Beltagi M. Unveiling the impact: COVID-19's influence on bacterial resistance in the Kingdom of Bahrain. *World J Virol* 2025; 14(1): 100501 [DOI: 10.5501/wjv.v14.i1.100501]

Observational Study

Pinheiro MG, Alves GGO, Conde MER, Costa SL, Sant'Anna RCS, Antunes IMF, Carneiro MC, Ronzei FS, Scaffo JC, Pinheiro FR, Andre LS, Povoa HC, Baltar VT, Giordani F, Hemerly ES, Alexandre GC, de Paula KC, Watanabe M, Nóbrega ACLD, Lobato JCP, Aguiar-Alves F. Serological surveillance for SARS-CoV-2 antibodies among students, faculty and staff within a large university system during the pandemic. *World J Virol* 2025; 14(1): 100338 [DOI: 10.5501/wjv.v14.i1.100338]

Basic Study

Thakur SK, Sinha AK, Sharma SK, Jahan A, Negi DK, Gupta R, Singh S. Prevalence of transfusion transmissible infections among various donor groups: A comparative analysis. *World J Virol* 2025; 14(1): 96098 [DOI: 10.5501/wjv.v14.i1.96098]

CASE REPORT

Patel S, Jay J, Pathak P, Antony MA, Thiriveedi M. Septic shock due to cytomegalovirus colitis associated with rituximab use: A case report. *World J Virol* 2025; 14(1): 99923 [DOI: 10.5501/wjv.v14.i1.99923]

LETTER TO THE EDITOR

Varama A. Revisiting dexamethasone dosage in COVID-19 management. *World J Virol* 2025; 14(1): 98359 [DOI: 10.5501/wjv.v14.i1.98359]

Sarker MS. Rhabdomyolysis-related acute kidney injury in COVID-19: A critical concern. *World J Virol* 2025; 14(1): 100160 [DOI: 10.5501/wjv.v14.i1.100160]

World Journal of Virology

Quarterly Volume 14 Number 1 March 25, 2025

Contents

ABOUT COVER

AIMS AND SCOPE

The primary aim of *World Journal of Virology* (*WJV*, *World J Virol*) is to provide scholars and readers from various fields of virology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJV mainly publishes articles reporting research results obtained in the field of virology and covering a wide range of topics including arbovirus infections, viral bronchiolitis, central nervous system viral diseases, coinfection, DNA virus infections, viral encephalitis, viral eye infections, chronic fatigue syndrome, animal viral hepatitis, human viral hepatitis, viral meningitis, opportunistic infections, viral pneumonia, RNA virus infections, sexually transmitted diseases, viral skin diseases, slow virus diseases, tumor virus infections, viremia, and zoonoses.

INDEXING/ABSTRACTING

The *WJV* is now abstracted and indexed in PubMed, PubMed Central, Reference Citation Analysis, China Science and Technology Journal Database, and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Virology

ISSN
ISSN 2220-3249 (online)

LAUNCH DATE
February 12, 2012

FREQUENCY
Quarterly

PUBLICATION DATE
March 25, 2025

PUBLISHING PARTNER
Department of Hepatology, Qilu Hospital of Shandong University

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

POLICY OF CO-AUTHORS
https://www.wjgnet.com/bpg/GerInfo/310

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

PUBLISHING PARTNER's OFFICIAL WEBSITE
https://www.qiluhospital.com/list-410-1.html

World Journal of Virology

Submit a Manuscript: https://www.f6publishing.com

World J Virol 2025 March 25; 14(1): 98765

DOI: 10.5501/wjv.v14.i1.98765

ISSN 2220-3249 (online)

EDITORIAL

Dexamethasone in coronavirus disease 2019 care: Dosage and utilization insights

Laiba Shamim, Imshaal Musharaf, Abdulqadir J Nashwan

Specialty type: Virology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade A, Grade B

Novelty: Grade A, Grade B

Creativity or Innovation: Grade A, Grade C

Scientific Significance: Grade A, Grade B

P-Reviewer: Tolentino JE

Received: July 4, 2024

Revised: November 4, 2024

Accepted: November 19, 2024

Published online: March 25, 2025

Processing time: 146 Days and 7.5 Hours

Laiba Shamim, Department of Clinical Medicine, Jinnah Sindh Medical University, Karachi 75510, Sindh, Pakistan

Imshaal Musharaf, Department of Medicine, Jinnah Sindh Medical University, Karachi 75510, Sindh, Pakistan

Abdulqadir J Nashwan, Department of Nursing and Midwifery Research, Hamad Medical Corporation, Doha 3050, Qatar

Corresponding author: Abdulqadir J Nashwan, MSc, PhD, Research Scientist, Department of Nursing and Midwifery Research, Hamad Medical Corporation, Rayyan Road, Doha 3050, Qatar. anashwan@hamad.qa

Abstract

Coronavirus disease 2019 (COVID-19) is a contagious disease caused by severe acute respiratory syndrome coronavirus 2. It was declared a global pandemic on March 11, 2020, by the World Health Organization. An excessive inflammatory response is a severe respiratory manifestation of COVID-19, which becomes predominant in later stages. Due to its immunosuppressive and anti-inflammatory properties, dexamethasone is the first systemic glucocorticoid to treat severe COVID-19 patients. This editorial reviews the efficacy and safety of high-dose *vs* low-dose dexamethasone in patients with COVID-19. Findings indicate that using low-dose dexamethasone is beneficial and emphasize the need for additional research on the use of high-dose dexamethasone. While the study provides a robust evidence base, it is limited by the lack of long-term data, focus on specific outcomes and heterogeneity of the included studies. Future research should focus on the long-term effects of dexamethasone and its impact across varying disease severities and patient populations to refine treatment strategies and improve patient care.

Key Words: COVID-19; Severe acute respiratory syndrome; Corticosteroid; Dexamethasone; Anti-inflammatory

Core Tip: This editorial evaluates a meta-analysis comparing high-dose and low-dose dexamethasone in the treatment of coronavirus disease 2019 (COVID-19) patients. The study reveals no significant differences in adverse effects and mortality between the dosing regimens. In line with the current guidelines, the study favors using low-dose dexamethasone but highlights the call for additional research on high-dose dexamethasone's benefits. The study includes limitations such as a lack of long-term data and heterogeneity of the included studies. It is crucial to address these gaps in the future to optimize treatment strategies for COVID-19.

Citation: Shamim L, Musharaf I, Nashwan AJ. Dexamethasone in coronavirus disease 2019 care: Dosage and utilization insights. *World J Virol* 2025; 14(1): 98765
URL: https://www.wjgnet.com/2220-3249/full/v14/i1/98765.htm
DOI: https://dx.doi.org/10.5501/wjv.v14.i1.98765

INTRODUCTION

The disease known as coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2)[1]. It is among the most deadly viruses in the history of humans, causing more than 6.8 million deaths globally since its discovery in December 2019[2]. COVID-19 was declared a global pandemic by the World Health Organization on March 11, 2020[3]. There are multiple stages of COVID-19, and each stage indicates the appropriate course of treatment. Stage 1 is the initial phase of early viral infections, including gastrointestinal or respiratory infections, fever, and lymphopenia. The second stage refers to the pulmonary stage. Multisystemic inflammatory syndrome (MIS) occurs in stage 3, and as a pathogenic characteristic, it is frequently associated with a cytokine storm. There is also the involvement of other mechanisms in the late stage of COVID-19, including neutrophil extracellular traps, edema and vascular leak, bradykinin storm, prothrombotic events, endothelins, and coagulation and complement cascade activation [4]. The clinical picture of COVID-19 shows the dominance of MIS and systemic inflammation over the original viral infection in advanced stages[4].

SYSTEMIC CORTICOSTEROIDS FOR COVID-19

Systemic corticosteroids are currently recommended by the National Institutes of Health COVID-19 treatment guidelines for COVID-19 patients who require respiratory support[5]. Systemic corticosteroids are the drugs available for treating inflammatory diseases, classified into mineralocorticoids and glucocorticoids. Dexamethasone is the first glucocorticoid to be used in severe COVID-19 patients with clinical benefits[6]. Dexamethasone is an anti-inflammatory and immunosuppressive agent. Inhibition of pro-inflammatory gene that encodes for cell adhesion molecules, cytokines, chemokines, and acute inflammatory response is the main anti-inflammatory effect of dexamethasone[7]. The primary causes of mortality related to COVID-19 such as cytokine storm induced by SARS-CoV-2, multiorgan failure, and severe acute respiratory distress syndrome can be suppressed by dexamethasone[8]. In a RECOVERY study, it was found that dexamethasone showed a reduction in 28-day mortality among COVID-19 patients requiring respiratory support when 6 mg of it was administered for up to 10 days.

In contrast, no benefit was shown in patients who did not require respiratory support[9]. We aim to evaluate the role of dexamethasone in the treatment of COVID-19 and various dose regimens and offer evidence-based recommendations for dexamethasone to improve patient care. The meta-analysis conducted by Sethi *et al*[10] focuses on the safety and efficacy of low-dose *vs* high-dose dexamethasone by investigating the impact of various dosing regimens of dexamethasone on the outcomes of COVID-19 patients.

Sethi *et al*[10] conducted a meta-analysis on the dosage and utilization of dexamethasone in the management of COVID-19. PRISMA guidelines were followed to conduct the systematic review and meta-analyses. Keywords related to corticosteroid dosing and COVID-19 were used to conduct a thorough literature search from databases such as MEDLINE, Google Scholar, and PubMed up to March 2024. Only randomized controlled trials were included, including dexamethasone-treated COVID-19 patients. The exclusion criteria included single-arm, non-randomized controlled trials, case reports, observational studies, and non-English articles. Initial screening involved reviewing the abstract and the title, after which the independent reviewers assessed the full text. Data extraction included participant demographics, study specifics, details of intervention, and outcomes. The Cochrane risk of bias tool and Newcastle-Ottawa scale were used to assess the potential biases and the quality of the study. A narrative synthesis approach was employed with meta-analyses utilizing a random-effect model where necessary. Forest plots were used to quantitatively synthesize and present the adverse events, hospital stay durations, and mortality outcomes.

Nine randomized controlled trials were analyzed in the study to compare the safety and efficacy of low-dose *vs* high-dose dexamethasone involving 2740 COVID-19 patients. Negligible differences in 28-day and 60-day all-cause mortality were found between the two dosing regimens. There was a slight reduction in average hospital stay when low-dose dexamethasone was administered to patients compared to high-dose dexamethasone administration. The reduction achieved no statistical significance. The rate of occurrence of the adverse effects, including infections, thrombosis, arrhythmias, and myocardial infarction, showed no significant differences and were similar between both groups. The

overall findings of the study support the use of low-dose dexamethasone and highlight the need for further research on high-dose dexamethasone and its potential benefits.

Similarly, Snow *et al*[11] and Kow *et al*[12] also conducted a meta-analysis and found no significant mortality benefit from high-dose dexamethasone compared to low-dose treatment. However, both studies noted a higher risk of hyperglycemia with a higher dose. This aligns with Sethi *et al*'s emphasis on the need for further investigation into high-dose dexamethasone, suggesting that its risks might outweigh the benefits without improving clinical outcomes[10].

The article covered a wide range of studies by offering an in-depth analysis. It provides a robust evidence base by synthesizing data from multiple studies and clinical trials. The patient cohort was directly compared by evenly dividing it between low-dose and high-dose treatments. The authors maintained a balanced perspective by considering both the potential risks and the advantages related to dexamethasone therapy.

The lack of long-term data on the dexamethasone usage is one significant limitation. Most of the studies included in the review are short-term, which may not fully reflect dexamethasone's potential side effects and long-term effects. Studies of varying quality were included in the review, which may impact the overall conclusion. The generalizability of the findings can be limited, and biases can be introduced by differences in methodologies, sample size, and study design. Studies with different patient populations were involved with disease severity of varying degrees. This variation makes it difficult to apply findings consistently to every patient group. The vaccination status of the patients was not discussed, which may influence the effectiveness of dexamethasone.

Moreover, the study focuses on hospitalized COVID-19 patients, which may have overlooked the effects of dexamethasone on less severe or non-hospitalized patients. Hospital stay duration and mortality were mainly assessed in the study, while other potential outcomes, such as functional recovery and quality of life, were not evaluated. Due to the emergence of new studies and treatment guidelines, some of the conclusions and data in the study may become outdated, given the evolving nature of COVID-19. To ensure the study remains accurate and relevant, there is a need for continuous updates to the review.

CONCLUSION

Low-dose dexamethasone has become the standard treatment for COVID-19 patients requiring oxygen support, showing reduced mortality. It is safer and more effective than higher doses. The risk of adverse events like hyperglycemia may rise with higher dosages, even if they might be just as effective as low doses in lowering mortality. The research by Sethi *et al* [10] provides valuable insights into the dosage of dexamethasone in COVID-19 patients. The evidence-based and comprehensive approach of the research is commendable. However, limitations such as heterogeneous patient population, rapidly evolving field, variability in study quality, and lack of long-term data must be acknowledged. Future research should address these limitations, assuring that treatment plans can be continuously improved and refined.

FOOTNOTES

Author contributions: Shamim L, Musharaf I and Nashwan AJ contributed to writing the draft and critically reviewing the literature.

Conflict-of-interest statement: The authors declare that they have no conflict of interest.

Country of origin: Qatar

ORCID number: Abdulqadir J Nashwan 0000-0003-4845-4119.

S-Editor: Fan M
L-Editor: A
P-Editor: Zheng XM

REFERENCES

1 **Majumder J**, Minko T. Recent Developments on Therapeutic and Diagnostic Approaches for COVID-19. *AAPS J* 2021; **23**: 14 [PMID: 33400058 DOI: 10.1208/s12248-020-00532-2]

2 **Zhu N**, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W; China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* 2020; **382**: 727-733 [PMID: 31978945 DOI: 10.1056/NEJMoa2001017]

3 **Cucinotta D**, Vanelli M. WHO Declares COVID-19 a Pandemic. *Acta Biomed* 2020; **91**: 157-160 [PMID: 32191675 DOI: 10.23750/abm.v91i1.9397]

4 **Szekanecz Z**, Balog A, Constantin T, Czirják L, Géher P, Kovács L, Kumánovics G, Nagy G, Rákóczi É, Szamosi S, Szűcs G, Vályi-Nagy I. COVID-19: autoimmunity, multisystemic inflammation and autoimmune rheumatic patients. *Expert Rev Mol Med* 2022; **24**: e13 [PMID: 35311631 DOI: 10.1017/erm.2022.10]

5 Coronavirus Disease 2019 (COVID-19) Treatment Guidelines [Internet]. Bethesda (MD): National Institutes of Health (US); 2021-Apr-21 [PMID: 34003615]

6 **Li G**, Hilgenfeld R, Whitley R, De Clercq E. Therapeutic strategies for COVID-19: progress and lessons learned. *Nat Rev Drug Discov* 2023; **22**: 449-475 [PMID: 37076602 DOI: 10.1038/s41573-023-00672-y]

7 **Ahmed MH**, Hassan A. Dexamethasone for the Treatment of Coronavirus Disease (COVID-19): a Review. *SN Compr Clin Med* 2020; **2**: 2637-2646 [PMID: 33163859 DOI: 10.1007/s42399-020-00610-8]

8 **Chen F**, Hao L, Zhu S, Yang X, Shi W, Zheng K, Wang T, Chen H. Potential Adverse Effects of Dexamethasone Therapy on COVID-19 Patients: Review and Recommendations. *Infect Dis Ther* 2021; **10**: 1907-1931 [PMID: 34296386 DOI: 10.1007/s40121-021-00500-z]

9 **RECOVERY Collaborative Group**, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, Staplin N, Brightling C, Ustianowski A, Elmahi E, Prudon B, Green C, Felton T, Chadwick D, Rege K, Fegan C, Chappell LC, Faust SN, Jaki T, Jeffery K, Montgomery A, Rowan K, Juszczak E, Baillie JK, Haynes R, Landray MJ. Dexamethasone in Hospitalized Patients with Covid-19. *N Engl J Med* 2021; **384**: 693-704 [PMID: 32678530 DOI: 10.1056/NEJMoa2021436]

10 **Sethi I**, Shaikh A, Sethi M, Chohan HK, Younus S, Khan SA, Surani S. Dosage and utilization of dexamethasone in the management of COVID-19: A critical review. *World J Virol* 2024; **13**: 95709 [PMID: 39323444 DOI: 10.5501/wjv.v13.i3.95709]

11 **Snow TAC**, Arulkumaran N, Singer M, Choi SH. Effect of dexamethasone dose on outcomes in acute COVID-19 disease: A systematic review and meta-analysis. *J Infect* 2023; **87**: 490-497 [PMID: 37757919 DOI: 10.1016/j.jinf.2023.09.008]

12 **Kow CS**, Ramachandram DS, Hasan SS. Intermediate- to high-dose dexamethasone versus low-dose dexamethasone in patients with COVID-19 requiring respiratory support: a systematic review and meta-analysis of randomized trials. *Inflammopharmacology* 2023; **31**: 2773-2779 [PMID: 37266814 DOI: 10.1007/s10787-023-01251-8]

World Journal of Virology

Submit a Manuscript: https://www.f6publishing.com

World J Virol 2025 March 25; 14(1): 99904

DOI: 10.5501/wjv.v14.i1.99904

ISSN 2220-3249 (online)

EDITORIAL

Convergence of COVID-19 and recurrent stroke: In-hospital mortality risks explored

Basavraj S Nagoba, Shree V Dhotre, Ajay M Gavkare, Sachin S Mumbre, Pradnya S Dhotre

Specialty type: Virology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B
Novelty: Grade B
Creativity or Innovation: Grade A
Scientific Significance: Grade B

P-Reviewer: Ong CT

Received: August 2, 2024
Revised: October 14, 2024
Accepted: October 24, 2024
Published online: March 25, 2025
Processing time: 117 Days and 18.4 Hours

Basavraj S Nagoba, Department of Microbiology, Maharashtra Institute of Medical Sciences and Research (Medical College), Latur 413531, India

Shree V Dhotre, Department of Microbiology, Ashwini Rural Medical College, Solapur 413001, India

Ajay M Gavkare, Department of Physiology, Maharashtra Institute of Medical Sciences and Research (Medical College), Latur 413531, India

Sachin S Mumbre, Department of Community Medicine, Ashwini Rural Medical College, Solapur 413001, India

Pradnya S Dhotre, Department of Biochemistry, Ashwini Rural Medical College, Solapur 413001, India

Corresponding author: Basavraj S Nagoba, PhD, Professor, Department of Microbiology, Maharashtra Institute of Medical Sciences and Research (Medical College), Vishwanathpuram, Ambajogai Road, Latur 413531, India. dr_bsnagoba@yahoo.com

Abstract

This editorial comments on the article by Desai *et al*, which investigates the impact of coronavirus disease 2019 (COVID-19) on in-hospital mortality among patients with recurrent stroke using data from the 2020 National Inpatient Sample. The findings reveal significantly higher mortality rates in COVID-19-positive patients compared to non-COVID-19 patients, particularly among middle-aged individuals, males, and ethnic minorities. This editorial explores the underlying mechanisms contributing to these outcomes and discusses the clinical implications for targeted management strategies in high-risk groups. The results emphasize the need for comprehensive approaches to mitigate the heightened risks faced by recurrent stroke patients during the COVID-19 pandemic.

Key Words: Recurrent stroke; COVID-19; In-hospital mortality; Nationwide analysis; Stroke admissions; Infectious diseases; Chronic health conditions; Hypercoagulability

Core Tip: This editorial highlighted the critical findings of heightened in-hospital mortality risk among recurrent stroke patients with coronavirus disease 2019 (COVID-19). Key findings include significantly higher mortality rates in COVID-19-positive patients, especially among middle-aged individuals, males, and ethnic minorities. The discussion explores the underlying mechanisms, such as inflammation and endothelial dysfunction, contributing to these outcomes. It also underscores the need for targeted management strategies for high-risk groups, emphasizing comprehensive approaches to mitigate risks for recurrent stroke patients during the COVID-19 pandemic. These insights aim to enhance clinical practices and improve patient outcomes in this vulnerable population.

Citation: Nagoba BS, Dhotre SV, Gavkare AM, Mumbre SS, Dhotre PS. Convergence of COVID-19 and recurrent stroke: In-hospital mortality risks explored. *World J Virol* 2025; 14(1): 99904
URL: https://www.wjgnet.com/2220-3249/full/v14/i1/99904.htm
DOI: https://dx.doi.org/10.5501/wjv.v14.i1.99904

INTRODUCTION

The intersection of infectious diseases and chronic health conditions has been starkly highlighted during the coronavirus disease 2019 (COVID-19) pandemic. The study by Desai *et al*[1] provides critical insights into how COVID-19 affects patients with recurrent stroke, a condition already associated with high morbidity and mortality. By analyzing nationwide data from 2020, this study offers a comprehensive overview of the additional risks posed by COVID-19 to this vulnerable population.

Recurrent stroke significantly increases the risk of severe complications and mortality, with nearly 25% of all strokes being recurrent[2]. The emergence of COVID-19 has exacerbated these risks, leading to heightened mortality rates among patients with comorbid conditions, including recurrent stroke[3,4]. Several studies have highlighted the prothrombotic nature of COVID-19, which contributes to increased stroke incidence and severity[5,6]. Desai *et al*[1] utilize data from the National Inpatient Sample to compare in-hospital mortality rates between COVID-19-positive and COVID-19-negative patients with recurrent stroke. The study reveals significantly higher mortality rates in COVID-19-positive patients, particularly among middle-aged individuals, males, and ethnic minorities, aligning with other research indicating disproportionate impacts on these groups[7,8].

This editorial explores the underlying mechanisms contributing to these outcomes, such as inflammation, endothelial dysfunction, and cytokine storms, which exacerbate the severity of recurrent strokes in COVID-19 patients[9,10]. Furthermore, the editorial discusses the clinical implications for targeted management strategies in high-risk groups, emphasizing comprehensive approaches to mitigate these heightened risks during the pandemic.

MECHANISMS OF RECURRENT STROKE ADMISSIONS WITH AND WITHOUT COVID-19 AND ASSOCIATED IN-HOSPITAL MORTALITY

The mechanisms underlying the increased mortality in recurrent stroke patients with COVID-19 are multifaceted, involving a complex interplay of viral pathogenesis and pre-existing cerebrovascular conditions. A study by Desai *et al*[1] highlights several key mechanisms that contribute to this heightened risk. Firstly, COVID-19 is known to induce a hypercoagulable state, which significantly elevates the risk of thrombotic events, including stroke[11]. The virus's ability to cause widespread endothelial injury leads to the activation of the coagulation cascade, resulting in an increased incidence of ischemic strokes. This is particularly detrimental in patients with a history of recurrent stroke, where the vascular system is already compromised[5]. Additionally, systemic inflammation plays a critical role. COVID-19 triggers a robust inflammatory response, often referred to as a cytokine storm, which can exacerbate pre-existing cerebrovascular conditions[12]. Inflammatory cytokines such as interleukin-6, interleukin-1β, and tumor necrosis factor alpha are elevated in COVID-19 patients, contributing to endothelial dysfunction and further increasing the risk of stroke[13]. The study also points to the role of direct viral invasion of the central nervous system (CNS). Severe acute respiratory syndrome coronavirus 2 can enter the CNS *via* the olfactory nerve or hematogenous spread, causing direct neuronal damage and further increasing the risk of neurological complications, including stroke[10]. This mechanism is particularly concerning for patients with a history of recurrent stroke, as their CNS may be more susceptible to viral invasion and subsequent damage. Moreover, the demographic disparities observed in the study by Desai *et al*[1] align with other research indicating that males, middle-aged individuals, and ethnic minorities are disproportionately affected by both COVID-19 and stroke[7]. These populations often have higher prevalence rates of comorbidities such as hypertension and diabetes, which are known risk factors for both stroke and severe COVID-19[8]. Lastly, delayed medical intervention due to overwhelmed healthcare systems during the pandemic has also been a significant factor. Many stroke patients with COVID-19 experienced delays in receiving timely medical care, which is crucial for reducing stroke-related morbidity and mortality[14]. This delay exacerbates the outcomes for recurrent stroke patients, leading to higher in-hospital mortality rates. By understanding these mechanisms, clinicians can develop targeted management strategies to better support recurrent stroke patients during the ongoing COVID-19 pandemic, thereby improving patient outcomes.

CLINICAL IMPLICATIONS

The study's findings have several significant clinical implications for managing recurrent stroke patients during and beyond the COVID-19 pandemic.

Enhanced risk stratification

Clinicians must integrate COVID-19 status into the risk assessment of patients with recurrent stroke. Given the higher mortality risk associated with COVID-19, especially in middle-aged individuals, males, and ethnic minorities, healthcare providers should prioritize rigorous monitoring and management of these high-risk groups.

Targeted interventions

There is a critical need for tailored interventions for recurrent stroke patients who test positive for COVID-19. This includes adjusting treatment plans to address COVID-19-specific complications, such as hypercoagulability and systemic inflammation, which significantly impact stroke outcomes.

Multidisciplinary approach

A collaborative, multidisciplinary approach involving neurologists, infectious disease specialists, and primary care providers is essential for managing patients with concurrent stroke and COVID-19. This team-based strategy can ensure comprehensive care that addresses both the neurological and infectious aspects of the disease.

Improved preventive measures

Emphasizing preventive strategies, such as vaccination against COVID-19 and adherence to stroke prevention protocols, is crucial. Given the higher risk of severe outcomes associated with COVID-19, ensuring that stroke patients are vaccinated and manage their chronic conditions effectively can help reduce mortality and morbidity.

Access to healthcare

Addressing disparities in healthcare access is vital. The study highlights that ethnic minorities and those from lower socioeconomic backgrounds are at higher risk of poor outcomes. Efforts should be made to ensure equitable access to care, including telemedicine options for patients who may face barriers to in-person visits.

Healthcare system adaptations

The increased burden on healthcare systems due to the pandemic necessitates adaptations to manage the dual challenge of recurrent stroke and COVID-19. This includes optimizing hospital resources, streamlining patient pathways, and ensuring adequate support for both acute and long-term care. By addressing these clinical implications, healthcare providers can better manage recurrent stroke patients during the ongoing pandemic, ultimately improving patient outcomes and reducing the overall impact of COVID-19 on this vulnerable population.

CONCLUSION

The study provides critical insights into the exacerbated in-hospital mortality risk for recurrent stroke patients with COVID-19. The findings underscore the severe impact of the pandemic on this vulnerable population, highlighting significant disparities based on age, gender, and ethnicity. COVID-19's role in heightening stroke-related mortality is intricately linked to its induction of hypercoagulability, systemic inflammation, and direct neural damage. These factors, compounded by delayed medical care and prevalent comorbidities, exacerbate outcomes for these patients.

The heightened mortality risk among middle-aged individuals, males, and ethnic minorities necessitates targeted management strategies to mitigate these risks. Implementing comprehensive care protocols that address both COVID-19 and cerebrovascular conditions is crucial. This includes enhancing preventive measures, optimizing treatment approaches, and ensuring equitable access to healthcare. As the pandemic continues, it is imperative for healthcare systems to adapt and refine strategies to improve outcomes for recurrent stroke patients, thereby reducing the adverse impact of COVID-19 on this high-risk group.

FOOTNOTES

Author contributions: Nagoba BS designed the overall concept and outline of the manuscript; Dhotre SV, Gavkare AM, Mumbre SS, and Dhotre PS contributed to the discussion and design of the manuscript; Nagoba BS, Dhotre SV, and Gavkare AM contributed to the writing, and editing and literature review of the manuscript; and all authors contributed to the finalizing the manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country of origin: India

ORCID number: Basavraj S Nagoba 0000-0001-5625-3777; Ajay M Gavkare 0000-0003-4711-5596; Sachin S Mumbre 0000-0002-9169-6001.

S-Editor: Bai Y
L-Editor: A
P-Editor: Guo X

REFERENCES

1 **Desai R**, Mellacheruvu SP, Akella SA, Mohammed AS, Hussain M, Mohammed AA, Saketha P, Sunkara P, Gummadi J, Ghantasala P. Recurrent stroke admissions with vs without COVID-19 and associated in-hospital mortality: A United States nationwide analysis, 2020. *World J Virol* 2024; **13**: 96453 [PMID: 39323442 DOI: 10.5501/wjv.v13.i3.96453]

2 **Benjamin EJ**, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Das SR, Delling FN, Djousse L, Elkind MSV, Ferguson JF, Fornage M, Jordan LC, Khan SS, Kissela BM, Knutson KL, Kwan TW, Lackland DT, Lewis TT, Lichtman JH, Longenecker CT, Loop MS, Lutsey PL, Martin SS, Matsushita K, Moran AE, Mussolino ME, O'Flaherty M, Pandey A, Perak AM, Rosamond WD, Roth GA, Sampson UKA, Satou GM, Schroeder EB, Shah SH, Spartano NL, Stokes A, Tirschwell DL, Tsao CW, Turakhia MP, VanWagner LB, Wilkins JT, Wong SS, Virani SS; American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. *Circulation* 2019; **139**: e56-e528 [PMID: 30700139 DOI: 10.1161/CIR.0000000000000659]

3 **Oxley TJ**, Mocco J, Majidi S, Kellner CP, Shoirah H, Singh IP, De Leacy RA, Shigematsu T, Ladner TR, Yaeger KA, Skliut M, Weinberger J, Dangayach NS, Bederson JB, Tuhrim S, Fifi JT. Large-Vessel Stroke as a Presenting Feature of Covid-19 in the Young. *N Engl J Med* 2020; **382**: e60 [PMID: 32343504 DOI: 10.1056/NEJMc2009787]

4 **Qureshi AI**, Baskett WI, Huang W, Shyu D, Myers D, Raju M, Lobanova I, Suri MFK, Naqvi SH, French BR, Siddiq F, Gomez CR, Shyu CR. Acute Ischemic Stroke and COVID-19: An Analysis of 27 676 Patients. *Stroke* 2021; **52**: 905-912 [PMID: 33535779 DOI: 10.1161/STROKEAHA.120.031786]

5 **Mao L**, Jin H, Wang M, Hu Y, Chen S, He Q, Chang J, Hong C, Zhou Y, Wang D, Miao X, Li Y, Hu B. Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. *JAMA Neurol* 2020; **77**: 683-690 [PMID: 32275288 DOI: 10.1001/jamaneurol.2020.1127]

6 **Avula A**, Nalleballe K, Narula N, Sapozhnikov S, Dandu V, Toom S, Glaser A, Elsayegh D. COVID-19 presenting as stroke. *Brain Behav Immun* 2020; **87**: 115-119 [PMID: 32360439 DOI: 10.1016/j.bbi.2020.04.077]

7 **Yancy CW**. COVID-19 and African Americans. *JAMA* 2020; **323**: 1891-1892 [PMID: 32293639 DOI: 10.1001/jama.2020.6548]

8 **Price-Haywood EG**, Burton J, Fort D, Seoane L. Hospitalization and Mortality among Black Patients and White Patients with Covid-19. *N Engl J Med* 2020; **382**: 2534-2543 [PMID: 32459916 DOI: 10.1056/NEJMsa2011686]

9 **Hess DC**, Eldahshan W, Rutkowski E. COVID-19-Related Stroke. *Transl Stroke Res* 2020; **11**: 322-325 [PMID: 32378030 DOI: 10.1007/s12975-020-00818-9]

10 **Baig AM**. Chronic COVID syndrome: Need for an appropriate medical terminology for long-COVID and COVID long-haulers. *J Med Virol* 2021; **93**: 2555-2556 [PMID: 33095459 DOI: 10.1002/jmv.26624]

11 **Tang N**, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost* 2020; **18**: 844-847 [PMID: 32073213 DOI: 10.1111/jth.14768]

12 **Goshua G**, Pine AB, Meizlish ML, Chang CH, Zhang H, Bahel P, Baluha A, Bar N, Bona RD, Burns AJ, Dela Cruz CS, Dumont A, Halene S, Hwa J, Koff J, Menninger H, Neparidze N, Price C, Siner JM, Tormey C, Rinder HM, Chun HJ, Lee AI. Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. *Lancet Haematol* 2020; **7**: e575-e582 [PMID: 32619411 DOI: 10.1016/S2352-3026(20)30216-7]

13 **Mehta P**, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ; HLH Across Speciality Collaboration, UK. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 2020; **395**: 1033-1034 [PMID: 32192578 DOI: 10.1016/S0140-6736(20)30628-0]

14 **Kansagra AP**, Goyal MS, Hamilton S, Albers GW. Collateral Effect of Covid-19 on Stroke Evaluation in the United States. *N Engl J Med* 2020; **383**: 400-401 [PMID: 32383831 DOI: 10.1056/NEJMc2014816]

World Journal of Virology

Submit a Manuscript: https://www.f6publishing.com

World J Virol 2025 March 25; 14(1): 100489

DOI: 10.5501/wjv.v14.i1.100489

ISSN 2220-3249 (online)

REVIEW

Pathogenesis and clinical management of arboviral diseases

Victoria Cenci Dietrich, Juan Marcos Caram Costa, Marina Maria Gomes Leite Oliveira, Carlos Eduardo Oliveira Aguiar, Luis Guilherme de Oliveira Silva, Marcel Silva Luz, Fabian Fellipe Bueno Lemos, Fabrício Freire de Melo

Specialty type: Virology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification
Scientific Quality: Grade A
Novelty: Grade A
Creativity or Innovation: Grade B
Scientific Significance: Grade A

P-Reviewer: Phuong TTT

Received: August 18, 2024
Revised: October 1, 2024
Accepted: October 28, 2024
Published online: March 25, 2025
Processing time: 102 Days and 0.8 Hours

Victoria Cenci Dietrich, Juan Marcos Caram Costa, Marina Maria Gomes Leite Oliveira, Carlos Eduardo Oliveira Aguiar, Luis Guilherme de Oliveira Silva, Marcel Silva Luz, Fabian Fellipe Bueno Lemos, Fabrício Freire de Melo, Instituto Multidisciplinar em Saúde, Universidade Federal da Bahia, Vitória da Conquista 45029-094, Bahia, Brazil

Co-corresponding authors: Fabian Fellipe Bueno Lemos and Fabrício Freire de Melo.

Corresponding author: Fabrício Freire de Melo, PhD, Assistant Professor, Instituto Multidisciplinar em Saúde, Universidade Federal da Bahia, 58 Rua Hormindo Barros, Quadra 17, Lote 58, Vitória da Conquista 45029-094, Bahia, Brazil. freiremeloufba@gmail.com

Abstract

Arboviral diseases are viral infections transmitted to humans through the bites of arthropods, such as mosquitoes, often causing a variety of pathologies associated with high levels of morbidity and mortality. Over the past decades, these infections have proven to be a significant challenge to health systems worldwide, particularly following the considerable geographic expansion of the dengue virus (DENV) and its most recent outbreak in Latin America as well as the difficult-to-control outbreaks of yellow fever virus (YFV), chikungunya virus (CHIKV), and Zika virus (ZIKV), leaving behind a substantial portion of the population with complications related to these infections. Currently, the world is experiencing a period of intense globalization, which, combined with global warming, directly contributes to wider dissemination of arbovirus vectors across the globe. Consequently, all continents remain on high alert for potential new outbreaks. Thus, this review aims to provide a comprehensive understanding of the pathogenesis of the four main arboviruses today (DENV, ZIKV, YFV, and CHIKV) discussing their viral characteristics, immune responses, and mechanisms of viral evasion, as well as important clinical aspects for patient management. This includes associated symptoms, laboratory tests, treatments, existing or developing vaccines and the main associated complications, thus integrating a broad historical, scientific and clinical approach.

Key Words: Arboviruses; Arbovirus infections; Dengue; Zika virus; Yellow fever; Chikungunya virus; Clinical diagnosis; Pathogenesis; Flavivirus; Togaviridae infections

Core Tip: This review delves into the historical characteristics, pathogenesis, and clinical management of the four major arboviruses that have triggered outbreaks worldwide: Dengue, Zika, yellow fever, and chikungunya fever. It aims to elucidate the viral characteristics, cellular tropism, and immune evasion mechanisms, as well as the primary clinical manifestations and their complications, laboratory diagnosis, treatment, prevention, and vaccines either currently available or under development. Thus, with a focus on the medical and scientific fields, this review enables the reader to acquire comprehensive and generalized knowledge about each of these arboviruses.

Citation: Cenci Dietrich V, Costa JMC, Oliveira MMGL, Aguiar CEO, Silva LGO, Luz MS, Lemos FFB, de Melo FF. Pathogenesis and clinical management of arboviral diseases. *World J Virol* 2025; 14(1): 100489
URL: https://www.wjgnet.com/2220-3249/full/v14/i1/100489.htm
DOI: https://dx.doi.org/10.5501/wjv.v14.i1.100489

INTRODUCTION

Arboviruses are an extensive group of viruses that have arthropods (insects and arachnids) as their primary vectors, transmitting the viruses to vertebrate hosts, such as humans, through their bites along with their saliva[1]. These infections are endemic to tropical and subtropical regions, where approximately 3.9 billion people live today, disproportionately affecting the poorest populations[2]. However, with rising temperatures due to global warming, there is a greater spread of the main urban vectors of arboviruses worldwide, such as mosquitoes of the genus *Aedes* [*Aedes aegypti* (*Ae. aegypti*) and *Ae. Albopictus*] and *Culex* [*Culex pipiens* (*Cx. Pipiens*), *Cx. quinquefasciatus*, and *Cx. tarsalis*], as these vectors tend to develop better in warm and humid environments[1,3-6]. Consequently, there is an increasing need for global collaborative efforts to prevent arbovirus outbreaks from becoming more common, with the first step in prevention being a better understanding of their main viral agents.

Currently, the main arboviruses with the potential to cause worldwide outbreaks are the dengue virus (DENV), with an estimated 96 million symptomatic cases and 40000 annual deaths in more than 129 countries[2], in addition to the Zika virus (ZIKV), the yellow fever (YFV) virus, and the chikungunya (CHIKV) virus (viruses which also have the capacity to generate morbidity and mortality). All these diseases have epidemic potential, as evidenced by their significant global outbreaks in the last decades[7,8].

According to the World Health Organization (WHO), arboviruses are responsible for 17% of all infectious diseases and cause approximately 700000 deaths annually worldwide[2], directly affecting the healthcare systems of developing and even developed countries. These diseases can overcrowd hospitals during outbreak periods and cause chronic complications in infected patients, leading to increased healthcare costs and considerable social damages. These impacts are further exacerbated by the limited knowledge about the viral characteristics and the suitable clinical management. Thus, this article aims to elucidate the pathogenic mechanisms of each of the four main arboviruses, with the intention of understanding aspects related to viral characteristics, tropism, immune response, and viral evasion, as well as the main symptoms, possible complications, treatment, vaccines, and prevention, highlighting the main points of appropriate patients' clinical management.

DENGUE FEVER

DENV is a positive-sense single-stranded RNA virus that belongs to the *Flavivirus* genus within the *Flaviviridae* family. There are four known serotypes spread globally: DENV-1, DENV-2, DENV-3, and DENV-4. Its RNA encodes 10 proteins, including three structural: Capsid (C), membrane (prM/M), and envelope (E); and seven non-structural, associated with RNA replication: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5[9,10].

DENV is primarily transmitted by *Ae. aegypti* and *Ae. albopictus* mosquitoes[11]. Initially, *Ae. aegypti* was thought to be the sole vector capable of causing significant outbreaks. However, recent research indicates that *Ae. albopictus* also plays a significant role in sustaining large outbreaks and has contributed to the resurgence of DENV, particularly in Southeast Asia[12]. Dengue fever, caused by DENV infection, is considered the most important mosquito-borne viral disease, spread globally[13], with 50-100 million people infected yearly[14]. Dengue incidence has started to notoriously increase since 1990[15], and numerous social and economic factors might explain this phenomenon[9], as well as global warming, seeing that it can contribute to the re-emergence of DENV by expanding the geographical range of mosquitoes and increasing infection rates[16].

Although most dengue cases present as mild or asymptomatic (anorexia, retro-orbital pain, myalgia and rash), approximately 5% progress to a more severe form[17], primarily attributed to immunological factors such as the cytokine storm and antibody-dependent enhancement[18], that cause an exacerbation of the inflammatory process, leading to increased vascular permeability and a risk for hemorrhage and shock[19], usually followed by persisting vomiting and abdominal pain, as well as other various warning signs[20]. Such severe cases are mostly seen in heterotypic secondary infections (infection with a different serotype) but can also occur primarily in children who inherit immunity from their mothers[21] correlating with an impaired adaptative immune response. Even though hard to predict, certain authors argue that elevated viremia is correlated with severity[22], while others refute this idea[23], and instead propose evaluating factors

like viral NS1 protein levels or host cytokine expression[24].

This complex host-pathogen interaction occurs within the context of the DENV's cyclical transmission process, involving human-mosquito and mosquito-human transmission. It is not very easy to predict sporadic outbreaks, as around 80% of DENV transmission occurs *via* asymptomatic hosts[25]. In this process, healthy mosquitoes acquire the virus by feeding on the blood of an infected host, and once the virus has infiltrated the vector's tissues, it becomes infected for life, and capable of transmitting the virus[26]. When an infected vector feeds on a naive host's blood, mosquito's saliva containing the virus is inoculated into the skin, initiating the infection.

Pathogenesis of dengue

Viral entry, replication, and release: Upon injection into the skin, cells of the monocyte lineage, such as macrophages, Langerhans cells and dendritic cells are among the primary targets[27]. Other potential targets include endothelial and epithelial cells, lymphocytes, hepatocytes, fibroblasts and keratinocytes[28] with reports of viral presence in various tissues, including the dermis (skin), blood, bone marrow, lymph nodes, liver and occasionally, the brain[29]. Cellular internalization of DENV occurs through envelope protein (E) interaction with numerous different receptors, including heparan sulfate proteoglycans[30], DC-Sing[31], heat shock protein (HSP) 70 and HSP90[32], triggering receptor-mediated endocytosis, that can be dependent or independent of clathrin. Fc and C1q receptors can also facilitate entry *via* antibody-mediated pathway[33]. Additionally, alternative entry pathways such as diffusion and macropinocytosis have been reported. The specific entry route for DENV also varies depending on the virus serotype[34,35].

Subsequently, the virus's E protein undergoes irreversible conformational changes due to the endosome's low pH, leading to fusion with the endosomal membrane[36], facilitated by hydrophobic peptides[36,37]. Following its release, the viral genome acts as a mRNA and gets translated into a single polypeptide, later processed and cleaved by host and viral proteases, resulting in the ten viral proteins. The seven non-structural proteins form a replication complex through invagination of the endoplasmic reticulum (ER) membrane, facilitating RNA replication and protecting viral particles from the host's innate immunity. This process relies on RNA-RNA, RNA-protein, and protein-protein interactions[38], with significant roles observed regarding NS3 and NS5[39,40].

During this process, numerous viral mechanisms have been observed to take place in order to maximize replication, including reprogramming protein synthesis in host cells to favor viral RNA translation[41], regulating cellular apoptosis through interaction between NS5 protein and the mechanistic target of rapamycin complex 2[42], binding between NS1 and Beclin 1[43], and maximizing ATP production by promoting anaerobic glycolysis *via* the hypoxic response[44] or activating lipophagy to enhance β-oxidation[45].

Once enough proteins are synthesized and the virion is assembled, it is again processed by the Golgi apparatus changing its surface from spiky to smooth[9,46]. Then, mature virions are secreted from the infected cell along with NS1 hexamers, that play a crucial role in the disease pathogenesis and severity[47].

Innate response and evasion: The innate immune response to DENV initiates upon the first cell infection and progresses concurrently with viral replication and release, rather than following these events[48]. Not surprisingly, the virus has developed multiple strategies to evade the host's innate response, primarily through its non-structural proteins[49].

When a dengue virion infects its initial target host cells, it activates pattern recognition receptors (PRRs) such as retinoic acid-inducible gene 1 (RIG1), melanoma differentiation-associated protein 5 (MDA5), toll-like receptor 3 (TLR-3), and TLR-7[50]. This sets off the signaling cascade of the innate response, leading to the release of cytokines and ultimately resulting in the expression of type I and III interferons (IFNs), recruitment of additional monocytes, and activation of the complement system[49]. Meanwhile, infected dendritic and Langerhans cells migrate to the lymph nodes and present viral antigens, initiating the adaptive response[51]. If successful, the antiviral response will counteract viral replication and infection. However, DENV evades it through its non-structural proteins (NS1 to NS5), which can disrupt receptor signaling, IFN synthesis, and regulation pathways[52-55]. Additionally, DENV's NS1 protein can inhibit the complement response by interacting with its protein complexes[56].

In a more passive way, DENV evades the immune response by forming the replication complex, that serves as a "barrier"[40], and by regulating cellular apoptosis, a common mechanism used by the immune system to suppress viral replication[57]. Furthermore, DENV's capability of infecting defense cells is itself a mean of dysregulating the host's antiviral response.

Despite its notable role in dengue pathogenesis, the innate response is not typically singled out as a factor in dengue severity, whereas the adaptive immune response has been pointed as the primary contributor, due to impaired immune responses against different serotypes[58].

Adaptative response and antibody-dependent enhancement: The adaptive immune response to DENV begins with antigen-presenting cells (APCs) presenting viral antigens to T cells in lymph nodes. This activation leads to differentiation of T lymphocytes into effector and memory T cells, providing long-term immunity against infections with the same serotype, but only short-term immunity against heterotypic infections[59]. DENV has shown capability of interacting with numerous factors regarding antigen-specific immunity, such as priming and activation of T and B cells[60], antibody production and neutralization properties, along with cytokine release[61]. The highlighted factors have a significant impact on the disease pathogenesis and will be further discussed.

DENV is able to impair T lymphocyte priming and activation either by antigenic variation or inducing apoptosis of APCs[62]. This leads to a compromised T cell response, regarding both CD8+ direct targeting of infected cells and CD4+ differentiation into helper T cells, the ladder being related to activation of B cells and antibody production. Anti-DENV neutralizing antibodies primarily target specific regions of the E and C proteins[63,64], and can also target the NS proteins, especially NS1[65]. However, genomic variations within a single serotype can cause alteration of these antigens,

disrupting the immune response even in homotypic infections[49]. Moreover, the antibody response to different DENV serotypes is less effective and can enhance viral infection through a process called antibody-dependent enhancement[66].

Antibody-dependent enhancement occurs when antibodies bind to the virus but fail to neutralize it, forming an antibody-virus complex[67]. This phenomenon is exacerbated by the original antigenic sin, where antibodies from the primary infection are more prevalent than those from the secondary infection. The antibody-virus complex can more efficiently enter monocytes by interacting with Fc and C1q receptors and triggering endocytosis, enhancing viral replication and dissemination[68]. This pathway also correlates with an increased inflammatory response by inhibiting the release of anti-inflammatory cytokines[69] and suppressing antiviral innate mediators[70]. Incomplete cleavage of the prM protein can also cause binding to prM antibodies and promote antibody-dependent enhancement to immature virions[71].

Recent studies have also demonstrated the potentiality of autoimmunity in dengue pathogenesis. Anti-NS1 antibodies can interact with endothelial cells (ECs), triggering inflammatory cytokine signaling and cellular apoptosis[72], and autoantibodies targeting ECs and platelets have also been observed, correlating to the increased vascular permeability seen in secondary dengue infections[73,74].

Overall, the adaptive immune response is a significant factor in dengue severity during secondary infections, especially regarding the process of antibody-dependent enhancement. These events, related to the evasion of the adaptive response, ultimately result in increased inflammatory process and vascular permeability, which are the main causes of dengue severity.

Cytokine storm and vascular permeability: Cytokine release is a common physiological event in the immunological system[75]; however, the exacerbated release of cytokines is one of the main events in severe cases of dengue. It is mostly observed in secondary infections[76], correlating with the impairment of the immune response. The molecular mechanisms through which the cytokine storm relates to dengue severity are still poorly understood, but numerous *in vitro* and *in vivo* experiments with animal models, as well as observational studies, have been conducted to better understand the role of different cytokines.

Events of the cytokine storm can be triggered by viral infection of both leukocytes and ECs[77], but also by the interaction of the NS1 protein with receptors such as TLR-4[78,79]. High levels of cytokines such as interleukin 1 (IL-1), IL-4, IL-8, IL-10, IL-13, and IL-17, as well as C-X-C motif chemokine ligand 10 (CXCL10), tumor necrosis factor-alpha (TNF-α), vascular endothelium growth factor A (VEGFA), macrophage migration inhibiting factor (MIF), IFN-β, and IFN-γ have largely associated with disease severity[61,80]. These cytokines are closely related to the increase of vascular permeability and subsequent plasma leak, and also to disrupting tight junctions[81] and inducing autophagy[82], with *in vitro* studies suggesting more specific roles of different cytokines, such as MIF's role in EC glycocalyx degradation[83,84] and the roles of CXCL10, VEGFA, and TNF-α in increased permeability and EC apoptosis[80,85,86]. For instance, a recent longitudinal study conducted by Bhatt *et al*[87] suggests that the analysis of cytokine levels in patients can be useful in predicting dengue severity.

Interestingly, anti-inflammatory cytokines such as IL-4 and IL-10 have been shown to paradoxically contribute to more inflammation, as they can suppress the immune response and impair viral clearance, increasing viral dissemination[88]. Moreover, cytokines that recruit more leukocytes to the site of inflammation, such as IL-8, IFN-γ, MIF, TNF-α, and CXCL10, can contribute to more infection and viral replication, since DENV has a tropism for these types of cells[89].

In summary, the cytokine storm is amplified during DENV infection due to a process of positive feedback, in which the release of cytokines and recruiting of leukocytes leads to even more inflammation. This ultimately results in events of vascular leak, which are maintained and amplified by DENV-induced coagulopathy and thrombocytopenia[90].

Coagulopathy and thrombocytopenia: Besides inducing vascular leak through endothelial damage and a cytokine storm, DENV employs numerous mechanisms to disrupt coagulation, which is meant to contain plasma leakage. Such events can occur *via* activating and dysregulating coagulation pathways, and also impairing various steps of the coagulation process[91].

In vitro and *in vivo* studies have demonstrated multiple ways DENV induces coagulopathy and thrombocytopenia. In bone marrow, the virus has been shown to suppress its activity and interfere with megakaryocyte maturation, either directly through infection and interaction with E protein[92] or indirectly *via* cytokines[93]. Apart from affecting megakaryopoiesis, early activation through DENV NS1 binding to TLR-4 on platelets can potentially cause apoptosis[79], platelet phagocytosis by macrophages and dysregulated clot formation[94], leading to platelet 'waste' and thrombocytopenia. DENV infection can also promote the production of cross-reactive antibodies that target platelets[95] and coagulation factors such as thrombin and plasminogen[73], resulting in imbalanced coagulation and fibrinolysis, ultimately leading to amplified vascular permeability. A simplified schematic representation of the discussed immunological aspects of dengue pathogenesis can be seen in Figure 1.

All of the highlighted aspects act dynamically and synergetically, ultimately resulting in increased vascular leak and risk for hemorrhage, correlating with dengue's clinical manifestations and major complications.

Clinical management of dengue

Clinical manifestations: Most cases of dengue infection are asymptomatic, but some patients can manifest symptoms after an incubation period of 3-15 days. Traditionally, dengue was classified into the stages of dengue fever (DF), dengue hemorrhagic fever, and dengue shock syndrome (DSS)[96], with plasma leakage identified as the main factor to disease severity[97]. However, in 2009, the WHO published an update, categorizing the disease as "dengue without warning signs" (DWS-), "dengue with warning signs" (DWS+), and "severe dengue" (SD), aiming to improve on the limitations of the previous classification and broaden the assessment and management of warning signs. Unusual manifestations and

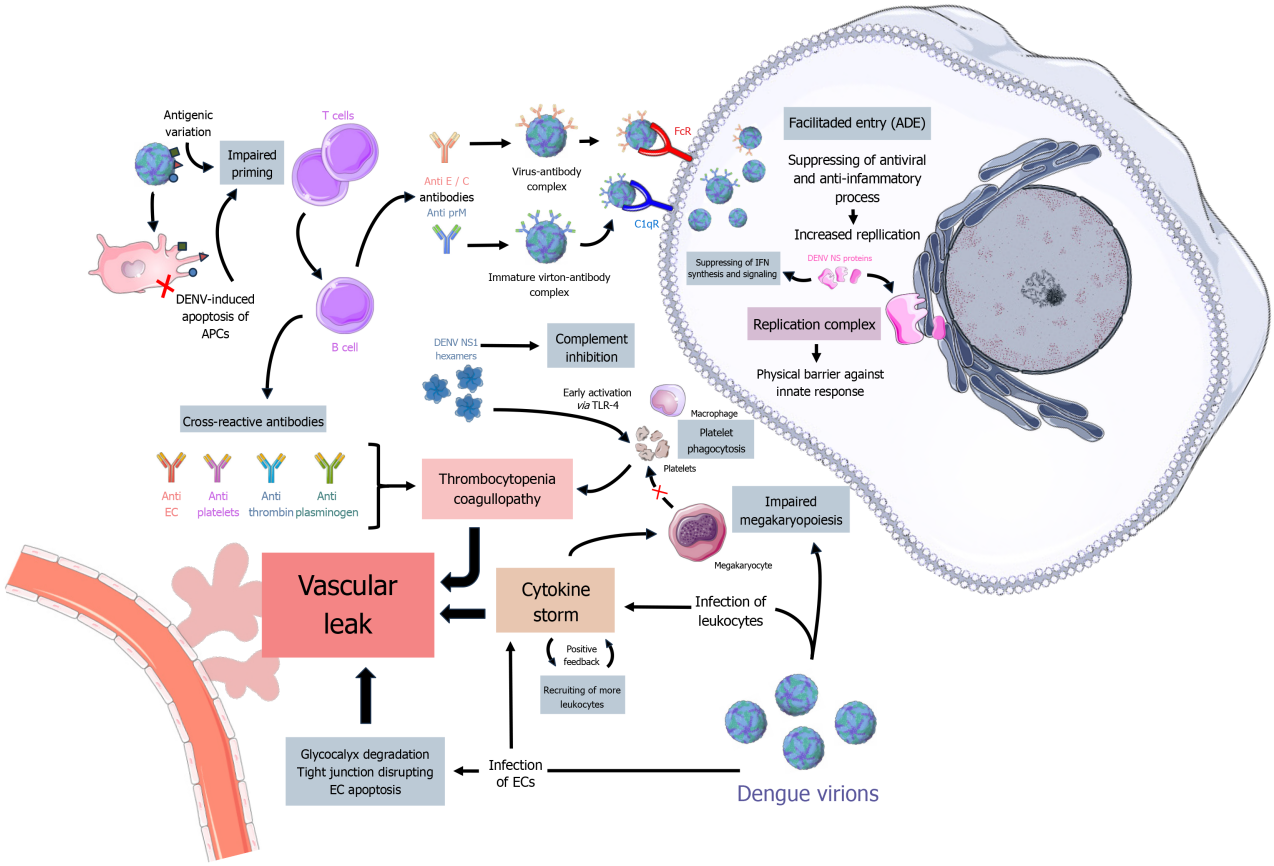


Figure 1 Simplified scheme of dengue pathogenesis' immunological aspects. APCs: Antigen-presenting cells; FcR: Fc receptor; C1qR: C1q receptor; ADE: Antibody-dependent enhancement; IFN: Interferon; TLR-4: Toll-like receptor 4; ECs: Endothelial cells. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license (Supplementary material).

involvement of various body systems beyond typical dengue symptoms are categorized under "extended dengue" (ED) [98]. Due to its importance and recent resurgence, the specific guidelines for dengue identifying and management have been reinforced worldwide, with the publishing of management manuals by important health organs.

Dengue presents dynamically with an abrupt onset of symptoms. It starts with a febrile phase, usually exceeding 38 °C [99] and lasting for 2-7 days, followed by symptoms such as anorexia, retro-orbital pain, myalgia, arthralgia, diarrhea, nausea, vomiting[100] and a maculopapular rash in 50% of cases, spreading from the face to the limbs[99]. The early phase of the disease can be nonspecific and challenging to differentiate from other febrile illnesses, especially arboviral diseases. In such cases, if the epidemiological factors are compatible, dengue management measures are recommended even if laboratory diagnosis has not been obtained, due to its potential for severe complications[99,101].

Most patients experience defervescence and recover within 3-7 days[100]. However, some patients may progress to a critical phase following fever reduction, characterized by increased plasma leakage. Symptoms can include abdominal pain, persistent vomiting, lethargy, bleeding tendencies, and fluid accumulation in the lungs (pleural effusion) or abdominal cavity (ascites) as well as disseminated intravascular coagulation[100]. Laboratory findings during this stage typically reveal an increase in hematocrit, thrombocytopenia, and leukopenia[102]. The critical phase usually lasts about 48 hours, with peak vascular leakage occurring approximately 24 hours after onset. Said vascular leak can lead to a hypovolemic shock, causing metabolic acidosis and multisystemic impairment, potentially fatal within only 24 hours or less, if left untreated[98,99].

Patients in use of non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen or aspirin may present instances of hemorrhage that are not directly related to thrombocytopenia[99], but to reduced thromboxane A2 synthesis *via* cyclooxygenase-1 inhibition[103]. Furthermore, ED presentations may include neurological, cardiac, and renal inflammation and dysfunction, that may present as encephalitis[104] myocarditis[105,106] and acute kidney failure[107]. Such complications have been documented in literature and require careful analysis and more specific interventions, although less common[108,109].

As of the listed symptoms, abdominal pain, persistent vomiting (three occurrences per hour or four occurrences in six hours), and mucosal bleeding are categorized as warning signs for DWS+, along with lethargy or restlessness, hepatomegaly (greater than 2 cm below the costal margin), increased hematocrit (observed in two consecutive measurements), and a decrease in platelet count (below 100000 cells/mm^3)[98,110]. These signs indicate a higher risk of developing severe dengue and necessitate careful monitoring.

If the patient survives the critical phase, the recovery is characterized by gradual improvement in well-being, return of appetite, fluid reabsorption, and hemodynamic stabilization. Additionally, hematocrit levels decrease and white blood

cell count will increase, in a returning to a normal state. However, it is important to note that DSS can also promote these hematological manifestations as a stress response[98], therefore, a careful analysis of these variables, alongside other clinical presentations, is crucial. Nonetheless, it is not unusual for recovering patients to present bradycardia, pruritus, polyuria, and islands of pallid areas in between the rash, indicating vascular recovery[98,99].

Diagnosis: Dengue diagnosis is crucial for decision-making in the management of suspected patients. It should be conducted carefully, considering the particularities of each specific case and context. In endemic areas, diagnosis can be based solely on clinical findings and the epidemiological context, not always requiring laboratory testing[99]. However, specific testing is also a very important tool in diagnosis, especially in cases with warning signs.

If a patient is suspected of dengue infection, tracking potential exposure by evaluating the presence of infected family members and recent travelling to endemic areas is crucial[98,99]. Vital signs, level of consciousness, and hemodynamic state must be assessed, as well as noting the presence of the most common dengue symptoms, discussed earlier (see "Clinical Manifestations"). A positive tourniquet test, along with other symptoms, suggests dengue infection, although it can yield negative results in obese patients or those already in shock, and should not be the only diagnostic tool[99,111]. All these factors should be paired with analysis of complementary laboratory findings, paying special attention to leukopenia, which has been shown to accurately indicate dengue infection[112]. It is extremely important to monitor for warning signs in newly admitted patients to promptly manage complications.

When specific testing is needed, the current stage of the disease and available resources should be considered. Up to 5 days from symptom onset, reverse transcription-polymerase chain reaction (RT-PCR) can be used, but as of requiring specialized work, it is usually not the first option[113]. Given that the acute viremic phase is sometimes missed because patients may seek medical assistance only after symptoms worsen[114], detection of NS1 antigen and IgM antibodies using enzyme-linked immunosorbent assay (ELISA), immunochromatographic assay or rapid diagnostic test kits can be useful. NS1 antigen levels are present from the beginning and typically peak around 6-10 days after disease onset[115], while IgM antibodies begin to rise after 6-14 days. These tests are simpler and provide quicker results, although they may cross-react with other flaviviruses[116]. In secondary infections, NS1 detection shows limited sensitivity[117], and IgM testing is complicated due to rapid IgG rise[114]. Distinguishing between primary and secondary infections is crucial not only for diagnostic purposes but also due to the risk of developing a more severe disease.

Therefore, detection of viral genome by RT-PCR is mostly recommended up until the first 5 days of symptoms, whereas detecting NS1 antigen and IgM antibodies with ELISA can be more effective for patients that have exceeded this mark, with higher serological levels at around 6-10 and 6-14 days, respectively[115-117]. Additionally, if a secondary infection is suspected, IgM and IgG detection can be conducted concomitantly for more specific results[116]. For every case, it is always important to pair laboratory findings with clinical and epidemiological factors in order to reassure the diagnosis.

Besides the most commonly used in clinical practice, the plaque reduction neutralization test (PRNT) is considered the gold standard for identifying DENV antibodies, seeing it is highly specific and can distinguish serotypes. However, it is not routinely used for clinical purposes, as it requires samples from both the febrile and convalescent phases with a minimum interval of 14 days, as well as specialized materials and professionals[99,118]. It is primarily used for research and surveillance purposes.

Treatment: Currently, there is no antiviral drug available for dengue, so disease treatment focuses on symptomatic control and managing complications. Patients can be categorized into groups for more specific management decisions based on their disease status, although specific guidelines for categorization may vary. The WHO categorizes patients as follows: DWS- (Group A); DWS+ and DF with co-existing risk factors or important social circumstances (Group B); and severe dengue (Group C)[98]. In contrast, the Brazilian dengue management manual categorizes patients as: Absence of warning signs (Group A); absence of warning signs but with spontaneous bleeding (Group B); presence of warning signs and/or risk factors (Group C); and presence of signs of shock, severe bleeding or organ disfunction (Group D)[99]. The Brazilian manual also emphasizes that a suspected case is enough for taking the according measurements, even if laboratory testing has not yet been done[99]. Although not always equal, the division into groups is important in order to optimize the use of hospital beds, especially in the context of large regional outbreaks, as well as how to specifically manage the disease complications. Even then, an individualized analysis to each context is important.

Patients without warning signs can be sent home, prescribed oral hydration, and advised to seek the nearest hospital if any warning signs appear. Paracetamol (acetaminophen) is usually the preferred drug for pain and fever control[98], though metamizole is also widely used in Latin American countries[99,119]. NSAIDs are not recommended, as they can increase the risk of internal bleeding and Reye's syndrome[100].

When warning signs are present and patients are hospitalized, intravenous fluid replacement is important to prevent further complications. If shock occurs, fluid resuscitation is necessary. Crystalloid solutions are primarily used, but if shock persists and progresses to a hypotensive state, a colloid solution may be required[98,99]. Careful attention to fluid administration is crucial, as fluid overload can cause or exacerbate complications. For that, laboratory indicators should be closely monitored, and IV fluid therapy should not exceed 48 hours[99,120].

There is a lack of consensus and clear evidence about some therapeutic measurements for dengue disease. Prophylactic platelet transfusion, for example, is sometimes conducted, but some procedures seem to be inappropriate[121]. Randomized trials and observational studies have shown that there is no evidence of prophylactic transfusion in bleeding prevention[122], and that unnecessary transfusions can be harmful[122]. Still in regard to bleeding, the WHO emphasizes that patients in use of anticoagulant therapy have a higher risk of severe hemorrhage[98], but no clear instructions are given to manage this situation. Temporary suspending of these medications by specific evaluation of clinicians appears to be safe, but more studies are needed to further specify this action[123].

Due to the immunological aspects of dengue, the idea of using corticosteroids to prevent severe cases pops to mind. These drugs could supposedly be useful in the intermediate phase of the disease, seeing that is when the immune system plays the bigger role in the pathogenesis[124]. However, there is no indication for the use of corticosteroids, as most studies conducted present inconclusive results or low-quality evidence[125]. All the discussed aspects highlight the importance of a careful and individualized analysis of each patient and case, even when following specific guidelines, as it is very hard to make general affirmations[124].

The development of drugs that target dengue structural and non-structural proteins emerges as a possibility for controlling viral entry and replication in hosts. Several *in vitro* and *in silico* studies have shown potentiality in this activity [126] but it is still hard to further correlate with clinical practice, as the available animal models for testing lack a mirroring to disease severity as seen in humans[127,128]. Targeting host cellular receptors that facilitate viral entry is also a possibility, but cytotoxicity should be taken into consideration, as these receptors also serve a purpose to the host[129].

Prevention: Various prevention methods have been implied as an attempt of controlling DENV vectors, encompassing biological, chemical and environmental techniques[130]. However, they present some serious issues that impair their capacity of controlling the disease transmission.

The releasing of mosquitoes infected with *Wolbachia,* as well as the sterile insect technique was believed to be an effective way of vector control for *Ae. aegypti* and *Ae. albopictus,* but further analysis revealed that by eliminating larvae competition, they could increase rates of surviving adults[131]. Trying to eliminate reproduction sites and using pesticides seem to not be very effective[132] and can lead to selection of resistant mosquitoes, while also being potentially harmful for the environment and for humans[133]. Moreover, behavioral methods such as the use of repellents and protective nets are usually recommended, but as of being dependent of community involvement, may not be effective in large scale[9]. The discussed topics do not mean that these attempts of vector control should not be implied at all, but they highlight the need for the development of effective vaccines.

Vaccines: The development of vaccines for dengue is specially difficulted due to the phenomenon of antibody-dependent enhancement[58]. Several different types, such as live attenuated, inactive virus, viral vector and DNA vaccines are now in development[134], but only live-attenuated vaccines have made into phase III trials, as registered in the ClinicalTrials.gov website, including DengVaxia© (CYD-TDV) by Sanofi Pasteur, QDenga© (TAK-003) by Takeda, the only approved and commercialized dengue vaccines as of today, and also a vaccine produced by Butantan Institute, not yet commercialized. These studies aim to evaluate the safety of dengue vaccines, either by themselves or concomitantly with other vaccines (Table 1).

DengVaxia© was the first dengue vaccine to be licensed. It is based on a YFV strain (YF17D) but with the prM and E regions substituted with those from DENV serotypes 1-4[135]. Conducted clinical trials have demonstrated that DengVaxia has a higher effectiveness against DENV-3 and DENV-4, than to the other viral serotypes[136]. As of today, the vaccination with DengVaxia© is restricted to children in age 9-16 that have been previously infected[137] although testing each individual for the confirmation of a previous dengue occurrence seems impracticable, seen that, after three years, the immunization with this vaccine has shown to increase hospitalization rates in dengue naive patients[138].

QDenga©, on the other hand, is based on a DENV-2 strain, with recombinant strains of the other 3 serotypes[139]. A phase III trial with patients across 8 endemic countries has demonstrated, after 4.5 years, that QDenga© is effective and safe for all 4 DENV serotypes in patients that have been previously infected, but only shows effectiveness against DENV-1 and DENV-2 for dengue naive patients[140]. However, there is no evidence of Qdenga© vaccinees having a higher risk of developing a severe illness, in contrast to what is observed to DengVaxia©. Considering that DengVaxia© is only capable of inducing anti-DENV antibodies against E and prM proteins, that might explain why QDenga© has a higher effectiveness, and also why DengVaxia© vaccinees can develop a higher risk of severity, seen that E and prM proteins are the ones involved in antibody-dependent enhancement.

ZIKA FEVER

ZIKV is an enveloped virus that upon infecting humans, primarily through the human-mosquito-human route, consequently causes the Zika fever[141].

This virus possesses a single-stranded, positive-sense RNA genome. It encodes proteins associated with the capsid (C), envelope (E), precursor of membrane protein (prM), and non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). Initially, these proteins are translated into a single polyprotein (5'-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3'), which is subsequently cleaved[142,143].

The envelope protein (E) is a surface protein essential for the adsorption and fusion of the viral envelope with the host cell's plasma membrane[144]. Consequently, it is the primary target for the majority of vaccines in development, as it elicits an innate immune response, triggering the neutralizing antibodies production (nAbs). These vaccines commonly utilize an alignment of the prM and E sequences (prM-E), which has already demonstrated favorable results, generating high levels of nAbs in both immunocompetent and immunocompromised mice[145-147].

The precursor of membrane protein (prM) plays a role in viral maturation and release from cells[148]. Therefore, its inhibition also yields a beneficial response by reducing viral multiplication in tissues. Additionally, the non-structural proteins are involved in the replication of the ZIKV and inhibit the expression of IFN I. As a result, they ensure a less effective immune response[149].

Table 1 Dengue live-attenuated vaccines phase III clinical trials-ClinicalTrials.gov database

Vaccine	Intervention/treatment	ClinicalTrials.gov ID	Status	Sponsor
DengVaxia©	CYD tetravalent dengue vaccine/human papillomavirus quadrivalent vaccine	NCT02993757	Completed	Sanofi Pasteur
	CYD tetravalent dengue vaccine/human papillomavirus bivalent vaccine	NCT02979535	Completed	Sanofi Pasteur
	CYD tetravalent dengue vaccine/yellow fever vaccine	NCT01436396	Completed	Sanofi Pasteur
	CYD tetravalent dengue vaccine/pentaxim™ vaccine	NCT01411241	Completed	Sanofi Pasteur
	Placebo/CYD tetravalent dengue vaccine	NCT01374516	Completed	Sanofi Pasteur
	Placebo/CYD tetravalent dengue vaccine	NCT01373281	Completed	Sanofi Pasteur
	Placebo/CYD tetravalent dengue vaccine	NCT01254422	Completed	Sanofi Pasteur
	Placebo/CYD tetravalent dengue vaccine	NCT01134263	Completed	Sanofi Pasteur
QDenga©	Placebo/TAK-003 tetravalent dengue vaccine	NCT06060067	Recruiting	Takeda
	9vHPV vaccine/TAK-003 tetravalent dengue vaccine	NCT04313244	Completed	Takeda
	Placebo/TAK-003 tetravalent dengue vaccine	NCT03999996	Completed	Takeda
	TAK-003 tetravalent dengue vaccine	NCT03771963	Completed	Takeda
	Placebo/TAK-003 tetravalent dengue vaccine/HAV vaccine	NCT03525119	Completed	Takeda
	Placebo/TAK-003 tetravalent dengue vaccine	NCT03423173	Completed	Takeda
	Placebo/TAK-003 tetravalent dengue vaccine/yellow fever vaccine	NCT03342898	Completed	Takeda
	Placebo/TAK-003 tetravalent dengue vaccine	NCT03341637	Completed	Takeda
	Placebo/TAK-003 tetravalent dengue vaccine	NCT02747927	Active, not recruiting	Takeda
Dengue Vaccine by Butantan	Placebo/butantan tetravalent dengue vaccine	NCT02406729	Active, not recruiting	Butantan Institute

The ZIKV was first isolated in 1947 from a febrile Rhesus monkey in Uganda's Zika Forest. This was followed by its isolation from *Ae. africanus* mosquitoes the next year[150]. Human infection was first documented in 1954 in Nigeria[6], with only 14 reported cases over the subsequent fifty years. After this long period, a significant outbreak occurred in 2007 on Yap Island, Micronesia, involving 45 confirmed cases associated with *Ae. hensilli* mosquitoes, where patients presented with fever, rash, and arthralgia[151] and subsequent outbreaks in French Polynesia revealed severe complications such as Guillain-Barré syndrome and microcephaly resulting from maternal-fetal transmission[147]. The virus rapidly spread across the Pacific[141] and into the Americas, culminating in a major outbreak in Brazil in 2015, with an estimated 440000 to 1.3 million suspected cases[151].

As of May 2024, the WHO reported through its epidemiological update that ninety-two countries in Africa, Oceania, America, Europe, and Asia presented current or previous ZIKV transmission. Sixty other countries across the aforementioned continents have established *Ae. aegypti* vectors but without known cases of viral transmission[152].

Main vectors of transmission: The vectors of ZIKV transmission, as with all other arboviruses, are arthropods, primarily mosquitoes of the family *Culicidae* and genus *Ae.* These mosquitoes inject the virus with their saliva when biting vertebrates such as humans, thereby infecting the host (horizontal transmission)[141,153,154]. These mosquitoes have both sylvatic and urban transmission cycles, with *Ae. aegypti* being the main cause of outbreaks in urban environments around the world[153]. Furthermore, other species within the family are also responsible for epidemics: *Ae. albopictus* is associated with the 2007 outbreak in Gabon[4]; *Ae. hensilli* was identified as the primary cause of the Zika outbreak on the island of Yap in the same year[5,155]. The ZIKV has also been detected in *Ae. polynesiensis*, *Ae. africanus*, and even in the common household mosquito *Cx. pipiens*, among others[150,153,156].

Non-vector means of transmission: The viral transmission is not only limited to horizontal transmission through mosquito bites, since it is also possible for the virus to be transmitted between humans through sexual intercourse[157-160], blood transfusions[161], and even maternal-fetal transmission (vertical transmission)[162-164].

There are several reports reinforcing the possibility of sexual transmission. It is likely that the viral presence in semen components and tissues associated with the reproductive organs contributes to this infection form[165], since some research has demonstrated that the viral presence in semen components can be substantial even weeks after infection onset[157,166]. The aforementioned reports provide data on confirmed symptomatic infections through positive serological tests in women residing in non-endemic countries for ZIKV, without recent travel or transfusions, but engaging

in unprotected sexual intercourse with partners who had traveled to countries where the virus is endemic. The traveling partners also exhibited symptoms and tested positive for ZIKV[158,159,167]. This type of transmission has already been reported from infected women to men[160] and even between men[168] and asymptomatic individuals[169]. Male-to-male transmission had the highest probability of transmission. After, male-to-female and female-to-male[160].

Therefore, considering the possibility of sexual transmission of ZIKV, the use of condoms during sexual intercourse, especially when pregnant, is extremely relevant. This not only helps prevent other sexually transmitted diseases but also reduces the possibility of sexually transmitting ZIKV, thus inhibiting the probability of developing another type of transmission: Vertical transmission (maternal-fetal).

Vertical transmission of pathogens is typically not facilitated, as the placenta acts as a protective barrier for the fetus against invading pathogens, preventing infection from crossing the placental barrier in most cases. The placenta is formed by cellular layers of syncytiotrophoblasts, originating from trophoblasts that merge to form syncytia. These initially penetrate the endometrium to secure the blastocyst and subsequently act as a barrier between maternal and fetal blood. Blastocysts are highly resistant to infections from various viruses and confer viral resistance to non-trophoblast paracrine cells through the release of effectors such as type III IFN[170,171].

However, one of the viruses capable of crossing the placenta is the ZIKV, and its invasion mechanisms remain unclear. The complexity of simulating vertical transmission, all stages of pregnancy, and the fact that the placentas of animals used for analysis usually have significant anatomical differences compared to the human placenta, make it challenging to conduct high-quality research[163,172,173].

Pathogenesis of Zika

Viral tropism, entry, replication and exocytosis: ZIKV has a capsule with surface glycoproteins that facilitate its adsorption by connecting with host cell receptors and subsequent phagocytosis[174]. In some cells, there are transmembrane receptors, such as TIM and TAM, that recognize phosphatidylserine, a signaling molecule for phagocytosis. The expression of these receptors in cells increases the infectivity of the ZIKV, as it has phosphatidylserine molecules in its envelope, along with the exposure of the transmembrane E protein, which also facilitates the virus's adsorption and phagocytosis[144,175]. Other cofactors present in the host cell, such as DC-SIGN and Hsp70, also assist in viral entry, giving the virus tropism for various cells in the human body[176,177].

The viral presence in rodents, humans, and other primates tissue analyses has been detected in placental cells, trophoblasts, endothelium, epithelium, immune cells, mature and progenitor neuronal cells, ocular tissues (cornea, retina, optic nerve, and aqueous humor), and bodily fluids such as tears, saliva, semen, cervical mucus, and urine. There is also infection evidence in the male reproductive system, including testicular cells (Sertoli cells, Leydig cells, and spermatogenic cells), as well as in the female reproductive system in vaginal epithelial cells and uterine fibroblasts[165,178].

Upon entering the cell, the virus begins its replication using the host's machinery to generate new viral copies. In the ER of the cell, the virus undergoes RNA replication and capsid assembly[179]. The cell then uses reticulophagy to degrade the ER and prevent the maturation of ZIKV. The reticulophagy receptor FAM134B is essential for this process. However, the viral protein NS2B3 cleaves and inactivates FAM134B, preventing reticulophagy and enhancing ZIKV replication[180].

After the virus is assembled in the ER, it is transported to the Golgi complex, undergoing maturation processes and conformational changes, facilitating the subsequent fusion of the mature virus with the plasma membrane and cellular exocytosis[181].

Host intrinsic defenses and IFN inhibition: Upon being infected, a significant portion of the host's cells have the capacity to release IFNs, particularly type 1 IFNs (IFN-α and IFN-β), which are glycoprotein cytokines capable of modifying the immune response through paracrine antiviral effects[182]. Thus, soon after the virus enters the cell, PRRs can recognize pathogen-associated patterns. PRRs such as RIG-I and MDA5 receptors (RIG-I-like receptors) detect the presence of viral RNA in the cytoplasm and are transported to the mitochondria after recognition, acting on the production of mitochondrial antiviral signaling proteins that will activate TANK-binding kinase 1 (TBK1). TBK1, in turn, phosphorylates transcription factors such as IRF3, 5, and 7, and NF-κB, which will then be responsible for activating the transcription of IFNs in the cell nucleus[183]. Finally, the produced IFN will induce the synthesis of enzymes through IFN-stimulated genes (ISGs) in neighboring cells, which will hinder viral replication in the respective stimulated cells [184].

However, the virus has the ability to inhibit the host's immune response through various mechanisms. The ZIKV has non-structural proteins responsible for several actions, including the inhibition of IFN production. The NS3 protein can bind to the 14-3-3 protein of RIG-I-like receptors, blocking the translocation of RIG-I and MDA5 to the mitochondria and thereby preventing the pathway for IFN transcription factors from occurring[185]. NS4A acts directly on the mitochondria, preventing the local action of RIG-I and MDA5[186]. NS1, NS2A, NS2B, and NS4B have been shown to inhibit TBK1, preventing the phosphorylation of transcription factors[149,187], and NS5 also has the ability to directly inactivate IRF3, reducing IFN-β synthesis[187]. All these mechanisms ensure that the virus evades the IFN-mediated immune response.

Adaptative response and the viral strains: The adaptive response against ZIKV is primarily mediated by CD4+ and CD8+ T cells, playing an important role in inhibiting viral replication, especially when there is inhibition of type I IFN [188]. CD4+ T cells predominantly differentiate into T helper 1 cells during Zika infection, increasing levels of cytokines such as IFN-γ, IL-2, TNF-α, and the transcription factor T-bet, given their crucial role in orchestrating the immune response through cytokine production[189]. CD8+ T cells, in turn, also aid in the release of cytokines like IFN-γ and TNF-α, along with higher expression of granzyme B[189]. B lymphocytes also participate in the adaptive immune response,

eventually transforming into plasma cells, ensuring humoral immunity by releasing antibodies against ZIKV. This action is also driven by CD4+ T cells, and one of the main antibodies released are EDIII-specific neutralizing antibodies that neutralize epitopes present in the virus's EDIII protein[190,191].

The inhibition of CD8+ T cells during infection has been correlated with increased viral infection in the central nervous system (CNS), but with higher survival rates and lower incidence of paralysis[192]. On the other hand, the decrease in CD4+ T cells resulted in paralysis in all studied mice, in addition to increasing the viral load in the CNS and reducing survival[193]. These findings highlight the significant role of CD8+ T cells in neuropathology, as it has been shown that these cells mediate the lysis of virus-infected neurons[189]. Conversely, CD4+ T cells have demonstrated a regulatory function by reducing the immunopathological effects triggered by CD8+ T cells.

Therefore, our immune system, which is responsible for defending the body against pathogens such as ZIKV, has its efficacy dependent on the virus's ability to evade immune signaling pathways and inhibit IFN production. The different strains of the ZIKV, which emerged after various genetic mutations, also differ in their capacity to manipulate this immune response and are essentially divided into two main lineages with considerably different pathogenic characteristics: The African and Asian lineages.

African strains (such as the East African MR766 and West African lineages) have been shown to induce more potent inflammatory reactions and possess greater virulence[182]. However, the Asian strains (which include contemporary strains from Asia, Oceania, and the Americas) are more strongly associated with neurological disorders and microcephaly present in congenital Zika syndrome[163,194]. The Asian lineage emerged following the viral migration from Africa to Southeast Asia, being detected in Malaysia (1966; P6-740), the Pacific Islands, and later spreading to the Americas[195].

In addition to inhibiting the production and signaling of type I IFN, which is also present in African strains, studies indicate that the Asian strains can prevent the transcription and translocation of INF to the cell nucleus through the actions of NS1, NS4B, and NS5 proteins. This can lead to much more prolonged infections, potentially lasting for months, in contrast to the African strains that typically result in self-limited febrile infections[193,196,197].

Cutaneous pruritus: Pruritus is a frequent symptom in ZIKV -infected patients potentially linked to mast cell degranulation *via* the IgE-dependent histaminergic itch pathway[164,198]. Mast cells, a type of innate immune system cell, are among the main culprits for the symptomatic manifestation of various inflammatory and allergic reactions, and their G protein-coupled receptors (mainly H1R and H4R) play an important role in the development of cutaneous itching[164, 198].

The ZIKV has already shown tropism for the HMC-1 Lineage of mast cells isolated from the human placenta, triggering histamine degranulation and releasing various cytokines, thus being identified as a potential cause of itching during infection[164]. This itching can be alleviated by administering antihistamine medications, thereby reducing this uncomfortable symptom.

Congenital Zika virus syndrome and neuronal tropism: The correlation between Zika infection in pregnant women and microcephaly in their offspring is widely known[199]. However, all the associated mechanisms are not yet completely elucidated. Although, ZIKV effectively replicates in immature neurons *in vivo*, as shown in studies infecting embryonic mouse brains with the Asian strain SZ01. This replication triggers apoptosis, disrupts the cell cycle, inhibits neural progenitor cell differentiation, and ultimately causes cerebral cortex thinning and microcephaly, a critical factor in the development of human microcephaly[194].

Wu *et al*[163] also demonstrated vertical transmission in immunocompetent pregnant mice through intraperitoneal injection containing the contemporary Asian strain (ZIKV SZ01) isolated in serum, infecting radial glial cells of the dorsal ventricular zone in offspring with decreased proliferation of cortical neuronal progenitor cells, which are their main target. The infection was shown to affect brain development, reducing the lateral ventricle cavity and cortical surface area. A considerable number increase of IL-17a receptors in brain samples was also observed, likely due to maternal immune activation in response to the virus.

The ZIKV can activate T cells (Th1, Th2, Th9, and Th17), increasing cytokine levels in the acute phase with significant elevation of interleukins IL-1b, IL-2, IL-4, IL-6, IL-9, IL-10, IL-13, and IL-17, decreasing their levels in the subsequent phase (subacute)[178]. However, the action of interleukins can extend beyond their antiviral role, as IL-17a is likely involved in the development of microcephaly in infected humans.

In 2016, a study subjected pregnant rodents to controlled immune response activation by the IL-17a pathway. The results demonstrated impairments in offspring cortical development alongside behavioral abnormalities similar to autism spectrum disorder, with impaired social interactions and considerable phenotypic changes. Blocking IL-17a activity through specific antibodies protected the offspring against brain damage, demonstrating the significant role of this cytokine in the observed alterations, such as fetal brain malformation. Therefore, in addition to the virus's direct action on CNS cells, cytokine reactions released due to maternal immune activation may also influence the brain damage caused by congenital Zika virus syndrome[200].

Guillain-Barré Syndrome and molecular mimicry: Adults can also develop neurological complications due to the virus's strong tropism for nervous tissues[165]. One of the most common complications of the infection is Guillain-Barré Syndrome (GBS), an immune-mediated polyradiculoneuropathy that results in axonal neuropathy and is the leading cause of flaccid paralysis worldwide[201].

Data from seven countries in Central and South America showed a significant 2.0 to 9.8-fold increase in GBS cases during ZIKV outbreaks, with subsequent decreases observed once outbreaks were controlled[202]. The presence of antiglycolipid antibodies, mainly against GA1, has been reported by Cao-Lormeau *et al*[203] in one-third of the analyzed patients diagnosed with GBS having anti-ZIKV IgG or IgM. Other analyses have demonstrated IgM and IgG antigan-

glioside antibodies in patients under similar conditions[204,205]. This immune response is likely triggered by molecular mimicry between viral structures and neuronal proteins, a situation where the patient's body begins to recognize parts of its own body as structures of the invading pathogen, causing self-damage.

Among the probable structures involved in molecular mimicry, the neuronal proteins associated with GBS (Heat Shock 70 kDa protein 12A and voltage-dependent L type Calcium channel subunit α-1C) and the glycan loop region of the viral envelope protein E may be responsible for the immune recognition error due to the conservation of an IVNDT motif present in both proteins[206]. This situation ultimately triggers the production of antibodies and an immune response against the myelin sheath of peripheral nerves, thus causing the axonal neuropathy associated with GBS.

Clinical management of Zika

Clinical manifestations and major complications: After being transmitted through arthropod bites, the ZIKV enters a period of incubation, which typically lasts between 3- and 12-day following transmission. Notably, only a minority of those infected (20% to 25%) develop symptoms after the incubation period[153,207].

Symptoms are generally mild, nonspecific, and often self-limiting[199], making them easily confusable with other infections, especially those caused by other arboviruses[153]. Therefore, diagnosis should not rely solely on symptoms but also on epidemiological factors, taking into account current endemic and epidemic conditions in the patient's residence and places they may have traveled.

Concurrent infections are not uncommon[208], especially in regions where common arbovirus vectors are endemic, such as parts of Africa, Asia, and Latin America[152]. Consequently, many symptoms may be erroneously attributed to another pathogen causing simultaneous infection in the host. Laboratory tests are essential allies in diagnosis, particularly for concurrent infections, allowing for the prediction and potential prevention of various complications associated with the involved pathogens, including gestational complications related to ZIKV[209].

Among the signs and symptoms, ZIKV infection can present with a maculopapular rash typically associated with pruritus. This rash usually has a centrifugal distribution, developing in proximal regions and later affecting distal limbs, lasting between one to four days. Fever tends to be mild (between 37.4-38.0 degrees Celsius), unlike the higher fevers associated with the DENV infection[153,199].

Other symptoms such as arthralgia, extremity edema, myalgia, fatigue, mild headache, dizziness, loss of appetite, digestive disturbances, auditory issues, and hypotension may also occur, rarely persisting for more than two weeks[153,199,207]. Retro-orbital pain, commonly linked with dengue, can also arise in ZIKV infections, and conjunctivitis or conjunctival hyperemia is more frequent in ZIKV infections compared to other arboviral infections[153]. Elevated intraocular pressure can also be a complication, potentially leading to glaucoma[210].

Genitourinary symptoms, including hematospermia, have been reported[167]. The virus's tropism for tissues in this region, as well as its presence in urine, semen, and secretions, is well-documented[157,165,166,199].

One of the most concerning complications of ZIKV infection is microcephaly. Reports from the 2013 outbreak in French Polynesia[211] to the 2015 outbreak in Brazil[212] have highlighted the detrimental impact of ZIKV on ongoing pregnancies. An increase from 20 cases of microcephaly per 10000 live births during the ZIKV outbreak in Brazil, compared to 0.5 per 10000 live births before the outbreak[213], underscores the virus's aggressive potential in disrupting fetal CNS development.

The virus's strong neurotropism also poses risks for adults, often underestimated. Besides potentially causing autoimmune complications such as Guillain-Barré Syndrome due to molecular mimicry[201,204,206,214], ZIKV infection has been associated with complications like transverse myelitis, encephalitis, chronic inflammatory demyelinating polyneuropathy, meningitis, seizures, optic neuropathy, and acute demyelinating polyneuropathy[215]. A case-control neuroimaging study demonstrated changes in gray matter volume in certain brain regions post-infection, thereby altering the functional organization and structure of the adult brain[216].

Deaths related to ZIKV infection are rare and typically linked to microcephaly[162]. In 2015, three deaths were reported in Brazil: An adult man with lupus erythematosus, rheumatoid arthritis, chronic corticosteroid use, and a history of alcoholism, a 16-year-old girl, and a newborn[217]. In Colombia, a 15-year-old girl with sickle cell anemia also died following ZIKV infection[218], indicating that comorbidities can increase mortality risk. When ZIKV infection is suspected, the WHO emphasizes the importance of asking the patient about the onset of symptoms. This information guides the selection of appropriate laboratory tests based on the duration of symptoms. A thorough travel history, especially if travel occurred within the past two weeks, should be obtained, including dates, locations, and duration of travel, as well as possible sexual contact with confirmed Zika cases, breastfeeding status, and recent vaccinations, particularly against other flaviviruses such as YFV, Japanese Encephalitis, and dengue[219].

Laboratory diagnosis: The laboratory diagnosis of ZIKV is typically performed using ELISAs to detect the presence of anti-ZIKV IgM and IgG antibodies. IgM is detectable from 5 days to 12 weeks after the onset of symptoms, whereas IgG antibodies appear a few days after IgM detection and usually remain detectable for over a year[220-222].

ELISA is generally a less complex, quicker, and cheaper method compared to other tests still used worldwide, such as the PRNT, fluorescent antibody test, hemagglutination-inhibition test, and complement fixation test[223]. However, false-positive and false-negative (particularly in immunocompromised patients with inadequate adaptive immune responses or when tests are conducted before the onset of the antibody-associated adaptive immune response) results for IgM and IgG tests are still significantly reported. Cross-reactivity, especially with viruses from the same family (Flaviviridae)[221], remains a common issue in these tests, potentially leading to incorrect diagnoses.

Therefore, PRNT can be used to confirm laboratory diagnoses by quantifying neutralizing antibodies in serum or cerebrospinal fluid (CSF) samples more specifically, serving as the gold standard for diagnosis[223]. For viral RNA detection, RT-PCR can be utilized during the infection, offering high sensitivity and specificity[141,155,223,224].

However, these tests are not very compatible with common outpatient settings, as they require appropriate infrastructure, trained professionals, and PCR thermocyclers[225,226]. Recently developed isothermal nucleic acid amplification technologies, which can amplify nucleic acids at constant temperatures, offer a faster alternative for diagnosing arboviruses. This technology provides results within minutes and does not require cycling equipment, potentially serving as a cheaper option for future outbreaks, particularly in regions with limited infrastructure[225].

According to the WHO[219], laboratory diagnosis can be conducted using serum, whole blood, or urine samples. Other samples may be considered when neurological complications related to ZIKV infection are suspected, including in cases of a sexual transmission suspected, as semen samples, since the virus tends to persist longer in urine and semen tests compared to blood analyses[166,227].

For patients within 7 days of symptom onset, nucleic acid tests (NAT), such as RT-PCR, are recommended to diagnose viral presence. For those with more than 7 days of symptoms, serological detection tests for IgM antibodies (ELISA), present in the acute phase of infection, can be used. NAT can also be employed, although negative results do not rule out infection since viremia may be low after one week of symptom onset[219]. IgG detection just indicates a past infection that elicited an immune memory response.

During outbreak periods, the WHO recommends prioritizing testing for a select group of patients[219] as there is normally a limited number of tests available to be used (Table 2).

Findings in complementary exams: During ZIKV infection, additional exams are commonly requested to assist in the clinical management of the patient. However, reports contain limited information about other laboratory exams performed. The results of a complete blood count and leukogram are usually normal, without leukopenia or thrombocytopenia, which are common in CHIKV and DENV infections[153]. The patient may show slight elevations in C-reactive protein, ferritin, and fibrinogen, as well as increased serum lactate dehydrogenase and liver enzymes, findings commonly seen in other viral infections[153,199]. These clinical data have little impact on the management of ZIKV infection, as the disease lacks research indicating a likelihood of a worse outcome even when the patient presents such altered exams.

Treatment: There is no specific treatment for ZIKV. However, appropriate management focuses on alleviating symptoms. The WHO recommends using antipyretics for patients with fever, antihistamines for those with itching, and analgesics for pain relief. Adequate hydration and rest can also aid in the patient's recovery[228].

The use of NSAIDs is contraindicated without completely ruling out DENV infection, as there is a risk of hemorrhagic complications[228]. Additionally, NSAIDs are contraindicated in pregnant women after the 32nd week of gestation regardless of the infecting virus, due to the risk of premature closure of the ductus arteriosus[229]. Administration of acetylsalicylic acid is also contraindicated, as it increases the risk of developing Reye's syndrome[229].

However, even if the current treatment is still just medication to alleviate the symptoms of the infection, various compounds have been studied in recent years as anti-flavivirus substances and may be available on the market for infection treatment in the coming years. An ideal drug should possess the ability to penetrate the blood-brain barrier, characterized by small and/or lipophilic molecules. Additionally, it should target the infection's host cells, such as fetal neural progenitor cells, exhibit placental penetrance to prevent vertical transmission, and be highly safe for administration to pregnant women[230].

The coinfection and cocirculation of flaviviruses like DENV, YFV, Mayaro, and Oropouche virus in various parts of the world infect hosts and cause immune responses with cross-reactivity. This has led researchers to conclude that the best option for developing an anti-ZIKV drug would also be one that protects against other flaviviruses. This is because the structures of several studied target proteins are similar, and recently developed preclinical compounds have shown activity against multiple flaviviruses, making them promising candidates for medication[230].

Consequently, considering the most promising proteins for the development of this medicine when examining host factors associated with infection, the TIM1 binding protein proves to be a significant factor for the binding of the viral phospholipid during ZIKV entry. However, it is also involved in the infection of DENV-2 and West Nile virus, making it an interesting target for a multi-flavivirus drug[230,231]. Inhibitors of the E protein binding to host receptors also show potential as drug targets, thus preventing viral adsorption. Nevertheless, one of the most promising targets for drug creation is certainly viral RNA-dependent RNA polymerases (RdRp), as they are extensively studied targets with substantial literature supporting their efficacy and safety[232]. By inhibiting RdRp, viral replication and the production of infectious particles are also inhibited.

To understand the importance of RdRp in the virus replication cycle, research shows that Zika, being part of a genus of single-stranded positive-sense RNA viruses, has RdRp proteins that synthesize a complementary (negative-sense) strand used to synthesize new positive-sense strands, which will later be used as mRNA in virus replication. In ZIKV, the RdRp protein responsible for creating new strands is NS5, which also suppresses type I IFN and is the focus of various drugs currently being developed.

The RdRp inhibitors have been successful in treating other viruses such as hepatitis C virus, HIV, and herpes simplex virus. A ProTide technology nucleoside analog for HCV, already approved by the FDA (Sofosbuvir), has shown significant effects and high safety as an anti-ZIKV agent both *in vivo* in mice (demonstrating prevention of vertical transmission and reduced morbidity) and *in vitro* in neuronal cells. Sofosbuvir is classified as category B for administration in pregnant women, meaning that while there are no controlled studies in pregnant women, animal studies have not shown risk to the fetus.

Conversely, analogs of Sofosbuvir (such as 2'-C-ethynyluridine aryoxyl phosphoramidate and 2'-C-methyluridine aryoxyl phosphoramidate) have demonstrated superior anti-ZIKV effects compared to the medication. However, clinical trials are also necessary to prove their safety in pregnant women and efficacy against sexual and vertical transmission.

Table 2 Recommendation for viral testing during Zika virus outbreak-World Health Organization

No.	Priority recommendation for viral testing during periods of ZIKV outbreak-WHO
1	Symptomatic patients who have had sexual relations with a partner with probable or confirmed infection
2	Suspected patients with neurological complications
3	Pregnant women with a travel history to endemic areas, residents in endemic areas or those in current outbreak regions
4	Pregnant women who have had sexual relations with a confirmed or probably infected patient
5	Pregnant women with suspected or confirmed fetal brain anomalies who have a travel history to endemic areas or reside in endemic areas or current outbreak regions
6	Women who have had miscarriages or stillbirths and traveled or resided in Zika virus-affected areas during pregnancy
7	Infants born with microcephaly or neurological complications whose mothers traveled or resided in endemic areas or current outbreak regions
8	Breastfeeding infants with mothers diagnosed with the viral infection

ZIKV: Zika virus; WHO: World Health Organization.

Finally, other potential preclinical compounds under investigation include protease inhibitors, viral assembly inhibitors, inhibitors of ZIKV fusion to the cell membrane, nucleoside biosynthesis inhibitors, and ZIKV antivirals targeting the host. These drugs are still being evaluated for safety and efficacy.

Prevention: Preventive measures range from personal care, such as using effective repellents containing DEET, IR3535, or icaridin, and wearing long clothing that covers as much skin as possible, especially in areas known to have ZIKV vectors, to collective measures, like eliminating potential breeding sites where water can accumulate. Special caution is advised during the peak activity times of the major urban vector, *Ae. aegypti* (early morning and late afternoon), using mosquito nets, window and door screens, and electric repellents at home to aid in prevention[228].

During pregnancy, women are advised not to travel to endemic or outbreak areas. Ultrasound scans to monitor fetal development are recommended every 3 to 4 weeks for patients with confirmed or suspected ZIKV infection, and newborns should be tested at birth[209].

Furthermore, regarding men, the WHO also advises that those who have traveled to endemic or outbreak areas should avoid sexual relations with their pregnant partners or use condoms for up to three months after exposure[228].

Vaccines in development: Successful flavivirus vaccines, such as the current dengue vaccine (Qdenga) from Takeda, along with knowledge of ZIKV pathogenesis and general characteristics, are key factors directly influencing the advancements in the development of various ZIKV vaccines. Nucleic acid vaccines (DNA and mRNA), inactivated virus vaccines, live attenuated virus vaccines, viral-vectored vaccines, virus-like particle (VLP) vaccines, protein antigen-based vaccines, and mosquito saliva antigen-based vaccines are all in preclinical or clinical stages of human testing[145,146].

Currently, more than 50 ZIKV vaccines are under development and a large portion is detailed on the ClinicalTrials.gov website, a global database maintained by the National Library of Medicine[233]. However, the WHO and the National Institutes of Health (NIH) report that Phase III field efficacy trials become unfeasible in the absence of a new outbreak, thus hindering effective human trials and subsequent approvals[234](Table 3). Finally, the lack of substantial investment from both private and governmental initiatives also delays the development process.

According to the WHO and the United Nations International Children's Emergency Fund (UNICEF), the target product against ZIKV should provide adequate protection against congenital Zika syndrome, particularly focusing on the immunization of women of childbearing age and pregnant women[235]. Innovative technologies recently developed, such as mRNA vaccines authorized to treat COVID-19[236], can be a promising alternative for ZIKV immunization, especially after the successful performance observed during the pandemic[237,238].

Inactivated virus vaccines are generally safe, even for pregnant women and immunocompromised individuals, making them an ideal candidate for preventing congenital Zika syndrome. However, this type of vaccine typically requires higher doses to ensure an adequate and long-lasting immune response. On the other hand, live attenuated virus vaccines usually elicit efficient immune responses from the first dose and tend to provide a much more durable immune response. However, given the pathogenesis of ZIKV, this type of vaccine may not be ideal for pregnant women due to the potential for maternal-fetal transmission and associated complications[239].

Probably, the combination of various types of vaccines that may become available on the market in the coming years will offer greater benefits to the population, as they have diverse indications, contraindications, and benefits.

YELLOW FEVER

Yellow fever (YF) is caused by the YFV and has historically been one of the world's most lethal and feared diseases. YFV belongs to the Flaviviridae family and is one of over 70 members of the Flavivirus genus, with "flavus" being the Latin word for yellow. The virion has a spherical shape with a diameter of 40-50 nm[143]. Its genome consists of a single-

Table 3 Zika vaccines currently in clinical trials-clinicalTrials.gov database

Vaccine technology platforms	Intervention/treatment	ClinicalTrials.gov ID	Phase	Status	Sponsor
DNA vaccine	Biological: VRC-ZKADNA090-00-VP; Other: VRC-PBSPLA043-00-VP	NCT03110770	2	Completed	NIAID
	Biological: VRC-ZKADNA090-00-VP	NCT02996461	1	Completed	NIAID
	Biological: VRC-ZKADNA085-00-VP	NCT02840487	1	Completed	NIAID
mRNA vaccine	Placebo/biological: mRNA-1325	NCT03014089	1	Completed	ModernaTX, Inc.
	Placebo/biological: mRNA-1893	NCT04064905	1	Completed	ModernaTX, Inc.
	Placebo/biological: mRNA-1893	NCT04917861	2	Active, Not Recruiting	ModernaTX, Inc.
Viral vectored vaccine	Placebo/biological: MV-ZIKA-RSP vaccinations (high or low doses)	NCT04033068	1	Completed	Themis Bioscience GmbH
	Biological: ChAdOx1 Zika	NCT04015648	1	Completed	University of Oxford
	Placebo/biological: MV-ZIKA	NCT02996890	1	Completed	Themis Bioscience GmbH
Live attenuated vaccine	Placebo/biological: rZIKV/D4Δ30-713	NCT03611946	1	Completed	NIAID
Purified inactivated vaccine	Placebo/biological: VLA1601	NCT03425149	1	Completed	Valneva Austria GmbH
	Placebo/biological: Zika virus purified inactivated vaccine	NCT02937233	1	Completed	Kathryn Stephenson
	Biological: VLA1601/CpG 1018®/3M-052-AF	NCT06334393	1	Recruiting	Valneva Austria GmbH
	Placebo/biological: PIZV	NCT03343626	1	Completed	Takeda
	Placebo/biological: Zika virus purified inactivated vaccine	NCT03008122	1	Completed	NIAID
	Placebo/biological: IXIARO; YF Vax 17D strain and Zika virus purified inactivated vaccine	NCT02963909	1	Completed	NIAID
	Drug: Saline/biological: Zika virus purified inactivated vaccine	NCT02952833	1	Completed	NIAID

NIAID: National Institute of Allergy and Infectious Diseases.

stranded positive-sense RNA of approximately 10.8 kb, containing an open reading frame that encodes a single polyprotein of 3411 amino acids[240], which is post-translationally cleaved to produce mature viral proteins. These viral proteins are classified as structural and non-structural. Despite having different genotypes, the YFV has a single serotype [241].

The structural proteins crucial for the formation and structure of the virion include the capsid protein C, the pre-membrane protein prM, and the envelope protein E, which together constitute the viral particle[242]. The transmembrane domains of the prM and E proteins act as localization signals to the ER, containing specific sequences that direct these proteins to the ER[243]. Notably, the E protein plays a crucial role in recognizing and binding to host cell receptors, facilitating viral entry[244], and is an important target of the immune system during YFV infection, triggering the production of neutralizing antibodies[245]. The non-structural proteins, including NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5, perform various essential functions in the viral replication cycle[246].

YF is a zoonotic viral disease endemic to various tropical regions in Africa and the Americas, characterized by a transmission dynamic involving non-human primates (NHP) reservoir and humans, mediated by mosquito vectors. The YFV is primarily transmitted to humans by primates through the bite of infected mosquitoes, posteriorly establishing a human-mosquito-human transmission cycle[247]. This interaction can result in a wide range of symptoms, from mild fever to severe liver disease and jaundice, the latter giving the disease its name[241]. Effective transmission control and a thorough understanding of the virus-host interaction are crucial for the management of the disease.

Although YF cannot be eradicated, epidemics can be prevented through mass immunization of the population and the maintenance of routine childhood vaccinations. Low vaccination coverage has led to significant outbreaks in Angola (2015-2016), the Democratic Republic of Congo (2016), and Brazil (2017-2018)[248], highlighting the urgent need to address this gap. Consequently, in 2016, to combat and eliminate the growing urban outbreaks of YFV and prevent its international spread, the WHO, in partnership with UNICEF and Gavi, launched the Eliminate Yellow Fever Epidemics

initiative. This initiative has strategic objectives to protect at-risk populations, prevent the international spread of YFV, and quickly contain outbreaks. One of its key actions is mass vaccination campaigns, with an estimate to have over one billion people properly vaccinated by 2026[249].

Phylogenetic analyses suggest that the virus, which gave rise to the currently circulating strains, originated in Africa within the last 1500 years and was introduced to the Americas around 300-400 years ago during the transatlantic slave trade[250]. The first recorded epidemic occurred in Yucatan in 1648[251]. The Spanish-American War highlighted the disease's impact, with more soldiers dying from YF than combat in Cuba invasions. Walter Reed's research between 1899 and 1901 confirmed mosquito transmission of the virus[251,252]. The isolation of the virus from a patient named Asibi in 1927 led to the development of the 17D vaccine by Max Theiler, who received a Nobel Prize for this achievement in 1936 [253].

As of 2023, the WHO reports that YF remains endemic in 34 African countries and 13 countries in Central and South America[254]. Ongoing vaccination efforts and surveillance are vital to prevent outbreaks and control the spread of the disease. The 17D vaccine continues to be a cornerstone in the fight against YF, highlighting the importance of immunization programs in endemic regions.

Pathogenesis of yellow fever

Transmission cycle: YF is a zoonotic infection transmitted from primates to humans through the bite of infected mosquitoes[241]. The virus is inoculated into the host *via* the mosquito's saliva. After an incubation period of 3 to 7 days, viremic hosts can infect other mosquitoes that feed on their blood, thus continuing the transmission cycle. Mosquitoes remain infected for life and can pass the virus to their eggs through transovarial transmission[255,256].

YFV has different transmission cycles: Sylvatic, urban, and intermediate. In the sylvatic cycle, transmission occurs in forested areas between blood-feeding mosquitoes and NHP. In the Americas, the primary vectors are mosquitoes of the *Haemagogus* and *Sabethes* genera, while in Africa, *Aedes* mosquitoes predominate. NHPs are the natural hosts of the virus. Following urbanization, YF entered the urban cycle, where transmission occurs among humans in urban and peri-urban areas, with *Ae. aegypti* being the primary vector[257]. There is a high risk of outbreaks when infected humans from forest areas travel to densely populated areas with low immunity to the virus, where vector mosquitoes are present[258].

In addition to the sylvatic cycle, there is an intermediate transmission cycle for YF. Small epidemics can occur at the edges of African savanna forests, in humid and semi-humid zones, known as emergent zones, where humans come into contact with the wild cycle. In this cycle, transmission occurs among NHPs, humans, and mosquitoes, such as *Ae. africanus*[257].

The recent detection of *Ae. albopictus* mosquitoes contaminated with YFV in urban and rural areas around the world is concerning and needs attention[259]. Additionally, transmission can occur through other routes, such as exposure to infected blood and aerosols in laboratories[260]. Cases of perinatal transmission to newborns have also been reported[261, 262]. Furthermore, the attenuated 17D vaccine strain of the virus has been reported to be transmissible through blood transfusion[263], organ transplantation[264] and, in rare cases, breastfeeding[265,266]. An investigation in the United States in 2021 revealed that transmission of the YF vaccine virus virus *via* organ transplantation and blood transfusion caused severe neurological disease and fatalities in four recipients. These findings suggest that after receiving a vaccine dose, blood donation should be delayed for at least two weeks[264]. Continuous surveillance and adequate vaccination are essential to prevent outbreaks.

Binding and entry: After the inoculation of the virus, the process of viral replication and dissemination to other tissues begins. In the Flaviviridae family, viral replication occurs through the synthesis of an antigenome template used for the production of genomic RNA and the synthesis of viral proteins[267]. The entry of flaviviruses into their target cells is mediated by the interaction of the E glycoprotein with receptor molecules on the cell surface, promoting fusion with the cell membrane for viral entry[268]. However, the host cell receptors that bind to the E glycoprotein have not yet been identified[269]. The DIII domain of the E protein is considered the receptor-binding domain of flaviviruses[244,270-273].

YFV binds non-specifically to heparan sulfate on the surface of host cells, such as hepatocytes and dendritic cells[274, 275]. Despite using different pathways, both wild-type and attenuated strains of YFV employ a pH-dependent entry mechanism[276]. The wild-type Asibi strain enters host cells through clathrin-mediated endocytosis, while the attenuated YFV-17D strain utilizes a clathrin-independent pathway[277]. The conformational rearrangement of the E glycoprotein occurs in the lower pH environment of the endosome, facilitating the fusion of the viral lipid envelope with the endosomal membrane[278].

It is important to note that the valosin-containing protein (VCP/p97), a cellular ATPase that unfolds and extracts ubiquitinated client proteins from large complexes, has been reported as a factor with various functions in flavivirus replication, such as viral uncoating[279]. Consequently, the viral genome is released into the host cell cytoplasm.

After the flavivirus RNA enters the host cell, the viral genome acts as messenger RNA (mRNA) and is translated at the rough ER into a polyprotein, anchored in the ER itself[280]. This polyprotein is cleaved by cellular peptidases and viral proteases NS2B/3[281-283].

The NS5 protein is particularly notable as it houses the methyltransferase and RdRp domains, which play a crucial role in viral RNA synthesis and regulation of replication processes[284], providing insights into targets for the development of therapeutic strategies[241]. Additionally, it is suggested that G protein-coupled receptor kinase 2 contributes to various stages of the virus life cycle by enhancing both viral entry and RNA synthesis, being a regulator of flavivirus infection [285].

Host factors play essential roles in this process: The signal peptidase complex associated with the ER (SPCS) is responsible for processing the Pr-E junction and secreting viral particles[286]; the DNAJC14 protein, a Hsp40 cochaperone, modulates flavivirus replication and may confer resistance to cell death during YFV infection by inhibiting viral

replication[287,288]; ribosomal proteins RPLP1 and RPLP2 facilitate viral genome translation, exhibiting pan-flaviviral activity[289]. These factors facilitate the processing and translation of the viral genome.

Following this processing, the viral RNA replication complex is assembled by non-structural proteins such as NS1, NS2B, NS3, NS4A, NS4B, and NS5. The NS4A protein induces rearrangements in the ER membrane, promoting active viral RNA replication, which is then packaged forming the virion[290]. These immature virions are secreted in vesicles and transported to the Golgi apparatus, where they traverse chambers with increasingly lower pH. The enzyme furin cleaves the viral envelope proteins, resulting in mature virions that are then released by exocytosis[291,292].

Immune response: The understanding of the pathogenic mechanisms of the YFV remains limited, primarily due to the lack of animal models that accurately reproduce the disease observed in humans[269]. Current knowledge is largely based on human tissue biopsies from fatal YF cases and indirect observations made in animal models[293], which do not fully capture the characteristics of human infection.

Upon being bitten, the infected mosquito injects the YFV into the skin. The virus is then recognized by APCs, such as dendritic cells, which activate PRRs, including RIG-I-like receptor (RLR) family[294] and TLRs such as TLR-2, 7, 8, and 9. This activation leads to the production of pro-inflammatory mediators and type I IFNs (such as IFN-α and IFN-β). These IFNs play a fundamental role in the antiviral response by promoting an antiviral state in adjacent cells and activating immune cells, which trigger multifaceted immune responses to present the antigen to CD4+ T lymphocytes in lymphoid tissues like the spleen and lymph nodes[295]. Plasmacytoid dendritic cells (pDCs) produce IFNs in response to YFV in a TLR-7-dependent manner, with this stimulation being more effective with immature viral particles[296]. In addition to this, antiviral response is mediated by RNA-sensing proteins like RIG-I and protein kinase R, which induce pro-inflammatory cytokines upon detection of viral genetic material independently of stress granules[297].

Studies have demonstrated that the viral non-structural protein NS5 interacts with the human transcription factor hSTAT2, induced by IFN-1, playing a crucial role in modulating the host's immune response, viral replication, and determining the tropism of YFV. This interaction allows the virus to evade type I IFN-mediated antiviral defenses[298,299]. YFV uses its NS5 protein to inhibit IFN-I signaling by interacting with STAT2, a process dependent on IFN-I-induced modifications, specifically STAT1 phosphorylation and NS5 polyubiquitination by TRIM23[300,301].

APCs like macrophages and dendritic cells are crucial in activating Th1-type CD4+ T lymphocytes, where antigens are presented on the surface of APCs *via* class II major histocompatibility complex molecules, recognized by T cell receptors [302]. The immune response against YFV begins with innate immunity, the body's first line of defense. Dendritic cells (S100+), present in tissues such as the skin and liver, are among the first to detect YFV. They phagocytize the virus and present its antigens to the adaptive immune system. Macrophages (CD68+), in addition to phagocytizing the virus and infected cells, secrete cytokines and inflammatory mediators, including reactive oxygen species, nitric oxide (NO), and TNF-α, which promote inflammation and recruit other immune cells to the infection site[303]. This release of inflammatory substances and cytokines, causes a "cytokine storm" that triggers disturbances. Consequently, there is midzonal apoptosis of hepatocytes, along with pathological changes like heart apoptosis and acute tubular necrosis in renal tissues [302]. Regarding serum markers, elevated levels of IL-6, MCP-1, IP-10, TNF-α, and IL-1RA were found in the sera of YFV-infected patients who had fatal outcomes compared to those with non-fatal outcomes[304].

Adaptive immunity is triggered when dendritic cells present viral antigens to CD4+ T cells. These helper T cells proliferate and release cytokines that activate other immune cells. In fatal YF cases, CD4+ T cells are predominant and crucial for coordinating the immune response[303]. CD8+ T lymphocytes target and lyse virus-infected liver cells, releasing viral particles that expose antigens to B lymphocyte-produced antibodies. B lymphocytes (CD20+), activated by helper T cells, produce specific antibodies that neutralize YFV, prevent viral entry into new cells, and mark the virus for destruction by macrophages[305].

Hepatic damage: The liver is particularly affected in YF. Immunohistochemical studies on the livers of patients who succumbed to the disease have revealed damage characterized by macro/microvesicular steatosis, apoptosis, and necrosis, especially in the intermediate zone of the liver, where viral antigens were most frequently observed[306].

Studies in animal models[293,307-310] and human tissue biopsies from fatal cases[303,306,311,312] highlight apoptosis as a central mechanism in the pathogenesis of YF, attributed not only to the cytopathic effect (CPE) induced by the virus but also to an imbalanced cytokine response.

The inflammatory infiltrate in the liver of fatal YF cases predominantly consists of CD4+ T lymphocytes, with smaller quantities of CD8+ T lymphocytes, macrophages CD68+, CD20+ B lymphocytes, NKT+ cells, and S100+ dendritic cells [303,311]. Cytokine expression is also notable, with significant numbers of cells expressing TGF-β, and to a lesser extent, TNF-α and IFN-γ[311]. Apoptosis, rather than necrosis, has been identified as the primary mechanism of cell death during infection, likely influenced by viral antigens and the presence of TGF-β, an apoptosis-inducing cytokine responsible for the downregulation of inflammatory infiltrates observed in the liver of fatal cases[303,306,311]. The interaction between the Fas receptor (CD95) and its ligand FasL, induced by TGF-β, is one of the mechanisms by which CD8+ T cells promote the apoptosis of infected cells, thereby limiting viral spread[312]. Other apoptotic markers are also found in the hepatic parenchyma, including CASPASE 3, CASPASE 8, BAX, GRANZYME B, and SURVIVIN[313]. These mechanisms contribute to the pathology observed in fatal cases and various symptoms associated such vascular leak syndrome, thrombocytopenia, changes in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, elevated blood urea nitrogen and creatinine, jaundice, vomiting, and hemorrhagic diathesis[302,311].

In situ studies suggest that viral infection activates the endothelium, exacerbating the inflammatory response in the liver. This occurs through the increased expression of adhesion molecules such as E-selectin, P-selectin, ICAM-1, VCAM-1, and VLA-4, which facilitate the adhesion and migration of inflammatory cells into the hepatic parenchyma, contributing to more severe tissue damage and potentially fatal outcomes in YF[314,315]. Complementing these findings, *in*

vitro tests indicate that the NS1 protein of flaviviruses can bind to and alter the permeability of ECs, particularly in the lungs and liver in the case of YF, resulting in increased vascular permeability in these tissues. This is associated with the virus's tropism[316].

Moreover, histopathological and immunohistochemical analyses of fatal YF patients revealed a predominant expression of Th17 cytokine markers in the midzonal region of the liver, the most affected area in the hepatic acinus, like ROR-γ, STAT3, IL-6, TGF-β, IL-17A e IL-23. These analyses showed significant cellular damage, with inflammatory infiltrates, councilman bodies (apoptotic hepatocytes)[317], steatosis, and necrosis[318].

Research indicates that inositol trisphosphate receptor type 3 (ITPR3), a calcium channel isoform, expressed in hepatocytes infected by the virus, can stimulate hepatocyte proliferation and reduce both steatosis and cell death, thereby protecting the organ against insufficiency. This protective mechanism implies that enhancing ITPR3 expression could be an effective therapeutic intervention to prevent the progression of liver failure and reduce mortality, potentially eliminating the need for liver transplants[319].

Clinical management of yellow fever

Clinical manifestation: The YFV is predominantly viscerotropic, primarily affecting the liver, as well as organs such as the kidneys, spleen, lymph nodes, and heart[241]. The disease classically manifests in three stages: Infection, remission, and intoxication. These stages are often not well demarcated[320].

In the first stage, the acute infection phase occurs approximately 3 to 6 days after the mosquito bite, presenting clinical characteristics such as high fever (approximately 39 °C), headache, malaise, photophobia, back pain, myalgia, irritability, restlessness, nausea, and vomiting, as well as conjunctival infection, white tongue with a red tip, and bradycardia (Faget's sign)[302,321]. During this phase, viremia occurs, where the virus replicates and disseminates through the bloodstream, making the host infectious to vectors. Laboratory findings may demonstrate leukopenia, neutropenia, and low C-reactive protein; elevated transaminases and proteinuria may also occur. Only about 45% of infected individuals exhibit symptoms[322].

Next, the remission stage begins, characterized by a temporary improvement in symptoms, lasting between 12 hours and 2 days. The final stage is the intoxication stage, marked by an exacerbated inflammatory response associated with hemodynamic collapse, where there is a sudden deterioration of the patient's clinical condition, with typical signs of liver and kidney failure, hemorrhagic fever, and multiple organ dysfunction. Liver dysfunction results from apoptosis with limited inflammation and manifests with elevated transaminases and bilirubin, as well as decreased synthesis of coagulation factors produced by the liver[241,303]. Patients may present with jaundice, oliguria or anuria, cardiovascular instability, and hemorrhagic diathesis[302,321]. Notably, about 12% of infected individuals reach this phase, of which approximately 47% succumb to the disease[322].

In the intoxication stage of YF, various laboratory and pathophysiological changes occur that reflect the severity of the disease, including thrombocytopenia with platelet counts below 50000 per milliliter; prolonged coagulation time and prothrombin time; decreased hepatic coagulation factors; decreased fibrinogen and factor VIII; and elevated fibrin degradation products, characteristic of disseminated intravascular coagulation[323,324].

The increase in AST/SGOT can exceed that of ALT/SGPT, which differs from other forms of viral hepatitis and may be related to skeletal or cardiac muscle injuries[241,325].

Laboratory diagnosis: Due to the numerous differential diagnoses stemming from the similarity of symptoms with a wide range of diseases, such as dengue, leptospirosis, viral hepatitis, malaria, and other hemorrhagic diseases, the clinical diagnosis of YF is challenging. Therefore, laboratory confirmation plays an essential role[320]. Laboratory diagnostic methods for YF are crucial to accurately identify the infection in suspected cases. According to the WHO, a suspected case is defined as any person with an acute onset of fever, accompanied by jaundice within 14 days of the onset of initial symptoms[326]. However, this categorization remains restrictive as the disease progresses to jaundice only in its more advanced stages (Table 4).

Laboratory tests for YF diagnosis, performed on blood, serum, CSF, urine, or other tissue samples, can be conducted through serological methods, viral isolation, viral genome detection, and viral antigen detection[259].

The most commonly used serological procedure is the detection of anti-YFV antibodies by IgM-ELISA, which offers a presumptive diagnosis of YF within a few hours[241]. The accuracy of this test for detecting YF is generally regarded as high, with a minimum precision of 90%[259]. However, case confirmation requires evaluation of the epidemiological context, detailed vaccination history of the individual, timing of sample collection, and potential co-circulation of other flaviviruses in the region[259]. It is important to consider that this type of test may present limitations, including cross-reactions with other flaviviruses and the need for additional confirmation[327].

NS1 antigen capture ELISA assays have proven efficient for early diagnosis of YF, with high sensitivity and no cross-reactions with other flaviviruses[328]. In serological tests, the PRNT, also known as the virus neutralization test, is more specific for the detection of anti-YFV antibodies compared to other assays and is considered the "gold standard" for differential diagnosis of flaviviruses[329]. However, the requirement for specific cell culture facilities, standardized controls, and well-trained personnel for reproducible results, as well as the 4-7 days needed for result analysis, limit the use of this method during outbreaks[320]. Another technique commonly used to identify IgM and IgG antibodies against the YFV is the indirect immunofluorescence[330].

For viral genome detection, the RT-PCR technique is highly sensitive, capable of detecting YFV in the early stages of infection, even before clinical symptoms manifest. Additionally, RT-PCR can distinguish between wild and vaccine strains of YFV, making it useful for monitoring viral spread and evaluating vaccination strategies[331,332]. Furthermore, other molecular approaches, such as loop-mediated isothermal amplification (LAMP)[333] and reverse transcription-mediated amplification (RT-LAMP)[334,335], offer the possibility of rapid and sensitive YFV diagnosis in field settings

Table 4 Final case classification of yellow fever

Classification	Criteria
Probable case	A suspected case and at least one of the following
	Presence of YF IgM antibody in the absence of YF immunization within 30 days of illness onset
	Epidemiological link to a confirmed case or an outbreak (*e.g.*, household members or persons in close proximity through work, residence in past month)
Confirmed case	A probable case and at least one of the following
	Negative results of differential neutralization testing with flaviviruses endemic in the area of exposure
	Seroconversion in appropriately paired samples tested by YF neutralization testing
	And absence of YF immunization within 30 days before onset of illness
	Or a suspected case and at least one of the following
	Detection of YFV genome in blood or other organs by real-time reverse transcriptase polymerase chain reaction
	Detection of YF antigen in liver or other organs by immunohistochemistry
	Isolation of YF virus
	And absence of YF immunization within 14 days before onset of illness
Discarded case	A person who tests negative for YF antibody testing (with specimen collected > 7 days post onset)
	Or negative immunohistochemistry on tissue samples

YF: Yellow fever; YFV: Yellow fever virus.

without requiring complex equipment.

Treatment, care and prophylaxis in the management of infection: YF presents a significant challenge for healthcare professionals, as it is a severe viral hemorrhagic disease requiring early diagnosis despite often having nonspecific symptoms. There is no specific therapeutic treatment for YF, and its management relies on supportive care. The Pan American Health Organization categorizes YF patients into three groups, corresponding to the classic phases of the disease[241] and reflecting its severity: Group A for mild cases, Group B for patients in remission, and Group C for severe forms with hepatic and renal complications[336].

Due to the potential for rapid disease progression, it is crucial to monitor the warning signs and risk factors associated with YF. For confirmed cases in the infection phase (Group A), attention should be paid to alarm signs such as dehydration, vomiting, diarrhea, abdominal pain, and mild bleeding. Clinical findings such as AST levels more than five times the upper limit of normal, platelet count below 50000/mm^3, and proteinuria are also significant[336]. These findings are associated with hepatic damage, hemorrhage risk, and renal impairment[337], respectively. Clinical management in this group includes oral and/or intravenous hydration, pain and fever control using dipyrone (maximum 8 g per day) and acetaminophen (maximum 2 g per day), taking into account the patient's hepatic condition and avoiding the use of NSAIDs. It is noteworthy that these medication recommendations may not be applicable worldwide, as the use of dipyrone is restricted in several countries.

For Groups B and C, the required level of attention increases due to the worsening of the patient's overall condition. Severe signs and symptoms at this stage include clinical findings such as jaundice, oliguria, mental confusion, seizures, hemorrhagic phenomena, tachypnea, hypotension, and signs of poor blood perfusion. Group B requires hospitalization, as patients in this group experience dehydration, multiple episodes of vomiting, nausea, diarrhea, altered urinary output, and hemodynamic instability, which may progress to shock. Finally, Group C patients exhibit characteristic signs of hepatic failure, acute renal failure, and hepatic encephalopathy, necessitating intensive care unit (ICU) support with advanced care[336].

Antiviral therapies have not yet been formally recommended for the treatment of YF. However, there is evidence supporting the efficacy of such therapies, including the use of sofosbuvir (an NS5 RNA-dependent RNA polymerase inhibitor), an antiviral drug used against hepatitis C, which has demonstrated antiviral activity against YFV *in vitro* and *in vivo*[338,339]. An off-label cohort study using sofosbuvir in YF patients suggested that this medication reduces the viral load of the virus in these patients[340].

Currently, there is no predictive model for the severity and mortality caused by the YFV[336]. However, during the YF epidemic in Brazil, several studies evaluated these parameters. A study conducted on patients admitted to a Brazilian ICU during the 2017 and 2018 outbreaks indicated that factors such as PT-INR, APACHE II, and grade IV hepatic encephalopathy were significant prognostic indicators of mortality risk, independent of other factors[341]. Another 2018 study in São Paulo found that advanced age, high neutrophil count, elevated creatinine, increased AST, and higher viral load were independently associated with higher mortality in YF cases[342]. Additionally, another study demonstrated a higher mortality rate for patients with a history of diabetes mellitus compared to those without this condition. This study also

showed that prophylactic use of anticonvulsants for patients with hepatic encephalopathy or arterial ammonia levels above 70 μmol/L reduced the frequency of seizures from 28% to 17%[343]. Furthermore, early aggressive hemodialysis, routine use of intravenous proton pump inhibitors, and plasma exchange were found to be beneficial[343].

Vaccines: Currently, all YF vaccines are derived from the 17D vaccine strain. Vaccination is the most important measure for preventing YF, being safe, accessible, and capable of providing lifelong immunity with a single dose. The YFV has seven genotypes, but only one serotype[241], ensuring that a single vaccine can protect individuals against all genotypes.

The immune response to the attenuated 17D virus (YF-17D) is mediated by both innate and adaptive immune mechanisms. The virus induces a strong innate immune response, triggered by the TLR-2, 7, 8, and 9 receptors on dendritic cells, leading to the production of pro-inflammatory mediators and type 1 IFNs[295]. The stimulation of these receptors induces a balanced Th1 and Th2 immune response[344]. Additionally, pDCs activate downstream signaling pathways that contribute to the antiviral state[252]. Notably, the complement system also plays a role in the innate immune response[345].

The adaptive immune response is characterized by the rapid and specific production of antibodies. The neutralizing IgM antibody is the first to be produced in the humoral response, peaking two weeks after vaccination and persisting for at least 18 months[344]. IgG antibodies are produced subsequently and can last up to 40 years in the body[252]. The vaccine provides effective immunity for 80%-100% of those vaccinated within 10 days and for over 99% within 30 days [254].

A randomized study conducted in Uganda demonstrated that a fractional dose (1/5 of the standard dose) was non-inferior to the standard dose in inducing seroconversion 28 days after vaccination[346]. These findings support the use of fractional doses during outbreaks when vaccine demand surges and in endemic regions where there is a shortage of YF vaccines.

According to WHO, people who are usually excluded from vaccination include[254]: (1) Infants aged less than 9 months; (2) Pregnant women-except during a YF outbreak when the risk of infection is high; (3) People with severe allergies to egg protein; and (4) People with severe immunodeficiency due to symptomatic HIV/AIDS or other causes, or who have a thymus disorder.

Despite the high reliability of the YF-17D vaccine, the occurrence of serious adverse events has been reported. These include vaccine-associated neurotropic disease, with a case fatality rate of 63%, and vaccine-associated viscerotropic disease, with a case fatality rate of less than 1.5%[241,344]. Additionally, anaphylaxis caused by the egg protein present in YF-17D vaccines is also a significant adverse effect[347].

The WHO recommends vaccination against YFV for travelers over nine months of age who are visiting areas at risk of YFV transmission. Additionally, with certain exceptions, vaccination is advised for all individuals residing in these regions, where it should be part of routine immunization programs. In the event of outbreaks, the WHO advocates for mass vaccination campaigns to achieve the necessary coverage to interrupt virus transmission[259] (Table 5).

CHIKUNGUNYA FEVER

CHIKV is a mosquito-borne virus responsible for periodic and explosive outbreaks of a febrile disease that is characterized by severe and sometimes prolonged polyarthritis[348]. CHIKV is in the *Togaviridae* family, genus *Alphavirus*. It is a small spherical enveloped virus, with a 60-70 nm diameter and its genome is a single strand RNA molecule of positive polarity, encoding four nonstructural (nsP1-4) and three structural proteins (C, E1, and E2)[349].

CHIKV is maintained in a complex sylvatic and rural cycle. The sylvatic cycles of CHIKV exist primarily in Africa between NHPs, rodents and possibly in bats and forest dwelling *Ae. species* (*Ae. albopictus*, *Ae. furcifer*, *Ae. africanus*, and *Ae. taylori*)[350]. Regarding rural and urban cycles, CHIKV transmission is primarily sustained within urban environments through human-to-human transmission. This occurs predominantly *via* the bite of infected mosquitoes such as *Ae. aegypti* or *Ae. Albopictus*[348,351,352].

Recent outbreaks of CHIKV in the Indian Ocean basin and Southeast Asia have been attributed to strains from the Indian Ocean lineage, a newly emerged subgroup within the ECSA clade[353]. This subgroup includes strains with an adaptive mutation (E1-A226V) that enhances viral fitness in *Ae. albopictus* mosquitoes, while maintaining replication efficiency in *Ae. aegypti*[354-357].

Thus, the intensification and expansion of vector-borne diseases are likely to become significant threats due to climate change. Although complicating factors such as mosquito range limits and viral evolution exist, climate change is expected to cause a substantial increase in exposure to *Aedes*-borne viruses. Moreover, several modeling studies predict that climate change will result in the expansion of vectors into temperate zones[358,359].

Despite the low mortality rates associated with CHIKV, it results in significant illness, severely affecting the quality of life for those affected and causing substantial economic losses, particularly in developing nations[360]. Recent outbreaks have heightened concerns about the potential public health impact of CHIKV in temperate regions. Factors such as urbanization, increased human mobility, viral adaptation, inadequate control measures, and the spread of new vectors are likely driving the resurgence of CHIKV infections[361].

CHIKV infection typically begins suddenly with a high fever, often accompanied by joint pain. Additional symptoms, though less common, can include severe polyarthralgia and arthritis, as well as rash, muscle pain, and headaches[348]. Acute symptomatic CHIKV disease often resembles other well-known arbovirus infections, such as DENV and ZIKV disease[362]. The simultaneous presence of these viruses in the same area complicates accurate diagnosis, as their symptoms can overlap and lead to frequent misdiagnoses.

Table 5 Yellow fever live-attenuated vaccines phase III and IV clinical trials-ClinicalTrials.gov database

Intervention/treatment	ClinicalTrials.gov ID	Status	Phase	Sponsor
Biological: 17D Yellow fever vaccine	NCT05332197	Unknown status	Phase 3	London School of Hygiene and Tropical Medicine
Biological: STAMARIL®/biological: Yellow fever vaccine, bio-manguinhos/biological: yellow fever vaccine, institut pasteur/biological: Yellow fever vaccine, chumakov institute (fractional doses)	NCT02991495	Completed	Phase 4	Epicentre
Biological: Yellow fever vaccine, institut pasteur (fractional doses)	NCT04059471	Completed	Phase 4	University of Oxford
Biological: 17DD yellow fever vaccine	NCT02555072	Completed	Phase 4	The Immunobiological Technology Institute (Bio-Manguinhos)/Oswaldo Cruz Foundation (Fiocruz)
Biological: SII yellow fever vaccine/biological: STAMARIL®	NCT05421611	Recruiting	Phase 3	Serum Institute of India Pvt. Ltd.
Biological: 17D yellow fever vaccine/other: Deuterad water	NCT01290055	Recruiting	Phase 4	Sri Edupuganti
Biological: SII yellow fever vaccine/biological: STAMARIL®	NCT05447377	Recruiting	Phase 3	Serum Institute of India Pvt. Ltd.
Biological: 17DD yellow fever vaccine (fractional doses)	NCT03725618	Unknown status	Phase 4	Centers for Disease Control and Prevention
Biological: YF-VAX®	NCT00694655	Recruiting	Phase 4	Emory University
Placebo/biological: CYD tetravalent dengue vaccine/biological: STAMARIL®	NCT01436396	Completed	Phase 3	Sanofi Pasteur
Biological: YF-VAX®	NCT05374317	Completed	Phase 4	United States Army Medical Research Institute of Infectious Diseases
Biological: STAMARIL®	NCT01426243	Completed	Phase 3	French National Agency for Research on AIDS and Viral Hepatitis
Biological: Yellow fever vaccine/biological: MMR vaccine	NCT03368495	Completed	Phase 4	Alba Maria Ropero
Dietary supplement: vitamin A/biological: candidate plasmodium falciparum malaria vaccine/biological: MR-Vac/biological: STAMARIL®	NCT02699099	Completed	Phase 3	GlaxoSmithKline
Biological: 17 DD yellow fever vaccine, biomanguinhos	NCT03132311	Recruiting	Phase 4	Oswaldo Cruz Foundation
Biological: Typhoid Vi polysaccharide vaccine/biological: Yellow fever vaccine/biological: Japanese encephalitis vaccine/biological: Rabies Vaccine/biological: MenACWY-CRM vaccine	NCT01466387	Completed	Phase 3	Novartis
Placebo/TAK-003 tetravalent dengue vaccine/YF-17D yellow fever vaccine	NCT03342898	Completed	Phase 3	Takeda
Placebo/biological: BCG vaccine/biological: Yellow fever vaccine/drug: Vancomycin/drug: neomycin	NCT06148025	Recruiting	Phase 4	South Australian Health and Medical Research Institute

Pathogenesis of chikungunya

Viral entry, replication and release: After the skin bite, the CHIKV enters subcutaneous capillaries and replicates in variety of susceptible cells, including dendritic cells, macrophages, synovial fibroblasts, ECs, and myocytes[362,363]. Additionally, it infects osteoblasts, contributing to the joint pathology and erosive disease observed in chronic arthritis patients[364]. To mediate the processes of entry and viral cell-cell spread, the fusion-related envelope glycoproteins of CHIKV, especially E1 and E2, expressed on the surface of the virion, are essential[365].

Thereby, multiple pathways and mechanisms are employed for CHIKV entry in a cell-type specific manner[366], such as then cell-adhesion molecule, matrix-remodeling-associated protein 8 (MXRA8), a multiple arthritogenic alphavirus receptor, widely expressed in epithelial and mesenchymal cells. Through the creation of various deletion variants, it has been demonstrated that the stalk region of MXRA8 is crucial for facilitating CHIKV entry as it binds in the ''canyon'' between two protomers of the E spike on the surface of the virion[365,367-369]. Besides, another host protein also identified as an entry factor for CHIKV is the CD147 complex, involved in its replication cycle. CD147 is widely expressed in various human cell types, including fibroblast and ECs. Interestingly, CD147 contains similar protein domains and

high structural homology as the previously mentioned alphavirus entry factor MXRA8[370].

Glycosaminoglycans were also shown to be host molecules involved in the binding of CHIKV, particularly heparin/heparan sulfate[371,372]. Some of the other already know CHIKV cell receptors are prohibitin[373], the phosphatidylserine receptor TIM-1[374], C-type calcium-dependent lectin DC-SIGN (DC-specific intercellular adhesion molecule-3-grabbing non-integrin)[375], and, more recently, the four-and-a-half LIM domain protein 1[376].

Furthermore, during the entry process, most reports indicate that CHIKV enters cells *via* clathrin-mediated endocytosis [377], although clathrin-independent pathways have been reported to mediate CHIKV entry to the target cells[378]. In addition, other pathways such as macropinocytosis[379,380], and the engulfment of apoptotic blebs[381]. Following entry, the virus's incubation period ranges from three to seven days[362].

Upon the early endosome's formation, clathrin molecules detach from the endocytic vesicle. This detachment, along with the endosome's pH acidification, prompts the fusion of the endosomal membrane with the viral membranes (*via* the E1 protein), leading to the release of genomic RNA. Immediately following this release, the ribosome translates the non-structural polyproteins (P1234 precursor). Then, the P1234 polyprotein is cleaved by nsP2, liberating the individual non-structural proteins, which forms the viral replicase complex. Consequently, this complex mediates the synthesis of negative-strand RNA, which serves as templates for both new positive-strand RNA and 26S subgenomic RNA. Next, the synthesis of these RNA forms takes place in specialized replication compartments known as spherules. The subgenomic RNA is then translated into the structural polyprotein precursor C-pE2-6K-E1 in the rough ER. The C protein, containing a protease domain, self-cleaves, dissociates from the polyprotein, and assembles with the genomic RNA to form the icosahedral nucleocapsid core in the cytoplasm. The pE2-6K-E1 precursor is directed to the RER lumen for maturation, culminating in the formation of E1-E2 heterodimers. These heterodimers are inserted into the cell membrane, creating the "virus budding microdomain". Finally, the assembled icosahedral nucleocapsid core migrates to this domain, where new viral particles are released extracellularly *via* the budding process[382-386].

Currently, the precise cellular mechanisms of the disease are still in need of elucidation. Although the structural details of the coat glycoproteins essential for viral entry are well understood, the potential target cell receptors and the exact mechanism of cell entry remain less well-known[387]. Lastly, numerous cell types, many of which are found at disease sites, are susceptible to CHIKV. As previously mentioned, these include chondrocytes, ECs, fibroblasts, hepatocytes, macrophages, monocytes, muscle satellite cells, myocytes, and osteoblasts[388-391].

Innate antiviral IFN response: Foremost, CHIKV infection induces systemic innate responses, primarily involving antiviral IFN-α, pro-inflammatory cytokines, and chemokines. This process is subsequently followed by the activation of adaptive immune responses[392].

When a chikungunya virion infects its initial target cells, it activates specific PRRs for RNA viruses, such as RLRs (RIG-I and MDA5) in the cytoplasm and TLRs, including TLR-3, TLR-7, and TLR-8 in the endosomal compartment[386,393]. This viral recognition initiates the signaling cascade of the innate immune response, activating IFN regulatory factors[394,395] and leading to the induction of type I-IFNs and various proinflammatory chemokines and cytokines. CHIKV infection has been demonstrated to induce the increased production of type I IFNs (IFN-α and IFN-β) as well as IFN-γ [394,396], that ultimately result in the induction of hundreds of ISGs that cooperatively establish an antiviral state within the infected and adjacent cells, including inhibition of viral replication and virion maturation[397-399].

Consequently, to persist within infected host cells, CHIKV has developed various strategies to evade IFN responses, much like many other viruses, such as CHIKV-encoded proteins strongly inhibiting the activation of the IFN-β and NF-κB promoters. Thus, CHIKV-encoded proteins may evade viral detection, resulting in a reduction of IFN responses[400-402].

Analysis of chemokines in CHIKV-infected patients shows a significant increase in IFN-γ-induced chemokines, such as CXCL9/MIG and CXCL10/IP-10, during the early days of infection[396]. IFN-γ, a primary cytokine for type 1 T helper cells, promotes the production of these chemokines, which are associated with the severity of CHIKV disease[403]. Additionally, elevated levels of CCL2/MCP-1, a known monocyte attractant, were detected, suggesting its involvement in disease progression and bone loss during chronic stages[404,405]. Furthermore, high levels of C5a anaphylatoxin were found early in the infection, a new observation. C5a's role in inflammation, similar to its effects in rheumatoid arthritis and dengue fever, indicates its potential as a therapeutic target for managing rheumatic complications associated with CHIKV[406-408].

Adaptative immune response: There is relatively more knowledge about innate immune responses to infection, which primarily react to the acute phase of the disease[409]. Research indicates that CD4 T cells are essential for mediating humoral immunity against CHIKV[410]. In patients who have chronic chikungunya infection or have recovered from it, IFN-γ-producing CD4+ T and CD8+ T cells were detectable in the majority (85%) of the patients 12 to 24 months post-infection[392]. These T cells were primarily directed against the nsP1 and E2 peptides[411]. However, the pathogenic roles of CD4+ T cells have also been demonstrated, as findings indicate that CD4+ T, and not CD8+ T cells, are responsible for the joint inflammation induced by CHIKV infection[412].

In patients with acute CHIKV infection, CD8+ T cells were shown to be activated, evidenced by increased expression of activation markers such as CD69, CD107a, perforin, and granzyme, which suggests that CD8+ T cells are active during the acute phase and mediate cytotoxic activities[413]. Results such as the increased activation of CD8+ T cells in patients with acute CHIKV infection, the accumulation of CHIKV-specific CD8+ T cells in mouse spleen and joint-associated tissues, and their ability to produce IFN-γ upon *ex vivo* stimulation, suggest that CD8+ T cells are functionally active during the acute phase[414]. However, the fact that these effector T cells did not reduce viremia levels, combined with the observation that pre-existing functional effector CD8+ T cells led to CHIKV clearance mainly in the spleen, suggests that CHIKV has developed strategies to evade CD8+ T cell recognition, allowing it to establish chronic infection in the joints [414].

Antibodies play a crucial role in controlling CHIKV infection[410]. Anti-CHIKV IgM levels gradually increase from as early as day 2-4 after symptom onset[415,416] and then remain stable for up to 4 months[417]. Similarly, anti-CHIKV IgG can be detected in the early convalescent stage, appearing around 10 days post-symptom onset in some patients, and persists for 2-3 months[418]. Early formation of IgG3 antibodies at day 10 correlates with reduced viremia levels and mitigates chronic and severe disease[418]. Moreover, early production of IgM antibodies, which possess neutralizing capabilities, contributes to lowering viremia levels, and the neutralizing activity of IgM complements early IgG antibodies and plays a critical role from days 4 to 10 post-symptom onset[416]. Additionally, the presence of both IgM and IgG is associated with modulation of cytokine and chemokine levels, suggesting their role in regulating immune responses in CHIKV-infected patients[418,419].

Antibodies directed against the E2 glycoprotein are critical in the immune response to CHIKV infection[410,420-422]. Furthermore, studies in CHIKV-infected patients have identified several monoclonal antibodies that neutralize the virus by targeting epitopes in the E1 and E2 glycoproteins, with E2-specific antibodies proving effective in protecting against lethal chikungunya infection[423]. These antibodies block viral fusion and release from infected cells[420]. Moreover, analysis of antibody responses in patients has revealed that CHIKV-specific IgG avidity increases over time, correlating with improved neutralizing capacity[414]. Additionally, patients in the acute phase show higher avidity against E1 and E2 proteins compared to those with chronic infections[424], suggesting that robust antibody responses may play a crucial role in preventing chronic disease progression.

Chronic chikungunya arthritis: It is roughly estimated that 30% to 40% of infected individuals experience some long-term sequelae. These sequelae include persistent arthralgia and/or arthritis, with severe pain present in about 37% of individuals suffering from persistent arthralgia[425]. Factors associated with the persistence of arthralgia in CHIKV-infected patients have not been fully explored. However, the few available studies indicate that patients over 40 years old, females, and those with higher levels of CXCL8 detected during the acute phase of the disease are more likely to experience persistent arthralgia[426,427].

Recent studies have identified human synovial tissues as sanctuaries for CHIKV, where viral RNA persists in joint fluid[428]. This persistence is thought to contribute to CHIKV -associated arthritis by infecting fibroblast-like synoviocytes and promoting the migration of primary human monocytes[429]. These monocytes/macrophages can then transform into osteoclast-like cells, producing high levels of TNF-α and IL-6 proinflammatory cytokines.

Despite advancements in our understanding of CHIKV infection, the exact immunopathogenic mechanisms that lead to CHIKV-induced arthralgia remain unclear. Cytokines and chemokines are key players in CHIKV immunopathology, considering that during the early acute phase, serum proinflammatory cytokines such as IFN-α, IFN-β, IFN-γ, CXCL10/IP-10, and IL-1β show a strong upregulation. IFN-α is detected early during infection, often on the first day, and its concentration correlates with viral load, which is significantly higher in elderly patients[430]. Additionally, the elevation of MCP-1, IL-6, IL-8, MIP-1α, and MIP-1β is most prominent in the chronic phase[431].

Furthermore, another study found that levels of CXCL9/MIG and CXCL10/IP-10, along with high concentrations of IgG, were associated with severe symptoms in CHIKV patients[432]. Moreover, TNF-α and IFN-γ-secreting NK-like T cells were elevated in patients with persistent CHIKV arthralgia[433]. In addition, a systematic meta-analysis of immune signatures in patients with acute CHIKV infection showed a correlation between increased proinflammatory cytokines and arthralgia[426].

Clinical management of chikungunya

CHIKV infection is typically classified into three stages: The acute stage (lasting from day 1 to day 21), the post-acute stage (spanning from day 21 to 3 months), and the chronic stage (lasting beyond 3 months)[434].

Acute phase of infection: During the acute stage, infected patients may undergo a viremic phase lasting 5 to 10 days, followed by a post-viremic phase lasting 6 to 21 days[434]. The acute viremic stage of CHIKV infection is characterized by an abrupt onset of high-grade fever (often > 39 °C), arthralgia, myalgia, headache, fatigue, nausea, vomiting, and arthritis. Additionally, other symptoms such as conjunctivitis, exanthema, and edema may also occur. The exanthema can manifest as a diffuse or focal skin rash. Despite these symptoms, the disease is self-limiting, and in most patients, it resolves within 7-10 days[430,435].

In the post-acute phase, symptoms exhibit diverse manifestations stemming from persistent initial acute symptoms, such as joint issues and fatigue, while fever typically subsides[436]. Additionally, polyarthritis tends to affect both sides of the body symmetrically, involving both small and large joints like knees, ankles, hands, and wrists and there may be periarticular involvement, including enthesitis, tenosynovitis, and bursitis[437,438]. Furthermore, acute CHIKV infection can potentially exacerbate pre-existing autoimmune arthritis[439].

Severe symptoms affecting vital organs can develop during CHIKV infection, including encephalitis, encephalopathy, and neuro-ocular diseases (such as uveitis, retinitis, and optic neuritis), as well as myelopathy and myelitis, Guillain-Barré syndrome, myocarditis, hepatitis, acute interstitial nephritis, severe sepsis, septic shock, and multi-organ failure [440-442]. Furthermore, individuals with comorbidities, the elderly, and infants are at a higher risk for these severe symptoms[442-444]. Additionally, perinatal CHIKV infection can result in sequelae such as microcephaly, cerebral palsy, and neurocognitive impairment[445].

Chronic or persistent phase of infection: The chronic stage of CHIKV infection is marked by symptoms that persist for over three months following the initial diagnosis of acute infection. Specifically, this chronic phase often affects distal joints due to continuous viral replication and inflammation. Moreover, the virus has been detected in several organs, including ECs in the liver, mononuclear cells in the spleen, macrophages in the synovial fluid and surrounding tissues, and satellite cells in the muscles[348].

Table 6 Chikungunya vaccines currently in clinical trials-clinicalTrials.gov database

Vaccine technology platforms	Intervention/treatment	ClinicalTrials.gov ID	Phase	Status	Sponsor
Virus-like particle vaccine	Biological: VRC-CHKVLP059-00-VP	NCT01489358	1	Completed	NIAID
	Biological: PXVX0317	NCT03992872	2	Completed	Bavarian Nordic
	Biological: CHIKV VLP/adjuvant	NCT05072080	3	Completed	Bavarian Nordic
	Biological: VRC-CHKVLP059-00-VP	NCT02562482	2	Completed	NIAID
Viral vectored vaccine	Biological: MV-CHIK/ Biological: MMR-vaccine	NCT03101111	2	Completed	Themis Bioscience GmbH
	Biological: ChAdOx1 Chik	NCT04440774	1	Completed	University of Oxford
Live attenuated vaccine	Biological: VLA1553	NCT04650399	3	Completed	Butantan Institute
	Biological: VLA1553	NCT03382964	1	Completed	Valneva Austria GmbH
	Biological: V184	NCT03807843	2	Completed	Themis Bioscience GmbH
Inactivated whole virion vaccine	Biological: BBV87	NCT04566484	3	Completed	International Vaccine Institute

NIAID: National Institute of Allergy and Infectious Diseases.

During the chronic stage, patients can suffer from unpredictable relapses of fever, fatigue, joint pain, and stiffness[446,447]. Furthermore, older individuals and those with a history of rheumatic or traumatic joint disorders are more likely to develop this chronic phase[447]. Age, female sex, and dehydration state during the acute phase are also important factors [448].

Diagnosis: The diagnosis of CHIKV infection can be conducted using various methods, including viral isolation, serological assays, and RNA detection through real-time quantitative reverse transcriptase PCR (qRT-PCR)[449]. RT-PCR and virus isolation are most effective when conducted near the onset of febrile illness, when viremia is at its peak. Serological tests to detect IgM and IgG antibodies are best performed approximately seven days after the onset of symptoms[386].

Diagnosing CHIKV infection solely based on clinical symptoms poses significant challenges, particularly in endemic regions where other arboviruses like DENV and ZIKV are also circulating. Healthcare providers should consider CHIKV infection in patients exhibiting acute febrile illness and polyarthralgia, especially among travelers returning from areas known for CHIKV transmission[386].

Virus isolation through cell culture is considered the gold standard for viral detection due to its high specificity[447]. To isolate CHIKV, various cell lines from humans, monkeys, and mosquitoes have been utilized[450]. CHIKV infection typically results in a noticeable CPE in culture, which can be observed as early as 24 hours after infection[363].

Nucleic acid detection is a rapid and highly sensitive assay used to diagnose CHIKV infection. One common method is qRT-PCR, which detects the CHIKV genome[451-454]. This technique can also be employed in multiplex PCR assays to simultaneously detect other arboviruses, including ZIKV[454]. A conserved region of the envelope E1 and E2 genes is the most common target for qRT-PCR, and other targets include nsP1 and nsP4 genes[451,452,455]. The viral RNA of CHIKV can be detected by the qRT-PCR method from 0 to 7 days of infection, after which qRT-PCR detection becomes unreliable [456].

Serological testing, simpler than qRT-PCR, can detect anti-CHIKV immunoglobulin M (IgM) and IgG. These antibodies are detectable using ELISA, immunofluorescence assays (IFA), and PRNT[457]. Among these, IgM ELISA is the most frequently utilized method for diagnosing CHIKV infection[458]. Serology-based diagnosis is constrained by potential cross-reactivity with other arboviruses[459]. CHIKV shares antigenic similarities with other viruses in the Alphavirus genus, such as Semliki forest virus, Mayaro virus, and o'nyong nyong virus[460].

A systematic review and meta-analysis found that IgM detection tests demonstrated over 90% diagnostic accuracy for ELISA-based tests, IFA, in-house developed tests, and samples collected more than seven days after symptom onset. However, sensitivity was lower for rapid tests (42.3%), commercial tests (78.6%), and samples collected within seven days before symptom onset (26.2%). Therefore, IgM detection tests are particularly recommended for use with samples obtained during the convalescent phase of CHIKV infection. The specificity of IgM detection tests remained above 90% across all test formats and sample collection times[461].

Serological tests for CHIKV E1/E2 antigen detection, including rapid assays[461,462], ELISA-based tests[456], and FLISA-based tests[458] have shown promising performance. However, challenges in developing antigen-based tests include variability in performance across different CHIKV genotypes[463]. Therefore, further evaluation in diverse geographical settings is crucial to validate these tests against all circulating CHIKV genotypes.

Treatment: At present, treatment for chikungunya primarily aims to alleviate symptoms, as there is no dedicated vaccine or specific treatment available. As a result, a range of drugs have been utilized with mixed effectiveness, primarily

targeting supportive care and the reduction of joint pain. It should be tailored to the clinical context and targeted at specific risk groups, focusing on managing fever and pain, addressing dehydration, providing organ support, and preventing iatrogenic complications and functional impairment[464]. Key pharmaceutical treatments for chikungunya include NSAIDs, disease-modifying antirheumatic drugs, and antivirals[465].

Analgesia using acetaminophen is the preferred treatment. Avoiding NSAIDs and salicylates within the initial 14 days of disease onset is advised due to the risk of bleeding complications associated with dengue fever, unless dengue has been definitively ruled out[464]. No specific NSAID class has demonstrated superiority in effectively managing post-chikungunya symptoms. In cases where conditions such as tenosynovitis, bursitis, tunnel syndrome, capsulitis, or synovitis are not adequately controlled by oral treatments, local anti-inflammatory therapy (either topical or *via* infiltration) should be prescribed to minimize excessive systemic medication use[434].

The use of corticosteroids is not recommended due to the risk of severe rebound arthritis and tenosynovitis. Therefore, systemic corticosteroids should only be considered for treating inflammatory polyarticular presentations, particularly when there is concurrent tenosynovitis or active synovitis, or when NSAIDs are ineffective or contraindicated[460].

In animal models of CHIKV infection, the use of CHIKV IgG or CHIKV-specific monoclonal antibodies for prophylaxis has demonstrated protective effects[466]. This highlights the potential of antibody-based therapies as a promising strategy for preventing severe CHIKV infection in at-risk individuals.

In cell-based screenings aimed at combating CHIKV infection, several drugs with antiviral properties have been identified. These drugs target distinct stages of the CHIKV replication cycle: Chloroquine[467] and chlorpromazine[468] act on virus entry, while harringtonine and homoharringtonine[469] affect viral protein translation. Others, such as trigocherriolide A[470], ribavirin[471], IFN-alpha[472], apigenin, and silybin[468], and inhibit virus replication.

Vaccines: Given the rapid spread of CHIKV across many countries, vaccinating the susceptible population remains the most effective method to control infection. CHIKV preclinical candidate vaccines under development encompass a variety of approaches, including a whole-virus inactivated vaccine[473], a VEE/CHIKV chimeric vaccine[474], a recombinant adenovirus vectored vaccine[475], a DNA-based CHIKV vaccine[476], a VLP vaccine[477], and a live-attenuated vaccine designed to elicit robust and enduring immune responses[478] (Table 6).

The leading vaccine candidate, a live-attenuated Δ5nsP3 (Valneva), has successfully completed Phase III trials and is now in the process of obtaining licensure. This vaccine stands out due to its superiority over single-dose regimens, achieving a remarkable 98.9% seroconversion in adults and older adults. It holds potential benefits for travelers planning to visit endemic areas. Nevertheless, further research is needed to determine its effectiveness in providing protection within endemic regions[479-481].

Another vaccine candidate, the adjuvanted VLP (CHIKV VLP) PXVX0317PXV, is non-self-replicating and known for its ability to elicit robust immunogenicity. However, it necessitates two doses, and the ongoing trial does not include participants from endemic areas. Therefore, this vaccine might be a viable option for immunocompromised individuals [482-485].

CONCLUSION

In summary, DENV, ZIKV, YFV, and CHIKV share several common characteristics, including their protein structure, tropism, evasion of the immune response, and various symptoms such as fever and rash. Moreover, they often have common transmission vectors and can exhibit cross-reactions in laboratory tests. Nonetheless, their individualities can significantly aid in differential diagnosis, necessitating healthcare professionals to adopt a clinical management approach tailored to the patients' symptoms and to prevent potential complications typically associated with a specific virus. For example, dengue is often correlated with hemorrhages, Zika with microcephaly, YFV with hepatitis, and CHIKV with polyarthralgia.

However, it is essential to highlight that the immune response of each patient and their symptoms are not always clear, and symptoms more commonly associated with one viral infection can also be present in others. Therefore, laboratory and epidemiological diagnoses, considering local endemics and current outbreaks, are crucial in accurately diagnosing the aforementioned arboviruses.

FOOTNOTES

Author contributions: Dietrich VC, Costa JMC, Oliveira MMGL, and Aguiar CEO researched the content, developed the article, and created the tables and illustrations; In addition, Dietrich VC, as the first author, also systematized the text, developed the abstract, introduction, conclusion, and handled the guidelines for the preparation and submission of the review; Lemos FFB, Silva LGO, Luz LMS, and de Melo FF reviewed the articles during the writing process, providing suggestions, and directing the research; Lemos FFB and de Melo FF contributed equally to this work as co-corresponding authors; de Melo FF, as the corresponding author, thoroughly reviewed the entire article; and all the cited authors contributed significantly to the production of the article.

Supported by the Permanecer Program (part of the actions of the Office of Affirmative Actions); Education and Diversity of the Dean of Student Assistance at the Federal University of Bahia (UFBA); and CNPq Research Productivity Fellow.

Conflict-of-interest statement: All authors declare no potential conflicts of interest.

Country of origin: Brazil

ORCID number: Victoria Cenci Dietrich 0009-0007-3627-6454; Juan Marcos Caram Costa 0009-0003-4045-9684; Marina Maria Gomes Leite Oliveira 0009-0000-5785-438X; Carlos Eduardo Oliveira Aguiar 0009-0000-7108-0640; Luis Guilherme de Oliveira Silva 0000-0001-7275-7182; Marcel Silva Luz 0000-0003-1650-5807; Fabian Fellipe Bueno Lemos 0000-0002-4686-7086; Fabrício Freire de Melo 0000-0002-5680-2753.

S-Editor: Chen YL
L-Editor: A
P-Editor: Xu ZH

REFERENCES

1 **Beckham JD**, Tyler KL. Arbovirus Infections. *Continuum (Minneap Minn)* 2015; **21**: 1599-1611 [PMID: 26633778 DOI: 10.1212/CON.0000000000000240]
2 **World Health Organization**. Vector-borne diseases. September 26, 2024. [cited 1 October 2024]. Available from: https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases
3 **Rodrigues-Alves ML**, Melo-Júnior OAO, Silveira P, Mariano RMDS, Leite JC, Santos TAP, Soares IS, Lair DF, Melo MM, Resende LA, da Silveira-Lemos D, Dutra WO, Gontijo NF, Araujo RN, Sant'Anna MRV, Andrade LAF, da Fonseca FG, Moreira LA, Giunchetti RC. Historical Perspective and Biotechnological Trends to Block Arboviruses Transmission by Controlling Aedes aegypti Mosquitos Using Different Approaches. *Front Med (Lausanne)* 2020; **7**: 275 [PMID: 32656216 DOI: 10.3389/fmed.2020.00275]
4 **Grard G**, Caron M, Mombo IM, Nkoghe D, Mboui Ondo S, Jiolle D, Fontenille D, Paupy C, Leroy EM. Zika virus in Gabon (Central Africa)--2007: a new threat from Aedes albopictus? *PLoS Negl Trop Dis* 2014; **8**: e2681 [PMID: 24516683 DOI: 10.1371/journal.pntd.0002681]
5 **Duffy MR**, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, Pretrick M, Marfel M, Holzbauer S, Dubray C, Guillaumot L, Griggs A, Bel M, Lambert AJ, Laven J, Kosoy O, Panella A, Biggerstaff BJ, Fischer M, Hayes EB. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med* 2009; **360**: 2536-2543 [PMID: 19516034 DOI: 10.1056/NEJMoa0805715]
6 **Laporta GZ**, Potter AM, Oliveira JFA, Bourke BP, Pecor DB, Linton YM. Global Distribution of Aedes aegypti and Aedes albopictus in a Climate Change Scenario of Regional Rivalry. *Insects* 2023; **14** [PMID: 36661976 DOI: 10.3390/insects14010049]
7 **Kading RC**, Brault AC, Beckham JD. Global Perspectives on Arbovirus Outbreaks: A 2020 Snapshot. *Trop Med Infect Dis* 2020; **5** [PMID: 32906771 DOI: 10.3390/tropicalmed5030142]
8 **Tajudeen YA**, Oladunjoye IO, Mustapha MO, Mustapha ST, Ajide-Bamigboye NT. Tackling the global health threat of arboviruses: An appraisal of the three holistic approaches to health. *Health Promot Perspect* 2021; **11**: 371-381 [PMID: 35079581 DOI: 10.34172/hpp.2021.48]
9 **Harapan H**, Michie A, Sasmono RT, Imrie A. Dengue: A Minireview. *Viruses* 2020; **12** [PMID: 32751561 DOI: 10.3390/v12080829]
10 **Sinha S**, Singh K, Ravi Kumar YS, Roy R, Phadnis S, Meena V, Bhattacharyya S, Verma B. Dengue virus pathogenesis and host molecular machineries. *J Biomed Sci* 2024; **31**: 43 [PMID: 38649998 DOI: 10.1186/s12929-024-01030-9]
11 **Mbanzulu KM**, Wumba R, Mboera LEG, Kayembe JN, Engbu D, Bojabwa MM, Zanga JK, Misinzo G, Kimera SI. Pattern of Aedes aegypti and Aedes albopictus Associated with Human Exposure to Dengue Virus in Kinshasa, the Democratic Republic of the Congo. *Trop Med Infect Dis* 2022; **7** [PMID: 36422943 DOI: 10.3390/tropicalmed7110392]
12 **Huang YS**, Higgs S, Vanlandingham DL. Emergence and re-emergence of mosquito-borne arboviruses. *Curr Opin Virol* 2019; **34**: 104-109 [PMID: 30743191 DOI: 10.1016/j.coviro.2019.01.001]
13 **World Health Organization**. Global strategy for dengue prevention and control 2012-2020. August 22, 2012. [cited 1 October 2024]. Available from: https://www.who.int/publications/i/item/9789241504034
14 **Guo C**, Zhou Z, Wen Z, Liu Y, Zeng C, Xiao D, Ou M, Han Y, Huang S, Liu D, Ye X, Zou X, Wu J, Wang H, Zeng EY, Jing C, Yang G. Global Epidemiology of Dengue Outbreaks in 1990-2015: A Systematic Review and Meta-Analysis. *Front Cell Infect Microbiol* 2017; **7**: 317 [PMID: 28748176 DOI: 10.3389/fcimb.2017.00317]
15 **Stanaway JD**, Shepard DS, Undurraga EA, Halasa YA, Coffeng LE, Brady OJ, Hay SI, Bedi N, Bensenor IM, Castañeda-Orjuela CA, Chuang TW, Gibney KB, Memish ZA, Rafay A, Ukwaja KN, Yonemoto N, Murray CJL. The global burden of dengue: an analysis from the Global Burden of Disease Study 2013. *Lancet Infect Dis* 2016; **16**: 712-723 [PMID: 26874619 DOI: 10.1016/S1473-3099(16)00026-8]
16 **Delrieu M**, Martinet JP, O'Connor O, Viennet E, Menkes C, Burtet-Sarramegna V, Frentiu FD, Dupont-Rouzeyrol M. Temperature and transmission of chikungunya, dengue, and Zika viruses: A systematic review of experimental studies on Aedes aegypti and Aedes albopictus. *Curr Res Parasitol Vector Borne Dis* 2023; **4**: 100139 [PMID: 37719233 DOI: 10.1016/j.crpvbd.2023.100139]
17 **Robinson M**, Einav S. Towards Predicting Progression to Severe Dengue. *Trends Microbiol* 2020; **28**: 478-486 [PMID: 31982232 DOI: 10.1016/j.tim.2019.12.003]
18 **Roy SK**, Bhattacharjee S. Dengue virus: epidemiology, biology, and disease aetiology. *Can J Microbiol* 2021; **67**: 687-702 [PMID: 34171205 DOI: 10.1139/cjm-2020-0572]
19 **Ngono AE**, Shresta S. Immune Response to Dengue and Zika. *Annu Rev Immunol* 2018; **36**: 279-308 [PMID: 29345964 DOI: 10.1146/annurev-immunol-042617-053142]
20 **Wong JM**, Adams LE, Durbin AP, Muñoz-Jordán JL, Poehling KA, Sánchez-González LM, Volkman HR, Paz-Bailey G. Dengue: A Growing Problem With New Interventions. *Pediatrics* 2022; **149** [PMID: 35543085 DOI: 10.1542/peds.2021-055522]
21 **O'Driscoll M**, Buddhari D, Huang AT, Waickman A, Kaewhirun S, Iamsirithaworn S, Khampaen D, Farmer A, Fernandez S, Rodriguez-Barraquer I, Srikiatkhachorn A, Thomas S, Endy T, Rothman AL, Anderson K, Cummings DAT, Salje H. Maternally derived antibody titer

dynamics and risk of hospitalized infant dengue disease. *Proc Natl Acad Sci U S A* 2023; **120**: e2308221120 [PMID: 37774093 DOI: 10.1073/pnas.2308221120]

22 **Vaughn DW**, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Endy TP, Raengsakulrach B, Rothman AL, Ennis FA, Nisalak A. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis* 2000; **181**: 2-9 [PMID: 10608744 DOI: 10.1086/315215]

23 **Perdomo-Celis F**, Salgado DM, Narváez CF. Magnitude of viremia, antigenemia and infection of circulating monocytes in children with mild and severe dengue. *Acta Trop* 2017; **167**: 1-8 [PMID: 27986543 DOI: 10.1016/j.actatropica.2016.12.011]

24 **Soo KM**, Khalid B, Ching SM, Tham CL, Basir R, Chee HY. Meta-analysis of biomarkers for severe dengue infections. *PeerJ* 2017; **5**: e3589 [PMID: 28929009 DOI: 10.7717/peerj.3589]

25 **Ten Bosch QA**, Clapham HE, Lambrechts L, Duong V, Buchy P, Althouse BM, Lloyd AL, Waller LA, Morrison AC, Kitron U, Vazquez-Prokopec GM, Scott TW, Perkins TA. Contributions from the silent majority dominate dengue virus transmission. *PLoS Pathog* 2018; **14**: e1006965 [PMID: 29723307 DOI: 10.1371/journal.ppat.1006965]

26 **Wu P**, Yu X, Wang P, Cheng G. Arbovirus lifecycle in mosquito: acquisition, propagation and transmission. *Expert Rev Mol Med* 2019; **21**: e1 [PMID: 30862324 DOI: 10.1017/erm.2018.6]

27 **Rodenhuis-Zybert IA**, Wilschut J, Smit JM. Dengue virus life cycle: viral and host factors modulating infectivity. *Cell Mol Life Sci* 2010; **67**: 2773-2786 [PMID: 20372965 DOI: 10.1007/s00018-010-0357-z]

28 **Urcuqui-Inchima S**, Patiño C, Torres S, Haenni AL, Díaz FJ. Recent developments in understanding dengue virus replication. *Adv Virus Res* 2010; **77**: 1-39 [PMID: 20951868 DOI: 10.1016/B978-0-12-385034-8.00001-6]

29 **Trivedi S**, Chakravarty A. Neurological Complications of Dengue Fever. *Curr Neurol Neurosci Rep* 2022; **22**: 515-529 [PMID: 35727463 DOI: 10.1007/s11910-022-01213-7]

30 **Chen Y**, Maguire T, Hileman RE, Fromm JR, Esko JD, Linhardt RJ, Marks RM. Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. *Nat Med* 1997; **3**: 866-871 [PMID: 9256277 DOI: 10.1038/nm0897-866]

31 **Navarro-Sanchez E**, Altmeyer R, Amara A, Schwartz O, Fieschi F, Virelizier JL, Arenzana-Seisdedos F, Desprès P. Dendritic-cell-specific ICAM3-grabbing non-integrin is essential for the productive infection of human dendritic cells by mosquito-cell-derived dengue viruses. *EMBO Rep* 2003; **4**: 723-728 [PMID: 12783086 DOI: 10.1038/sj.embor.embor866]

32 **Reyes-Del Valle J**, Chávez-Salinas S, Medina F, Del Angel RM. Heat shock protein 90 and heat shock protein 70 are components of dengue virus receptor complex in human cells. *J Virol* 2005; **79**: 4557-4567 [PMID: 15795242 DOI: 10.1128/JVI.79.8.4557-4567.2005]

33 **Khandia R**, Munjal A, Dhama K, Karthik K, Tiwari R, Malik YS, Singh RK, Chaicumpa W. Modulation of Dengue/Zika Virus Pathogenicity by Antibody-Dependent Enhancement and Strategies to Protect Against Enhancement in Zika Virus Infection. *Front Immunol* 2018; **9**: 597 [PMID: 29740424 DOI: 10.3389/fimmu.2018.00597]

34 **Nanaware N**, Banerjee A, Mullick Bagchi S, Bagchi P, Mukherjee A. Dengue Virus Infection: A Tale of Viral Exploitations and Host Responses. *Viruses* 2021; **13** [PMID: 34696397 DOI: 10.3390/v13101967]

35 **Cruz-Oliveira C**, Freire JM, Conceição TM, Higa LM, Castanho MA, Da Poian AT. Receptors and routes of dengue virus entry into the host cells. *FEMS Microbiol Rev* 2015; **39**: 155-170 [PMID: 25725010 DOI: 10.1093/femsre/fuu004]

36 **Marzinek JK**, Holdbrook DA, Huber RG, Verma C, Bond PJ. Pushing the Envelope: Dengue Viral Membrane Coaxed into Shape by Molecular Simulations. *Structure* 2016; **24**: 1410-1420 [PMID: 27396828 DOI: 10.1016/j.str.2016.05.014]

37 **Kuhn RJ**, Zhang W, Rossmann MG, Pletnev SV, Corver J, Lenches E, Jones CT, Mukhopadhyay S, Chipman PR, Strauss EG, Baker TS, Strauss JH. Structure of dengue virus: implications for flavivirus organization, maturation, and fusion. *Cell* 2002; **108**: 717-725 [PMID: 11893341 DOI: 10.1016/s0092-8674(02)00660-8]

38 **Tay MYF**, Vasudevan SG. The Transactions of NS3 and NS5 in Flaviviral RNA Replication. *Adv Exp Med Biol* 2018; **1062**: 147-163 [PMID: 29845531 DOI: 10.1007/978-981-10-8727-1_11]

39 **Teramoto T**, Balasubramanian A, Choi KH, Padmanabhan R. Serotype-specific interactions among functional domains of dengue virus 2 nonstructural proteins (NS) 5 and NS3 are crucial for viral RNA replication. *J Biol Chem* 2017; **292**: 9465-9479 [PMID: 28396347 DOI: 10.1074/jbc.M117.775643]

40 **Osawa T**, Aoki M, Ehara H, Sekine SI. Structures of dengue virus RNA replicase complexes. *Mol Cell* 2023; **83**: 2781-2791.e4 [PMID: 37478848 DOI: 10.1016/j.molcel.2023.06.023]

41 **Singh K**, Martinez MG, Lin J, Gregory J, Nguyen TU, Abdelaal R, Kang K, Brennand K, Grünweller A, Ouyang Z, Phatnani H, Kielian M, Wendel HG. Transcriptional and Translational Dynamics of Zika and Dengue Virus Infection. *Viruses* 2022; **14** [PMID: 35891396 DOI: 10.3390/v14071418]

42 **Carter CC**, Mast FD, Olivier JP, Bourgeois NM, Kaushansky A, Aitchison JD. Dengue activates mTORC2 signaling to counteract apoptosis and maximize viral replication. *Front Cell Infect Microbiol* 2022; **12**: 979996 [PMID: 36171757 DOI: 10.3389/fcimb.2022.979996]

43 **Chaudhary N**, Srivastava S, Gupta S, Menon MB, Patel AK. Dengue virus induced autophagy is mediated by HMGB1 and promotes viral propagation. *Int J Biol Macromol* 2023; **229**: 624-635 [PMID: 36587643 DOI: 10.1016/j.ijbiomac.2022.12.299]

44 **Frakolaki E**, Kaimou P, Moraiti M, Kalliampakou KI, Karampetsou K, Dotsika E, Liakos P, Vassilacopoulou D, Mavromara P, Bartenschlager R, Vassilaki N. The Role of Tissue Oxygen Tension in Dengue Virus Replication. *Cells* 2018; **7** [PMID: 30513781 DOI: 10.3390/cells7120241]

45 **Liang S**, Wu YS, Li DY, Tang JX, Liu HF. Autophagy in Viral Infection and Pathogenesis. *Front Cell Dev Biol* 2021; **9**: 766142 [PMID: 34722550 DOI: 10.3389/fcell.2021.766142]

46 **Barnard TR**, Abram QH, Lin QF, Wang AB, Sagan SM. Molecular Determinants of Flavivirus Virion Assembly. *Trends Biochem Sci* 2021; **46**: 378-390 [PMID: 33423940 DOI: 10.1016/j.tibs.2020.12.007]

47 **Alcon-LePoder S**, Drouet MT, Roux P, Frenkiel MP, Arborio M, Durand-Schneider AM, Maurice M, Le Blanc I, Gruenberg J, Flamand M. The secreted form of dengue virus nonstructural protein NS1 is endocytosed by hepatocytes and accumulates in late endosomes: implications for viral infectivity. *J Virol* 2005; **79**: 11403-11411 [PMID: 16103191 DOI: 10.1128/JVI.79.17.11403-11411.2005]

48 **Uno N**, Ross TM. Dengue virus and the host innate immune response. *Emerg Microbes Infect* 2018; **7**: 167 [PMID: 30301880 DOI: 10.1038/s41426-018-0168-0]

49 **Lee MF**, Voon GZ, Lim HX, Chua ML, Poh CL. Innate and adaptive immune evasion by dengue virus. *Front Cell Infect Microbiol* 2022; **12**: 1004608 [PMID: 36189361 DOI: 10.3389/fcimb.2022.1004608]

50 **Nasirudeen AM**, Wong HH, Thien P, Xu S, Lam KP, Liu DX. RIG-I, MDA5 and TLR3 synergistically play an important role in restriction of

dengue virus infection. *PLoS Negl Trop Dis* 2011; **5**: e926 [PMID: 21245912 DOI: 10.1371/journal.pntd.0000926]

51 **Mapalagamage M**, Weiskopf D, Sette A, De Silva AD. Current Understanding of the Role of T Cells in Chikungunya, Dengue and Zika Infections. *Viruses* 2022; **14** [PMID: 35215836 DOI: 10.3390/v14020242]

52 **Castillo Ramirez JA**, Urcuqui-Inchima S. Dengue Virus Control of Type I IFN Responses: A History of Manipulation and Control. *J Interferon Cytokine Res* 2015; **35**: 421-430 [PMID: 25629430 DOI: 10.1089/jir.2014.0129]

53 **Dalrymple NA**, Cimica V, Mackow ER. Dengue Virus NS Proteins Inhibit RIG-I/MAVS Signaling by Blocking TBK1/IRF3 Phosphorylation: Dengue Virus Serotype 1 NS4A Is a Unique Interferon-Regulating Virulence Determinant. *mBio* 2015; **6**: e00553-e00515 [PMID: 25968648 DOI: 10.1128/mBio.00553-15]

54 **Aguirre S**, Luthra P, Sanchez-Aparicio MT, Maestre AM, Patel J, Lamothe F, Fredericks AC, Tripathi S, Zhu T, Pintado-Silva J, Webb LG, Bernal-Rubio D, Solovyov A, Greenbaum B, Simon V, Basler CF, Mulder LC, García-Sastre A, Fernandez-Sesma A. Dengue virus NS2B protein targets cGAS for degradation and prevents mitochondrial DNA sensing during infection. *Nat Microbiol* 2017; **2**: 17037 [PMID: 28346446 DOI: 10.1038/nmicrobiol.2017.37]

55 **Potisopon S**, Priet S, Collet A, Decroly E, Canard B, Selisko B. The methyltransferase domain of dengue virus protein NS5 ensures efficient RNA synthesis initiation and elongation by the polymerase domain. *Nucleic Acids Res* 2014; **42**: 11642-11656 [PMID: 25209234 DOI: 10.1093/nar/gku666]

56 **Thiemmeca S**, Tamdet C, Punyadee N, Prommool T, Songjaeng A, Noisakran S, Puttikhunt C, Atkinson JP, Diamond MS, Ponlawat A, Avirutnan P. Secreted NS1 Protects Dengue Virus from Mannose-Binding Lectin-Mediated Neutralization. *J Immunol* 2016; **197**: 4053-4065 [PMID: 27798151 DOI: 10.4049/jimmunol.1600323]

57 **Okamoto T**, Suzuki T, Kusakabe S, Tokunaga M, Hirano J, Miyata Y, Matsuura Y. Regulation of Apoptosis during Flavivirus Infection. *Viruses* 2017; **9** [PMID: 28846635 DOI: 10.3390/v9090243]

58 **Shukla R**, Ramasamy V, Shanmugam RK, Ahuja R, Khanna N. Antibody-Dependent Enhancement: A Challenge for Developing a Safe Dengue Vaccine. *Front Cell Infect Microbiol* 2020; **10**: 572681 [PMID: 33194810 DOI: 10.3389/fcimb.2020.572681]

59 **Halstead S**. Recent advances in understanding dengue. *F1000Res* 2019; **8** [PMID: 31448083 DOI: 10.12688/f1000research.19197.1]

60 **Khanam A**, Gutiérrez-Barbosa H, Lyke KE, Chua JV. Immune-Mediated Pathogenesis in Dengue Virus Infection. *Viruses* 2022; **14** [PMID: 36423184 DOI: 10.3390/v14112575]

61 **Kuczera D**, Assolini JP, Tomiotto-Pellissier F, Pavanelli WR, Silveira GF. Highlights for Dengue Immunopathogenesis: Antibody-Dependent Enhancement, Cytokine Storm, and Beyond. *J Interferon Cytokine Res* 2018; **38**: 69-80 [PMID: 29443656 DOI: 10.1089/jir.2017.0037]

62 **Olagnier D**, Peri S, Steel C, van Montfoort N, Chiang C, Beljanski V, Slifker M, He Z, Nichols CN, Lin R, Balachandran S, Hiscott J. Cellular oxidative stress response controls the antiviral and apoptotic programs in dengue virus-infected dendritic cells. *PLoS Pathog* 2014; **10**: e1004566 [PMID: 25521078 DOI: 10.1371/journal.ppat.1004566]

63 **Cockburn JJ**, Navarro Sanchez ME, Goncalvez AP, Zaitseva E, Stura EA, Kikuti CM, Duquerroy S, Dussart P, Chernomordik LV, Lai CJ, Rey FA. Structural insights into the neutralization mechanism of a higher primate antibody against dengue virus. *EMBO J* 2012; **31**: 767-779 [PMID: 22139356 DOI: 10.1038/emboj.2011.439]

64 **Anandarao R**, Swaminathan S, Khanna N. The identification of immunodominant linear epitopes of dengue type 2 virus capsid and NS4a proteins using pin-bound peptides. *Virus Res* 2005; **112**: 60-68 [PMID: 16022901 DOI: 10.1016/j.virusres.2005.03.022]

65 **Biering SB**, Akey DL, Wong MP, Brown WC, Lo NTN, Puerta-Guardo H, Tramontini Gomes de Sousa F, Wang C, Konwerski JR, Espinosa DA, Bockhaus NJ, Glasner DR, Li J, Blanc SF, Juan EY, Elledge SJ, Mina MJ, Beatty PR, Smith JL, Harris E. Structural basis for antibody inhibition of flavivirus NS1-triggered endothelial dysfunction. *Science* 2021; **371**: 194-200 [PMID: 33414220 DOI: 10.1126/science.abc0476]

66 **Bournazos S**, Gupta A, Ravetch JV. The role of IgG Fc receptors in antibody-dependent enhancement. *Nat Rev Immunol* 2020; **20**: 633-643 [PMID: 32782358 DOI: 10.1038/s41577-020-00410-0]

67 **Xu Z**, Zhang H, Yang D, Wei D, Demongeot J, Zeng Q. The Mathematical Modeling of the Host-Virus Interaction in Dengue Virus Infection: A Quantitative Study. *Viruses* 2024; **16** [PMID: 38399992 DOI: 10.3390/v16020216]

68 **Waldran MJ**, Wegman AD, Bahr LE, Roy NH, Currier JR, Waickman AT. Soluble NS1 Antagonizes IgG- and IgA- Mediated Monocytic Phagocytosis of DENV Infected Cells. *J Infect Dis* 2023; **228**: 70-79 [PMID: 37103221 DOI: 10.1093/infdis/jiad122]

69 **Chareonsirisuthigul T**, Kalayanarooj S, Ubol S. Dengue virus (DENV) antibody-dependent enhancement of infection upregulates the production of anti-inflammatory cytokines, but suppresses anti-DENV free radical and pro-inflammatory cytokine production, in THP-1 cells. *J Gen Virol* 2007; **88**: 365-375 [PMID: 17251552 DOI: 10.1099/vir.0.82537-0]

70 **Malavige GN**, Jeewandara C, Alles KM, Salimi M, Gomes L, Kamaladasa A, Jayaratne SD, Ogg GS. Suppression of virus specific immune responses by IL-10 in acute dengue infection. *PLoS Negl Trop Dis* 2013; **7**: e2409 [PMID: 24040431 DOI: 10.1371/journal.pntd.0002409]

71 **Dowd KA**, Sirohi D, Speer SD, VanBlargan LA, Chen RE, Mukherjee S, Whitener BM, Govero J, Aleshnick M, Larman B, Sukupolvi-Petty S, Sevvana M, Miller AS, Klose T, Zheng A, Koenig S, Kielian M, Kuhn RJ, Diamond MS, Pierson TC. prM-reactive antibodies reveal a role for partially mature virions in dengue virus pathogenesis. *Proc Natl Acad Sci U S A* 2023; **120**: e2218899120 [PMID: 36638211 DOI: 10.1073/pnas.2218899120]

72 **Tsai CL**, Sun DS, Su MT, Lien TS, Chen YH, Lin CY, Huang CH, King CC, Li CR, Chen TH, Chiu YH, Lu CC, Chang HH. Suppressed humoral immunity is associated with dengue nonstructural protein NS1-elicited anti-death receptor antibody fractions in mice. *Sci Rep* 2020; **10**: 6294 [PMID: 32286343 DOI: 10.1038/s41598-020-62958-0]

73 **Chuang YC**, Lin YS, Liu HS, Yeh TM. Molecular mimicry between dengue virus and coagulation factors induces antibodies to inhibit thrombin activity and enhance fibrinolysis. *J Virol* 2014; **88**: 13759-13768 [PMID: 25231318 DOI: 10.1128/JVI.02166-14]

74 **Lin YS**, Lin CF, Lei HY, Liu HS, Yeh TM, Chen SH, Liu CC. Antibody-mediated endothelial cell damage *via* nitric oxide. *Curr Pharm Des* 2004; **10**: 213-221 [PMID: 14754400 DOI: 10.2174/1381612043453469]

75 **Liu C**, Chu D, Kalantar-Zadeh K, George J, Young HA, Liu G. Cytokines: From Clinical Significance to Quantification. *Adv Sci (Weinh)* 2021; **8**: e2004433 [PMID: 34114369 DOI: 10.1002/advs.202004433]

76 **Guzman MG**, Alvarez M, Halstead SB. Secondary infection as a risk factor for dengue hemorrhagic fever/dengue shock syndrome: an historical perspective and role of antibody-dependent enhancement of infection. *Arch Virol* 2013; **158**: 1445-1459 [PMID: 23471635 DOI: 10.1007/s00705-013-1645-3]

77 **Srikiatkhachorn A**, Mathew A, Rothman AL. Immune-mediated cytokine storm and its role in severe dengue. *Semin Immunopathol* 2017; **39**: 563-574 [PMID: 28401256 DOI: 10.1007/s00281-017-0625-1]

78 **Modhiran N**, Watterson D, Muller DA, Panetta AK, Sester DP, Liu L, Hume DA, Stacey KJ, Young PR. Dengue virus NS1 protein activates

cells *via* Toll-like receptor 4 and disrupts endothelial cell monolayer integrity. *Sci Transl Med* 2015; **7**: 304ra142 [PMID: 26355031 DOI: 10.1126/scitranslmed.aaa3863]

79 **Chao CH**, Wu WC, Lai YC, Tsai PJ, Perng GC, Lin YS, Yeh TM. Dengue virus nonstructural protein 1 activates platelets *via* Toll-like receptor 4, leading to thrombocytopenia and hemorrhage. *PLoS Pathog* 2019; **15**: e1007625 [PMID: 31009511 DOI: 10.1371/journal.ppat.1007625]

80 **Ferreira RA**, de Oliveira SA, Gandini M, Ferreira Lda C, Correa G, Abiraude FM, Reid MM, Cruz OG, Kubelka CF. Circulating cytokines and chemokines associated with plasma leakage and hepatic dysfunction in Brazilian children with dengue fever. *Acta Trop* 2015; **149**: 138-147 [PMID: 25944351 DOI: 10.1016/j.actatropica.2015.04.023]

81 **Pan P**, Li G, Shen M, Yu Z, Ge W, Lao Z, Fan Y, Chen K, Ding Z, Wang W, Wan P, Shereen MA, Luo Z, Chen X, Zhang Q, Lin L, Wu J. DENV NS1 and MMP-9 cooperate to induce vascular leakage by altering endothelial cell adhesion and tight junction. *PLoS Pathog* 2021; **17**: e1008603 [PMID: 34310658 DOI: 10.1371/journal.ppat.1008603]

82 **Chen HR**, Chuang YC, Lin YS, Liu HS, Liu CC, Perng GC, Yeh TM. Dengue Virus Nonstructural Protein 1 Induces Vascular Leakage through Macrophage Migration Inhibitory Factor and Autophagy. *PLoS Negl Trop Dis* 2016; **10**: e0004828 [PMID: 27409803 DOI: 10.1371/journal.pntd.0004828]

83 **Chen HR**, Chao CH, Liu CC, Ho TS, Tsai HP, Perng GC, Lin YS, Wang JR, Yeh TM. Macrophage migration inhibitory factor is critical for dengue NS1-induced endothelial glycocalyx degradation and hyperpermeability. *PLoS Pathog* 2018; **14**: e1007033 [PMID: 29702687 DOI: 10.1371/journal.ppat.1007033]

84 **Puerta-Guardo H**, Glasner DR, Harris E. Dengue Virus NS1 Disrupts the Endothelial Glycocalyx, Leading to Hyperpermeability. *PLoS Pathog* 2016; **12**: e1005738 [PMID: 27416066 DOI: 10.1371/journal.ppat.1005738]

85 **Seet RC**, Chow AW, Quek AM, Chan YH, Lim EC. Relationship between circulating vascular endothelial growth factor and its soluble receptors in adults with dengue virus infection: a case-control study. *Int J Infect Dis* 2009; **13**: e248-e253 [PMID: 19261502 DOI: 10.1016/j.ijid.2008.11.028]

86 **Cardier JE**, Mariño E, Romano E, Taylor P, Liprandi F, Bosch N, Rothman AL. Proinflammatory factors present in sera from patients with acute dengue infection induce activation and apoptosis of human microvascular endothelial cells: possible role of TNF-alpha in endothelial cell damage in dengue. *Cytokine* 2005; **30**: 359-365 [PMID: 15935956 DOI: 10.1016/j.cyto.2005.01.021]

87 **Bhatt P**, Varma M, Sood V, Ambikan A, Jayaram A, Babu N, Gupta S, Mukhopadhyay C, Neogi U. Temporal cytokine storm dynamics in dengue infection predicts severity. *Virus Res* 2024; **341**: 199306 [PMID: 38176525 DOI: 10.1016/j.virusres.2023.199306]

88 **Malavige GN**, Jeewandara C, Ogg GS. Dysfunctional Innate Immune Responses and Severe Dengue. *Front Cell Infect Microbiol* 2020; **10**: 590004 [PMID: 33194836 DOI: 10.3389/fcimb.2020.590004]

89 **Schmid MA**, Harris E. Monocyte recruitment to the dermis and differentiation to dendritic cells increases the targets for dengue virus replication. *PLoS Pathog* 2014; **10**: e1004541 [PMID: 25474197 DOI: 10.1371/journal.ppat.1004541]

90 **Simon AY**, Sutherland MR, Pryzdial EL. Dengue virus binding and replication by platelets. *Blood* 2015; **126**: 378-385 [PMID: 25943787 DOI: 10.1182/blood-2014-09-598029]

91 **de Azeredo EL**, Monteiro RQ, de-Oliveira Pinto LM. Thrombocytopenia in Dengue: Interrelationship between Virus and the Imbalance between Coagulation and Fibrinolysis and Inflammatory Mediators. *Mediators Inflamm* 2015; **2015**: 313842 [PMID: 25999666 DOI: 10.1155/2015/313842]

92 **Lin GL**, Chang HH, Lien TS, Chen PK, Chan H, Su MT, Liao CY, Sun DS. Suppressive effect of dengue virus envelope protein domain III on megakaryopoiesis. *Virulence* 2017; **8**: 1719-1731 [PMID: 28622093 DOI: 10.1080/21505594.2017.1343769]

93 **Lai JH**, Wu DW, Wu CH, Hung LF, Huang CY, Ka SM, Chen A, Chang ZF, Ho LJ. Mitochondrial CMPK2 mediates immunomodulatory and antiviral activities through IFN-dependent and IFN-independent pathways. *iScience* 2021; **24**: 102498 [PMID: 34142025 DOI: 10.1016/j.isci.2021.102498]

94 **Ojha A**, Nandi D, Batra H, Singhal R, Annarapu GK, Bhattacharyya S, Seth T, Dar L, Medigeshi GR, Vrati S, Vikram NK, Guchhait P. Platelet activation determines the severity of thrombocytopenia in dengue infection. *Sci Rep* 2017; **7**: 41697 [PMID: 28139770 DOI: 10.1038/srep41697]

95 **Wan SW**, Yang YW, Chu YT, Lin CF, Chang CP, Yeh TM, Anderson R, Lin YS. Anti-dengue virus nonstructural protein 1 antibodies contribute to platelet phagocytosis by macrophages. *Thromb Haemost* 2016; **115**: 646-656 [PMID: 26632672 DOI: 10.1160/TH15-06-0498]

96 **Tayal A**, Kabra SK, Lodha R. Management of Dengue: An Updated Review. *Indian J Pediatr* 2023; **90**: 168-177 [PMID: 36574088 DOI: 10.1007/s12098-022-04394-8]

97 **Srichaikul T**, Nimmannitya S. Haematology in dengue and dengue haemorrhagic fever. *Baillieres Best Pract Res Clin Haematol* 2000; **13**: 261-276 [PMID: 10942625 DOI: 10.1053/beha.2000.0073]

98 **World Health Organization**. UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. Handbook for clinical management of dengue. 2012. [cited 1 October 2024]. Available from: https://iris.who.int/handle/10665/76887

99 **Ministry of Health**. Dengue: Diagnóstico e manejo clínico: adulto e criança. 2023. [cited 1 October 2024]. Available from: https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/svsa/dengue/dengue-diagnostico-e-manejo-clinico-adulto-e-crianca/view

100 **World Health Organization**. Dengue and severe dengue. April 23, 2024. [cited 1 October 2024]. Available from: https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue

101 **Pan American Health Organization**. Guidelines for the clinical diagnosis and treatment of dengue, chikungunya, and Zika. March 24, 2022. [cited 1 October 2024]. Available from: https://www.paho.org/en/documents/guidelines-clinical-diagnosis-and-treatment-dengue-chikungunya-and-zika

102 **Chuansumrit A**, Chaiyaratana W. Hemostatic derangement in dengue hemorrhagic fever. *Thromb Res* 2014; **133**: 10-16 [PMID: 24120237 DOI: 10.1016/j.thromres.2013.09.028]

103 **Moore N**, Pollack C, Butkerait P. Adverse drug reactions and drug-drug interactions with over-the-counter NSAIDs. *Ther Clin Risk Manag* 2015; **11**: 1061-1075 [PMID: 26203254 DOI: 10.2147/TCRM.S79135]

104 **Hussain H**, Janaka KVC, Gunasekara H, Krishnan M, Perera I. Acute Psychosis Presenting With Dengue Fever Complicated by Dengue Encephalitis. *Cureus* 2024; **16**: e55628 [PMID: 38586783 DOI: 10.7759/cureus.55628]

105 **Sandeep M**, Padhi BK, Yella SST, Sruthi KG, Venkatesan RG, Krishna Sasanka KBS, Satapathy P, Mohanty A, Al-Tawfiq JA, Iqhrammullah M, Rabaan AA, Kabi A, Sah S, Rustagi S, Al-Qaim ZH, Barboza JJ, Waheed Y, Harapan H, Sah R. Myocarditis manifestations in dengue cases: A systematic review and meta-analysis. *J Infect Public Health* 2023; **16**: 1761-1768 [PMID: 37738692 DOI: 10.1016/j.jiph.2023.08.005]

106 **Khan AA**, Khan FU, Akhtar SA, Ghaffar R. Dengue beyond fever-fatal dengue myocarditis and complete heart block: A case report and brief

overview of cardiac manifestations of dengue fever. *SAGE Open Med Case Rep* 2023; **11**: 2050313X231193983 [PMID: 37605746 DOI: 10.1177/2050313X231193983]

107 **Ahmed M**, Wasim MA, Kazi AN, Akber H, Sheikh M, Patel MJ. A curious case of expanded dengue syndrome. *Trop Doct* 2024; **54**: 179-181 [PMID: 38225193 DOI: 10.1177/00494755231224493]

108 **Kularatne SA**, Dalugama C. Dengue infection: Global importance, immunopathology and management. *Clin Med (Lond)* 2022; **22**: 9-13 [PMID: 35078789 DOI: 10.7861/clinmed.2021-0791]

109 **Kok BH**, Lim HT, Lim CP, Lai NS, Leow CY, Leow CH. Dengue virus infection-a review of pathogenesis, vaccines, diagnosis and therapy. *Virus Res* 2023; **324**: 199018 [PMID: 36493993 DOI: 10.1016/j.virusres.2022.199018]

110 **Khetarpal N**, Khanna I. Dengue Fever: Causes, Complications, and Vaccine Strategies. *J Immunol Res* 2016; **2016**: 6803098 [PMID: 27525287 DOI: 10.1155/2016/6803098]

111 **Grande AJ**, Reid H, Thomas E, Foster C, Darton TC. Tourniquet Test for Dengue Diagnosis: Systematic Review and Meta-analysis of Diagnostic Test Accuracy. *PLoS Negl Trop Dis* 2016; **10**: e0004888 [PMID: 27486661 DOI: 10.1371/journal.pntd.0004888]

112 **Gregory CJ**, Lorenzi OD, Colón L, García AS, Santiago LM, Rivera RC, Bermúdez LJ, Báez FO, Aponte DV, Tomashek KM, Gutierrez J, Alvarado L. Utility of the tourniquet test and the white blood cell count to differentiate dengue among acute febrile illnesses in the emergency room. *PLoS Negl Trop Dis* 2011; **5**: e1400 [PMID: 22163057 DOI: 10.1371/journal.pntd.0001400]

113 **Ahmed NH**, Broor S. Comparison of NS1 antigen detection ELISA, real time RT-PCR and virus isolation for rapid diagnosis of dengue infection in acute phase. *J Vector Borne Dis* 2014; **51**: 194-199 [PMID: 25253212]

114 **Ohst C**, Saschenbrecker S, Stiba K, Steinhagen K, Probst C, Radzimski C, Lattwein E, Komorowski L, Stöcker W, Schlumberger W. Reliable Serological Testing for the Diagnosis of Emerging Infectious Diseases. *Adv Exp Med Biol* 2018; **1062**: 19-43 [PMID: 29845523 DOI: 10.1007/978-981-10-8727-1_3]

115 **Xu H**, Di B, Pan YX, Qiu LW, Wang YD, Hao W, He LJ, Yuen KY, Che XY. Serotype 1-specific monoclonal antibody-based antigen capture immunoassay for detection of circulating nonstructural protein NS1: Implications for early diagnosis and serotyping of dengue virus infections. *J Clin Microbiol* 2006; **44**: 2872-2878 [PMID: 16891505 DOI: 10.1128/JCM.00777-06]

116 **Aquino VH**, Fumagalli MJ, Silva A, de Moura Negrini BV, Rojas A, Guillen Y, Bernal C, Figueiredo LTM. Linear epitope mapping in the E and NS1 proteins of dengue and Zika viruses: Prospection of peptides for vaccines and diagnostics. *PLoS One* 2023; **18**: e0292451 [PMID: 37788262 DOI: 10.1371/journal.pone.0292451]

117 **Goncalves A**, Peeling RW, Chu MC, Gubler DJ, de Silva AM, Harris E, Murtagh M, Chua A, Rodriguez W, Kelly C, Wilder-Smith A. Innovative and New Approaches to Laboratory Diagnosis of Zika and Dengue: A Meeting Report. *J Infect Dis* 2018; **217**: 1060-1068 [PMID: 29294035 DOI: 10.1093/infdis/jix678]

118 **Thomas SJ**, Nisalak A, Anderson KB, Libraty DH, Kalayanarooj S, Vaughn DW, Putnak R, Gibbons RV, Jarman R, Endy TP. Dengue plaque reduction neutralization test (PRNT) in primary and secondary dengue virus infections: How alterations in assay conditions impact performance. *Am J Trop Med Hyg* 2009; **81**: 825-833 [PMID: 19861618 DOI: 10.4269/ajtmh.2009.08-0625]

119 **Cascorbi I**. The Uncertainties of Metamizole Use. *Clin Pharmacol Ther* 2021; **109**: 1373-1375 [PMID: 34038591 DOI: 10.1002/cpt.2258]

120 **Yacoub S**, Trung TH, Lam PK, Thien VHN, Hai DHT, Phan TQ, Nguyet OPK, Quyen NTH, Simmons CP, Broyd C, Screaton GR, Wills B. Cardio-haemodynamic assessment and venous lactate in severe dengue: Relationship with recurrent shock and respiratory distress. *PLoS Negl Trop Dis* 2017; **11**: e0005740 [PMID: 28692675 DOI: 10.1371/journal.pntd.0005740]

121 **Chaurasia R**, Zaman S, Chatterjee K, Das B. Retrospective Review of Platelet Transfusion Practices during 2013 Dengue Epidemic of Delhi, India. *Transfus Med Hemother* 2015; **42**: 227-231 [PMID: 26557814 DOI: 10.1159/000371500]

122 **Lee TH**, Wong JG, Leo YS, Thein TL, Ng EL, Lee LK, Lye DC. Potential Harm of Prophylactic Platelet Transfusion in Adult Dengue Patients. *PLoS Negl Trop Dis* 2016; **10**: e0004576 [PMID: 27015272 DOI: 10.1371/journal.pntd.0004576]

123 **Chia PY**, Htun HL, Leo YS, Lye DC. Safety of temporary interruption of antiplatelet therapy in dengue fever with thrombocytopenia. *J Infect* 2021; **82**: 270-275 [PMID: 33271172 DOI: 10.1016/j.jinf.2020.10.038]

124 **Bandara SMR**, Herath HMMTB. Effectiveness of corticosteroid in the treatment of dengue-A systemic review. *Heliyon* 2018; **4**: e00816 [PMID: 30258999 DOI: 10.1016/j.heliyon.2018.e00816]

125 **Zhang F**, Kramer CV. Corticosteroids for dengue infection. *Cochrane Database Syst Rev* 2014; **2014**: CD003488 [PMID: 24984082 DOI: 10.1002/14651858.CD003488.pub3]

126 **Lee MF**, Wu YS, Poh CL. Molecular Mechanisms of Antiviral Agents against Dengue Virus. *Viruses* 2023; **15** [PMID: 36992414 DOI: 10.3390/v15030705]

127 **Kayesh MEH**, Tsukiyama-Kohara K. Mammalian animal models for dengue virus infection: a recent overview. *Arch Virol* 2022; **167**: 31-44 [PMID: 34761286 DOI: 10.1007/s00705-021-05298-2]

128 **Watanabe S**, Low JG, Vasudevan SG. Preclinical Antiviral Testing for Dengue Virus Infection in Mouse Models and Its Association with Clinical Studies. *ACS Infect Dis* 2018; **4**: 1048-1057 [PMID: 29756760 DOI: 10.1021/acsinfecdis.8b00054]

129 **Mahajan S**, Choudhary S, Kumar P, Tomar S. Antiviral strategies targeting host factors and mechanisms obliging +ssRNA viral pathogens. *Bioorg Med Chem* 2021; **46**: 116356 [PMID: 34416512 DOI: 10.1016/j.bmc.2021.116356]

130 **Rather IA**, Parray HA, Lone JB, Paek WK, Lim J, Bajpai VK, Park YH. Prevention and Control Strategies to Counter Dengue Virus Infection. *Front Cell Infect Microbiol* 2017; **7**: 336 [PMID: 28791258 DOI: 10.3389/fcimb.2017.00336]

131 **Yakob L**, Funk S, Camacho A, Brady O, Edmunds WJ. Aedes aegypti Control Through Modernized, Integrated Vector Management. *PLoS Curr* 2017; **9** [PMID: 28286698 DOI: 10.1371/currents.outbreaks.45deb8e03a438c4d088afb4fafae8747]

132 **Buhler C**, Winkler V, Runge-Ranzinger S, Boyce R, Horstick O. Environmental methods for dengue vector control-A systematic review and meta-analysis. *PLoS Negl Trop Dis* 2019; **13**: e0007420 [PMID: 31295250 DOI: 10.1371/journal.pntd.0007420]

133 **Dalpadado R**, Gunathilaka N, Amarasinghe D, Udayanaga L. A Challenge for a Unique Dengue Vector Control Programme: Assessment of the Spatial Variation of Insecticide Resistance Status amongst Aedes aegypti and Aedes albopictus Populations in Gampaha District, Sri Lanka. *Biomed Res Int* 2021; **2021**: 6619175 [PMID: 33884266 DOI: 10.1155/2021/6619175]

134 **Akter R**, Tasneem F, Das S, Soma MA, Georgakopoulos-Soares I, Juthi RT, Sazed SA. Approaches of dengue control: vaccine strategies and future aspects. *Front Immunol* 2024; **15**: 1362780 [PMID: 38487527 DOI: 10.3389/fimmu.2024.1362780]

135 **Guy B**, Barrere B, Malinowski C, Saville M, Teyssou R, Lang J. From research to phase III: preclinical, industrial and clinical development of the Sanofi Pasteur tetravalent dengue vaccine. *Vaccine* 2011; **29**: 7229-7241 [PMID: 21745521 DOI: 10.1016/j.vaccine.2011.06.094]

136 **Hou J**, Ye W, Chen J. Current Development and Challenges of Tetravalent Live-Attenuated Dengue Vaccines. *Front Immunol* 2022; **13**:

840104 [PMID: 35281026 DOI: 10.3389/fimmu.2022.840104]
137 Dengue vaccine: WHO position paper, September 2018-Recommendations. *Vaccine* 2019; **37**: 4848-4849 [PMID: 30424888 DOI: 10.1016/j.vaccine.2018.09.063]
138 **Sridhar S**, Luedtke A, Langevin E, Zhu M, Bonaparte M, Machabert T, Savarino S, Zambrano B, Moureau A, Khromava A, Moodie Z, Westling T, Mascareñas C, Frago C, Cortés M, Chansinghakul D, Noriega F, Bouckenooghe A, Chen J, Ng SP, Gilbert PB, Gurunathan S, DiazGranados CA. Effect of Dengue Serostatus on Dengue Vaccine Safety and Efficacy. *N Engl J Med* 2018; **379**: 327-340 [PMID: 29897841 DOI: 10.1056/NEJMoa1800820]
139 **Angelin M**, Sjölin J, Kahn F, Ljunghill Hedberg A, Rosdahl A, Skorup P, Werner S, Woxenius S, Askling HH. Qdenga®-A promising dengue fever vaccine; can it be recommended to non-immune travelers? *Travel Med Infect Dis* 2023; **54**: 102598 [PMID: 37271201 DOI: 10.1016/j.tmaid.2023.102598]
140 **Tricou V**, Yu D, Reynales H, Biswal S, Saez-Llorens X, Sirivichayakul C, Lopez P, Borja-Tabora C, Bravo L, Kosalaraksa P, Vargas LM, Alera MT, Rivera L, Watanaveeradej V, Dietze R, Fernando L, Wickramasinghe VP, Moreira ED Jr, Fernando AD, Gunasekera D, Luz K, Oliveira AL, Tuboi S, Escudero I, Hutagalung Y, Lloyd E, Rauscher M, Zent O, Folschweiller N, LeFevre I, Espinoza F, Wallace D. Long-term efficacy and safety of a tetravalent dengue vaccine (TAK-003): 4·5-year results from a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet Glob Health* 2024; **12**: e257-e270 [PMID: 38245116 DOI: 10.1016/S2214-109X(23)00522-3]
141 **Musso D**, Gubler DJ. Zika Virus. *Clin Microbiol Rev* 2016; **29**: 487-524 [PMID: 27029595 DOI: 10.1128/CMR.00072-15]
142 **Kuno G**, Chang GJ. Full-length sequencing and genomic characterization of Bagaza, Kedougou, and Zika viruses. *Arch Virol* 2007; **152**: 687-696 [PMID: 17195954 DOI: 10.1007/s00705-006-0903-z]
143 **Chambers TJ**, Hahn CS, Galler R, Rice CM. Flavivirus genome organization, expression, and replication. *Annu Rev Microbiol* 1990; **44**: 649-688 [PMID: 2174669 DOI: 10.1146/annurev.mi.44.100190.003245]
144 **Heinz FX**, Stiasny K. The Antigenic Structure of Zika Virus and Its Relation to Other Flaviviruses: Implications for Infection and Immunoprophylaxis. *Microbiol Mol Biol Rev* 2017; **81** [PMID: 28179396 DOI: 10.1128/MMBR.00055-16]
145 **Wang Y**, Ling L, Zhang Z, Marin-Lopez A. Current Advances in Zika Vaccine Development. *Vaccines (Basel)* 2022; **10** [PMID: 36366325 DOI: 10.3390/vaccines10111816]
146 **Pattnaik A**, Sahoo BR, Pattnaik AK. Current Status of Zika Virus Vaccines: Successes and Challenges. *Vaccines (Basel)* 2020; **8** [PMID: 32486368 DOI: 10.3390/vaccines8020266]
147 **Richner JM**, Himansu S, Dowd KA, Butler SL, Salazar V, Fox JM, Julander JG, Tang WW, Shresta S, Pierson TC, Ciaramella G, Diamond MS. Modified mRNA Vaccines Protect against Zika Virus Infection. *Cell* 2017; **168**: 1114-1125.e10 [PMID: 28222903 DOI: 10.1016/j.cell.2017.02.017]
148 **Li L**, Lok SM, Yu IM, Zhang Y, Kuhn RJ, Chen J, Rossmann MG. The flavivirus precursor membrane-envelope protein complex: structure and maturation. *Science* 2008; **319**: 1830-1834 [PMID: 18369147 DOI: 10.1126/science.1153263]
149 **Wu Y**, Liu Q, Zhou J, Xie W, Chen C, Wang Z, Yang H, Cui J. Zika virus evades interferon-mediated antiviral response through the co-operation of multiple nonstructural proteins in vitro. *Cell Discov* 2017; **3**: 17006 [PMID: 28373913 DOI: 10.1038/celldisc.2017.6]
150 **Dick GW**, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. *Trans R Soc Trop Med Hyg* 1952; **46**: 509-520 [PMID: 12995440 DOI: 10.1016/0035-9203(52)90042-4]
151 **Cao-Lormeau VM**, Roche C, Teissier A, Robin E, Berry AL, Mallet HP, Sall AA, Musso D. Zika virus, French polynesia, South pacific, 2013. *Emerg Infect Dis* 2014; **20**: 1085-1086 [PMID: 24856001 DOI: 10.3201/eid2006.140138]
152 **World Health Organization**. Zika epidemiology update. June 3, 2024. [cited 1 October 2024]. Available from: https://www.who.int/publications/m/item/zika-epidemiology-update-may-2024
153 **Ioos S**, Mallet HP, Leparc Goffart I, Gauthier V, Cardoso T, Herida M. Current Zika virus epidemiology and recent epidemics. *Med Mal Infect* 2014; **44**: 302-307 [PMID: 25001879 DOI: 10.1016/j.medmal.2014.04.008]
154 **Smith CE**. Arthropod-borne viruses. *Br Med Bull* 1959; **15**: 235-239 [PMID: 13831911 DOI: 10.1093/oxfordjournals.bmb.a069771]
155 **Lanciotti RS**, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, Stanfield SM, Duffy MR. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis* 2008; **14**: 1232-1239 [PMID: 18680646 DOI: 10.3201/eid1408.080287]
156 **Boccolini D**, Toma L, Di Luca M, Severini F, Romi R, Remoli ME, Sabbatucci M, Venturi G, Rezza G, Fortuna C. Experimental investigation of the susceptibility of Italian Culex pipiens mosquitoes to Zika virus infection. *Euro Surveill* 2016; **21** [PMID: 27605056 DOI: 10.2807/1560-7917.ES.2016.21.35.30328]
157 **Mansuy JM**, Dutertre M, Mengelle C, Fourcade C, Marchou B, Delobel P, Izopet J, Martin-Blondel G. Zika virus: high infectious viral load in semen, a new sexually transmitted pathogen? *Lancet Infect Dis* 2016; **16**: 405 [PMID: 26949027 DOI: 10.1016/S1473-3099(16)00138-9]
158 **Musso D**, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau VM. Potential sexual transmission of Zika virus. *Emerg Infect Dis* 2015; **21**: 359-361 [PMID: 25625872 DOI: 10.3201/eid2102.141363]
159 **D'Ortenzio E**, Matheron S, Yazdanpanah Y, de Lamballerie X, Hubert B, Piorkowski G, Maquart M, Descamps D, Damond F, Leparc-Goffart I. Evidence of Sexual Transmission of Zika Virus. *N Engl J Med* 2016; **374**: 2195-2198 [PMID: 27074370 DOI: 10.1056/NEJMc1604449]
160 **Major CG**, Paz-Bailey G, Hills SL, Rodriguez DM, Biggerstaff BJ, Johansson M. Risk Estimation of Sexual Transmission of Zika Virus-United States, 2016-2017. *J Infect Dis* 2021; **224**: 1756-1764 [PMID: 33822107 DOI: 10.1093/infdis/jiab173]
161 **Magnus MM**, Espósito DLA, Costa VAD, Melo PS, Costa-Lima C, Fonseca BALD, Addas-Carvalho M. Risk of Zika virus transmission by blood donations in Brazil. *Hematol Transfus Cell Ther* 2018; **40**: 250-254 [PMID: 30128434 DOI: 10.1016/j.htct.2018.01.011]
162 **Paixao ES**, Cardim LL, Costa MCN, Brickley EB, de Carvalho-Sauer RCO, Carmo EH, Andrade RFS, Rodrigues MS, Veiga RV, Costa LC, Moore CA, França GVA, Smeeth L, Rodrigues LC, Barreto ML, Teixeira MG. Mortality from Congenital Zika Syndrome-Nationwide Cohort Study in Brazil. *N Engl J Med* 2022; **386**: 757-767 [PMID: 35196428 DOI: 10.1056/NEJMoa2101195]
163 **Wu KY**, Zuo GL, Li XF, Ye Q, Deng YQ, Huang XY, Cao WC, Qin CF, Luo ZG. Vertical transmission of Zika virus targeting the radial glial cells affects cortex development of offspring mice. *Cell Res* 2016; **26**: 645-654 [PMID: 27174054 DOI: 10.1038/cr.2016.58]
164 **Rabelo K**, Gonçalves AJDS, Souza LJ, Sales AP, Lima SMB, Trindade GF, Ciambarella BT, Amorim Tasmo NR, Diaz BL, Carvalho JJ, Duarte MPO, Paes MV. Zika Virus Infects Human Placental Mast Cells and the HMC-1 Cell Line, and Triggers Degranulation, Cytokine Release and Ultrastructural Changes. *Cells* 2020; **9** [PMID: 32316163 DOI: 10.3390/cells9040975]
165 **Miner JJ**, Diamond MS. Zika Virus Pathogenesis and Tissue Tropism. *Cell Host Microbe* 2017; **21**: 134-142 [PMID: 28182948 DOI: 10.1016/j.chom.2017.01.004]

166 **Atkinson B**, Hearn P, Afrough B, Lumley S, Carter D, Aarons EJ, Simpson AJ, Brooks TJ, Hewson R. Detection of Zika Virus in Semen. *Emerg Infect Dis* 2016; **22**: 940 [PMID: 27088817 DOI: 10.3201/eid2205.160107]

167 **Foy BD**, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, Haddow AD, Lanciotti RS, Tesh RB. Probable non-vector-borne transmission of Zika virus, Colorado, USA. *Emerg Infect Dis* 2011; **17**: 880-882 [PMID: 21529401 DOI: 10.3201/eid1705.101939]

168 **Deckard DT**, Chung WM, Brooks JT, Smith JC, Woldai S, Hennessey M, Kwit N, Mead P. Male-to-Male Sexual Transmission of Zika Virus--Texas, January 2016. *MMWR Morb Mortal Wkly Rep* 2016; **65**: 372-374 [PMID: 27078057 DOI: 10.15585/mmwr.mm6514a3]

169 **Fréour T**, Mirallié S, Hubert B, Splingart C, Barrière P, Maquart M, Leparc-Goffart I. Sexual transmission of Zika virus in an entirely asymptomatic couple returning from a Zika epidemic area, France, April 2016. *Euro Surveill* 2016; **21** [PMID: 27311680 DOI: 10.2807/1560-7917.ES.2016.21.23.30254]

170 **Bayer A**, Lennemann NJ, Ouyang Y, Bramley JC, Morosky S, Marques ET Jr, Cherry S, Sadovsky Y, Coyne CB. Type III Interferons Produced by Human Placental Trophoblasts Confer Protection against Zika Virus Infection. *Cell Host Microbe* 2016; **19**: 705-712 [PMID: 27066743 DOI: 10.1016/j.chom.2016.03.008]

171 **Ouyang Y**, Bayer A, Chu T, Tyurin VA, Kagan VE, Morelli AE, Coyne CB, Sadovsky Y. Isolation of human trophoblastic extracellular vesicles and characterization of their cargo and antiviral activity. *Placenta* 2016; **47**: 86-95 [PMID: 27780544 DOI: 10.1016/j.placenta.2016.09.008]

172 **Arora N**, Sadovsky Y, Dermody TS, Coyne CB. Microbial Vertical Transmission during Human Pregnancy. *Cell Host Microbe* 2017; **21**: 561-567 [PMID: 28494237 DOI: 10.1016/j.chom.2017.04.007]

173 **Mesci P**, Macia A, Moore SM, Shiryaev SA, Pinto A, Huang CT, Tejwani L, Fernandes IR, Suarez NA, Kolar MJ, Montefusco S, Rosenberg SC, Herai RH, Cugola FR, Russo FB, Sheets N, Saghatelian A, Shresta S, Momper JD, Siqueira-Neto JL, Corbett KD, Beltrão-Braga PCB, Terskikh AV, Muotri AR. Blocking Zika virus vertical transmission. *Sci Rep* 2018; **8**: 1218 [PMID: 29352135 DOI: 10.1038/s41598-018-19526-4]

174 **Hamel R**, Dejarnac O, Wichit S, Ekchariyawat P, Neyret A, Luplertlop N, Perera-Lecoin M, Surasombatpattana P, Talignani L, Thomas F, Cao-Lormeau VM, Choumet V, Briant L, Desprès P, Amara A, Yssel H, Missé D. Biology of Zika Virus Infection in Human Skin Cells. *J Virol* 2015; **89**: 8880-8896 [PMID: 26085147 DOI: 10.1128/JVI.00354-15]

175 **Meertens L**, Carnec X, Lecoin MP, Ramdasi R, Guivel-Benhassine F, Lew E, Lemke G, Schwartz O, Amara A. The TIM and TAM families of phosphatidylserine receptors mediate dengue virus entry. *Cell Host Microbe* 2012; **12**: 544-557 [PMID: 23084921 DOI: 10.1016/j.chom.2012.08.009]

176 **Pujhari S**, Brustolin M, Macias VM, Nissly RH, Nomura M, Kuchipudi SV, Rasgon JL. Heat shock protein 70 (Hsp70) mediates Zika virus entry, replication, and egress from host cells. *Emerg Microbes Infect* 2019; **8**: 8-16 [PMID: 30866755 DOI: 10.1080/22221751.2018.1557988]

177 **Garcia-Vallejo JJ**, van Kooyk Y. The physiological role of DC-SIGN: a tale of mice and men. *Trends Immunol* 2013; **34**: 482-486 [PMID: 23608151 DOI: 10.1016/j.it.2013.03.001]

178 **Tappe D**, Pérez-Girón JV, Zammarchi L, Rissland J, Ferreira DF, Jaenisch T, Gómez-Medina S, Günther S, Bartoloni A, Muñoz-Fontela C, Schmidt-Chanasit J. Cytokine kinetics of Zika virus-infected patients from acute to reconvalescent phase. *Med Microbiol Immunol* 2016; **205**: 269-273 [PMID: 26702627 DOI: 10.1007/s00430-015-0445-7]

179 **Mohd Ropidi MI**, Khazali AS, Nor Rashid N, Yusof R. Endoplasmic reticulum: a focal point of Zika virus infection. *J Biomed Sci* 2020; **27**: 27 [PMID: 31959174 DOI: 10.1186/s12929-020-0618-6]

180 **Lennemann NJ**, Coyne CB. Dengue and Zika viruses subvert reticulophagy by NS2B3-mediated cleavage of FAM134B. *Autophagy* 2017; **13**: 322-332 [PMID: 28102736 DOI: 10.1080/15548627.2016.1265192]

181 **Sirohi D**, Kuhn RJ. Zika Virus Structure, Maturation, and Receptors. *J Infect Dis* 2017; **216**: S935-S944 [PMID: 29267925 DOI: 10.1093/infdis/jix515]

182 **Beaver JT**, Lelutiu N, Habib R, Skountzou I. Evolution of Two Major Zika Virus Lineages: Implications for Pathology, Immune Response, and Vaccine Development. *Front Immunol* 2018; **9**: 1640 [PMID: 30072993 DOI: 10.3389/fimmu.2018.01640]

183 **Loo YM**, Gale M Jr. Immune signaling by RIG-I-like receptors. *Immunity* 2011; **34**: 680-692 [PMID: 21616437 DOI: 10.1016/j.immuni.2011.05.003]

184 **Schneider WM**, Chevillotte MD, Rice CM. Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol* 2014; **32**: 513-545 [PMID: 24555472 DOI: 10.1146/annurev-immunol-032713-120231]

185 **Riedl W**, Acharya D, Lee JH, Liu G, Serman T, Chiang C, Chan YK, Diamond MS, Gack MU. Zika Virus NS3 Mimics a Cellular 14-3-3-Binding Motif to Antagonize RIG-I- and MDA5-Mediated Innate Immunity. *Cell Host Microbe* 2019; **26**: 493-503.e6 [PMID: 31600501 DOI: 10.1016/j.chom.2019.09.012]

186 **Ma J**, Ketkar H, Geng T, Lo E, Wang L, Xi J, Sun Q, Zhu Z, Cui Y, Yang L, Wang P. Zika Virus Non-structural Protein 4A Blocks the RLR-MAVS Signaling. *Front Microbiol* 2018; **9**: 1350 [PMID: 29988497 DOI: 10.3389/fmicb.2018.01350]

187 **Xia H**, Luo H, Shan C, Muruato AE, Nunes BTD, Medeiros DBA, Zou J, Xie X, Giraldo MI, Vasconcelos PFC, Weaver SC, Wang T, Rajsbaum R, Shi PY. An evolutionary NS1 mutation enhances Zika virus evasion of host interferon induction. *Nat Commun* 2018; **9**: 414 [PMID: 29379028 DOI: 10.1038/s41467-017-02816-2]

188 **Winkler CW**, Myers LM, Woods TA, Messer RJ, Carmody AB, McNally KL, Scott DP, Hasenkrug KJ, Best SM, Peterson KE. Adaptive Immune Responses to Zika Virus Are Important for Controlling Virus Infection and Preventing Infection in Brain and Testes. *J Immunol* 2017; **198**: 3526-3535 [PMID: 28330900 DOI: 10.4049/jimmunol.1601949]

189 **Lee LJ**, Komarasamy TV, Adnan NAA, James W, Rmt Balasubramaniam V. Hide and Seek: The Interplay Between Zika Virus and the Host Immune Response. *Front Immunol* 2021; **12**: 750365 [PMID: 34745123 DOI: 10.3389/fimmu.2021.750365]

190 **Stettler K**, Beltramello M, Espinosa DA, Graham V, Cassotta A, Bianchi S, Vanzetta F, Minola A, Jaconi S, Mele F, Foglierini M, Pedotti M, Simonelli L, Dowall S, Atkinson B, Percivalle E, Simmons CP, Varani L, Blum J, Baldanti F, Cameroni E, Hewson R, Harris E, Lanzavecchia A, Sallusto F, Corti D. Specificity, cross-reactivity, and function of antibodies elicited by Zika virus infection. *Science* 2016; **353**: 823-826 [PMID: 27417494 DOI: 10.1126/science.aaf8505]

191 **Wang Q**, Yang H, Liu X, Dai L, Ma T, Qi J, Wong G, Peng R, Liu S, Li J, Li S, Song J, Liu J, He J, Yuan H, Xiong Y, Liao Y, Li J, Yang J, Tong Z, Griffin BD, Bi Y, Liang M, Xu X, Qin C, Cheng G, Zhang X, Wang P, Qiu X, Kobinger G, Shi Y, Yan J, Gao GF. Molecular determinants of human neutralizing antibodies isolated from a patient infected with Zika virus. *Sci Transl Med* 2016; **8**: 369ra179 [PMID: 27974667 DOI: 10.1126/scitranslmed.aai8336]

192 **Jurado KA**, Yockey LJ, Wong PW, Lee S, Huttner AJ, Iwasaki A. Antiviral CD8 T cells induce Zika-virus-associated paralysis in mice. *Nat Microbiol* 2018; **3**: 141-147 [PMID: 29158604 DOI: 10.1038/s41564-017-0060-z]

193 **Hassert M**, Wolf KJ, Schwetye KE, DiPaolo RJ, Brien JD, Pinto AK. CD4+T cells mediate protection against Zika associated severe disease in a mouse model of infection. *PLoS Pathog* 2018; **14**: e1007237 [PMID: 30212537 DOI: 10.1371/journal.ppat.1007237]

194 **Li C**, Xu D, Ye Q, Hong S, Jiang Y, Liu X, Zhang N, Shi L, Qin CF, Xu Z. Zika Virus Disrupts Neural Progenitor Development and Leads to Microcephaly in Mice. *Cell Stem Cell* 2016; **19**: 672 [PMID: 27814481 DOI: 10.1016/j.stem.2016.10.017]

195 **Gatherer D**, Kohl A. Zika virus: a previously slow pandemic spreads rapidly through the Americas. *J Gen Virol* 2016; **97**: 269-273 [PMID: 26684466 DOI: 10.1099/jgv.0.000381]

196 **Paz-Bailey G**, Rosenberg ES, Doyle K, Munoz-Jordan J, Santiago GA, Klein L, Perez-Padilla J, Medina FA, Waterman SH, Gubern CG, Alvarado LI, Sharp TM. Persistence of Zika Virus in Body Fluids-Final Report. *N Engl J Med* 2018; **379**: 1234-1243 [PMID: 28195756 DOI: 10.1056/NEJMoa1613108]

197 **Mladinich MC**, Schwedes J, Mackow ER. Zika Virus Persistently Infects and Is Basolaterally Released from Primary Human Brain Microvascular Endothelial Cells. *mBio* 2017; **8** [PMID: 28698279 DOI: 10.1128/mBio.00952-17]

198 **Song J**, Xian D, Yang L, Xiong X, Lai R, Zhong J. Pruritus: Progress toward Pathogenesis and Treatment. *Biomed Res Int* 2018; **2018**: 9625936 [PMID: 29850592 DOI: 10.1155/2018/9625936]

199 **Plourde AR**, Bloch EM. A Literature Review of Zika Virus. *Emerg Infect Dis* 2016; **22**: 1185-1192 [PMID: 27070380 DOI: 10.3201/eid2207.151990]

200 **Choi GB**, Yim YS, Wong H, Kim S, Kim H, Kim SV, Hoeffer CA, Littman DR, Huh JR. The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science* 2016; **351**: 933-939 [PMID: 26822608 DOI: 10.1126/science.aad0314]

201 **Shahrizaila N**, Lehmann HC, Kuwabara S. Guillain-Barré syndrome. *Lancet* 2021; **397**: 1214-1228 [PMID: 33647239 DOI: 10.1016/S0140-6736(21)00517-1]

202 **Dos Santos T**, Rodriguez A, Almiron M, Sanhueza A, Ramon P, de Oliveira WK, Coelho GE, Badaró R, Cortez J, Ospina M, Pimentel R, Masis R, Hernandez F, Lara B, Montoya R, Jubithana B, Melchor A, Alvarez A, Aldighieri S, Dye C, Espinal MA. Zika Virus and the Guillain-Barré Syndrome-Case Series from Seven Countries. *N Engl J Med* 2016; **375**: 1598-1601 [PMID: 27579558 DOI: 10.1056/NEJMc1609015]

203 **Cao-Lormeau VM**, Blake A, Mons S, Lastère S, Roche C, Vanhomwegen J, Dub T, Baudouin L, Teissier A, Larre P, Vial AL, Decam C, Choumet V, Halstead SK, Willison HJ, Musset L, Manuguerra JC, Despres P, Fournier E, Mallet HP, Musso D, Fontanet A, Neil J, Ghawché F. Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *Lancet* 2016; **387**: 1531-1539 [PMID: 26948433 DOI: 10.1016/S0140-6736(16)00562-6]

204 **Rivera-Correa J**, de Siqueira IC, Mota S, do Rosário MS, Pereira de Jesus PA, Alcantara LCJ, Ernst JD, Rodriguez A. Anti-ganglioside antibodies in patients with Zika virus infection-associated Guillain-Barré Syndrome in Brazil. *PLoS Negl Trop Dis* 2019; **13**: e0007695 [PMID: 31527907 DOI: 10.1371/journal.pntd.0007695]

205 **Nico D**, Conde L, Rivera-Correa JL, Vasconcelos-Dos-Santos A, Mesentier-Louro L, Freire-de-Lima L, Arruda MB, Freire-de-Lima CG, Ferreira ODC Jr, Lopes Moreira ME, Zin AA, Vasconcelos ZFM, Otero RM, Palatnik-de-Sousa CB, Tanuri A, Todeschini AR, Savino W, Rodriguez A, Morrot A. Prevalence of IgG Autoantibodies against GD3 Ganglioside in Acute Zika Virus Infection. *Front Med (Lausanne)* 2018; **5**: 25 [PMID: 29594116 DOI: 10.3389/fmed.2018.00025]

206 **Lebeau G**, Frumence E, Turpin J, Begue F, Hoarau JJ, Gadea G, Krejbich-Trotot P, Desprès P, Viranaicken W. Zika E Glycan Loop Region and Guillain-Barré Syndrome-Related Proteins: A Possible Molecular Mimicry to Be Taken in Account for Vaccine Development. *Vaccines (Basel)* 2021; **9** [PMID: 33808706 DOI: 10.3390/vaccines9030283]

207 **Pielnaa P**, Al-Saadawe M, Saro A, Dama MF, Zhou M, Huang Y, Huang J, Xia Z. Zika virus-spread, epidemiology, genome, transmission cycle, clinical manifestation, associated challenges, vaccine and antiviral drug development. *Virology* 2020; **543**: 34-42 [PMID: 32056845 DOI: 10.1016/j.virol.2020.01.015]

208 **Baba SS**, Fagbami AH, Ojeh CK. Preliminary studies on the use of solid-phase immunosorbent techniques for the rapid detection of Wesselsbron virus (WSLV) IgM by haemagglutination-inhibition. *Comp Immunol Microbiol Infect Dis* 1999; **22**: 71-79 [PMID: 10099030 DOI: 10.1016/s0147-9571(98)00003-4]

209 **Oduyebo T**, Igbinosa I, Petersen EE, Polen KN, Pillai SK, Ailes EC, Villanueva JM, Newsome K, Fischer M, Gupta PM, Powers AM, Lampe M, Hills S, Arnold KE, Rose LE, Shapiro-Mendoza CK, Beard CB, Muñoz JL, Rao CY, Meaney-Delman D, Jamieson DJ, Honein MA. Update: Interim Guidance for Health Care Providers Caring for Pregnant Women with Possible Zika Virus Exposure-United States, July 2016. *MMWR Morb Mortal Wkly Rep* 2016; **65**: 739-744 [PMID: 27467820 DOI: 10.15585/mmwr.mm6529e1]

210 **De Moraes CG**, Pettito M, Yepez JB, Sakuntabhai A, Simon-Loriere E, Zaidi MB, Prot M, Ruffie C, Kim SS, Allikmets R, Terwilliger JD, Lee JH, Maestre GE. Optic neuropathy and congenital glaucoma associated with probable Zika virus infection in Venezuelan patients. *JMM Case Rep* 2018; **5**: e005145 [PMID: 29896405 DOI: 10.1099/jmmcr.0.005145]

211 **Cauchemez S**, Besnard M, Bompard P, Dub T, Guillemette-Artur P, Eyrolle-Guignot D, Salje H, Van Kerkhove MD, Abadie V, Garel C, Fontanet A, Mallet HP. Association between Zika virus and microcephaly in French Polynesia, 2013-15: a retrospective study. *Lancet* 2016; **387**: 2125-2132 [PMID: 26993883 DOI: 10.1016/S0140-6736(16)00651-6]

212 **Hennessey M**, Fischer M, Staples JE. Zika Virus Spreads to New Areas-Region of the Americas, May 2015-January 2016. *MMWR Morb Mortal Wkly Rep* 2016; **65**: 55-58 [PMID: 26820163 DOI: 10.15585/mmwr.mm6503e1]

213 **Schuler-Faccini L**, Ribeiro EM, Feitosa IM, Horovitz DD, Cavalcanti DP, Pessoa A, Doriqui MJ, Neri JI, Neto JM, Wanderley HY, Cernach M, El-Husny AS, Pone MV, Serao CL, Sanseverino MT; Brazilian Medical Genetics Society-Zika Embryopathy Task Force. Possible Association Between Zika Virus Infection and Microcephaly-Brazil, 2015. *MMWR Morb Mortal Wkly Rep* 2016; **65**: 59-62 [PMID: 26820244 DOI: 10.15585/mmwr.mm6503e2]

214 **Muñoz LS**, Barreras P, Pardo CA. Zika Virus-Associated Neurological Disease in the Adult: Guillain-Barré Syndrome, Encephalitis, and Myelitis. *Semin Reprod Med* 2016; **34**: 273-279 [PMID: 27612158 DOI: 10.1055/s-0036-1592066]

215 **Halani S**, Tombindo PE, O'Reilly R, Miranda RN, Erdman LK, Whitehead C, Bielecki JM, Ramsay L, Ximenes R, Boyle J, Krueger C, Willmott S, Morris SK, Murphy KE, Sander B. Clinical manifestations and health outcomes associated with Zika virus infections in adults: A systematic review. *PLoS Negl Trop Dis* 2021; **15**: e0009516 [PMID: 34252102 DOI: 10.1371/journal.pntd.0009516]

216 **Bido-Medina R**, Wirsich J, Rodríguez M, Oviedo J, Miches I, Bido P, Tusen L, Stoeter P, Sadaghiani S. Impact of Zika Virus on adult human brain structure and functional organization. *Ann Clin Transl Neurol* 2018; **5**: 752-762 [PMID: 29928658 DOI: 10.1002/acn3.575]

217 **Pan American Health Organization**. Epidemiological Alert, Neurological syndrome, congenital malformations, and Zika virus infection. Implications for public health in the American. December 1, 2015. [cited 1 October 2024]. Available from: https://iris.paho.org/handle/10665.2/50697

218 **Arzuza-Ortega L**, Polo A, Pérez-Tatis G, López-García H, Parra E, Pardo-Herrera LC, Rico-Turca AM, Villamil-Gómez W, Rodríguez-Morales AJ. Fatal Sickle Cell Disease and Zika Virus Infection in Girl from Colombia. *Emerg Infect Dis* 2016; **22**: 925-927 [PMID: 27089120 DOI: 10.3201/eid2205.151934]

219 **World Health Organization**. Testes de laboratório para a infecção pelo vírus Zika. March 23, 2016. [cited 1 October 2024]. Available from: https://iris.who.int/bitstream/handle/10665/204671/WHO_ZIKV_LAB_16.1_por.pdf?sequence=6&isAllowed=y

220 **Johnson AJ**, Martin DA, Karabatsos N, Roehrig JT. Detection of anti-arboviral immunoglobulin G by using a monoclonal antibody-based capture enzyme-linked immunosorbent assay. *J Clin Microbiol* 2000; **38**: 1827-1831 [PMID: 10790108 DOI: 10.1128/JCM.38.5.1827-1831.2000]

221 **Sirikajornpan K**, Suntarattiwong P, Suwanpakdee D, Tabprasit S, Buddhari D, Thaisomboonsuk B, Klungthong C, Poolpanichupatam Y, Buathong R, Srikiatkhachorn A, Jones A, Fernandez S, Hunsawong T. Standardization and Evaluation of an Anti-ZIKV IgM ELISA Assay for the Serological Diagnosis of Zika Virus Infection. *Am J Trop Med Hyg* 2021; **105**: 936-941 [PMID: 34339380 DOI: 10.4269/ajtmh.21-0163]

222 **Martin DA**, Muth DA, Brown T, Johnson AJ, Karabatsos N, Roehrig JT. Standardization of immunoglobulin M capture enzyme-linked immunosorbent assays for routine diagnosis of arboviral infections. *J Clin Microbiol* 2000; **38**: 1823-1826 [PMID: 10790107 DOI: 10.1128/JCM.38.5.1823-1826.2000]

223 **Roehrig JT**, Hombach J, Barrett AD. Guidelines for Plaque-Reduction Neutralization Testing of Human Antibodies to Dengue Viruses. *Viral Immunol* 2008; **21**: 123-132 [PMID: 18476771 DOI: 10.1089/vim.2008.0007]

224 **Faye O**, Faye O, Diallo D, Diallo M, Weidmann M, Sall AA. Quantitative real-time PCR detection of Zika virus and evaluation with field-caught mosquitoes. *Virol J* 2013; **10**: 311 [PMID: 24148652 DOI: 10.1186/1743-422X-10-311]

225 **Varghese J**, De Silva I, Millar DS. Latest Advances in Arbovirus Diagnostics. *Microorganisms* 2023; **11** [PMID: 37317133 DOI: 10.3390/microorganisms11051159]

226 **Pedlar M**, Emery MJ, Warburton PJ. Amplifying PCR productivity and environmental sustainability through shortened cycling protocols. *Biochimie* 2024; **221**: 60-64 [PMID: 38262587 DOI: 10.1016/j.biochi.2024.01.013]

227 **Gourinat AC**, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of Zika virus in urine. *Emerg Infect Dis* 2015; **21**: 84-86 [PMID: 25530324 DOI: 10.3201/eid2101.140894]

228 **World Health Organization**. Zika virus. December 8, 20222022. [cited 1 October 2024]. Available from: https://www.who.int/news-room/fact-sheets/detail/zika-virus

229 **Falcao MB**, Cimerman S, Luz KG, Chebabo A, Brigido HA, Lobo IM, Timerman A, Angerami RN, da Cunha CA, Bacha HA, Alves JR, Barbosa AN, Teixeira RF, Weissmann L, Oliveira PR, Cyrillo MA, Bandeira AC. Management of infection by the Zika virus. *Ann Clin Microbiol Antimicrob* 2016; **15**: 57 [PMID: 27686610 DOI: 10.1186/s12941-016-0172-y]

230 **Bernatchez JA**, Tran LT, Li J, Luan Y, Siqueira-Neto JL, Li R. Drugs for the Treatment of Zika Virus Infection. *J Med Chem* 2020; **63**: 470-489 [PMID: 31549836 DOI: 10.1021/acs.jmedchem.9b00775]

231 **Jemielity S**, Wang JJ, Chan YK, Ahmed AA, Li W, Monahan S, Bu X, Farzan M, Freeman GJ, Umetsu DT, Dekruyff RH, Choe H. TIM-family proteins promote infection of multiple enveloped viruses through virion-associated phosphatidylserine. *PLoS Pathog* 2013; **9**: e1003232 [PMID: 23555248 DOI: 10.1371/journal.ppat.1003232]

232 **Menéndez-Arias L**, Andino R. Viral polymerases. *Virus Res* 2017; **234**: 1-3 [PMID: 28188807 DOI: 10.1016/j.virusres.2017.02.003]

233 **Lin HH**, Yip BS, Huang LM, Wu SC. Zika virus structural biology and progress in vaccine development. *Biotechnol Adv* 2018; **36**: 47-53 [PMID: 28916391 DOI: 10.1016/j.biotechadv.2017.09.004]

234 **Vannice KS**, Cassetti MC, Eisinger RW, Hombach J, Knezevic I, Marston HD, Wilder-Smith A, Cavaleri M, Krause PR. Demonstrating vaccine effectiveness during a waning epidemic: A WHO/NIH meeting report on approaches to development and licensure of Zika vaccine candidates. *Vaccine* 2019; **37**: 863-868 [PMID: 30639461 DOI: 10.1016/j.vaccine.2018.12.040]

235 **World Health Organization**. WHO/UNICEF Zika Virus (ZIKV) Vaccine Target Product Profile (TPP). February 8, 2017. [cited 1 October 2024]. Available from: https://www.who.int/publications/m/item/who-unicef-zika-virus-(zikv)-vaccine-target-product-profile-(tpp)

236 **Fortner A**, Schumacher D. First COVID-19 Vaccines Receiving the US FDA and EMA Emergency Use Authorization. *Discoveries (Craiova)* 2021; **9**: e122 [PMID: 33969180 DOI: 10.15190/d.2021.1]

237 **Pardi N**, Hogan MJ, Porter FW, Weissman D. mRNA vaccines-a new era in vaccinology. *Nat Rev Drug Discov* 2018; **17**: 261-279 [PMID: 29326426 DOI: 10.1038/nrd.2017.243]

238 **Fiolet T**, Kherabi Y, MacDonald CJ, Ghosn J, Peiffer-Smadja N. Comparing COVID-19 vaccines for their characteristics, efficacy and effectiveness against SARS-CoV-2 and variants of concern: a narrative review. *Clin Microbiol Infect* 2022; **28**: 202-221 [PMID: 34715347 DOI: 10.1016/j.cmi.2021.10.005]

239 **Pollard AJ**, Bijker EM. A guide to vaccinology: from basic principles to new developments. *Nat Rev Immunol* 2021; **21**: 83-100 [PMID: 33353987 DOI: 10.1038/s41577-020-00479-7]

240 **Rice CM**, Lenches EM, Eddy SR, Shin SJ, Sheets RL, Strauss JH. Nucleotide sequence of yellow fever virus: implications for flavivirus gene expression and evolution. *Science* 1985; **229**: 726-733 [PMID: 4023707 DOI: 10.1126/science.4023707]

241 **Monath TP**, Vasconcelos PF. Yellow fever. *J Clin Virol* 2015; **64**: 160-173 [PMID: 25453327 DOI: 10.1016/j.jcv.2014.08.030]

242 **Mukhopadhyay S**, Kuhn RJ, Rossmann MG. A structural perspective of the flavivirus life cycle. *Nat Rev Microbiol* 2005; **3**: 13-22 [PMID: 15608696 DOI: 10.1038/nrmicro1067]

243 **Op De Beeck A**, Rouillé Y, Caron M, Duvet S, Dubuisson J. The transmembrane domains of the prM and E proteins of yellow fever virus are endoplasmic reticulum localization signals. *J Virol* 2004; **78**: 12591-12602 [PMID: 15507646 DOI: 10.1128/JVI.78.22.12591-12602.2004]

244 **Davis EH**, Wang B, White M, Huang YS, Sarathy VV, Wang T, Bourne N, Higgs S, Barrett ADT. Impact of yellow fever virus envelope protein on wild-type and vaccine epitopes and tissue tropism. *NPJ Vaccines* 2022; **7**: 39 [PMID: 35322047 DOI: 10.1038/s41541-022-00460-6]

245 **Davis EH**, Barrett ADT. Structure-Function of the Yellow Fever Virus Envelope Protein: Analysis of Antibody Epitopes. *Viral Immunol* 2020; **33**: 12-21 [PMID: 31682201 DOI: 10.1089/vim.2019.0107]

246 **Lindenbach BD**, Rice CM. Molecular biology of flaviviruses. *Adv Virus Res* 2003; **59**: 23-61 [PMID: 14696326 DOI: 10.1016/s0065-3527(03)59002-9]

247 **Hanley KA**, Monath TP, Weaver SC, Rossi SL, Richman RL, Vasilakis N. Fever versus fever: the role of host and vector susceptibility and interspecific competition in shaping the current and future distributions of the sylvatic cycles of dengue virus and yellow fever virus. *Infect Genet Evol* 2013; **19**: 292-311 [PMID: 23523817 DOI: 10.1016/j.meegid.2013.03.008]

248 **Ahmed QA**, Memish ZA. Yellow fever from Angola and Congo: a storm gathers. *Trop Doct* 2017; **47**: 92-96 [PMID: 28424031 DOI:

10.1177/0049475517699726]
249 **World Health Organization**. Eliminate Yellow Fever Epidemics (EYE) 2017-2026. January 21, 2018. [cited 1 October 2024]. Available from: https://www.who.int/publications/i/item/9789241513661
250 **Bryant JE**, Holmes EC, Barrett AD. Out of Africa: a molecular perspective on the introduction of yellow fever virus into the Americas. *PLoS Pathog* 2007; **3**: e75 [PMID: 17511518 DOI: 10.1371/journal.ppat.0030075]
251 **Staples JE**, Monath TP. Yellow fever: 100 years of discovery. *JAMA* 2008; **300**: 960-962 [PMID: 18728272 DOI: 10.1001/jama.300.8.960]
252 **Pulendran B**. Learning immunology from the yellow fever vaccine: innate immunity to systems vaccinology. *Nat Rev Immunol* 2009; **9**: 741-747 [PMID: 19763148 DOI: 10.1038/nri2629]
253 **Lloyd W**, Theiler M, Ricci N. Modification of the virulence of yellow fever virus by cultivation in tissues in vitro. *T Roy Soc Trop Med H* 1936; **29**: 481-529 [DOI: 10.1016/s0035-9203(36)90002-0]
254 **World Health Organization**. Yellow fever [Internet]. 2023. [cited 1 October 2024]. Available from: https://www.who.int/news-room/fact-sheets/detail/yellow-fever
255 **Aitken TH**, Tesh RB, Beaty BJ, Rosen L. Transovarial transmission of yellow fever virus by mosquitoes (Aedes aegypti). *Am J Trop Med Hyg* 1979; **28**: 119-121 [PMID: 434305 DOI: 10.4269/ajtmh.1979.28.119]
256 **Dutary BE**, Leduc JW. Transovarial transmission of yellow fever virus by a sylvatic vector, Haemagogus equinus. *Trans R Soc Trop Med Hyg* 1981; **75**: 128 [PMID: 6115487 DOI: 10.1016/0035-9203(81)90036-5]
257 **Barrett AD**, Higgs S. Yellow fever: a disease that has yet to be conquered. *Annu Rev Entomol* 2007; **52**: 209-229 [PMID: 16913829 DOI: 10.1146/annurev.ento.52.110405.091454]
258 **Gardner CL**, Ryman KD. Yellow fever: a reemerging threat. *Clin Lab Med* 2010; **30**: 237-260 [PMID: 20513550 DOI: 10.1016/j.cll.2010.01.001]
259 **World Health Organization**. Laboratory manual for yellow fever. January 25, 2024. [cited 1 October 2024]. Available from: https://www.who.int/publications/i/item/9789240084476
260 **Pedrosa PB**, Cardoso TA. Viral infections in workers in hospital and research laboratory settings: a comparative review of infection modes and respective biosafety aspects. *Int J Infect Dis* 2011; **15**: e366-e376 [PMID: 21497126 DOI: 10.1016/j.ijid.2011.03.005]
261 **Bentlin MR**, de Barros Almeida RA, Coelho KI, Ribeiro AF, Siciliano MM, Suzuki A, Fortaleza CM. Perinatal transmission of yellow fever, Brazil, 2009. *Emerg Infect Dis* 2011; **17**: 1779-1780 [PMID: 21888828 DOI: 10.3201/eid1709.110242]
262 **Diniz LMO**, Romanelli RMC, de Carvalho AL, Teixeira DC, de Carvalho LFA, Ferreira Cury V, Filho MPL, Perígolo G, Heringer TP. Perinatal Yellow Fever: A Case Report. *Pediatr Infect Dis J* 2019; **38**: 300-301 [PMID: 29634624 DOI: 10.1097/INF.0000000000002051]
263 **Centers for Disease Control and Prevention (CDC)**. Transfusion-related transmission of yellow fever vaccine virus--California, 2009. *MMWR Morb Mortal Wkly Rep* 2010; **59**: 34-37 [PMID: 20094025]
264 **Gould CV**, Free RJ, Bhatnagar J, Soto RA, Royer TL, Maley WR, Moss S, Berk MA, Craig-Shapiro R, Kodiyanplakkal RPL, Westblade LF, Muthukumar T, Puius YA, Raina A, Hadi A, Gyure KA, Trief D, Pereira M, Kuehnert MJ, Ballen V, Kessler DA, Dailey K, Omura C, Doan T, Miller S, Wilson MR, Lehman JA, Ritter JM, Lee E, Silva-Flannery L, Reagan-Steiner S, Velez JO, Laven JJ, Fitzpatrick KA, Panella A, Davis EH, Hughes HR, Brault AC, St George K, Dean AB, Ackelsberg J, Basavaraju SV, Chiu CY, Staples JE; Yellow Fever Vaccine Virus Transplant and Transfusion Investigation Team. Transmission of yellow fever vaccine virus through blood transfusion and organ transplantation in the USA in 2021: report of an investigation. *Lancet Microbe* 2023; **4**: e711-e721 [PMID: 37544313 DOI: 10.1016/S2666-5247(23)00170-2]
265 **Hassan T**, Bashir RA, Abdelrahman DN, Madni H, M El Hussein AR, Elkidir IM, Enan KA. Transmission of yellow fever vaccine virus from breast feeding mothers to their infants: reporting of yellow fever virus (YFV) RNA detection in milk specimens. *F1000Res* 2022; **11**: 76 [PMID: 38106444 DOI: 10.12688/f1000research.74576.3]
266 **Centers for Disease Control and Prevention (CDC)**. Transmission of yellow fever vaccine virus through breast-feeding-Brazil, 2009. *MMWR Morb Mortal Wkly Rep* 2010; **59**: 130-132 [PMID: 20150888]
267 **Simmonds P**, Becher P, Bukh J, Gould EA, Meyers G, Monath T, Muerhoff S, Pletnev A, Rico-Hesse R, Smith DB, Stapleton JT; Ictv Report Consortium. ICTV Virus Taxonomy Profile: Flaviviridae. *J Gen Virol* 2017; **98**: 2-3 [PMID: 28218572 DOI: 10.1099/jgv.0.000672]
268 **Kaufmann B**, Rossmann MG. Molecular mechanisms involved in the early steps of flavivirus cell entry. *Microbes Infect* 2011; **13**: 1-9 [PMID: 20869460 DOI: 10.1016/j.micinf.2010.09.005]
269 **Douam F**, Ploss A. Yellow Fever Virus: Knowledge Gaps Impeding the Fight Against an Old Foe. *Trends Microbiol* 2018; **26**: 913-928 [PMID: 29933925 DOI: 10.1016/j.tim.2018.05.012]
270 **Chávez JH**, Silva JR, Amarilla AA, Moraes Figueiredo LT. Domain III peptides from flavivirus envelope protein are useful antigens for serologic diagnosis and targets for immunization. *Biologicals* 2010; **38**: 613-618 [PMID: 20817489 DOI: 10.1016/j.biologicals.2010.07.004]
271 **Lee E**, Hall RA, Lobigs M. Common E protein determinants for attenuation of glycosaminoglycan-binding variants of Japanese encephalitis and West Nile viruses. *J Virol* 2004; **78**: 8271-8280 [PMID: 15254199 DOI: 10.1128/JVI.78.15.8271-8280.2004]
272 **Hung JJ**, Hsieh MT, Young MJ, Kao CL, King CC, Chang W. An external loop region of domain III of dengue virus type 2 envelope protein is involved in serotype-specific binding to mosquito but not mammalian cells. *J Virol* 2004; **78**: 378-388 [PMID: 14671119 DOI: 10.1128/jvi.78.1.378-388.2004]
273 **Huang YJ**, Nuckols JT, Horne KM, Vanlandingham D, Lobigs M, Higgs S. Mutagenesis analysis of T380R mutation in the envelope protein of yellow fever virus. *Virol J* 2014; **11**: 60 [PMID: 24678844 DOI: 10.1186/1743-422X-11-60]
274 **Germi R**, Crance JM, Garin D, Guimet J, Lortat-Jacob H, Ruigrok RW, Zarski JP, Drouet E. Heparan sulfate-mediated binding of infectious dengue virus type 2 and yellow fever virus. *Virology* 2002; **292**: 162-168 [PMID: 11878919 DOI: 10.1006/viro.2001.1232]
275 **Lee E**, Lobigs M. E protein domain III determinants of yellow fever virus 17D vaccine strain enhance binding to glycosaminoglycans, impede virus spread, and attenuate virulence. *J Virol* 2008; **82**: 6024-6033 [PMID: 18400851 DOI: 10.1128/JVI.02509-07]
276 **Harrison SC**. The pH sensor for flavivirus membrane fusion. *J Cell Biol* 2008; **183**: 177-179 [PMID: 18936246 DOI: 10.1083/jcb.200809175]
277 **Fernandez-Garcia MD**, Meertens L, Chazal M, Hafirassou ML, Dejarnac O, Zamborlini A, Despres P, Sauvonnet N, Arenzana-Seisdedos F, Jouvenet N, Amara A. Vaccine and Wild-Type Strains of Yellow Fever Virus Engage Distinct Entry Mechanisms and Differentially Stimulate Antiviral Immune Responses. *mBio* 2016; **7**: e01956-e01915 [PMID: 26861019 DOI: 10.1128/mBio.01956-15]
278 **Bressanelli S**, Stiasny K, Allison SL, Stura EA, Duquerroy S, Lescar J, Heinz FX, Rey FA. Structure of a flavivirus envelope glycoprotein in its low-pH-induced membrane fusion conformation. *EMBO J* 2004; **23**: 728-738 [PMID: 14963486 DOI: 10.1038/sj.emboj.7600064]
279 **Ramanathan HN**, Zhang S, Douam F, Mar KB, Chang J, Yang PL, Schoggins JW, Ploss A, Lindenbach BD. A Sensitive Yellow Fever Virus

Entry Reporter Identifies Valosin-Containing Protein (VCP/p97) as an Essential Host Factor for Flavivirus Uncoating. *mBio* 2020; **11** [PMID: 32291299 DOI: 10.1128/mBio.00467-20]

280 **Westaway EG**, Brinton MA, Gaidamovich SYa, Horzinek MC, Igarashi A, Kääriäinen L, Lvov DK, Porterfield JS, Russell PK, Trent DW. Flaviviridae. *Intervirology* 1985; **24**: 183-192 [PMID: 3000978 DOI: 10.1159/000149642]

281 **Amberg SM**, Rice CM. Mutagenesis of the NS2B-NS3-mediated cleavage site in the flavivirus capsid protein demonstrates a requirement for coordinated processing. *J Virol* 1999; **73**: 8083-8094 [PMID: 10482557 DOI: 10.1128/JVI.73.10.8083-8094.1999]

282 **Chambers TJ**, Droll DA, Tang Y, Liang Y, Ganesh VK, Murthy KHM, Nickells M. Yellow fever virus NS2B-NS3 protease: characterization of charged-to-alanine mutant and revertant viruses and analysis of polyprotein-cleavage activities. *J Gen Virol* 2005; **86**: 1403-1413 [PMID: 15831952 DOI: 10.1099/vir.0.80427-0]

283 **Chambers TJ**, Nestorowicz A, Rice CM. Mutagenesis of the yellow fever virus NS2B/3 cleavage site: determinants of cleavage site specificity and effects on polyprotein processing and viral replication. *J Virol* 1995; **69**: 1600-1605 [PMID: 7853494 DOI: 10.1128/JVI.69.3.1600-1605.1995]

284 **Dubankova A**, Boura E. Structure of the yellow fever NS5 protein reveals conserved drug targets shared among flaviviruses. *Antiviral Res* 2019; **169**: 104536 [PMID: 31202975 DOI: 10.1016/j.antiviral.2019.104536]

285 **Le Sommer C**, Barrows NJ, Bradrick SS, Pearson JL, Garcia-Blanco MA. G protein-coupled receptor kinase 2 promotes flaviviridae entry and replication. *PLoS Negl Trop Dis* 2012; **6**: e1820 [PMID: 23029581 DOI: 10.1371/journal.pntd.0001820]

286 **Zhang R**, Miner JJ, Gorman MJ, Rausch K, Ramage H, White JP, Zuiani A, Zhang P, Fernandez E, Zhang Q, Dowd KA, Pierson TC, Cherry S, Diamond MS. A CRISPR screen defines a signal peptide processing pathway required by flaviviruses. *Nature* 2016; **535**: 164-168 [PMID: 27383988 DOI: 10.1038/nature18625]

287 **Yi Z**, Sperzel L, Nürnberger C, Bredenbeek PJ, Lubick KJ, Best SM, Stoyanov CT, Law LM, Yuan Z, Rice CM, MacDonald MR. Identification and characterization of the host protein DNAJC14 as a broadly active flavivirus replication modulator. *PLoS Pathog* 2011; **7**: e1001255 [PMID: 21249176 DOI: 10.1371/journal.ppat.1001255]

288 **Bozzacco L**, Yi Z, Andreo U, Conklin CR, Li MM, Rice CM, MacDonald MR. Chaperone-Assisted Protein Folding Is Critical for Yellow Fever Virus NS3/4A Cleavage and Replication. *J Virol* 2016; **90**: 3212-3228 [PMID: 26739057 DOI: 10.1128/JVI.03077-15]

289 **Campos RK**, Wong B, Xie X, Lu YF, Shi PY, Pompon J, Garcia-Blanco MA, Bradrick SS. RPLP1 and RPLP2 Are Essential Flavivirus Host Factors That Promote Early Viral Protein Accumulation. *J Virol* 2017; **91** [PMID: 27974556 DOI: 10.1128/JVI.01706-16]

290 **Klaitong P**, Smith DR. Roles of Non-Structural Protein 4A in Flavivirus Infection. *Viruses* 2021; **13** [PMID: 34696510 DOI: 10.3390/v13102077]

291 **van den Elsen K**, Quek JP, Luo D. Molecular Insights into the Flavivirus Replication Complex. *Viruses* 2021; **13** [PMID: 34064113 DOI: 10.3390/v13060956]

292 **Roby JA**, Setoh YX, Hall RA, Khromykh AA. Post-translational regulation and modifications of flavivirus structural proteins. *J Gen Virol* 2015; **96**: 1551-1569 [PMID: 25711963 DOI: 10.1099/vir.0.000097]

293 **Ferreira MS**, Sousa JR, Bezerra Júnior PS, Cerqueira VD, Oliveira Júnior CA, Rivero GRC, Castro PHG, Silva GA, Muniz JAPC, da Silva EVP, Casseb SMM, Pagliari C, Martins LC, Tesh RB, Quaresma JAS, Vasconcelos PFC. Experimental Yellow Fever in Squirrel Monkey: Characterization of Liver In Situ Immune Response. *Viruses* 2023; **15** [PMID: 36851765 DOI: 10.3390/v15020551]

294 **Guo HY**, Zhang XC, Jia RY. Toll-Like Receptors and RIG-I-Like Receptors Play Important Roles in Resisting Flavivirus. *J Immunol Res* 2018; **2018**: 6106582 [PMID: 29888293 DOI: 10.1155/2018/6106582]

295 **Querec T**, Bennouna S, Alkan S, Laouar Y, Gorden K, Flavell R, Akira S, Ahmed R, Pulendran B. Yellow fever vaccine YF-17D activates multiple dendritic cell subsets *via* TLR2, 7, 8, and 9 to stimulate polyvalent immunity. *J Exp Med* 2006; **203**: 413-424 [PMID: 16461338 DOI: 10.1084/jem.20051720]

296 **Sinigaglia L**, Gracias S, Décembre E, Fritz M, Bruni D, Smith N, Herbeuval JP, Martin A, Dreux M, Tangy F, Jouvenet N. Immature particles and capsid-free viral RNA produced by Yellow fever virus-infected cells stimulate plasmacytoid dendritic cells to secrete interferons. *Sci Rep* 2018; **8**: 10889 [PMID: 30022130 DOI: 10.1038/s41598-018-29235-7]

297 **Beauclair G**, Streicher F, Chazal M, Bruni D, Lesage S, Gracias S, Bourgeau S, Sinigaglia L, Fujita T, Meurs EF, Tangy F, Jouvenet N. Retinoic Acid Inducible Gene I and Protein Kinase R, but Not Stress Granules, Mediate the Proinflammatory Response to Yellow Fever Virus. *J Virol* 2020; **94** [PMID: 32878892 DOI: 10.1128/JVI.00403-20]

298 **Miorin L**, Laurent-Rolle M, Pisanelli G, Co PH, Albrecht RA, García-Sastre A, Morrison J. Host-Specific NS5 Ubiquitination Determines Yellow Fever Virus Tropism. *J Virol* 2019; **93** [PMID: 31043530 DOI: 10.1128/JVI.00151-19]

299 **Laurent-Rolle M**, Morrison J, Rajsbaum R, Macleod JML, Pisanelli G, Pham A, Ayllon J, Miorin L, Martinez C, tenOever BR, García-Sastre A. The interferon signaling antagonist function of yellow fever virus NS5 protein is activated by type I interferon. *Cell Host Microbe* 2014; **16**: 314-327 [PMID: 25211074 DOI: 10.1016/j.chom.2014.07.015]

300 **Airo AM**, Felix-Lopez A, Mancinelli V, Evseev D, Lopez-Orozco J, Shire K, Paszkowski P, Frappier L, Magor KE, Hobman TC. Flavivirus Capsid Proteins Inhibit the Interferon Response. *Viruses* 2022; **14** [PMID: 35632712 DOI: 10.3390/v14050968]

301 **Zoladek J**, Nisole S. Mosquito-borne flaviviruses and type I interferon: catch me if you can! *Front Microbiol* 2023; **14**: 1257024 [PMID: 37965539 DOI: 10.3389/fmicb.2023.1257024]

302 **Quaresma JA**, Pagliari C, Medeiros DB, Duarte MI, Vasconcelos PF. Immunity and immune response, pathology and pathologic changes: progress and challenges in the immunopathology of yellow fever. *Rev Med Virol* 2013; **23**: 305-318 [PMID: 23873723 DOI: 10.1002/rmv.1752]

303 **Quaresma JA**, Barros VL, Pagliari C, Fernandes ER, Andrade HF Jr, Vasconcelos PF, Duarte MI. Hepatocyte lesions and cellular immune response in yellow fever infection. *Trans R Soc Trop Med Hyg* 2007; **101**: 161-168 [PMID: 16872652 DOI: 10.1016/j.trstmh.2006.02.019]

304 **ter Meulen J**, Sakho M, Koulemou K, Magassouba N, Bah A, Preiser W, Daffis S, Klewitz C, Bae HG, Niedrig M, Zeller H, Heinzel-Gutenbrunner M, Koivogui L, Kaufmann A. Activation of the cytokine network and unfavorable outcome in patients with yellow fever. *J Infect Dis* 2004; **190**: 1821-1827 [PMID: 15499539 DOI: 10.1086/425016]

305 **Alberts B**, Johnson A, Lewis J. Helper T Cells and Lymphocyte Activation. In: Molecular Biology of the Cell. 4th edition. New York: Garland Science, 2002

306 **Quaresma JA**, Barros VL, Fernandes ER, Pagliari C, Takakura C, da Costa Vasconcelos PF, de Andrade HF Jr, Duarte MI. Reconsideration of histopathology and ultrastructural aspects of the human liver in yellow fever. *Acta Trop* 2005; **94**: 116-127 [PMID: 15829426 DOI: 10.1016/j.actatropica.2005.03.003]

307 **Engelmann F**, Josset L, Girke T, Park B, Barron A, Dewane J, Hammarlund E, Lewis A, Axthelm MK, Slifka MK, Messaoudi I.

Pathophysiologic and transcriptomic analyses of viscerotropic yellow fever in a rhesus macaque model. *PLoS Negl Trop Dis* 2014; **8**: e3295 [PMID: 25412185 DOI: 10.1371/journal.pntd.0003295]

308 **Monath TP**, Brinker KR, Chandler FW, Kemp GE, Cropp CB. Pathophysiologic correlations in a rhesus monkey model of yellow fever with special observations on the acute necrosis of B cell areas of lymphoid tissues. *Am J Trop Med Hyg* 1981; **30**: 431-443 [PMID: 7235133 DOI: 10.4269/ajtmh.1981.30.431]

309 **Xiao SY**, Zhang H, Guzman H, Tesh RB. Experimental yellow fever virus infection in the Golden hamster (Mesocricetus auratus). II. Pathology. *J Infect Dis* 2001; **183**: 1437-1444 [PMID: 11319680 DOI: 10.1086/320200]

310 **Hudson NP**. The Pathology of Experimental Yellow Fever in the Macacus Rhesus: I. Gross Pathology. *Am J Pathol* 1928; **4**: 395-406.1 [PMID: 19969807]

311 **Quaresma JA**, Barros VL, Pagliari C, Fernandes ER, Guedes F, Takakura CF, Andrade HF Jr, Vasconcelos PF, Duarte MI. Revisiting the liver in human yellow fever: virus-induced apoptosis in hepatocytes associated with TGF-beta, TNF-alpha and NK cells activity. *Virology* 2006; **345**: 22-30 [PMID: 16278000 DOI: 10.1016/j.virol.2005.09.058]

312 **Quaresma JA**, Barros VL, Fernandes ER, Pagliari C, Guedes F, da Costa Vasconcelos PF, de Andrade Junior HF, Duarte MI. Immunohistochemical examination of the role of Fas ligand and lymphocytes in the pathogenesis of human liver yellow fever. *Virus Res* 2006; **116**: 91-97 [PMID: 16219382 DOI: 10.1016/j.virusres.2005.08.019]

313 **da Costa Lopes J**, Falcão LFM, Martins Filho AJ, Carvalho MLG, Mendes CCH, Olímpio FA, do Socorro Cabral Miranda V, Dos Santos LC, Chiang JO, Cruz ACR, Galúcio VCA, do Socorro da Silva Azevedo R, Martins LC, Duarte MIS, de Sousa JR, da Costa Vasconcelos PF, Quaresma JAS. Factors Involved in the Apoptotic Cell Death Mechanism in Yellow Fever Hepatitis. *Viruses* 2022; **14** [PMID: 35746675 DOI: 10.3390/v14061204]

314 **Olímpio FA**, Falcão LFM, Carvalho MLG, da Costa Lopes J, Mendes CCH, Filho AJM, da Silva CAM, Miranda VDSC, Santos LCD, da Silva Vilacoert FS, Cruz ACR, Galúcio VCA, da Silva Azevedo RDS, Martins LC, Duarte MIS, de Sousa JR, da Costa Vasconcelos PF, Quaresma JAS. Endothelium Activation during Severe Yellow Fever Triggers an Intense Cytokine-Mediated Inflammatory Response in the Liver Parenchyma. *Pathogens* 2022; **11** [PMID: 35056050 DOI: 10.3390/pathogens11010101]

315 **Futosi K**, Fodor S, Mócsai A. Neutrophil cell surface receptors and their intracellular signal transduction pathways. *Int Immunopharmacol* 2013; **17**: 638-650 [PMID: 23994464 DOI: 10.1016/j.intimp.2013.06.034]

316 **Puerta-Guardo H**, Glasner DR, Espinosa DA, Biering SB, Patana M, Ratnasiri K, Wang C, Beatty PR, Harris E. Flavivirus NS1 Triggers Tissue-Specific Vascular Endothelial Dysfunction Reflecting Disease Tropism. *Cell Rep* 2019; **26**: 1598-1613.e8 [PMID: 30726741 DOI: 10.1016/j.celrep.2019.01.036]

317 **Vieira WT**, Gayotto LC, de Lima CP, de Brito T. Histopathology of the human liver in yellow fever with special emphasis on the diagnostic role of the Councilman body. *Histopathology* 1983; **7**: 195-208 [PMID: 6303935 DOI: 10.1111/j.1365-2559.1983.tb02235.x]

318 **Carvalho MLG**, Falcão LFM, Lopes JDC, Mendes CCH, Olímpio FA, Miranda VDSC, Santos LCD, de Moraes DDP, Bertonsin Filho MV, da Costa LD, da Silva Azevedo RDS, Cruz ACR, Galúcio VCA, Martins LC, Duarte MIS, Martins Filho AJ, Sousa JR, Vasconcelos PFDC, Quaresma JAS. Role of Th17 Cytokines in the Liver's Immune Response during Fatal Yellow Fever: Triggering Cell Damage Mechanisms. *Cells* 2022; **11** [PMID: 35805137 DOI: 10.3390/cells11132053]

319 **Lemos FO**, França A, Lima Filho ACM, Florentino RM, Santos ML, Missiaggia DG, Rodrigues GOL, Dias FF, Souza Passos IB, Teixeira MM, Andrade AMF, Lima CX, Vidigal PVT, Costa VV, Fonseca MC, Nathanson MH, Leite MF. Molecular Mechanism for Protection Against Liver Failure in Human Yellow Fever Infection. *Hepatol Commun* 2020; **4**: 657-669 [PMID: 32363317 DOI: 10.1002/hep4.1504]

320 **Domingo C**, Charrel RN, Schmidt-Chanasit J, Zeller H, Reusken C. Yellow fever in the diagnostics laboratory. *Emerg Microbes Infect* 2018; **7**: 129 [PMID: 30002363 DOI: 10.1038/s41426-018-0128-8]

321 **Monath TP**. Yellow fever: an update. *Lancet Infect Dis* 2001; **1**: 11-20 [PMID: 11871403 DOI: 10.1016/S1473-3099(01)00016-0]

322 **Johansson MA**, Vasconcelos PF, Staples JE. The whole iceberg: estimating the incidence of yellow fever virus infection from the number of severe cases. *Trans R Soc Trop Med Hyg* 2014; **108**: 482-487 [PMID: 24980556 DOI: 10.1093/trstmh/tru092]

323 **Franco MB**, Jardim LL, de Carvalho BN, Basques F, Ribeiro DD, Pereira LS, Rezende SM. Deficiency of coagulation factors is associated with the bleeding diathesis of severe yellow fever. *Ann Hematol* 2023; **102**: 1939-1949 [PMID: 37226021 DOI: 10.1007/s00277-023-05262-x]

324 **Vasconcelos PF**. [Yellow Fever]. *Rev Soc Bras Med Trop* 2003; **36**: 275-293 [PMID: 12806465 DOI: 10.1590/s0037-86822003000200012]

325 **Waggoner JJ**, Rojas A, Pinsky BA. Yellow Fever Virus: Diagnostics for a Persistent Arboviral Threat. *J Clin Microbiol* 2018; **56** [PMID: 30021822 DOI: 10.1128/JCM.00827-18]

326 **World Health Organization**. Yellow Fever Vaccine-Preventable Diseases Surveillance Standards. April 30, 2020 [cited 1 October 2024]. Available from: https://www.who.int/publications/m/item/vaccine-preventable-diseases-surveillance-standards-yellow-fever

327 **Houghton-Triviño N**, Montaña D, Castellanos J. Dengue-yellow fever sera cross-reactivity; challenges for diagnosis. *Rev Salud Publica (Bogota)* 2008; **10**: 299-307 [PMID: 19039426 DOI: 10.1590/s0124-00642008000200010]

328 **Liu D**, Chen D, Zhang T, Yu N, Ren R, Chen Y, Wang C. Preparation and application of yellow fever virus NS1 protein-specific monoclonal antibodies. *J Med Virol* 2021; **93**: 3374-3382 [PMID: 32841419 DOI: 10.1002/jmv.26455]

329 **Dia M**, Bob NS, Talla C, Dupressoir A, Escadafal C, Thiam MS, Diallo A, Ndiaye O, Heraud JM, Faye O, Sall AA, Faye O, Fall G. Performance assessment and validation of a plaque reduction neutralization test (PRNT) in support to yellow fever diagnostic and vaccine clinical trials. *J Med Virol* 2023; **95**: e28700 [PMID: 36951314 DOI: 10.1002/jmv.28700]

330 **Niedrig M**, Kürsteiner O, Herzog C, Sonnenberg K. Evaluation of an indirect immunofluorescence assay for detection of immunoglobulin M (IgM) and IgG antibodies against yellow fever virus. *Clin Vaccine Immunol* 2008; **15**: 177-181 [PMID: 18045884 DOI: 10.1128/CVI.00078-07]

331 **Hughes HR**, Russell BJ, Mossel EC, Kayiwa J, Lutwama J, Lambert AJ. Development of a Real-Time Reverse Transcription-PCR Assay for Global Differentiation of Yellow Fever Virus Vaccine-Related Adverse Events from Natural Infections. *J Clin Microbiol* 2018; **56** [PMID: 29643198 DOI: 10.1128/JCM.00323-18]

332 **Fischer C**, Torres MC, Patel P, Moreira-Soto A, Gould EA, Charrel RN, de Lamballerie X, Nogueira RMR, Sequeira PC, Rodrigues CDS, Kümmerer BM, Drosten C, Landt O, Bispo de Filippis AM, Drexler JF. Lineage-Specific Real-Time RT-PCR for Yellow Fever Virus Outbreak Surveillance, Brazil. *Emerg Infect Dis* 2017; **23**: 1867-1871 [PMID: 28949285 DOI: 10.3201/eid2311.171131]

333 **Huang B**, Montgomery BL, Adamczyk R, Ehlers G, van den Hurk AF, Warrilow D. A LAMP-based colorimetric assay to expedite field surveillance of the invasive mosquito species Aedes aegypti and Aedes albopictus. *PLoS Negl Trop Dis* 2020; **14**: e0008130 [PMID: 32130209 DOI: 10.1371/journal.pntd.0008130]

334 **Nunes MR**, Vianez JL Jr, Nunes KN, da Silva SP, Lima CP, Guzman H, Martins LC, Carvalho VL, Tesh RB, Vasconcelos PF. Analysis of a Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP) for yellow fever diagnostic. *J Virol Methods* 2015; **226**: 40-51 [PMID: 26459206 DOI: 10.1016/j.jviromet.2015.10.003]

335 **Kwallah Ao**, Inoue S, Muigai AW, Kubo T, Sang R, Morita K, Mwau M. A real-time reverse transcription loop-mediated isothermal amplification assay for the rapid detection of yellow fever virus. *J Virol Methods* 2013; **193**: 23-27 [PMID: 23692685 DOI: 10.1016/j.jviromet.2013.05.004]

336 **Pan American Health Organization**. Clinical Management of Yellow Fever in the Region of the Americas. Experiences and Recommendations for Health Services. 2023. [cited 1 October 2024]. Available from: https://iris.paho.org/handle/10665.2/57318

337 **Abbate M**, Zoja C, Remuzzi G. How does proteinuria cause progressive renal damage? *J Am Soc Nephrol* 2006; **17**: 2974-2984 [PMID: 17035611 DOI: 10.1681/ASN.2006040377]

338 **de Freitas CS**, Higa LM, Sacramento CQ, Ferreira AC, Reis PA, Delvecchio R, Monteiro FL, Barbosa-Lima G, James Westgarth H, Vieira YR, Mattos M, Rocha N, Hoelz LVB, Leme RPP, Bastos MM, Rodrigues GOL, Lopes CEM, Queiroz-Junior CM, Lima CX, Costa VV, Teixeira MM, Bozza FA, Bozza PT, Boechat N, Tanuri A, Souza TML. Yellow fever virus is susceptible to sofosbuvir both *in vitro* and *in vivo*. *PLoS Negl Trop Dis* 2019; **13**: e0007072 [PMID: 30699122 DOI: 10.1371/journal.pntd.0007072]

339 **Mendes ÉA**, Pilger DRB, Santos Nastri ACS, Malta FM, Pascoalino BDS, Carneiro D'Albuquerque LA, Balan A, Freitas LHG Jr, Durigon EL, Carrilho FJ, Rebello Pinho JR. Sofosbuvir inhibits yellow fever virus in vitro and in patients with acute liver failure. *Ann Hepatol* 2019; **18**: 816-824 [PMID: 31594756 DOI: 10.1016/j.aohep.2019.09.001]

340 **Rezende IM**, Mendonça DC, Costa TA, de Oliveria GFG, Arruda MS, Gonçalves AP, Alves PA, Calzavara-Silva CE, Martins-Filho OA, Teixeira-Carvalho A, Bonjardim CA, Monath TP, LaBeaud AD, Drumond BP, Pascoal-Xavier MA, Pereira LS, Ramalho DB. Sofosbuvir Off-label Treatment of Yellow Fever Patients During an Outbreak in Brazil, 2018: A Cohort Study. *Open Forum Infect Dis* 2024; **11**: ofae312 [PMID: 38933737 DOI: 10.1093/ofid/ofae312]

341 **de Ávila RE**, José Fernandes H, Barbosa GM, Araújo AL, Gomes TCC, Barros TG, Moreira RLF, Silva GLC, de Oliveira NR. Clinical profiles and factors associated with mortality in adults with yellow fever admitted to an intensive care unit in Minas Gerais, Brazil. *Int J Infect Dis* 2020; **93**: 90-97 [PMID: 32004691 DOI: 10.1016/j.ijid.2020.01.039]

342 **Kallas EG**, D'Elia Zanella LGFAB, Moreira CHV, Buccheri R, Diniz GBF, Castiñeiras ACP, Costa PR, Dias JZC, Marmorato MP, Song ATW, Maestri A, Borges IC, Joelsons D, Cerqueira NB, Santiago E Souza NC, Morales Claro I, Sabino EC, Levi JE, Avelino-Silva VI, Ho YL. Predictors of mortality in patients with yellow fever: an observational cohort study. *Lancet Infect Dis* 2019; **19**: 750-758 [PMID: 31104909 DOI: 10.1016/S1473-3099(19)30125-2]

343 **Ho YL**, Joelsons D, Leite GFC, Malbouisson LMS, Song ATW, Perondi B, Andrade LC, Pinto LF, D'Albuquerque LAC, Segurado AAC; Hospital das Clínicas Yellow Fever Assistance Group. Severe yellow fever in Brazil: clinical characteristics and management. *J Travel Med* 2019; **26** [PMID: 31150098 DOI: 10.1093/jtm/taz040]

344 **Barrett AD**, Teuwen DE. Yellow fever vaccine-how does it work and why do rare cases of serious adverse events take place? *Curr Opin Immunol* 2009; **21**: 308-313 [PMID: 19520559 DOI: 10.1016/j.coi.2009.05.018]

345 **Querec TD**, Akondy RS, Lee EK, Cao W, Nakaya HI, Teuwen D, Pirani A, Gernert K, Deng J, Marzolf B, Kennedy K, Wu H, Bennouna S, Oluoch H, Miller J, Vencio RZ, Mulligan M, Aderem A, Ahmed R, Pulendran B. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. *Nat Immunol* 2009; **10**: 116-125 [PMID: 19029902 DOI: 10.1038/ni.1688]

346 **Juan-Giner A**, Kimathi D, Grantz KH, Hamaluba M, Kazooba P, Njuguna P, Fall G, Dia M, Bob NS, Monath TP, Barrett AD, Hombach J, Mulogo EM, Ampeire I, Karanja HK, Nyehangane D, Mwanga-Amumpaire J, Cummings DAT, Bejon P, Warimwe GM, Grais RF. Immunogenicity and safety of fractional doses of yellow fever vaccines: a randomised, double-blind, non-inferiority trial. *Lancet* 2021; **397**: 119-127 [PMID: 33422245 DOI: 10.1016/S0140-6736(20)32520-4]

347 **Cançado BLB**, Aranda CS, Mallozi MC, Weckx LY, Solé D. Egg allergy and yellow fever vaccination. *J Pediatr (Rio J)* 2024; **100**: 60-66 [PMID: 37597532 DOI: 10.1016/j.jped.2023.07.004]

348 **Silva LA**, Dermody TS. Chikungunya virus: epidemiology, replication, disease mechanisms, and prospective intervention strategies. *J Clin Invest* 2017; **127**: 737-749 [PMID: 28248203 DOI: 10.1172/JCI84417]

349 **Caglioti C**, Lalle E, Castilletti C, Carletti F, Capobianchi MR, Bordi L. Chikungunya virus infection: an overview. *New Microbiol* 2013; **36**: 211-227 [PMID: 23912863 DOI: 10.1007/s40475-015-0038-y]

350 **Althouse BM**, Guerbois M, Cummings DAT, Diop OM, Faye O, Faye A, Diallo D, Sadio BD, Sow A, Faye O, Sall AA, Diallo M, Benefit B, Simons E, Watts DM, Weaver SC, Hanley KA. Role of monkeys in the sylvatic cycle of chikungunya virus in Senegal. *Nat Commun* 2018; **9**: 1046 [PMID: 29535306 DOI: 10.1038/s41467-018-03332-7]

351 **Horwood PF**, Buchy P. Chikungunya. *Rev Sci Tech* 2015; **34**: 479-489 [PMID: 26601450 DOI: 10.20506/rst.34.2.2373]

352 **Weaver SC**. Evolutionary influences in arboviral disease. *Curr Top Microbiol Immunol* 2006; **299**: 285-314 [PMID: 16568903 DOI: 10.1007/3-540-26397-7_10]

353 **Volk SM**, Chen R, Tsetsarkin KA, Adams AP, Garcia TI, Sall AA, Nasar F, Schuh AJ, Holmes EC, Higgs S, Maharaj PD, Brault AC, Weaver SC. Genome-scale phylogenetic analyses of chikungunya virus reveal independent emergences of recent epidemics and various evolutionary rates. *J Virol* 2010; **84**: 6497-6504 [PMID: 20410280 DOI: 10.1128/JVI.01603-09]

354 **Tsetsarkin KA**, Chen R, Sherman MB, Weaver SC. Chikungunya virus: evolution and genetic determinants of emergence. *Curr Opin Virol* 2011; **1**: 310-317 [PMID: 21966353 DOI: 10.1016/j.coviro.2011.07.004]

355 **Tsetsarkin KA**, Chen R, Leal G, Forrester N, Higgs S, Huang J, Weaver SC. Chikungunya virus emergence is constrained in Asia by lineage-specific adaptive landscapes. *Proc Natl Acad Sci U S A* 2011; **108**: 7872-7877 [PMID: 21518887 DOI: 10.1073/pnas.1018344108]

356 **Tsetsarkin KA**, McGee CE, Volk SM, Vanlandingham DL, Weaver SC, Higgs S. Epistatic roles of E2 glycoprotein mutations in adaption of chikungunya virus to Aedes albopictus and Ae. aegypti mosquitoes. *PLoS One* 2009; **4**: e6835 [PMID: 19718263 DOI: 10.1371/journal.pone.0006835]

357 **Tsetsarkin KA**, Vanlandingham DL, McGee CE, Higgs S. A single mutation in chikungunya virus affects vector specificity and epidemic potential. *PLoS Pathog* 2007; **3**: e201 [PMID: 18069894 DOI: 10.1371/journal.ppat.0030201]

358 **Ryan SJ**, Carlson CJ, Mordecai EA, Johnson LR. Global expansion and redistribution of Aedes-borne virus transmission risk with climate change. *PLoS Negl Trop Dis* 2019; **13**: e0007213 [PMID: 30921321 DOI: 10.1371/journal.pntd.0007213]

359 **Messina JP**, Brady OJ, Pigott DM, Golding N, Kraemer MU, Scott TW, Wint GR, Smith DL, Hay SI. The many projected futures of dengue. *Nat Rev Microbiol* 2015; **13**: 230-239 [PMID: 25730702 DOI: 10.1038/nrmicro3430]

360 **Arif M**, Tauran P, Kosasih H, Pelupessy NM, Sennang N, Mubin RH, Sudarmono P, Tjitra E, Murniati D, Alam A, Gasem MH, Aman AT, Lokida D, Hadi U, Parwati KTM, Lau CY, Neal A, Karyana M. Chikungunya in Indonesia: Epidemiology and diagnostic challenges. *PLoS Negl Trop Dis* 2020; **14**: e0008355 [PMID: 32479497 DOI: 10.1371/journal.pntd.0008355]

361 **Petersen LR**, Powers AM. Chikungunya: epidemiology. *F1000Res* 2016; **5** [PMID: 26918158 DOI: 10.12688/f1000research.7171.1]

362 **de Lima Cavalcanti TYV**, Pereira MR, de Paula SO, Franca RFO. A Review on Chikungunya Virus Epidemiology, Pathogenesis and Current Vaccine Development. *Viruses* 2022; **14** [PMID: 35632709 DOI: 10.3390/v14050969]

363 **Sourisseau M**, Schilte C, Casartelli N, Trouillet C, Guivel-Benhassine F, Rudnicka D, Sol-Foulon N, Le Roux K, Prevost MC, Fsihi H, Frenkiel MP, Blanchet F, Afonso PV, Ceccaldi PE, Ozden S, Gessain A, Schuffenecker I, Verhasselt B, Zamborlini A, Saïb A, Rey FA, Arenzana-Seisdedos F, Desprès P, Michault A, Albert ML, Schwartz O. Characterization of reemerging chikungunya virus. *PLoS Pathog* 2007; **3**: e89 [PMID: 17604450 DOI: 10.1371/journal.ppat.0030089]

364 **Noret M**, Herrero L, Rulli N, Rolph M, Smith PN, Li RW, Roques P, Gras G, Mahalingam S. Interleukin 6, RANKL, and osteoprotegerin expression by chikungunya virus-infected human osteoblasts. *J Infect Dis* 2012; **206** 455-7: 457-9 [PMID: 22634878 DOI: 10.1093/infdis/jis368]

365 **Sun Q**, Du X, Cheng W. Structures Unveil the Invasion Mechanism of Chikungunya Virus. *Trends Microbiol* 2019; **27**: 656-658 [PMID: 31221449 DOI: 10.1016/j.tim.2019.05.011]

366 **Weber C**, Berberich E, von Rhein C, Henß L, Hildt E, Schnierle BS. Identification of Functional Determinants in the Chikungunya Virus E2 Protein. *PLoS Negl Trop Dis* 2017; **11**: e0005318 [PMID: 28114368 DOI: 10.1371/journal.pntd.0005318]

367 **Basore K**, Kim AS, Nelson CA, Zhang R, Smith BK, Uranga C, Vang L, Cheng M, Gross ML, Smith J, Diamond MS, Fremont DH. Cryo-EM Structure of Chikungunya Virus in Complex with the Mxra8 Receptor. *Cell* 2019; **177**: 1725-1737.e16 [PMID: 31080061 DOI: 10.1016/j.cell.2019.04.006]

368 **Kim AS**, Zimmerman O, Fox JM, Nelson CA, Basore K, Zhang R, Durnell L, Desai C, Bullock C, Deem SL, Oppenheimer J, Shapiro B, Wang T, Cherry S, Coyne CB, Handley SA, Landis MJ, Fremont DH, Diamond MS. An Evolutionary Insertion in the Mxra8 Receptor-Binding Site Confers Resistance to Alphavirus Infection and Pathogenesis. *Cell Host Microbe* 2020; **27**: 428-440.e9 [PMID: 32075743 DOI: 10.1016/j.chom.2020.01.008]

369 **Song H**, Zhao Z, Chai Y, Jin X, Li C, Yuan F, Liu S, Gao Z, Wang H, Song J, Vazquez L, Zhang Y, Tan S, Morel CM, Yan J, Shi Y, Qi J, Gao F, Gao GF. Molecular Basis of Arthritogenic Alphavirus Receptor MXRA8 Binding to Chikungunya Virus Envelope Protein. *Cell* 2019; **177**: 1714-1724.e12 [PMID: 31080063 DOI: 10.1016/j.cell.2019.04.008]

370 **De Caluwé L**, Coppens S, Vereecken K, Daled S, Dhaenens M, Van Ostade X, Deforce D, Ariën KK, Bartholomeeusen K. The CD147 Protein Complex Is Involved in Entry of Chikungunya Virus and Related Alphaviruses in Human Cells. *Front Microbiol* 2021; **12**: 615165 [PMID: 33717005 DOI: 10.3389/fmicb.2021.615165]

371 **Tanaka A**, Tumkosit U, Nakamura S, Motooka D, Kishishita N, Priengprom T, Sa-Ngasang A, Kinoshita T, Takeda N, Maeda Y. Genome-Wide Screening Uncovers the Significance of N-Sulfation of Heparan Sulfate as a Host Cell Factor for Chikungunya Virus Infection. *J Virol* 2017; **91** [PMID: 28404855 DOI: 10.1128/JVI.00432-17]

372 **McAllister N**, Liu Y, Silva LM, Lentscher AJ, Chai W, Wu N, Griswold KA, Raghunathan K, Vang L, Alexander J, Warfield KL, Diamond MS, Feizi T, Silva LA, Dermody TS. Chikungunya Virus Strains from Each Genetic Clade Bind Sulfated Glycosaminoglycans as Attachment Factors. *J Virol* 2020; **94** [PMID: 32999033 DOI: 10.1128/JVI.01500-20]

373 **Wintachai P**, Wikan N, Kuadkitkan A, Jaimipuk T, Ubol S, Pulmanausahakul R, Auewarakul P, Kasinrerk W, Weng WY, Panyasrivanit M, Paemanee A, Kittisenachai S, Roytrakul S, Smith DR. Identification of prohibitin as a Chikungunya virus receptor protein. *J Med Virol* 2012; **84**: 1757-1770 [PMID: 22997079 DOI: 10.1002/jmv.23403]

374 **Moller-Tank S**, Kondratowicz AS, Davey RA, Rennert PD, Maury W. Role of the phosphatidylserine receptor TIM-1 in enveloped-virus entry. *J Virol* 2013; **87**: 8327-8341 [PMID: 23698310 DOI: 10.1128/JVI.01025-13]

375 **Prado Acosta M**, Geoghegan EM, Lepenies B, Ruzal S, Kielian M, Martinez MG. Surface (S) Layer Proteins of Lactobacillus acidophilus Block Virus Infection *via* DC-SIGN Interaction. *Front Microbiol* 2019; **10**: 810 [PMID: 31040840 DOI: 10.3389/fmicb.2019.00810]

376 **Meertens L**, Hafirassou ML, Couderc T, Bonnet-Madin L, Kril V, Kümmerer BM, Labeau A, Brugier A, Simon-Loriere E, Burlaud-Gaillard J, Doyen C, Pezzi L, Goupil T, Rafasse S, Vidalain PO, Bertrand-Legout A, Gueneau L, Juntas-Morales R, Ben Yaou R, Bonne G, de Lamballerie X, Benkirane M, Roingeard P, Delaugerre C, Lecuit M, Amara A. FHL1 is a major host factor for chikungunya virus infection. *Nature* 2019; **574**: 259-263 [PMID: 31554973 DOI: 10.1038/s41586-019-1578-4]

377 **Hoornweg TE**, van Duijl-Richter MKS, Ayala Nuñez NV, Albulescu IC, van Hemert MJ, Smit JM. Dynamics of Chikungunya Virus Cell Entry Unraveled by Single-Virus Tracking in Living Cells. *J Virol* 2016; **90**: 4745-4756 [PMID: 26912616 DOI: 10.1128/JVI.03184-15]

378 **Bernard E**, Solignat M, Gay B, Chazal N, Higgs S, Devaux C, Briant L. Endocytosis of chikungunya virus into mammalian cells: role of clathrin and early endosomal compartments. *PLoS One* 2010; **5**: e11479 [PMID: 20628602 DOI: 10.1371/journal.pone.0011479]

379 **Izumida M**, Hayashi H, Tanaka A, Kubo Y. Cathepsin B Protease Facilitates Chikungunya Virus Envelope Protein-Mediated Infection *via* Endocytosis or Macropinocytosis. *Viruses* 2020; **12** [PMID: 32635194 DOI: 10.3390/v12070722]

380 **Lee CHR**, Mohamed Hussain K, Chu JJH. Macropinocytosis dependent entry of Chikungunya virus into human muscle cells. *PLoS Negl Trop Dis* 2019; **13**: e0007610 [PMID: 31449523 DOI: 10.1371/journal.pntd.0007610]

381 **Krejbich-Trotot P**, Denizot M, Hoarau JJ, Jaffar-Bandjee MC, Das T, Gasque P. Chikungunya virus mobilizes the apoptotic machinery to invade host cell defenses. *FASEB J* 2011; **25**: 314-325 [PMID: 20881210 DOI: 10.1096/fj.10-164178]

382 **Constant LEC**, Rajsfus BF, Carneiro PH, Sisnande T, Mohana-Borges R, Allonso D. Overview on Chikungunya Virus Infection: From Epidemiology to State-of-the-Art Experimental Models. *Front Microbiol* 2021; **12**: 744164 [PMID: 34675908 DOI: 10.3389/fmicb.2021.744164]

383 **Sharma R**, Kesari P, Kumar P, Tomar S. Structure-function insights into chikungunya virus capsid protein: Small molecules targeting capsid hydrophobic pocket. *Virology* 2018; **515**: 223-234 [PMID: 29306785 DOI: 10.1016/j.virol.2017.12.020]

384 **Brown RS**, Anastasakis DG, Hafner M, Kielian M. Multiple capsid protein binding sites mediate selective packaging of the alphavirus genomic RNA. *Nat Commun* 2020; **11**: 4693 [PMID: 32943634 DOI: 10.1038/s41467-020-18447-z]

385 **Chmielewski D**, Schmid MF, Simmons G, Jin J, Chiu W. Chikungunya virus assembly and budding visualized in situ using cryogenic electron tomography. *Nat Microbiol* 2022; **7**: 1270-1279 [PMID: 35773421 DOI: 10.1038/s41564-022-01164-2]

386 **Hakim MS**, Aman AT. Understanding the Biology and Immune Pathogenesis of Chikungunya Virus Infection for Diagnostic and Vaccine Development. *Viruses* 2022; **15** [PMID: 36680088 DOI: 10.3390/v15010048]

387 **Weaver SC**, Lecuit M. Chikungunya virus and the global spread of a mosquito-borne disease. *N Engl J Med* 2015; **372**: 1231-1239 [PMID: 25806915 DOI: 10.1056/NEJMra1406035]

388 **Hoarau JJ**, Jaffar Bandjee MC, Krejbich Trotot P, Das T, Li-Pat-Yuen G, Dassa B, Denizot M, Guichard E, Ribera A, Henni T, Tallet F, Moiton MP, Gauzère BA, Bruniquet S, Jaffar Bandjee Z, Morbidelli P, Martigny G, Jolivet M, Gay F, Grandadam M, Tolou H, Vieillard V, Debré P, Autran B, Gasque P. Persistent chronic inflammation and infection by Chikungunya arthritogenic alphavirus in spite of a robust host immune response. *J Immunol* 2010; **184**: 5914-5927 [PMID: 20404278 DOI: 10.4049/jimmunol.0900255]

389 **Couderc T**, Chrétien F, Schilte C, Disson O, Brigitte M, Guivel-Benhassine F, Touret Y, Barau G, Cayet N, Schuffenecker I, Desprès P, Arenzana-Seisdedos F, Michault A, Albert ML, Lecuit M. A mouse model for Chikungunya: young age and inefficient type-I interferon signaling are risk factors for severe disease. *PLoS Pathog* 2008; **4**: e29 [PMID: 18282093 DOI: 10.1371/journal.ppat.0040029]

390 **Labadie K**, Larcher T, Joubert C, Mannioui A, Delache B, Brochard P, Guigand L, Dubreil L, Lebon P, Verrier B, de Lamballerie X, Suhrbier A, Cherel Y, Le Grand R, Roques P. Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. *J Clin Invest* 2010; **120**: 894-906 [PMID: 20179353 DOI: 10.1172/JCI40104]

391 **Gardner J**, Anraku I, Le TT, Larcher T, Major L, Roques P, Schroder WA, Higgs S, Suhrbier A. Chikungunya virus arthritis in adult wild-type mice. *J Virol* 2010; **84**: 8021-8032 [PMID: 20519386 DOI: 10.1128/JVI.02603-09]

392 **Wauquier N**, Becquart P, Nkoghe D, Padilla C, Ndjoyi-Mbiguino A, Leroy EM. The acute phase of Chikungunya virus infection in humans is associated with strong innate immunity and T CD8 cell activation. *J Infect Dis* 2011; **204**: 115-123 [PMID: 21628665 DOI: 10.1093/infdis/jiq006]

393 **Her Z**, Teng TS, Tan JJ, Teo TH, Kam YW, Lum FM, Lee WW, Gabriel C, Melchiotti R, Andiappan AK, Lulla V, Lulla A, Win MK, Chow A, Biswas SK, Leo YS, Lecuit M, Merits A, Rénia L, Ng LF. Loss of TLR3 aggravates CHIKV replication and pathology due to an altered virus-specific neutralizing antibody response. *EMBO Mol Med* 2015; **7**: 24-41 [PMID: 25452586 DOI: 10.15252/emmm.201404459]

394 **Rudd PA**, Wilson J, Gardner J, Larcher T, Babarit C, Le TT, Anraku I, Kumagai Y, Loo YM, Gale M Jr, Akira S, Khromykh AA, Suhrbier A. Interferon response factors 3 and 7 protect against Chikungunya virus hemorrhagic fever and shock. *J Virol* 2012; **86**: 9888-9898 [PMID: 22761364 DOI: 10.1128/JVI.00956-12]

395 **Cook LE**, Locke MC, Young AR, Monte K, Hedberg ML, Shimak RM, Sheehan KCF, Veis DJ, Diamond MS, Lenschow DJ. Distinct Roles of Interferon Alpha and Beta in Controlling Chikungunya Virus Replication and Modulating Neutrophil-Mediated Inflammation. *J Virol* 2019; **94** [PMID: 31619554 DOI: 10.1128/JVI.00841-19]

396 **Tanabe ISB**, Santos EC, Tanabe ELL, Souza SJM, Santos FEF, Taniele-Silva J, Ferro JFG, Lima MC, Moura AA, Anderson L, Bassi ÊJ. Cytokines and chemokines triggered by Chikungunya virus infection in human patients during the very early acute phase. *Trans R Soc Trop Med Hyg* 2019; **113**: 730-733 [PMID: 31365117 DOI: 10.1093/trstmh/trz065]

397 **Wichit S**, Hamel R, Yainoy S, Gumpangseth N, Panich S, Phuadraksa T, Saetear P, Monteil A, Morales Vargas R, Missé D. Interferon-inducible protein (IFI) 16 regulates Chikungunya and Zika virus infection in human skin fibroblasts. *EXCLI J* 2019; **18**: 467-476 [PMID: 31423125 DOI: 10.17179/excli2019-1271]

398 **Nair SR**, Abraham R, Sundaram S, Sreekumar E. Interferon regulated gene (IRG) expression-signature in a mouse model of chikungunya virus neurovirulence. *J Neurovirol* 2017; **23**: 886-902 [PMID: 29067635 DOI: 10.1007/s13365-017-0583-3]

399 **Wichit S**, Hamel R, Zanzoni A, Diop F, Cribier A, Talignani L, Diack A, Ferraris P, Liegeois F, Urbach S, Ekchariyawat P, Merits A, Yssel H, Benkirane M, Missé D. SAMHD1 Enhances Chikungunya and Zika Virus Replication in Human Skin Fibroblasts. *Int J Mol Sci* 2019; **20** [PMID: 30959732 DOI: 10.3390/ijms20071695]

400 **Valdés-López JF**, Fernandez GJ, Urcuqui-Inchima S. Interleukin 27 as an inducer of antiviral response against chikungunya virus infection in human macrophages. *Cell Immunol* 2021; **367**: 104411 [PMID: 34325085 DOI: 10.1016/j.cellimm.2021.104411]

401 **Bae S**, Lee JY, Myoung J. Chikungunya Virus nsP2 Impairs MDA5/RIG-I-Mediated Induction of NF-κB Promoter Activation: A Potential Target for Virus-Specific Therapeutics. *J Microbiol Biotechnol* 2020; **30**: 1801-1809 [PMID: 33323678 DOI: 10.4014/jmb.2012.12005]

402 **Bae S**, Lee JY, Myoung J. Chikungunya Virus-Encoded nsP2, E2 and E1 Strongly Antagonize the Interferon-β Signaling Pathway. *J Microbiol Biotechnol* 2019; **29**: 1852-1859 [PMID: 31635445 DOI: 10.4014/jmb.1910.10014]

403 **Teng TS**, Kam YW, Lee B, Hapuarachchi HC, Wimal A, Ng LC, Ng LF. A Systematic Meta-analysis of Immune Signatures in Patients With Acute Chikungunya Virus Infection. *J Infect Dis* 2015; **211**: 1925-1935 [PMID: 25635123 DOI: 10.1093/infdis/jiv049]

404 **Michlmayr D**, Pak TR, Rahman AH, Amir ED, Kim EY, Kim-Schulze S, Suprun M, Stewart MG, Thomas GP, Balmaseda A, Wang L, Zhu J, Suaréz-Fariñas M, Wolinsky SM, Kasarskis A, Harris E. Comprehensive innate immune profiling of chikungunya virus infection in pediatric cases. *Mol Syst Biol* 2018; **14**: e7862 [PMID: 30150281 DOI: 10.15252/msb.20177862]

405 **Chen W**, Foo SS, Taylor A, Lulla A, Merits A, Hueston L, Forwood MR, Walsh NC, Sims NA, Herrero LJ, Mahalingam S. Bindarit, an inhibitor of monocyte chemotactic protein synthesis, protects against bone loss induced by chikungunya virus infection. *J Virol* 2015; **89**: 581-593 [PMID: 25339772 DOI: 10.1128/JVI.02034-14]

406 **Hornum L**, Hansen AJ, Tornehave D, Fjording MS, Colmenero P, Wätjen IF, Søe Nielsen NH, Bliddal H, Bartels EM. C5a and C5aR are elevated in joints of rheumatoid and psoriatic arthritis patients, and C5aR blockade attenuates leukocyte migration to synovial fluid. *PLoS One* 2017; **12**: e0189017 [PMID: 29220376 DOI: 10.1371/journal.pone.0189017]

407 **Andersson C**, Wenander CS, Usher PA, Hebsgaard JB, Sondergaard BC, Rønø B, Mackay C, Friedrichsen B, Chang C, Tang R, Hornum L. Rapid-onset clinical and mechanistic effects of anti-C5aR treatment in the mouse collagen-induced arthritis model. *Clin Exp Immunol* 2014; **177**: 219-233 [PMID: 24665841 DOI: 10.1111/cei.12338]

408 **Conroy AL**, Gélvez M, Hawkes M, Rajwans N, Tran V, Liles WC, Villar-Centeno LA, Kain KC. Host biomarkers are associated with progression to dengue haemorrhagic fever: a nested case-control study. *Int J Infect Dis* 2015; **40**: 45-53 [PMID: 26255888 DOI: 10.1016/j.ijid.2015.07.027]

409 **Mahauad-Fernandez WD**, Jones PH, Okeoma CM. Critical role for bone marrow stromal antigen 2 in acute Chikungunya virus infection. *J Gen Virol* 2014; **95**: 2450-2461 [PMID: 25053563 DOI: 10.1099/vir.0.068643-0]

410 **Lum FM**, Teo TH, Lee WW, Kam YW, Rénia L, Ng LF. An essential role of antibodies in the control of Chikungunya virus infection. *J Immunol* 2013; **190**: 6295-6302 [PMID: 23670192 DOI: 10.4049/jimmunol.1300304]

411 **Hoarau JJ**, Gay F, Pellé O, Samri A, Jaffar-Bandjee MC, Gasque P, Autran B. Identical strength of the T cell responses against E2, nsP1 and capsid CHIKV proteins in recovered and chronic patients after the epidemics of 2005-2006 in La Reunion Island. *PLoS One* 2013; **8**: e84695 [PMID: 24376836 DOI: 10.1371/journal.pone.0084695]

412 **Teo TH**, Lum FM, Claser C, Lulla V, Lulla A, Merits A, Rénia L, Ng LF. A pathogenic role for CD4+ T cells during Chikungunya virus

infection in mice. *J Immunol* 2013; **190**: 259-269 [PMID: 23209328 DOI: 10.4049/jimmunol.1202177]

413 **Dias CNS**, Gois BM, Lima VS, Guerra-Gomes IC, Araújo JMG, Gomes JAS, Araújo DAM, Medeiros IA, Azevedo FLAA, Veras RC, Janebro DI, Amaral IPGD, Keesen TSL. Human CD8 T-cell activation in acute and chronic chikungunya infection. *Immunology* 2018; **155**: 499-504 [PMID: 30099739 DOI: 10.1111/imm.12992]

414 **Davenport BJ**, Bullock C, McCarthy MK, Hawman DW, Murphy KM, Kedl RM, Diamond MS, Morrison TE. Chikungunya Virus Evades Antiviral CD8(+) T Cell Responses To Establish Persistent Infection in Joint-Associated Tissues. *J Virol* 2020; **94** [PMID: 32102875 DOI: 10.1128/JVI.02036-19]

415 **Jain J**, Okabayashi T, Kaur N, Nakayama E, Shioda T, Gaind R, Kurosu T, Sunil S. Evaluation of an immunochromatography rapid diagnosis kit for detection of chikungunya virus antigen in India, a dengue-endemic country. *Virol J* 2018; **15**: 84 [PMID: 29751761 DOI: 10.1186/s12985-018-1000-0]

416 **Chua CL**, Sam IC, Chiam CW, Chan YF. The neutralizing role of IgM during early Chikungunya virus infection. *PLoS One* 2017; **12**: e0171989 [PMID: 28182795 DOI: 10.1371/journal.pone.0171989]

417 **Prince HE**, Seaton BL, Matud JL, Batterman HJ. Chikungunya virus RNA and antibody testing at a National Reference Laboratory since the emergence of Chikungunya virus in the Americas. *Clin Vaccine Immunol* 2015; **22**: 291-297 [PMID: 25540275 DOI: 10.1128/CVI.00720-14]

418 **Kam YW**, Simarmata D, Chow A, Her Z, Teng TS, Ong EK, Rénia L, Leo YS, Ng LF. Early appearance of neutralizing immunoglobulin G3 antibodies is associated with chikungunya virus clearance and long-term clinical protection. *J Infect Dis* 2012; **205**: 1147-1154 [PMID: 22389226 DOI: 10.1093/infdis/jis033]

419 **Reddy V**, Mani RS, Desai A, Ravi V. Correlation of plasma viral loads and presence of Chikungunya IgM antibodies with cytokine/chemokine levels during acute Chikungunya virus infection. *J Med Virol* 2014; **86**: 1393-1401 [PMID: 24523146 DOI: 10.1002/jmv.23875]

420 **Tumkosit U**, Siripanyaphinyo U, Takeda N, Tsuji M, Maeda Y, Ruchusatsawat K, Shioda T, Mizushima H, Chetanachan P, Wongjaroen P, Matsuura Y, Tatsumi M, Tanaka A. Anti-Chikungunya Virus Monoclonal Antibody That Inhibits Viral Fusion and Release. *J Virol* 2020; **94** [PMID: 32699087 DOI: 10.1128/JVI.00252-20]

421 **Kam YW**, Lum FM, Teo TH, Lee WW, Simarmata D, Harjanto S, Chua CL, Chan YF, Wee JK, Chow A, Lin RT, Leo YS, Le Grand R, Sam IC, Tong JC, Roques P, Wiesmüller KH, Rénia L, Rötzschke O, Ng LF. Early neutralizing IgG response to Chikungunya virus in infected patients targets a dominant linear epitope on the E2 glycoprotein. *EMBO Mol Med* 2012; **4**: 330-343 [PMID: 22389221 DOI: 10.1002/emmm.201200213]

422 **Henss L**, Yue C, Von Rhein C, Tschismarov R, Lewis-Ximenez LL, Dölle A, Baylis SA, Schnierle BS. Analysis of Humoral Immune Responses in Chikungunya Virus (CHIKV)-Infected Patients and Individuals Vaccinated With a Candidate CHIKV Vaccine. *J Infect Dis* 2020; **221**: 1713-1723 [PMID: 31828322 DOI: 10.1093/infdis/jiz658]

423 **Quiroz JA**, Malonis RJ, Thackray LB, Cohen CA, Pallesen J, Jangra RK, Brown RS, Hofmann D, Holtsberg FW, Shulenin S, Nyakatura EK, Durnell LA, Rayannavar V, Daily JP, Ward AB, Aman MJ, Dye JM, Chandran K, Diamond MS, Kielian M, Lai JR. Human monoclonal antibodies against chikungunya virus target multiple distinct epitopes in the E1 and E2 glycoproteins. *PLoS Pathog* 2019; **15**: e1008061 [PMID: 31697791 DOI: 10.1371/journal.ppat.1008061]

424 **Anfasa F**, Lim SM, Fekken S, Wever R, Osterhaus ADME, Martina BEE. Characterization of antibody response in patients with acute and chronic chikungunya virus disease. *J Clin Virol* 2019; **117**: 68-72 [PMID: 31229935 DOI: 10.1016/j.jcv.2019.06.001]

425 **Murillo-Zamora E**, Mendoza-Cano O, Trujillo-Hernández B, Guzmán-Esquivel J, Higareda-Almaraz E, Higareda-Almaraz MA, Sánchez-Piña RA, Lugo-Radillo A. Persistent Arthralgia and Related Risks Factors: A Cohort Study at 12 Months from Laboratory-Confirmed Chikungunya Infection. *Arch Med Res* 2018; **49**: 65-73 [PMID: 29703609 DOI: 10.1016/j.arcmed.2018.04.008]

426 **Jacob-Nascimento LC**, Carvalho CX, Silva MMO, Kikuti M, Anjos RO, Fradico JRB, Campi-Azevedo AC, Tauro LB, Campos GS, Moreira PSDS, Portilho MM, Martins-Filho OA, Ribeiro GS, Reis MG. Acute-Phase Levels of CXCL8 as Risk Factor for Chronic Arthralgia Following Chikungunya Virus Infection. *Front Immunol* 2021; **12**: 744183 [PMID: 34659240 DOI: 10.3389/fimmu.2021.744183]

427 **Sissoko D**, Malvy D, Ezzedine K, Renault P, Moscetti F, Ledrans M, Pierre V. Post-epidemic Chikungunya disease on Reunion Island: course of rheumatic manifestations and associated factors over a 15-month period. *PLoS Negl Trop Dis* 2009; **3**: e389 [PMID: 19274071 DOI: 10.1371/journal.pntd.0000389]

428 **Suhrbier A**. Rheumatic manifestations of chikungunya: emerging concepts and interventions. *Nat Rev Rheumatol* 2019; **15**: 597-611 [PMID: 31481759 DOI: 10.1038/s41584-019-0276-9]

429 **Chang AY**, Martins KAO, Encinales L, Reid SP, Acuña M, Encinales C, Matranga CB, Pacheco N, Cure C, Shukla B, Ruiz Arteta T, Amdur R, Cazares LH, Gregory M, Ward MD, Porras A, Rico Mendoza A, Dong L, Kenny T, Brueggemann E, Downey LG, Kamalapathy P, Lichtenberger P, Falls O, Simon GL, Bethony JM, Firestein GS. Chikungunya Arthritis Mechanisms in the Americas: A Cross-Sectional Analysis of Chikungunya Arthritis Patients Twenty-Two Months After Infection Demonstrating No Detectable Viral Persistence in Synovial Fluid. *Arthritis Rheumatol* 2018; **70**: 585-593 [PMID: 29266856 DOI: 10.1002/art.40383]

430 **Borgherini G**, Poubeau P, Staikowsky F, Lory M, Le Moullec N, Becquart JP, Wengling C, Michault A, Paganin F. Outbreak of chikungunya on Reunion Island: early clinical and laboratory features in 157 adult patients. *Clin Infect Dis* 2007; **44**: 1401-1407 [PMID: 17479933 DOI: 10.1086/517537]

431 **Chaaitanya IK**, Muruganandam N, Sundaram SG, Kawalekar O, Sugunan AP, Manimunda SP, Ghosal SR, Muthumani K, Vijayachari P. Role of proinflammatory cytokines and chemokines in chronic arthropathy in CHIKV infection. *Viral Immunol* 2011; **24**: 265-271 [PMID: 21830898 DOI: 10.1089/vim.2010.0123]

432 **Kelvin AA**, Banner D, Silvi G, Moro ML, Spataro N, Gaibani P, Cavrini F, Pierro A, Rossini G, Cameron MJ, Bermejo-Martin JF, Paquette SG, Xu L, Danesh A, Farooqui A, Borghetto I, Kelvin DJ, Sambri V, Rubino S. Inflammatory cytokine expression is associated with chikungunya virus resolution and symptom severity. *PLoS Negl Trop Dis* 2011; **5**: e1279 [PMID: 21858242 DOI: 10.1371/journal.pntd.0001279]

433 **Thanapati S**, Ganu M, Giri P, Kulkarni S, Sharma M, Babar P, Ganu A, Tripathy AS. Impaired NK cell functionality and increased TNF-α production as biomarkers of chronic chikungunya arthritis and rheumatoid arthritis. *Hum Immunol* 2017; **78**: 370-374 [PMID: 28213049 DOI: 10.1016/j.humimm.2017.02.006]

434 **Simon F**, Javelle E, Cabie A, Bouquillard E, Troisgros O, Gentile G, Leparc-Goffart I, Hoen B, Gandjbakhch F, Rene-Corail P, Franco JM, Caumes E, Combe B, Poiraudeau S, Gane-Troplent F, Djossou F, Schaerverbeke T, Criquet-Hayot A, Carrere P, Malvy D, Gaillard P, Wendling D; Société de pathologie infectieuse de langue francaise. French guidelines for the management of chikungunya (acute and persistent presentations). November 2014. *Med Mal Infect* 2015; **45**: 243-263 [PMID: 26119684 DOI: 10.1016/j.medmal.2015.05.007]

435 **Simon F**, Parola P, Grandadam M, Fourcade S, Oliver M, Brouqui P, Hance P, Kraemer P, Mohamed AA, de Lamballerie X, Charrel R, Tolou

H. Chikungunya infection: an emerging rheumatism among travelers returned from Indian Ocean islands. Report of 47 cases. *Medicine (Baltimore)* 2007; **86**: 123-137 [PMID: 17505252 DOI: 10.1097/MD/0b013e31806010a5]

436 **Zaid A**, Gérardin P, Taylor A, Mostafavi H, Malvy D, Mahalingam S. Chikungunya Arthritis: Implications of Acute and Chronic Inflammation Mechanisms on Disease Management. *Arthritis Rheumatol* 2018; **70**: 484-495 [PMID: 29287308 DOI: 10.1002/art.40403]

437 **Javelle E**, Tiong TH, Leparc-Goffart I, Savini H, Simon F. Inflammation of the external ear in acute chikungunya infection: Experience from the outbreak in Johor Bahru, Malaysia, 2008. *J Clin Virol* 2014; **59**: 270-273 [PMID: 24556566 DOI: 10.1016/j.jcv.2014.01.011]

438 **Blettery M**, Brunier L, Polomat K, Moinet F, Deligny C, Arfi S, Jean-Baptiste G, De Bandt M. Brief Report: Management of Chronic Post-Chikungunya Rheumatic Disease: The Martinican Experience. *Arthritis Rheumatol* 2016; **68**: 2817-2824 [PMID: 27273928 DOI: 10.1002/art.39775]

439 **Godaert L**, Najioullah F, Bartholet S, Colas S, Yactayo S, Cabié A, Fanon JL, Césaire R, Dramé M. Atypical Clinical Presentations of Acute Phase Chikungunya Virus Infection in Older Adults. *J Am Geriatr Soc* 2017; **65**: 2510-2515 [PMID: 28940357 DOI: 10.1111/jgs.15004]

440 **Mehta R**, Gerardin P, de Brito CAA, Soares CN, Ferreira MLB, Solomon T. The neurological complications of chikungunya virus: A systematic review. *Rev Med Virol* 2018; **28**: e1978 [PMID: 29671914 DOI: 10.1002/rmv.1978]

441 **Torres JR**, Leopoldo Códova G, Castro JS, Rodríguez L, Saravia V, Arvelaez J, Ríos-Fabra A, Longhi MA, Marcano M. Chikungunya fever: Atypical and lethal cases in the Western hemisphere: A Venezuelan experience. *IDCases* 2015; **2**: 6-10 [PMID: 26793440 DOI: 10.1016/j.idcr.2014.12.002]

442 **Economopoulou A**, Dominguez M, Helynck B, Sissoko D, Wichmann O, Quenel P, Germonneau P, Quatresous I. Atypical Chikungunya virus infections: clinical manifestations, mortality and risk factors for severe disease during the 2005-2006 outbreak on Réunion. *Epidemiol Infect* 2009; **137**: 534-541 [PMID: 18694529 DOI: 10.1017/S0950268808001167]

443 **Imad HA**, Phadungsombat J, Nakayama EE, Suzuki K, Ibrahim AM, Afaa A, Azeema A, Nazfa A, Yazfa A, Ahmed A, Saeed A, Waheed A, Shareef F, Islam MM, Anees SM, Saleem S, Aroosha A, Afzal I, Leaungwutiwong P, Piyaphanee W, Phumratanaprapin W, Shioda T. Clinical Features of Acute Chikungunya Virus Infection in Children and Adults during an Outbreak in the Maldives. *Am J Trop Med Hyg* 2021; **105**: 946-954 [PMID: 34339379 DOI: 10.4269/ajtmh.21-0189]

444 **Tandale BV**, Sathe PS, Arankalle VA, Wadia RS, Kulkarni R, Shah SV, Shah SK, Sheth JK, Sudeep AB, Tripathy AS, Mishra AC. Systemic involvements and fatalities during Chikungunya epidemic in India, 2006. *J Clin Virol* 2009; **46**: 145-149 [PMID: 19640780 DOI: 10.1016/j.jcv.2009.06.027]

445 **Ramos R**, Viana R, Brainer-Lima A, FloreÂncio T, Carvalho MD, van der Linden V, Amorim A, Rocha MA, Medeiros F. Perinatal Chikungunya Virus-associated Encephalitis Leading to Postnatal-Onset Microcephaly and Optic Atrophy. *Pediatr Infect Dis J* 2018; **37**: 94-95 [PMID: 28737626 DOI: 10.1097/INF.0000000000001690]

446 **Simon F**, Javelle E, Oliver M, Leparc-Goffart I, Marimoutou C. Chikungunya virus infection. *Curr Infect Dis Rep* 2011; **13**: 218-228 [PMID: 21465340 DOI: 10.1007/s11908-011-0180-1]

447 **Mohan A**, Kiran DH, Manohar IC, Kumar DP. Epidemiology, clinical manifestations, and diagnosis of Chikungunya fever: lessons learned from the re-emerging epidemic. *Indian J Dermatol* 2010; **55**: 54-63 [PMID: 20418981 DOI: 10.4103/0019-5154.60355]

448 **Bertolotti A**, Thioune M, Abel S, Belrose G, Calmont I, Césaire R, Cervantes M, Fagour L, Javelle É, Lebris C, Najioullah F, Pierre-François S, Rozé B, Vigan M, Laouénan C, Cabié A; Chronic Chikungunya working group of University Medical Center of Martinique. Prevalence of chronic chikungunya and associated risks factors in the French West Indies (La Martinique): A prospective cohort study. *PLoS Negl Trop Dis* 2020; **14**: e0007327 [PMID: 32163420 DOI: 10.1371/journal.pntd.0007327]

449 **Johnson BW**, Russell BJ, Goodman CH. Laboratory Diagnosis of Chikungunya Virus Infections and Commercial Sources for Diagnostic Assays. *J Infect Dis* 2016; **214**: S471-S474 [PMID: 27920176 DOI: 10.1093/infdis/jiw274]

450 **Wikan N**, Sakoonwatanyoo P, Ubol S, Yoksan S, Smith DR. Chikungunya virus infection of cell lines: analysis of the East, Central and South African lineage. *PLoS One* 2012; **7**: e31102 [PMID: 22299053 DOI: 10.1371/journal.pone.0031102]

451 **Thirion L**, Pezzi L, Corcostegui I, Dubot-Pérès A, Falchi A, de Lamballerie X, Charrel RN. Development and Evaluation of a Duo Chikungunya Virus Real-Time RT-PCR Assay Targeting Two Regions within the Genome. *Viruses* 2019; **11** [PMID: 31443243 DOI: 10.3390/v11080755]

452 **Edwards T**, Del Carmen Castillo Signor L, Williams C, Larcher C, Espinel M, Theaker J, Donis E, Cuevas LE, Adams ER. Analytical and clinical performance of a Chikungunya qRT-PCR for Central and South America. *Diagn Microbiol Infect Dis* 2017; **89**: 35-39 [PMID: 28633900 DOI: 10.1016/j.diagmicrobio.2017.06.001]

453 **Chiam CW**, Chan YF, Loong SK, Yong SS, Hooi PS, Sam IC. Real-time polymerase chain reaction for diagnosis and quantitation of negative strand of chikungunya virus. *Diagn Microbiol Infect Dis* 2013; **77**: 133-137 [PMID: 23886793 DOI: 10.1016/j.diagmicrobio.2013.06.018]

454 **Broeders S**, Garlant L, Fraiture MA, Vandermassen E, Suin V, Vanhomwegen J, Dupont-Rouzeyrol M, Rousset D, Van Gucht S, Roosens N. A new multiplex RT-qPCR method for the simultaneous detection and discrimination of Zika and chikungunya viruses. *Int J Infect Dis* 2020; **92**: 160-170 [PMID: 31884173 DOI: 10.1016/j.ijid.2019.12.028]

455 **Giry C**, Roquebert B, Li-Pat-Yuen G, Gasque P, Jaffar-Bandjee MC. Improved detection of genus-specific Alphavirus using a generic TaqMan® assay. *BMC Microbiol* 2017; **17**: 164 [PMID: 28738838 DOI: 10.1186/s12866-017-1080-9]

456 **Appassakij H**, Khuntikij P, Kemapunmanus M, Wutthanarungsan R, Silpapojakul K. Viremic profiles in asymptomatic and symptomatic chikungunya fever: a blood transfusion threat? *Transfusion* 2013; **53**: 2567-2574 [PMID: 23176378 DOI: 10.1111/j.1537-2995.2012.03960.x]

457 **Manzoor KN**, Javed F, Ejaz M, Ali M, Mujaddadi N, Khan AA, Khattak AA, Zaib A, Ahmad I, Saeed WK, Manzoor S. The global emergence of Chikungunya infection: An integrated view. *Rev Med Virol* 2022; **32**: e2287 [PMID: 34428335 DOI: 10.1002/rmv.2287]

458 **Andrew A**, Navien TN, Yeoh TS, Citartan M, Mangantig E, Sum MSH, Ch'ng ES, Tang TH. Diagnostic accuracy of serological tests for the diagnosis of Chikungunya virus infection: A systematic review and meta-analysis. *PLoS Negl Trop Dis* 2022; **16**: e0010152 [PMID: 35120141 DOI: 10.1371/journal.pntd.0010152]

459 **Clements TL**, Rossi CA, Irish AK, Kibuuka H, Eller LA, Robb ML, Kataaha P, Michael NL, Hensley LE, Schoepp RJ. Chikungunya and O'nyong-nyong Viruses in Uganda: Implications for Diagnostics. *Open Forum Infect Dis* 2019; **6**: ofz001 [PMID: 31660384 DOI: 10.1093/ofid/ofz001]

460 **Fischer C**, Bozza F, Merino Merino XJ, Pedroso C, de Oliveira Filho EF, Moreira-Soto A, Schwalb A, de Lamballerie X, Netto EM, Bozza PT, Sarno M, Brites C, Gotuzzo E, Talledo M, Drexler JF. Robustness of Serologic Investigations for Chikungunya and Mayaro Viruses following Coemergence. *mSphere* 2020; **5** [PMID: 32024703 DOI: 10.1128/mSphere.00915-19]

461 **Huits R**, Okabayashi T, Cnops L, Barbé B, Van Den Berg R, Bartholomeeusen K, Ariën KK, Jacobs J, Bottieau E, Nakayama EE, Shioda T, Van Esbroeck M. Diagnostic accuracy of a rapid E1-antigen test for chikungunya virus infection in a reference setting. *Clin Microbiol Infect*

2018; **24**: 78-81 [PMID: 28606643 DOI: 10.1016/j.cmi.2017.06.004]

462 **Reddy A**, Bosch I, Salcedo N, Herrera BB, de Puig H, Narváez CF, Caicedo-Borrero DM, Lorenzana I, Parham L, García K, Mercado M, Turca AMR, Villar-Centeno LA, Gélvez-Ramírez M, Ríos NAG, Hiley M, García D, Diamond MS, Gehrke L. Development and Validation of a Rapid Lateral Flow E1/E2-Antigen Test and ELISA in Patients Infected with Emerging Asian Strain of Chikungunya Virus in the Americas. *Viruses* 2020; **12** [PMID: 32882998 DOI: 10.3390/v12090971]

463 **Tuekprakhon A**, Nakayama EE, Bartholomeeusen K, Puiprom O, Sasaki T, Huits R, Luplertlop N, Kosoltanapiwat N, Maneekan P, Ariën KK, Shioda T, Leaungwutiwong P. Variation at position 350 in the Chikungunya virus 6K-E1 protein determines the sensitivity of detection in a rapid E1-antigen test. *Sci Rep* 2018; **8**: 1094 [PMID: 29348674 DOI: 10.1038/s41598-018-19174-8]

464 **Vairo F**, Haider N, Kock R, Ntoumi F, Ippolito G, Zumla A. Chikungunya: Epidemiology, Pathogenesis, Clinical Features, Management, and Prevention. *Infect Dis Clin North Am* 2019; **33**: 1003-1025 [PMID: 31668189 DOI: 10.1016/j.idc.2019.08.006]

465 **Millsapps EM**, Underwood EC, Barr KL. Development and Application of Treatment for Chikungunya Fever. *Res Rep Trop Med* 2022; **13**: 55-66 [PMID: 36561535 DOI: 10.2147/RRTM.S370046]

466 **Couderc T**, Khandoudi N, Grandadam M, Visse C, Gangneux N, Bagot S, Prost JF, Lecuit M. Prophylaxis and therapy for Chikungunya virus infection. *J Infect Dis* 2009; **200**: 516-523 [PMID: 19572805 DOI: 10.1086/600381]

467 **Khan M**, Santhosh SR, Tiwari M, Lakshmana Rao PV, Parida M. Assessment of in vitro prophylactic and therapeutic efficacy of chloroquine against Chikungunya virus in vero cells. *J Med Virol* 2010; **82**: 817-824 [PMID: 20336760 DOI: 10.1002/jmv.21663]

468 **Pohjala L**, Utt A, Varjak M, Lulla A, Merits A, Ahola T, Tammela P. Inhibitors of alphavirus entry and replication identified with a stable Chikungunya replicon cell line and virus-based assays. *PLoS One* 2011; **6**: e28923 [PMID: 22205980 DOI: 10.1371/journal.pone.0028923]

469 **Kaur P**, Thiruchelvan M, Lee RC, Chen H, Chen KC, Ng ML, Chu JJ. Inhibition of chikungunya virus replication by harringtonine, a novel antiviral that suppresses viral protein expression. *Antimicrob Agents Chemother* 2013; **57**: 155-167 [PMID: 23275491 DOI: 10.1128/AAC.01467-12]

470 **Bourjot M**, Leyssen P, Neyts J, Dumontet V, Litaudon M. Trigocherrierin A, a potent inhibitor of chikungunya virus replication. *Molecules* 2014; **19**: 3617-3627 [PMID: 24662077 DOI: 10.3390/molecules19033617]

471 **Albulescu IC**, Tas A, Scholte FEM, Snijder EJ, van Hemert MJ. An in vitro assay to study chikungunya virus RNA synthesis and the mode of action of inhibitors. *J Gen Virol* 2014; **95**: 2683-2692 [PMID: 25135884 DOI: 10.1099/vir.0.069690-0]

472 **Schilte C**, Couderc T, Chretien F, Sourisseau M, Gangneux N, Guivel-Benhassine F, Kraxner A, Tschopp J, Higgs S, Michault A, Arenzana-Seisdedos F, Colonna M, Peduto L, Schwartz O, Lecuit M, Albert ML. Type I IFN controls chikungunya virus *via* its action on nonhematopoietic cells. *J Exp Med* 2010; **207**: 429-442 [PMID: 20123960 DOI: 10.1084/jem.20090851]

473 **Kumar M**, Sudeep AB, Arankalle VA. Evaluation of recombinant E2 protein-based and whole-virus inactivated candidate vaccines against chikungunya virus. *Vaccine* 2012; **30**: 6142-6149 [PMID: 22884660 DOI: 10.1016/j.vaccine.2012.07.072]

474 **Wang E**, Kim DY, Weaver SC, Frolov I. Chimeric Chikungunya viruses are nonpathogenic in highly sensitive mouse models but efficiently induce a protective immune response. *J Virol* 2011; **85**: 9249-9252 [PMID: 21697494 DOI: 10.1128/JVI.00844-11]

475 **López-Camacho C**, Kim YC, Blight J, Lazaro Moreli M, Montoya-Diaz E, Huiskonen JT, Kümmerer BM, Reyes-Sandoval A. Assessment of Immunogenicity and Neutralisation Efficacy of Viral-Vectored Vaccines Against Chikungunya Virus. *Viruses* 2019; **11** [PMID: 30987160 DOI: 10.3390/v11040322]

476 **Tretyakova I**, Hearn J, Wang E, Weaver S, Pushko P. DNA vaccine initiates replication of live attenuated chikungunya virus in vitro and elicits protective immune response in mice. *J Infect Dis* 2014; **209**: 1882-1890 [PMID: 24585894 DOI: 10.1093/infdis/jiu114]

477 **Zhao Z**, Deng Y, Niu P, Song J, Wang W, Du Y, Huang B, Wang W, Zhang L, Zhao P, Tan W. Co-Immunization With CHIKV VLP and DNA Vaccines Induces a Promising Humoral Response in Mice. *Front Immunol* 2021; **12**: 655743 [PMID: 33868299 DOI: 10.3389/fimmu.2021.655743]

478 **Plante KS**, Rossi SL, Bergren NA, Seymour RL, Weaver SC. Extended Preclinical Safety, Efficacy and Stability Testing of a Live-attenuated Chikungunya Vaccine Candidate. *PLoS Negl Trop Dis* 2015; **9**: e0004007 [PMID: 26340754 DOI: 10.1371/journal.pntd.0004007]

479 **Schneider M**, Narciso-Abraham M, Hadl S, McMahon R, Toepfer S, Fuchs U, Hochreiter R, Bitzer A, Kosulin K, Larcher-Senn J, Mader R, Dubischar K, Zoihsl O, Jaramillo JC, Eder-Lingelbach S, Buerger V, Wressnigg N. Safety and immunogenicity of a single-shot live-attenuated chikungunya vaccine: a double-blind, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet* 2023; **401**: 2138-2147 [PMID: 37321235 DOI: 10.1016/S0140-6736(23)00641-4]

480 **Roques P**, Fritzer A, Dereuddre-Bosquet N, Wressnigg N, Hochreiter R, Bossevot L, Pascal Q, Guehenneux F, Bitzer A, Corbic Ramljak I, Le Grand R, Lundberg U, Meinke A. Effectiveness of CHIKV vaccine VLA1553 demonstrated by passive transfer of human sera. *JCI Insight* 2022; **7** [PMID: 35700051 DOI: 10.1172/jci.insight.160173]

481 **Wressnigg N**, Hochreiter R, Zoihsl O, Fritzer A, Bézay N, Klingler A, Lingnau K, Schneider M, Lundberg U, Meinke A, Larcher-Senn J, Čorbic-Ramljak I, Eder-Lingelbach S, Dubischar K, Bender W. Single-shot live-attenuated chikungunya vaccine in healthy adults: a phase 1, randomised controlled trial. *Lancet Infect Dis* 2020; **20**: 1193-1203 [PMID: 32497524 DOI: 10.1016/S1473-3099(20)30238-3]

482 **Bennett SR**, McCarty JM, Ramanathan R, Mendy J, Richardson JS, Smith J, Alexander J, Ledgerwood JE, de Lame PA, Royalty Tredo S, Warfield KL, Bedell L. Safety and immunogenicity of PXVX0317, an aluminium hydroxide-adjuvanted chikungunya virus-like particle vaccine: a randomised, double-blind, parallel-group, phase 2 trial. *Lancet Infect Dis* 2022; **22**: 1343-1355 [PMID: 35709798 DOI: 10.1016/S1473-3099(22)00226-2]

483 **Chen GL**, Coates EE, Plummer SH, Carter CA, Berkowitz N, Conan-Cibotti M, Cox JH, Beck A, O'Callahan M, Andrews C, Gordon IJ, Larkin B, Lampley R, Kaltovich F, Gall J, Carlton K, Mendy J, Haney D, May J, Bray A, Bailer RT, Dowd KA, Brockett B, Gordon D, Koup RA, Schwartz R, Mascola JR, Graham BS, Pierson TC, Donastorg Y, Rosario N, Pape JW, Hoen B, Cabié A, Diaz C, Ledgerwood JE; VRC 704 Study Team. Effect of a Chikungunya Virus-Like Particle Vaccine on Safety and Tolerability Outcomes: A Randomized Clinical Trial. *JAMA* 2020; **323**: 1369-1377 [PMID: 32286643 DOI: 10.1001/jama.2020.2477]

484 **Chang LJ**, Dowd KA, Mendoza FH, Saunders JG, Sitar S, Plummer SH, Yamshchikov G, Sarwar UN, Hu Z, Enama ME, Bailer RT, Koup RA, Schwartz RM, Akahata W, Nabel GJ, Mascola JR, Pierson TC, Graham BS, Ledgerwood JE; VRC 311 Study Team. Safety and tolerability of chikungunya virus-like particle vaccine in healthy adults: a phase 1 dose-escalation trial. *Lancet* 2014; **384**: 2046-2052 [PMID: 25132507 DOI: 10.1016/S0140-6736(14)61185-5]

485 **Akahata W**, Yang ZY, Andersen H, Sun S, Holdaway HA, Kong WP, Lewis MG, Higgs S, Rossmann MG, Rao S, Nabel GJ. A virus-like particle vaccine for epidemic Chikungunya virus protects nonhuman primates against infection. *Nat Med* 2010; **16**: 334-338 [PMID: 20111039 DOI: 10.1038/nm.2105]

World Journal of Virology

Submit a Manuscript: https://www.f6publishing.com

World J Virol 2025 March 25; 14(1): 99249

DOI: 10.5501/wjv.v14.i1.99249

ISSN 2220-3249 (online)

MINIREVIEWS

Insights into gastrointestinal manifestation of human immunodeficiency virus: A narrative review

Pratiksha Moliya, Anmol Singh, Navdeep Singh, Vikash Kumar, Aalam Sohal

Specialty type: Virology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade D
Novelty: Grade C
Creativity or Innovation: Grade C
Scientific Significance: Grade B

P-Reviewer: Zhai M

Received: July 18, 2024
Revised: October 15, 2024
Accepted: November 4, 2024
Published online: March 25, 2025
Processing time: 132 Days and 23.5 Hours

Pratiksha Moliya, Department of Transplant Hepatology, University of Nebraska Medical Center, Omaha, NE 69198, United States

Anmol Singh, Department of Medicine, Tristar Centennial Medical Center, Nashville, TN 37203, United States

Navdeep Singh, Department of Medicine, Government Medical College, Amritsar 143001, Punjab, India

Vikash Kumar, Aalam Sohal, Department of Gastroenterology and Hepatology, Creighton University School of Medicine, Phoenix, AZ 85012, United States

Co-corresponding authors: Anmol Singh and Aalam Sohal.

Corresponding author: Aalam Sohal, MBBS, Academic Fellow, Department of Gastroenterology and Hepatology, Creighton University School of Medicine, 3216 NE 45th Pl Suite 212, Phoenix, AZ 85012, United States. aalamsohal@gmail.com

Abstract

Human immunodeficiency virus (HIV) modifies CD4-positive cells, resulting in immunodeficiency and a wide range of gastrointestinal (GI) manifestations. The burden of HIV-related GI illnesses has significantly evolved with the widespread use of antiretroviral therapy (ART). While ART has effectively reduced the occurrence of opportunistic infections, it has led to an increase in therapy-related GI illnesses. Common esophageal conditions in HIV patients include gastroesophageal reflux disease, idiopathic esophageal ulcers, herpes simplex virus, cytomegalovirus (CMV), and candidal esophagitis. Kaposi's sarcoma, a hallmark of acquired immunodeficiency syndrome, may affect the entire GI system. Gastritis and peptic ulcer disease are also frequently seen in patients with HIV. Diarrhea, often linked to both opportunistic infections and ART, requires careful evaluation. Bloody diarrhea, often a sign of colitis caused by bacterial infections such as Shigella or Clostridium difficile, is prevalent. Small bowel lymphoma, although rare, is increasing in prevalence. Anorectal disorders, including proctitis, fissures, and anal squamous cell carcinoma, are particularly relevant in homosexual men, underlining the importance of timely diagnosis. This review comprehensively explores the epidemiology, pathogenesis, and treatment considerations for the various GI disorders associated with HIV, highlighting the importance of accurate diagnosis and effective treatment to improve outcomes for HIV-infected patients.

Key Words: Human immunodeficiency virus; Opportunistic infections; Antiretroviral treatment; Gastrointestinal

Core Tip: Human immunodeficiency virus (HIV) modifies CD4-positive cells, causing immunodeficiency and leading to various gastrointestinal (GI) issues. Highly active antiretroviral therapy (ART) has shifted the burden of HIV-related GI illnesses, reducing opportunistic infections while increasing GI problems associated with therapy, such as gastroesophageal reflux disease, non-infectious diarrhea, and HIV enteropathy. Diarrhea, linked to both infections and ART, requires careful evaluation to identify the etiology. Conditions such as colitis-related bloody diarrhea, small bowel lymphoma, and anorectal disorders, including proctitis and anal squamous cell carcinoma, also require timely diagnosis and management, especially in at-risk populations.

Citation: Moliya P, Singh A, Singh N, Kumar V, Sohal A. Insights into gastrointestinal manifestation of human immunodeficiency virus: A narrative review. *World J Virol* 2025; 14(1): 99249
URL: https://www.wjgnet.com/2220-3249/full/v14/i1/99249.htm
DOI: https://dx.doi.org/10.5501/wjv.v14.i1.99249

INTRODUCTION

The epidemiology of human immunodeficiency virus (HIV) has changed significantly over the past few decades, particularly with the introduction and widespread adoption of active antiretroviral therapy (ART). As of 2017, an estimated 36.8 million people worldwide were living with HIV, with the highest prevalence in southern sub-Saharan Africa. The incidence of new HIV cases peaked in 1999 at 3.16 million but decreased to 1.94 million by 2017. Similarly, HIV-related mortality reached its highest point in 2006 with 1.95 million deaths, dropping to 0.95 million deaths by 2017[1]. HIV interacts with host cells that express the CD4 surface glycoprotein in order to be recognized, making CD4-expressing T cells and macrophages the main targets of the virus. The envelope glycoprotein on the viral surface facilitates viral entry into host cells. Chemokine coreceptors, CCR5 and CXCR4, present on the host cell surface, induce a conformational change in the envelope protein, resulting in the fusion of viral and host cell membranes. This membrane fusion releases the viral capsid into the host cell. HIV manipulates these CD4-positive cells to facilitate its multiplication, resulting in their elimination, disruption of T cell homeostasis, and subsequent immunodeficiency[2].

Multiple opportunistic infections and some cancers are characteristics of AIDS that clearly define it as a disease based on cell-mediated immunity. HIV can cause a wide range of conditions in the gastrointestinal (GI) tract, leading to symptoms ranging from dysphagia to bloody diarrhea and tenesmus. GI problems are quite prevalent in HIV patients and might result from immunosuppressed states that make them more vulnerable to opportunistic infections, medication-induced consequences, or other underlying etiologies. It has been suggested that more than 70% of the patients will have GI manifestations during their disease course[3]. HIV-related GI disorders have changed significantly since the mid-1990s with the introduction of highly ART. As of now, 50%-70% of HIV patients reported GI symptoms. With the increased use of ART, the prevalence of opportunistic infections has declined, changing the burden of GI diseases in HIV patients.

In addition to the increased risk of infections, HIV may also lead to the development of tumors secondary to immunosuppression. HIV-associated neoplasms and infections are the two primary categories of these GI illnesses. Squamous cell carcinoma, non-Hodgkin's lymphoma, and Kaposi sarcoma (KS) are examples of HIV-acquired neoplasms, while common infections include ailments like cytomegalovirus and tuberculosis (TB)[4,5]. The presentation of these disorders varies significantly leading to much diagnostic confusion. Anorectal disorders are also common, which may present as tumors, warts, ulcers, and infections in the anorectal area[6]. The complex link between HIV infection and GI diseases is highlighted by these clinical problems, underscoring the need for a thorough knowledge of these manifestations to effectively treat patients and improve their quality of life.

To diagnose the underlying illness and provide the right medication, HIV patients who appear with GI problems must be evaluated using a comprehensive strategy that includes a full medical history, a thorough physical examination, and targeted diagnostic testing[3]. This review aims to provide insight into the dynamic nature of HIV-related GI illnesses and the critical role that contemporary diagnostic and treatment approaches play in the management of this condition.

The objective of conducting this literature review was to assess the various GI manifestations noted in HIV patients. Various databases, including PubMed, Google Scholar, EMBASE and MEDLINE, were used (from Inception to May 2024) to locate sources for this literary analysis. Search terms used for this review included "HIV" "gastrointestinal manifestations" "antiretroviral therapy" "opportunistic infections" "esophagitis" "Kaposi's sarcoma" and "diarrhea". The studies were reviewed by two independent authors (Moliya P and Singh A), and if there was any conflict regarding the inclusion/exclusion of the study in the literature review, the opinion of the third author (Sohal A) was taken into account. Studies were included if they focused on GI disorders in HIV patients and were published in English. Exclusion criteria were studies not focusing on HIV-associated GI conditions or those with incomplete data. To minimize selection bias, studies were evaluated based on their relevance, quality, and recency.

ESOPHAGEAL MANIFESTATIONS IN HIV

Patients with HIV/acquired immunodeficiency syndrome (AIDS) often exhibit various upper GI symptoms, ranging from odynophagia (discomfort on swallowing) to dysphagia (trouble swallowing). Opportunistic infections are more prevalent in HIV patients with low CD4 counts and the severity of the symptoms is directly correlated with CD4 count. The most common opportunistic manifestations in the esophagus include Herpes simplex virus (HSV), cytomegalovirus (CMV), and Candida species[7]. Additionally, HIV patients may also be affected by a disorder called idiopathic esophageal ulcers (IEU), which may be brought on by immunological factors or direct consequences of HIV. Non-infectious conditions and malignancies such as lymphoma and Kaposi's sarcoma are also possible manifestations. Before the advent of ART, the burden of esophageal manifestations secondary to opportunistic infections was high. However, after the advent of ART and protease inhibitors (PIs) in 1996, there has been an observed increase in esophageal conditions such as gastroesophageal reflux disease (GERD), Pill esophagitis, *etc.* in this patient population[8].

While odynophagia is usually a sign of esophageal ulcerative illness, dysphagia is often linked to candidal esophagitis [9]. Considering that some patients with HIV may have both odynophagia and dysphagia, a thorough evaluation is essential (Figure 1, Table 1).

Candidal esophagitis

The most frequent cause of esophagitis in HIV patients is candidal esophagitis with the prevalence of Candidal esophagitis being 11.2% in HIV-infected patients while 2.9% in non-HIV-infected patients[10]. Although other Candida species may potentially infect the esophagus, *Candida albicans* are often the culprit, accounting for 88% of cases. Patients who have esophageal symptoms, particularly dysphagia, odynophagia, and retrosternal pain with a CD4+ lymphocyte count of less than 100 cells/μL, should be suspected of having this condition. Systemic antifungals like Fluconazole are useful empirical therapy with 82% of patients showing improvement[11]. Fluconazole is taken orally in loading doses of 200 mg and then 100 mg every day for a period of 10 to 14 days[12]. A two-week regimen of isavuconazole, administered orally, is as effective as fluconazole for treating uncomplicated esophageal candidiasis. This regimen includes options for a loading dose of 200 mg followed by 50 mg daily, a loading dose of 400 mg followed by 100 mg daily, or 400 mg weekly [13]. However, patients with severe dysphagia may face challenges swallowing oral drugs.

While symptoms of esophageal candidiasis can be mimicked by infections such as CMV or HSV esophagitis, initiating antifungal therapy is generally recommended as both a diagnostic and therapeutic approach before considering endoscopy. If there is no response to the antifungal treatment, endoscopy is recommended to investigate other causes of esophagitis or drug-resistant Candida (Figure 1). Most patients respond quickly to antifungal therapy; signs and symptoms typically improve within 48 to 72 hours. The refractory disease affects around 4% to 5% of HIV-positive patients with esophageal candidiasis, particularly those with CD4 counts below 50 cells/mm^3 and extensive azole treatment history. For such cases, Posaconazole oral suspension (400 mg twice daily for 28 days) is effective in 75% of patients. Alternatives include anidulafungin, caspofungin, micafungin, or voriconazole for azole-refractory esophageal candidiasis[14].

CMV esophagitis

CMV is the most frequent viral opportunistic infection in individuals with AIDS, with the esophagus being the second most commonly affected site in the GI tract after the colon. Patients are most vulnerable to opportunistic infections when their CD4+ T lymphocyte cell count falls below 50 cells/mm^3[15]. Dysphagia and odynophagia are symptoms associated with CMV esophagitis. Endoscopy and biopsy are the gold standard for diagnosing the condition, which might reveal well-demarcated, vertical, single, or multiple ulcers or widespread esophagitis at the mid to distal esophagus. The primary treatments for CMV esophagitis are ganciclovir and valganciclovir. Treatment begins with induction therapy using intravenous ganciclovir at 10-15 mg/kg daily in divided doses for 3 to 6 weeks, depending on the patient's condition. Maintenance therapy involves daily intravenous ganciclovir at 5 mg/kg, particularly for those with concurrent retinitis or recurring symptoms. Relapse is common due to underlying severe immune deficiency, and treatment suppresses rather than eradicates the virus. If resistance develops, foscarnet is an alternative, and in patients with monotherapy, combination therapy with ganciclovir and foscarnet is beneficial[16]. CMV esophagitis in immunocompromised patients is associated with considerable morbidity and mortality, with some studies indicating up to a 25% mortality rate within one year[17].

HSV esophagitis

HSV esophagitis mostly occurs in immunocompromised hosts, particularly HIV patients, and it mostly co-exists with herpes labialis or oropharyngeal ulcers; it is rare in immunocompetent patients. Most cases of HSV esophagitis typically occur at an average age of 35 years, mainly in males, and are caused by HSV-1, though a few cases of HSV-2 esophagitis have also been reported[18]. HSV esophagitis may present either as a primary infection or as a reactivation, typically featuring acute systemic symptoms and widespread erosive-ulcerative lesions in the mid to distal esophagus. Patients often experience an initial prodrome of systemic symptoms such as fever, nausea, or vomiting, which precedes the onset of esophageal symptoms. The GI manifestations commonly include odynophagia, dysphagia, heartburn, epigastric pain, and occasionally atypical symptoms such as chest pain.

HSV esophagitis is typically identified through endoscopic findings, with further confirmation from histopathology. Endoscopically, it appears as fragile mucosa dotted with distinct vesicles and “volcano” ulcers, usually 1-3 mm in size, predominantly located in the distal esophagus. For the most accurate diagnostic results, biopsies should be taken from the ulcer margins, where signs of viral activity are most evident. Histological examination often reveals multinucleated

Table 1 Various esophageal manifestations in patients with human immunodeficiency virus

Type	CD4 counts	Endoscopic findings	Biopsy findings	First line therapy	Alternative treatment(s)
Candidal esophagitis	< 200/uL	White plaques, exudates, mucosal lesions	Yeast with pseudohyphae, parakeratosis	Fluconazole: 200 mg loading dose, followed by 100 mg daily for 10-14 days	Isavuconazole: (200 mg load, then 50 mg daily; 400 mg load, then 100 mg daily; or 400 mg weekly), posaconazole for refractory cases (400 mg twice daily for 28 days)
CMV esophagitis	< 50/uL	Well-demarcated vertical ulcers, single or multiple	Intracellular: Inclusions with clear halo- "owl's eye" appearance	Ganciclovir: 10-15 mg/kg daily in divided doses for 3-6 weeks	Valganciclovir (oral), foscarnet for resistance, combination therapy with ganciclovir and foscarnet in case of failure
HSV esophagitis	< 200/uL	Fragile mucosa with distinct vesicles and "volcano" ulcers	Multinucleated giant cells, cowdry a inclusion bodies	Acyclovir 200 mg five times a day or 400 mg three times a day for 7-10 days	Famciclovir, valacyclovir
Idiopathic esophageal ulcers		Large single ulcers, profound depth, located mid-esophagus	Negative for infections or malignancy	Oral steroids dosages vary based on severity and patient response	Thalidomide (as a therapeutic trial in severe cases)
Pill esophagitis		Varied ulcerations along the esophageal lining	Granulation tissue, necrotic squamous epithelium, and intrae-pithelial eosinophils	Behavioral changes like taking medication with enough water, avoiding lying down immediately after taking pills	Prevention is key; treatment focuses on behavioral changes)

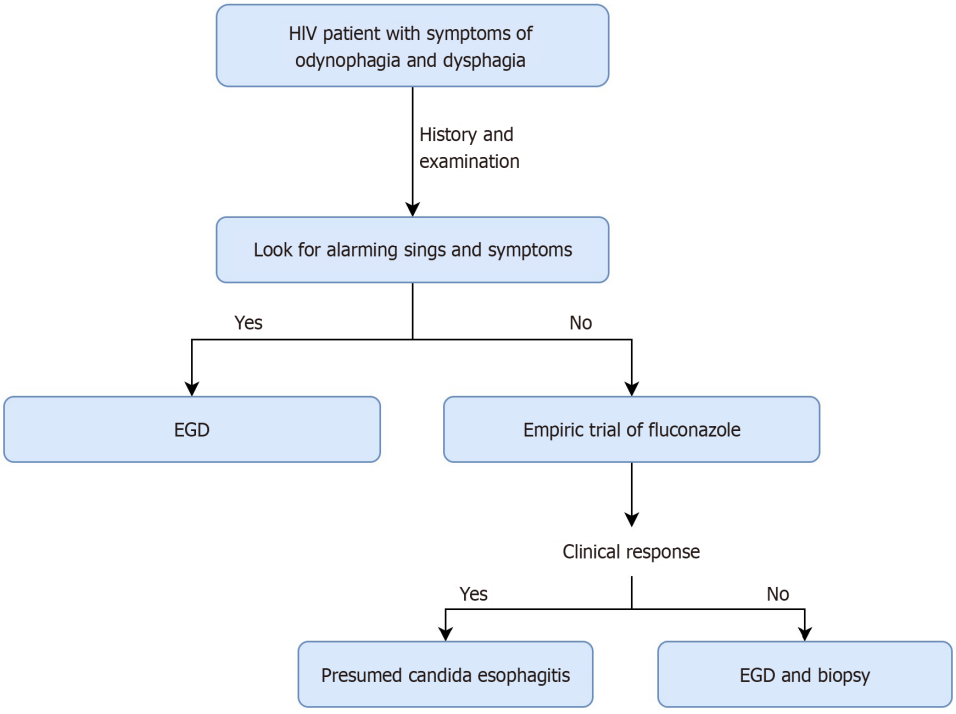


Figure 1 Clinical strategy for management of human immunodeficiency virus positive patients presenting with complaints of dysphagia and odynophagia. EGD: Esophagogastroduodenoscopy.

giant cells with ground-glass nuclei and eosinophilic inclusions, which are characteristic of this condition. Acute HSV-1 infections can be identified through serological analysis, showing positive IgM and negative IgG. This pattern may also be observed in HSV-2 infections, though less frequently[18]. A treatment course with oral acyclovir 200 mg five times a day or 400 mg three times a day for 7-10 days can lead to faster improvement. Famciclovir (500 mg two times daily) or Valacyclovir (1 g two times daily) can also be considered alternative oral therapy, although there is limited experience with these drugs in HSV esophagitis[18,19].

IEUs

IEUs occur in around 10% of HIV patients, either during acute retroviral syndrome or in advanced HIV (CD4 < 50/uL) due to severe immunodeficiency. They are typically seen in the middle esophagus and present as a single ulcer that is

enormous (5-10 cm) in size, oval, and has a profound depth. The exact pathogenesis is unknown, and the diagnosis is based on exclusion, necessitating repeated negative biopsies that are negative for infectious processes. Odynophagia is the main symptom of IEUs. A review of the current literature shows that there are no prospective, placebo-controlled, randomized, double-blind trials on the specific therapy of IEU, but according to case reports, oral corticosteroid treatment appears to be a reliable and secure therapy for HIV-related IEU. The response rate to steroids can be 92%-96%. Alternatively, thalidomide 200 mg orally daily for a 28-day course has been reported to be effective[20].

Pill esophagitis

Pill-induced esophagitis in HIV patients is an under-recognized condition that requires greater awareness among healthcare providers. Often, the high burden of antiretroviral treatment, combined with improper techniques like taking pills before sleeping or lying down and insufficient water intake, leads to damage to the esophageal mucosal lining. Zidovudine, Didanosine, and Stavudine, commonly used in highly ART (HAART), can lead to esophagitis. Common symptoms include dysphagia, odynophagia, chest discomfort, heartburn, and retrosternal irritation. To reduce the risk of such injuries, it is crucial for patients to take medications with plenty of water and to remain upright–sitting or standing–for at least 30 minutes afterward[21].

Esophageal malignancies and other conditions

Esophageal malignancies, such as KS and lymphoma, provide a major challenge to HIV patients because of their aggressive nature and complicated therapy. KS is the most common GI malignancy in AIDS, affecting 40% of patients, and is often asymptomatic[22]. The best imaging modality for diagnosing GI KS is port venous-enhanced computed tomography (CT). CT scans of individuals with disseminated illness show enhanced lymph nodes in around 80% of cases [23]. KS masses usually have a polypoid appearance and are usually less than 3 cm, while bigger masses may sometimes arise. Radiological evaluations, endoscopy, and biopsy are necessary for the diagnosis. Radiation treatment, chemotherapy, surgery, and antiretroviral therapy are examples of management techniques[24].

In addition to infections and ulcers, HIV patients may also have problems with neuropathic pain and esophageal motility. HIV neuropathy can lead to GI discomfort and is usually managed symptomatically with metoclopramide and other prokinetic medicines. HIV's neurotrophic properties may also cause irregularities in esophageal motility, which can lead to autonomic dysfunction in the GI, and neurologic plexus[25].

GASTRIC DISORDERS

While the stomach is usually unaffected by opportunistic infections, HIV patients often experience abdominal discomfort, nausea, or vomiting. Common gastric conditions in these patients, like the general population, include gastritis, GERD, and peptic ulcer[26]. The incidence of *Helicobacter pylori*, a frequent cause of PUD in non-HIV positive people, seems to be lower in HIV patients; instead, CMV is the leading cause of peptic ulcer disease in these patients. Chronic gastritis is a common finding, however, the incidence of acute gastritis in the gastric antrum is lower in severely immunodeficient patients compared to their HIV-negative counterparts[27]. These patients also have concurrent elevated pH, which influences drug absorption and can predispose patients to gastric colonization by pathogens like *Candida albicans*[28]. Proton-pump inhibitors and histamine-2 blockers which are commonly used for symptomatic management might also interfere with HAART[8]. Thus, caution must be given when prescribing these Dyspepsia is a prevalent complaint among HIV-infected individuals on HAART, often accompanied by mucosal alterations such as erythema, erosion, and ulcers upon endoscopic evaluation[29]. Despite these observations, conflicting findings exist regarding the extent of gastric acid impairment in non-AIDS HIV-1-infected individuals. While some studies have reported no significant alterations, others have documented notable changes in reduced gastric acid output[30].

Studies have also shown that patients with HIV have motor activity disturbances secondary to damage to the peripheral and central autonomic nervous system. As a result, various patterns of gastric emptying, with delayed emptying of solids and accelerated emptying of liquids, have been observed in HIV-positive individuals compared to healthy controls[31]. The other common gastric pathologies include gastric lymphoma, KS, and a few opportunistic infections (including CMV, TB, toxoplasmosis, and cryptococcosis)[26]. GI toxoplasmosis is relatively rare, affecting only 6%-20% of patients with disseminated disease. The symptoms can range from diarrhea and abdominal pain to nausea, vomiting, anorexia, and ascites. Typical endoscopic findings include thickened gastric folds, ulcerative lesions, and general inflammation[32]. Gastric cryptococcus is primarily asymptomatic but, when symptoms do occur, it includes odynophagia, diarrhea, nausea, vomiting, and melena. Gastric cryptococcus is most identified during post-mortem autopsies[33].

DIARRHEA IN HIV PATIENTS

Acute and chronic diarrhea are major complications of HIV infection and AIDS, which leads to significant morbidity and increased healthcare costs[34,35]. Diarrhea has been noted in up to 50% of the AIDS patients in North America, with numbers approaching 100% in the developing world. Previous studies have shown that patients are more likely to experience diarrhea compared to patients without HIV (28% *vs* 7%, $P < 0.001$)[34]. The etiology of diarrhea in HIV-infected patients is multi-factorial. Infections, HIV-enteropathy, HAART-associated diarrhea, autonomic neuropathy, and

chronic pancreatitis are among the common causes (Table 2). With the introduction of ART, a decline in infectious diarrhea along with a rise in non-infectious diarrhea has been noted. In this section, we will focus on infectious etiologies and HAART-associated diarrhea[35]. The impact on quality of life is illustrated in a national survey in which 40% of HIV patients reported that diarrhea negatively affected their social lives, causing them to alter their daily schedules and develop feelings of shame. Therefore, thorough workup is essential when an HIV patient presents with diarrhea (Figure 2).

Infectious diarrhea

Previous studies have noted that approximately 44% to 82% of cases of chronic diarrhea in HIV patients have an identifiable infectious etiology[36-39]. However, in resource risk settings, the infectious etiology has been noted in as low as 12% of patients with diarrhea[40]. However, infections continue to be highly endemic in resource-limited settings, with chronic diarrheal disease being used as a predictor for HIV-seropositivity in certain populations. Table 2 highlights the various infectious causes of diarrhea in HIV patients along with the recommended management options.

HAART-associated diarrhea

Since the introduction of HAART therapy, there has been a significant reduction in the incidence of infectious diarrhea. However, at the same time, there has been a steady increase in the incidence of medication-induced, such that it is the leading diagnostic consideration when diarrhea is the sole complaint, particularly when there is a temporal association [40]. Up to 12.5% of patients taking post-exposure prophylaxis experience diarrhea[41]. GI complications continue to be one of the major reasons for discontinuing retroviral treatment and associated reduced quality of life. Up to 19% of the patients treated with HAART reported moderate to potentially life-threatening diarrhea, which can be related to the study drug[42].

Diarrhea has been noted with all main classes of ARVs: Nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitor, PIs, and integrase inhibitors[43,44]. Among these medications, ritonavir, which is used in combination with other PIs, is most commonly associated with diarrhea. Lopinavir/ritonavir and fosamprenavir/ritonavir have been noted to have the highest incidence (10%-15%) compared to the other combinations[45,46]. Poor adherence and switching regimes also increase the risk of development of resistance to virological treatment. Various mechanisms have been proposed for the occurrence of diarrhea. Nefinavir is noted to enhance calcium signaling in the secretory epithelial cells leading to the loss of chloride ions across the epithelial membrane, leading to secretory diarrhea [47]. Ritonavir on the other hand has demonstrated cell apoptosis and disruption of intestinal epithelial barrier integrity leading to diarrhea[48].

Management options included probiotics, antimotility, and antisecretory agents. Crofelemer is an antisecretory drug that inhibits chloride channels in the gut, which are responsible for large-volume water loss. In a randomized controlled trial of 376 HIV patients on HAART, Crofelemer was noted to significantly reduce diarrheal symptoms compared with placebo (clinical response defined as ≤ 2 watery stools/week during ≥ 2 of 4 weeks; 17.6 *vs* 8.0%; P ¼ 0.01)[49]. Pharmacokinetic studies have shown that systemic absorption of Crofelemer is minimal, with over 95% of subjects having serum levels below the quantifiable threshold[50]. Importantly, Crofelemer does not exhibit significant interactions with antiretroviral therapies[51].

HIV enteropathy

HIV enteropathy is an idiopathic form of diarrhea in HIV-infected individuals, characterized by the absence of identifiable pathogens and diagnosed by exclusion. It is associated with increased inflammation and immune activation and decreased mucosal repair and regeneration. It is also known as the 'Slim disease', in recognition of the severity of weight loss caused[52]. Chronic diarrhea, malabsorption, increased intestinal permeability and malnutrition are common clinical manifestations. The primary goal of managing HIV enteropathy is to prevent further depletion of CD4+ T lymphocytes, incorporating the initiation of HAART alongside supportive care. Previous studies highlighting the improvement in clinical signs and symptoms support the initiation of HAART[53]. Additionally, adjunctive therapies, including antimotility agents, antisecretory drugs (Crofelemer as discussed in the previous section), adsorbents, and opioids, are utilized to manage symptoms[54].

COLITIS

Bloody diarrhea and tenesmus are frequent symptoms of colitis in HIV patients. These conditions are often brought on by bacterial infections such as *Shigella*, *E. coli*, *Campylobacter jejuni*, and *Clostridium difficile*. HIV individuals are much more likely to get salmonellosis, for which ciprofloxacin is used as a therapy[55]. While *Campylobacter jejuni* usually causes watery diarrhea, *Shigella* presents similarly. Ciprofloxacin is a medication that treats a variety of enteric diseases, including *E. coli* infections[56]. When *Clostridium difficile*-related colitis is detected by toxin assay and treated with metronidazole or tinidazole, it may happen even in the absence of recent antibiotic medication. Amphotericin B and itraconazole are used to treat fungus-related diarrhea, which is uncommon and often occurs with systemic diseases such as GI histoplasmosis. HIV patients may also have GI problems from non-infectious reasons, such as drug-induced diarrhea from antiretroviral drugs, inflammatory bowel disorders, lymphoma, and Kaposi's sarcoma[57]. Each of these conditions calls for a different diagnosis and treatment strategy.

Table 2 Common opportunistic pathogens in human immunodeficiency virus-associated diarrhea-location, presentation, diagnosis and treatment

Pathogen	Location	Clinical features	Diagnosis	Chemoprophylaxis	Treatment
Cytomegalovirus	Gastric, small bowel (ileum), and large bowel (colon)	Bloody diarrhea, fever, weight loss, fever, anorexia, abdominal pain	DNA PCR from stool sample	None recommended	IV ganciclovir for 21 to 42 days (can with to oral valganciclovir once the patient can tolerate)
Herpes simplex	Anorectal	Tenesmus, rectal pain, hematochezia, proctitis	DNA PCR for cutaneous lesion	None recommended	Acyclovir/Valacyclovir PO for 5 to 10 days
Cryptosporidium	Gastric, small bowel (ileum), and large bowel	Acute/Subacute onset watery diarrhea, nausea/vomiting, lower abdominal cramping	Microscopic identification of the oocysts in stool with acid-fast staining or direct immunofluorescence	None recommended	Nitazoxanide (500/1000 mg POD BID for 14 days) or paromomycin (500 mg PO QID for 14 to 21 days)
Microsporidia	Small and large bowel	Diarrhea with cramps and abdominal pain, wasting, malnutrition	Microscopic identification of the spores in stool with trichrome staining	None recommended	For *Enterocytozoon bieneusi* –nitazoxanide 500 mg BID for 14 days. For intestinal infection caused by microsporidia other than *E. bieneusi*–albendazole 400 mg BID for 14 days
Isospora belli	Gastric	Watery diarrhea, with abdominal pain, cramping, nausea/vomiting, low-grade fever, dehydration	Microscopic identification of the oocysts in stool with acid-fast staining or UV fluorescence microscopy	None recommended. Indirect evidence of a protective effect of TMP-SMX	TMP-SMX (160/800mg QID for 10 days)
Cyclospora	Small bowel	Watery diarrhea, weight loss, abdominal cramping, low-grade fever	Microscopic identification of the oocysts in stool with acid-fast staining	None recommended	TMP-SMX (160/800 mg PO BID for 14 days) or nitazoxanide (500 mg PO BID for 7 days)
Entamoeba histolytica	Colon	Diarrhea with blood and mucus, cramping lower abdomen pain, bloating, fever, chills	DNA PCR from stool sample, stool antigen test, microscopic identification of the cysts/trophozoites in stool	None recommended	Metronidazole 500-750 mg PO TID for 5-10 days or tinidazole 2 g PO once daily for 3 days, followed by a luminal agent
Histoplasmosis	Terminal ileum and colon	Intermittent bloody diarrhea, abdominal pain, fever, weight loss	EIA for urine or serum antigen. Histopathological examination using GMS stain	Itraconazole for patients with CD4 counts < 150/mm^3	IV liposomal amphotericin B (3 mg/kg daily) for ≥ 2 weeks or clinical improvement with stepdown to oral itraconazole
Mycobacterium tuberculosis	Terminal ileum and cecum	Abdominal pain, diarrhea, fever, weight loss, night sweats, fatigue	Acid-fast staining	None recommended	Initial phase (2 months): Isoniazid 300 mg daily, rifampin 600 mg daily, pyrazinamide 25 mg/kg daily, and ethambutol 15 mg/kg daily. Continuation phase (4-7 months): Isoniazid 300 mg daily and rifampin 600 mg daily
Mycobacterium avium complex	Small bowel and large bowel	Fever, abdominal pain, weight loss, night sweats, fatigue	Acid-fast staining	Azithromycin for patients with CD4 count < 50/mm^3	Clarithromycin (7.5 to 15 mg/kg PO BID for at least 12 months)

PCR: Polymerase chain reaction; PO: Per Os (by mouth/orally); BID: Bis in die (twice daily); QID: Quater in die (four times daily); TMP-SMX: Trimethoprim-Sulfamethoxazole; GMS: Grocott's methenamine Silver; EIA: Enzyme immunoassay.

SMALL BOWEL LYMPHOMA

Although primary small bowel lymphoma is an uncommon condition, its prevalence is rising, especially in those living with HIV. As you go proximally in the small intestine, the incidence of this lymphoma decreases, mostly affecting the terminal ileum. AIDS is often diagnosed in people with non-Hodgkin's lymphoma or small bowel lymphoma[58].

Spoken thickening of the intestinal wall (between 1 and 7 cm), fungating masses, tumor infiltration of the myenteric nerve plexus resulting in aneurysmal intestine dilatation, and, less often, solid mass lesions are among the radiological characteristics of small bowel lymphoma. In one case, for example, a 50-year-old man with a CD4 count of 190 cells/μL showed signs of lymphadenopathy, upstream small intestinal dilatation, circumferential thickening of the terminal ileum and caecum, and lymphomatous infiltration of the ileocaecal valve. Further pathological examination confirmed the non-Hodgkin's lymphoma diagnosis[59]. This manifestation emphasizes how crucial it is to keep an eye on and manage GI issues in HIV patients, as these illnesses may have detrimental effects and call for specific diagnosis and treatment plans.

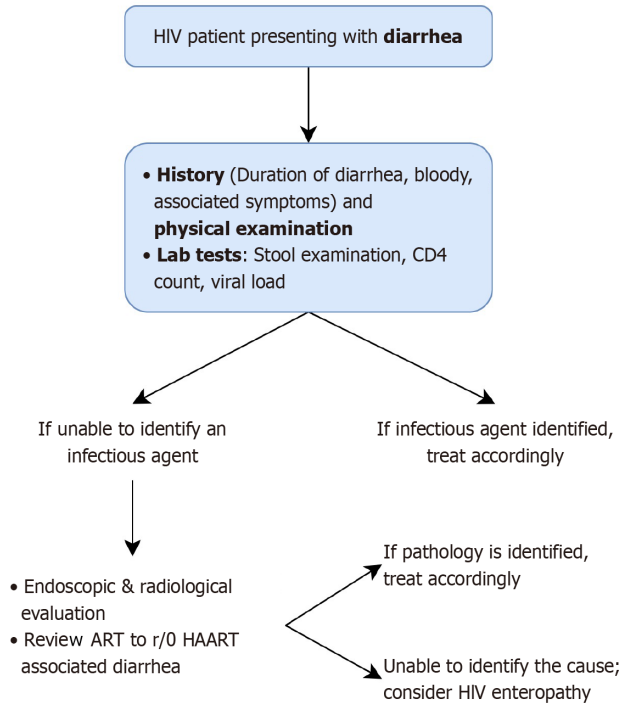


Figure 2 Clinical strategy for management of human immunodeficiency virus positive patients presenting with complaints of diarrhea.
ART: Antiretroviral therapy; HAART: Highly active ART; HIV: Human immunodeficiency virus.

ANORECTAL DISORDERS

The frequency of anorectal disease is unchanged by ART, which makes it a significant issue for HIV patients, especially gay men. Numerous anorectal diseases affect this population, such as proctitis, fissures, ulcerations, perirectal abscesses, and anal fistulas. Add to this the possibility of anal neoplasms associated with the human papillomavirus (HPV) and other variables, and anorectal carcinoma ranks fourth among malignancies in HIV patients; it is more prevalent among homosexual men who are HIV positive[59]. Anorectal cytology may identify anal squamous cell carcinoma, which is similar to cervical cancer screening in that it is often linked to squamous intraepithelial neoplasia associated with HPV. While anoscopy combined with biopsy is still the preferred method of diagnosis, anorectal cytology may be a helpful screening tool[6].

Other common anorectal symptoms in the HIV population include proctitis, which is frequently brought on by infectious agents such as gonorrhea, herpes simplex, chlamydia, and syphilis and requires the use of an appropriate antibiotic or antiviral therapy, and anal condyloma associated with HPV infection, which can be treated with a variety of surgical options[60]. Furthermore, overlapping clinical presentations may result from colitis caused by various sources, such as lymphogranuloma venereum. Essential diagnostic instruments for evaluating anorectal disorders, identifying opportunistic infections, and directing treatment choices include associative sigmoidoscopy and anoscopy combined with mucosal biopsies.

ANAL SQUAMOUS CELL CARCINOMA

HPV infection is a common cause of anal squamous cell carcinoma, which is more common in HIV-positive persons because of the higher risk of co-infections, especially *via* anoreceptive sexual relations[60]. While HIV is not a direct cause, it is a marker for increased vulnerability to STDs such as HPV, with those who are positive for the virus seven times more likely to have persistent HPV[5]. Magnetic resonance imaging (MRI) is the recommended imaging modality for evaluating anal malignancies. It offers comprehensive details on the tumor's location, size, and local invasion. When using T1-weighted imaging, T2-weighted imaging, or short tau inversion recovery sequences, malignant tissue located inside the anal canal usually exhibits low signal intensity, intermediate signal intensity, or lower signal intensity than ischioanal fat[61].

Anal squamous cell carcinoma appears on CT images as a solid, enhancing mass that becomes larger and more amorphous. In one example, T2-weighted imaging revealed a massive soft tissue mass with significant soft tissue invasion in a 42-year-old male HIV-positive patient with a CD4 level of 16 cells/μL[59]. The mass's signal strength was moderate, meaning it was less than that of the adipose tissue around it. This manifestation highlights how crucial it is for HIV-positive people to get routine monitoring and early identification to treat potentially dangerous GI side effects including anal squamous cell carcinoma.

GI TB

Global health concerns about TB persist, particularly concerning those living with HIV. Up to 70% of HIV patients are predicted to have TB at some time in their life. When the CD4 count falls, TB risk rises dramatically, reaching a crucial threshold of around 200 cells/μL. In those who are HIV-positive, lung TB often leads to intestinal TB[55].

Although TB in the abdomen may infect any section of the GI system, this is the area most often affected because of the large concentration of lymphoid tissue in the terminal ileum. Abdominal TB manifests on CT or MRI scans as circumferential thickening of the afflicted intestine segment, sometimes with adjacent lymphadenopathy[56]. These imaging characteristics, however, are often non-specific and may be confused with illnesses like cancer or inflammatory bowel disease.

Asymmetric thickening of the medial wall of the caecum and terminal ileum, together with significant lymphadenopathy with central regions of diminished attenuation, are indicative signs. Research involving two patients with abdominal TB revealed that at the time of presentation, their CD4 Levels were both less than 100 cells/μL[59]. These instances underscore the need to identify GI TB as a plausible complication in patients living with HIV/AIDS and the necessity of exercising caution in both diagnosis and treatment.

PANCREATITIS

The interplay between HIV and the pancreas encompasses a complex array of direct viral effects, indirect consequences, and complications arising from ART. Notably, HIV-infected patients may present with various pancreatic abnormalities, including acute pancreatitis, which is influenced by factors such as prolonged HIV seropositivity, exposure to PIs, immunodeficiency, AIDS, chronic liver and/or biliary disease, and hypertriglyceridemia[62]. This underscores the ongoing concern regarding the management and assessment of pancreatitis risk in such settings. Compared to the general population, HIV-infected individuals exhibit a heightened incidence of acute pancreatitis, with risk factors including severe immunosuppression, female gender, and specific medication usage such as stavudine and aerosolized pentamidine[63]. PIs used in ART have been implicated in inducing insulin resistance and diminishing insulin secretion by pancreatic beta cells *via* inhibition of glucose translocation through glucose transporter 4[64].

Moreover, an inverse relationship exists between serum pancreatic enzyme levels and CD4 Lymphocyte counts, suggesting a link between immunosuppression and pancreatic inflammation[57]. Non-infectious complications, notably non-Hodgkin's lymphoma, can also involve the pancreas in HIV-infected patients[65]. Furthermore, exocrine pancreatic insufficiency has been identified in HIV-infected individuals, contributing to malabsorption and growth alterations, particularly in pediatric populations[64,66]. The prevalence of exocrine pancreatic insufficiency among HIV-infected patients on suppressive ART remains significant, with some responding to pancreatic enzyme replacement therapy[67]. In summary, HIV infection impacts the pancreas through diverse mechanisms, including direct viral effects, ART-related toxicity, and associated opportunistic infections or malignancies. Clinicians should maintain vigilance for signs of pancreatic involvement in HIV-infected patients, as it can significantly contribute to morbidity and complicate the management of HIV disease.

CONCLUSION

In conclusion, there is a complex connection between HIV infection and a variety of GI symptoms. It highlights how important it is to comprehend and treat these GI disorders, which can range from diarrhea, enteritis, colitis, and esophageal problems to anorectal diseases, anal squamous cell carcinoma, GI tuberculosis, liver disorders, and pancreatitis. Early diagnosis of HIV and identifying the underlying causes of associated GI conditions are essential. The research also highlights the evolving patterns of GI disease since the introduction of HAART, with a decline in infectious GI complications in developing countries and a rise in those related to medication side effects and direct viral injury. Recognizing how factors like medication use and local dietary habits influence these changing patterns is key to effectively managing these patients. Addressing these GI issues is crucial for improving the quality of life in HIV-positive individuals and reducing the burden of these complications as HIV treatments continue to advance.

FOOTNOTES

Author contributions: Moliya P, and Sohal A conceptualized and designed the study. Moliya P, Singh A and Singh N conducted the literature review, interpreted the data, and drafted the original manuscript; Kumar V and Sohal A supervised the study and made critical revisions. All authors have read and approved the final manuscript.

Conflict-of-interest statement: The authors declare no conflict of interest for this article.

Country of origin: United States

ORCID number: Aalam Sohal 0000-0001-8365-7240.

S-Editor: Liu H
L-Editor: A
P-Editor: Zhang L

REFERENCES

1 **GBD 2017 HIV collaborators**. Global, regional, and national incidence, prevalence, and mortality of HIV, 1980-2017, and forecasts to 2030, for 195 countries and territories: a systematic analysis for the Global Burden of Diseases, Injuries, and Risk Factors Study 2017. *Lancet HIV* 2019; **6**: e831-e859 [PMID: 31439534 DOI: 10.1016/S2352-3018(19)30196-1]
2 **Meissner ME**, Talledge N, Mansky LM. Molecular Biology and Diversification of Human Retroviruses. *Front Virol* 2022; **2** [PMID: 35783361 DOI: 10.3389/fviro.2022.872599]
3 **Serlin MH**, Dieterich D. Gastrointestinal Disorders in HIV. *Glob HIV/AIDS Med* 2008 [DOI: 10.1016/b978-1-4160-2882-6.50027-7]
4 **Sousa RH**, Souza LL, Guedes PT, Prado-Ribeiro AC, Rodrigues-Oliveira L, Brandão TB, Alves BW, Lopes MA, Santos-Silva AR, Monteiro JC, Gonçalves TT, Almeida OP. Oral Kaposi sarcoma development is associated with HIV viral load, CD4+ count and CD4+/CD8+ ratio. *Med Oral Patol Oral Cir Bucal* 2021; **26**: e748-e753 [PMID: 34704977 DOI: 10.4317/medoral.24708]
5 **Dandapani SV**, Eaton M, Thomas CR Jr, Pagnini PG. HIV- positive anal cancer: an update for the clinician. *J Gastrointest Oncol* 2010; **1**: 34-44 [PMID: 22811803 DOI: 10.3978/j.issn.2078-6891.2010.005]
6 **Friedlander MA**, Stier E, Lin O. Anorectal cytology as a screening tool for anal squamous lesions: cytologic, anoscopic, and histologic correlation. *Cancer* 2004; **102**: 19-26 [PMID: 14968414 DOI: 10.1002/cncr.11888]
7 **Connolly GM**, Hawkins D, Harcourt-Webster JN, Parsons PA, Husain OA, Gazzard BG. Oesophageal symptoms, their causes, treatment, and prognosis in patients with the acquired immunodeficiency syndrome. *Gut* 1989; **30**: 1033-1039 [PMID: 2548933 DOI: 10.1136/gut.30.8.1033]
8 **Mönkemüller K**. Declining prevalence of opportunistic gastrointestinal disease in the era of combination antiretroviral therapy. *Am J Gastroenterol* 2000; **95**: 457-462 [PMID: 10685750 DOI: 10.1016/s0002-9270(99)00827-8]
9 **Abdullah HM**, Ullah W, Abdallah M, Khan U, Hurairah A, Atiq M. Clinical presentations, management, and outcomes of acute esophageal necrosis: a systemic review. *Expert Rev Gastroenterol Hepatol* 2019; **13**: 507-514 [PMID: 30933549 DOI: 10.1080/17474124.2019.1601555]
10 **Takahashi Y**, Nagata N, Shimbo T, Nishijima T, Watanabe K, Aoki T, Sekine K, Okubo H, Watanabe K, Sakurai T, Yokoi C, Mimori A, Oka S, Uemura N, Akiyama J. Upper Gastrointestinal Symptoms Predictive of Candida Esophagitis and Erosive Esophagitis in HIV and Non-HIV Patients: An Endoscopy-Based Cross-Sectional Study of 6011 Patients. *Medicine (Baltimore)* 2015; **94**: e2138 [PMID: 26632738 DOI: 10.1097/MD.0000000000002138]
11 **Wilcox CM**, Alexander LN, Clark WS, Thompson SE 3rd. Fluconazole compared with endoscopy for human immunodeficiency virus-infected patients with esophageal symptoms. *Gastroenterology* 1996; **110**: 1803-1809 [PMID: 8964406 DOI: 10.1053/gast.1996.v110.pm8964406]
12 **Koletar SL**, Russell JA, Fass RJ, Plouffe JF. Comparison of oral fluconazole and clotrimazole troches as treatment for oral candidiasis in patients infected with human immunodeficiency virus. *Antimicrob Agents Chemother* 1990; **34**: 2267-2268 [PMID: 2073120 DOI: 10.1128/AAC.34.11.2267]
13 **Viljoen J**, Azie N, Schmitt-Hoffmann AH, Ghannoum M. A phase 2, randomized, double-blind, multicenter trial to evaluate the safety and efficacy of three dosing regimens of isavuconazole compared with fluconazole in patients with uncomplicated esophageal candidiasis. *Antimicrob Agents Chemother* 2015; **59**: 1671-1679 [PMID: 25561337 DOI: 10.1128/AAC.04586-14]
14 **Skiest DJ**, Vazquez JA, Anstead GM, Graybill JR, Reynes J, Ward D, Hare R, Boparai N, Isaacs R. Posaconazole for the treatment of azole-refractory oropharyngeal and esophageal candidiasis in subjects with HIV infection. *Clin Infect Dis* 2007; **44**: 607-614 [PMID: 17243069 DOI: 10.1086/511039]
15 **Li L**, Chakinala RC. Cytomegalovirus Esophagitis. 2023 May 1. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan- [PMID: 32310570]
16 **Parente F**, Bianchi Porro G. Treatment of cytomegalovirus esophagitis in patients with acquired immune deficiency syndrome: a randomized controlled study of foscarnet versus ganciclovir. The Italian Cytomegalovirus Study Group. *Am J Gastroenterol* 1998; **93**: 317-322 [PMID: 9517631 DOI: 10.1111/j.1572-0241.1998.00317.x]
17 **Marques S**, Carmo J, Pinto D, Bispo M, Ramos S, Chagas C. Cytomegalovirus Disease of the Upper Gastrointestinal Tract: A 10-Year Retrospective Study. *GE Port J Gastroenterol* 2017; **24**: 262-268 [PMID: 29255766 DOI: 10.1159/000479232]
18 **Shah R**, Patel S, Henriquez R, Parikh J, Mandalia A. Herpes Simplex Virus Esophagitis in an Immunocompetent Patient. *Cureus* 2023; **15**: e44668 [PMID: 37799253 DOI: 10.7759/cureus.44668]
19 **McBane RD**, Gross JB Jr. Herpes esophagitis: clinical syndrome, endoscopic appearance, and diagnosis in 23 patients. *Gastrointest Endosc* 1991; **37**: 600-603 [PMID: 1756917 DOI: 10.1016/s0016-5107(91)70862-6]
20 **Wilcox CM**, Schwartz DA. A pilot study of oral corticosteroid therapy for idiopathic esophageal ulcerations associated with human immunodeficiency virus infection. *Am J Med* 1992; **93**: 131-134 [PMID: 1497008 DOI: 10.1016/0002-9343(92)90041-9]
21 **Kwak HA**, Hart J. The Many Faces of Medication-Related Injury in the Gastrointestinal Tract. *Surg Pathol Clin* 2017; **10**: 887-908 [PMID: 29103538 DOI: 10.1016/j.path.2017.07.007]
22 **Kaposi**. Idiopathisches multiples Pigmentsarkom der Haut. *Arch f Dermat* 1872; **4**: 265-273 [DOI: 10.1007/bf01830024]
23 **Restrepo CS**, Martínez S, Lemos JA, Carrillo JA, Lemos DF, Ojeda P, Koshy P. Imaging manifestations of Kaposi sarcoma. *Radiographics* 2006; **26**: 1169-1185 [PMID: 16844940 DOI: 10.1148/rg.264055129]
24 **Chai J**, Jamal MM. Esophageal malignancy: a growing concern. *World J Gastroenterol* 2012; **18**: 6521-6526 [PMID: 23236223 DOI: 10.3748/wjg.v18.i45.6521]

25 **Zalar AE**, Olmos MA, Piskorz EL, Magnanini FL. Esophageal motility disorders in HIV patients. *Dig Dis Sci* 2003; **48**: 962-967 [PMID: 12772797 DOI: 10.1023/a:1023063916026]

26 **Chiu HM**, Wu MS, Hung CC, Shun CT, Lin JT. Low prevalence of Helicobacter pylori but high prevalence of cytomegalovirus-associated peptic ulcer disease in AIDS patients: Comparative study of symptomatic subjects evaluated by endoscopy and CD4 counts. *J Gastroenterol Hepatol* 2004; **19**: 423-428 [PMID: 15012780 DOI: 10.1111/j.1440-1746.2003.03278.x]

27 **Kullberg BJ**, Arendrup MC. Invasive Candidiasis. *N Engl J Med* 2015; **373**: 1445-1456 [PMID: 26444731 DOI: 10.1056/NEJMra1315399]

28 **Welage LS**, Carver PL, Revankar S, Pierson C, Kauffman CA. Alterations in gastric acidity in patients infected with human immunodeficiency virus. *Clin Infect Dis* 1995; **21**: 1431-1438 [PMID: 8749628 DOI: 10.1093/clinids/21.6.1431]

29 **Werneck-Silva AL**, Prado IB. Dyspepsia in HIV-infected patients under highly active antiretroviral therapy. *J Gastroenterol Hepatol* 2007; **22**: 1712-1716 [PMID: 17559368 DOI: 10.1111/j.1440-1746.2007.04897.x]

30 **Hellerstein MK**, Grunfeld C, Wu K, Christiansen M, Kaempfer S, Kletke C, Shackleton CH. Increased de novo hepatic lipogenesis in human immunodeficiency virus infection. *J Clin Endocrinol Metab* 1993; **76**: 559-565 [PMID: 8445011 DOI: 10.1210/jcem.76.3.8445011]

31 **Konturek JW**, Fischer H, van der Voort IR, Domschke W. Disturbed gastric motor activity in patients with human immunodeficiency virus infection. *Scand J Gastroenterol* 1997; **32**: 221-225 [PMID: 9085458 DOI: 10.3109/00365529709000198]

32 **Merzianu M**, Gorelick SM, Paje V, Kotler DP, Sian C. Gastric toxoplasmosis as the presentation of acquired immunodeficiency syndrome. *Arch Pathol Lab Med* 2005; **129**: e87-e90 [PMID: 15794696 DOI: 10.5858/2005-129-e87-GTATPO]

33 **Dhillon R**, Sohal A, Wormser V, Li L. Disseminated cryptococcosis with gastric and thyroid involvement. *IDCases* 2021; **24**: e01141 [PMID: 34012773 DOI: 10.1016/j.idcr.2021.e01141]

34 **Siddiqui U**, Bini EJ, Chandarana K, Leong J, Ramsetty S, Schiliro D, Poles M. Prevalence and impact of diarrhea on health-related quality of life in HIV-infected patients in the era of highly active antiretroviral therapy. *J Clin Gastroenterol* 2007; **41**: 484-490 [PMID: 17450031 DOI: 10.1097/01.mcg.0000225694.46874.fc]

35 **Lubeck DP**, Bennett CL, Mazonson PD, Fifer SK, Fries JF. Quality of life and health service use among HIV-infected patients with chronic diarrhea. *J Acquir Immune Defic Syndr (1988)* 1993; **6**: 478-484 [PMID: 8483111 DOI: 10.1097/00126334-199305000-00008]

36 **Laughon BE**, Druckman DA, Vernon A, Quinn TC, Polk BF, Modlin JF, Yolken RH, Bartlett JG. Prevalence of enteric pathogens in homosexual men with and without acquired immunodeficiency syndrome. *Gastroenterology* 1988; **94**: 984-993 [PMID: 2831107 DOI: 10.1016/0016-5085(88)90557-4]

37 **Smith PD**, Lane HC, Gill VJ, Manischewitz JF, Quinnan GV, Fauci AS, Masur H. Intestinal infections in patients with the acquired immunodeficiency syndrome (AIDS). Etiology and response to therapy. *Ann Intern Med* 1988; **108**: 328-333 [PMID: 2829677 DOI: 10.7326/0003-4819-108-3-328]

38 **Wilcox CM**, Schwartz DA, Cotsonis G, Thompson SE 3rd. Chronic unexplained diarrhea in human immunodeficiency virus infection: determination of the best diagnostic approach. *Gastroenterology* 1996; **110**: 30-37 [PMID: 8536874 DOI: 10.1053/gast.1996.v110.pm8536874]

39 **Blanshard C**, Francis N, Gazzard BG. Investigation of chronic diarrhoea in acquired immunodeficiency syndrome. A prospective study of 155 patients. *Gut* 1996; **39**: 824-832 [PMID: 9038664 DOI: 10.1136/gut.39.6.824]

40 **Knox TA**, Spiegelman D, Skinner SC, Gorbach S. Diarrhea and abnormalities of gastrointestinal function in a cohort of men and women with HIV infection. *Am J Gastroenterol* 2000; **95**: 3482-3489 [PMID: 11151881 DOI: 10.1111/j.1572-0241.2000.03365.x]

41 **Reijers MH**, Weigel HM, Hart AA, Ten Kate RW, Mulder JW, Reiss P, Schuitemaker H, Hoetelmans RM, Weverling GJ, Lange JM. Toxicity and drug exposure in a quadruple drug regimen in HIV-1 infected patients participating in the ADAM study. *AIDS* 2000; **14**: 59-67 [PMID: 10714568 DOI: 10.1097/00002030-200001070-00007]

42 **Hill A**, Balkin A. Risk Factors for Gastrointestinal Adverse Events in HIV Treated and Untreated Patients. *AIDS Rev* 2009; **11**: 30-38 [PMID: 19290032]

43 **Silverberg MJ**, Gore ME, French AL, Gandhi M, Glesby MJ, Kovacs A, Wilson TE, Young MA, Gange SJ. Prevalence of clinical symptoms associated with highly active antiretroviral therapy in the Women's Interagency HIV Study. *Clin Infect Dis* 2004; **39**: 717-724 [PMID: 15356788 DOI: 10.1086/423181]

44 **Feasey NA**, Healey P, Gordon MA. Review article: the aetiology, investigation and management of diarrhoea in the HIV-positive patient. *Aliment Pharmacol Ther* 2011; **34**: 587-603 [PMID: 21777262 DOI: 10.1111/j.1365-2036.2011.04781.x]

45 **Hicks CB**, DeJesus E, Sloan LM, Sension MG, Wohl DA, Liao Q, Ross LL, Pakes GE, Pappa KA, Lancaster CT; COL100758 Study Team. Comparison of once-daily fosamprenavir boosted with either 100 or 200 mg of ritonavir, in combination with abacavir/lamivudine: 96-week results from COL100758. *AIDS Res Hum Retroviruses* 2009; **25**: 395-403 [PMID: 19320570 DOI: 10.1089/aid.2008.0231]

46 **Johnson M**, Grinsztejn B, Rodriguez C, Coco J, DeJesus E, Lazzarin A, Lichtenstein K, Rightmire A, Sankoh S, Wilber R. Atazanavir plus ritonavir or saquinavir, and lopinavir/ritonavir in patients experiencing multiple virological failures. *AIDS* 2005; **19**: 685-694 [PMID: 15821394 DOI: 10.1097/01.aids.0000166091.39317.99]

47 **Wu X**, Li Y, Peng K, Zhou H. HIV protease inhibitors in gut barrier dysfunction and liver injury. *Curr Opin Pharmacol* 2014; **19**: 61-66 [PMID: 25105480 DOI: 10.1016/j.coph.2014.07.008]

48 **Braga Neto MB**, Aguiar CV, Maciel JG, Oliveira BM, Sevilleja JE, Oriá RB, Brito GA, Warren CA, Guerrant RL, Lima AA. Evaluation of HIV protease and nucleoside reverse transcriptase inhibitors on proliferation, necrosis, apoptosis in intestinal epithelial cells and electrolyte and water transport and epithelial barrier function in mice. *BMC Gastroenterol* 2010; **10**: 90 [PMID: 20701796 DOI: 10.1186/1471-230X-10-90]

49 **Macarthur RD**, Hawkins TN, Brown SJ, Lamarca A, Clay PG, Barrett AC, Bortey E, Paterson C, Golden PL, Forbes WP. Efficacy and safety of crofelemer for noninfectious diarrhea in HIV-seropositive individuals (ADVENT trial): a randomized, double-blind, placebo-controlled, two-stage study. *HIV Clin Trials* 2013; **14**: 261-273 [PMID: 24334179 DOI: 10.1310/hct1406-261]

50 **Frampton JE**. Crofelemer: a review of its use in the management of non-infectious diarrhoea in adult patients with HIV/AIDS on antiretroviral therapy. *Drugs* 2013; **73**: 1121-1129 [PMID: 23807722 DOI: 10.1007/s40265-013-0083-6]

51 **Gao WY**. Center for Drug Evaluation and Research. 2012. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/202292Orig1s000MedR.pdf

52 **Serwadda D**, Mugerwa RD, Sewankambo NK, Lwegaba A, Carswell JW, Kirya GB, Bayley AC, Downing RG, Tedder RS, Clayden SA, Weiss RA, Dalgleish AG. Slim disease: a new disease in Uganda and its association with HTLV-III infection. *Lancet* 1985; **2**: 849-852 [PMID: 2864575 DOI: 10.1016/S0140-6736(85)90122-9]

53 **Canani RB**, Spagnuolo MI, Cirillo P, Guarino A. Ritonavir combination therapy restores intestinal function in children with advanced HIV disease. *J Acquir Immune Defic Syndr* 1999; **21**: 307-312 [PMID: 10428109 DOI: 10.1097/00126334-199908010-00008]

54 **MacArthur RD**, DuPont HL. Etiology and pharmacologic management of noninfectious diarrhea in HIV-infected individuals in the highly active antiretroviral therapy era. *Clin Infect Dis* 2012; **55**: 860-867 [PMID: 22700829 DOI: 10.1093/cid/cis544]

55 **Hsu RB**, Tsay YG, Chen RJ, Chu SH. Risk factors for primary bacteremia and endovascular infection in patients without acquired immunodeficiency syndrome who have nontyphoid salmonellosis. *Clin Infect Dis* 2003; **36**: 829-834 [PMID: 12652381 DOI: 10.1086/367932]

56 **Dryden MS**, Gabb RJ, Wright SK. Empirical treatment of severe acute community-acquired gastroenteritis with ciprofloxacin. *Clin Infect Dis* 1996; **22**: 1019-1025 [PMID: 8783703 DOI: 10.1093/clinids/22.6.1019]

57 **Sherman DS**, Fish DN. Management of protease inhibitor-associated diarrhea. *Clin Infect Dis* 2000; **30**: 908-914 [PMID: 10854364 DOI: 10.1086/313826]

58 **Ghai S**, Pattison J, Ghai S, O'Malley ME, Khalili K, Stephens M. Primary gastrointestinal lymphoma: spectrum of imaging findings with pathologic correlation. *Radiographics* 2007; **27**: 1371-1388 [PMID: 17848697 DOI: 10.1148/rg.275065151]

59 **Renn A**, Kazmi F, Khan N, Rawal B, O'Boyle E. The HIV manifestations within the gastrointestinal tract: A pictorial review. *SA J Radiol* 2017; **21**: 1233 [PMID: 31754480 DOI: 10.4102/sajr.v21i2.1233]

60 **Klausner JD**, Kohn R, Kent C. Etiology of clinical proctitis among men who have sex with men. *Clin Infect Dis* 2004; **38**: 300-302 [PMID: 14699467 DOI: 10.1086/380838]

61 **Koh DM**, Dzik-Jurasz A, O'Neill B, Tait D, Husband JE, Brown G. Pelvic phased-array MR imaging of anal carcinoma before and after chemoradiation. *Br J Radiol* 2008; **81**: 91-98 [PMID: 18238920 DOI: 10.1259/bjr/96187638]

62 **Manfredi R**, Calza L. HIV infection and the pancreas: risk factors and potential management guidelines. *Int J STD AIDS* 2008; **19**: 99-105 [PMID: 18334062 DOI: 10.1258/ijsa.2007.007076]

63 **Riedel DJ**, Gebo KA, Moore RD, Lucas GM. A ten-year analysis of the incidence and risk factors for acute pancreatitis requiring hospitalization in an urban HIV clinical cohort. *AIDS Patient Care STDS* 2008; **22**: 113-121 [PMID: 18260802 DOI: 10.1089/apc.2007.0034]

64 **Kibirige D**, Ssekitoleko R. Endocrine and metabolic abnormalities among HIV-infected patients: a current review. *Int J STD AIDS* 2013; **24**: 603-611 [PMID: 23970578 DOI: 10.1177/0956462413479895]

65 **Rickels MR**, Bellin M, Toledo FG, Robertson RP, Andersen DK, Chari ST, Brand R, Frulloni L, Anderson MA, Whitcomb DC; PancreasFest Recommendation Conference Participants. Detection, evaluation and treatment of diabetes mellitus in chronic pancreatitis: recommendations from PancreasFest 2012. *Pancreatology* 2013; **13**: 336-342 [PMID: 23890130 DOI: 10.1016/j.pan.2013.05.002]

66 **Carroccio A**, Fontana M, Spagnuolo MI, Zuin G, Montalto G, Canani RB, Verghi F, Di Martino D, Bastoni K, Buffardi F, Guarino A. Pancreatic dysfunction and its association with fat malabsorption in HIV infected children. *Gut* 1998; **43**: 558-563 [PMID: 9824586 DOI: 10.1136/gut.43.4.558]

67 **Yilmaz A**, Hagberg L. Exocrine pancreatic insufficiency is common in people living with HIV on effective antiretroviral therapy. *Infect Dis (Lond)* 2018; **50**: 193-199 [PMID: 28838283 DOI: 10.1080/23744235.2017.1370126]

World Journal of Virology

Submit a Manuscript: https://www.f6publishing.com

World J Virol 2025 March 25; 14(1): 100003

DOI: 10.5501/wjv.v14.i1.100003

ISSN 2220-3249 (online)

MINIREVIEWS

Crimean-Congo hemorrhagic fever: Pathogenesis, transmission and public health challenges

Sita Kumari Karanam, Kandra Nagvishnu, Praveen Kumar Uppala, Sandhya Edhi, Srinivasa Rao Varri

Specialty type: Virology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B
Novelty: Grade C
Creativity or Innovation: Grade C
Scientific Significance: Grade C

P-Reviewer: Ramos-de-Souza J

Received: August 5, 2024
Revised: October 11, 2024
Accepted: November 5, 2024
Published online: March 25, 2025
Processing time: 114 Days and 20.4 Hours

Sita Kumari Karanam, Department of Pharmaceutical Biotechnology, Maharajah's College of Pharmacy, Vizianagaram 535002, Andhra Pradesh, India

Kandra Nagvishnu, Department of Pharmacology, Santhiram Medical College and General Hospital, Nandyal 518501, Andhra Pradesh, India

Praveen Kumar Uppala, Department of Pharmacology, Maharajah's College of Pharmacy, Vizianagaram 535002, Andhra Pradesh, India

Sandhya Edhi, Department of Pharmacy, Maharajah's College of Pharmacy, Vizianagaram 535002, Andhra Pradesh, India

Srinivasa Rao Varri, Department of Pharmaceutical Analysis, Maharajah's College of Pharmacy, Vizianagaram 535002, Andhra Pradesh, India

Corresponding author: Praveen Kumar Uppala, PhD, Assistant Professor, Research Fellow, Department of Pharmacology, Maharajah's College of Pharmacy, Phool Baugh, Vizianagaram 535002, Andhra Pradesh, India. praveen.chintu32@gmail.com

Abstract

The dangerous Crimean-Congo hemorrhagic fever virus (CCHFV), an encapsulated negative-sense RNA virus of the family Nairoviridae, is transmitted from person to person *via* ticks. With a case fatality rate between 10% to 40%, the most common ways that the disease may spread to humans are *via* tick bites or coming into touch with infected animals' blood or tissues. Furthermore, the transfer of bodily fluids between individuals is another potential route of infection. There is a wide range of symptoms experienced by patients throughout each stage, from myalgia and fever to extreme bruising and excess bleeding. Tick management measures include minimising the spread of ticks from one species to another and from people to animals *via* the use of protective clothing, repellents, and proper animal handling. In order to prevent the spread of illness, healthcare workers must adhere to stringent protocols. Despite the lack of an authorised vaccine, the main components of treatment now consist of preventative measures and supportive care, which may include the antiviral medicine ribavirin. We still don't know very much about the virus's mechanisms, even though advances in molecular virology and animal models have improved our understanding of the pathogenesis of CCHFV. A critical need for vaccination that is both safe and effective, as well as for quick diagnosis and efficient treatments to lessen the disease's impact in areas where it is most prevalent. Important steps towards

lowering Crimean-Congo hemorrhagic fever mortality and morbidity rates were to anticipatethe future availability of immunoglobulin products.

Key Words: Crimean-congo haemorrhagic fever; Tick-borne illness; Immunoglobulins; Viral hemorrhagic fever; Antiviral therapy

Core Tip: This review provides a comprehensive overview of Crimean-Congo hemorrhagic fever (CCHF), a severe tick-borne viral disease with significant public health implications. The article discusses the virus's transmission dynamics, clinical manifestations, current diagnostic techniques, and available treatments, including the use of antiviral therapy. It emphasizes the urgent need for vaccine development, better diagnostic tools, and efficient therapies. By addressing gaps in knowledge and highlighting the importance of a one health approach, this review serves as a critical resource for researchers and healthcare professionals seeking to improve CCHF control and prevention strategies.

Citation: Karanam SK, Nagvishnu K, Uppala PK, Edhi S, Varri SR. Crimean-Congo hemorrhagic fever: Pathogenesis, transmission and public health challenges. *World J Virol* 2025; 14(1): 100003
URL: https://www.wjgnet.com/2220-3249/full/v14/i1/100003.htm
DOI: https://dx.doi.org/10.5501/wjv.v14.i1.100003

INTRODUCTION

Between 10%-40% of infected people die during Crimean-Congo hemorrhagic fever virus (CCHFV) outbreaks of severe viral hemorrhagic fever. As a febrile sickness, the disease was first known as Crimean haemorrhagic fever when it was initially discovered in 1944 among troops on the Crimean Peninsula (near the Black Sea)[1]. The present name of the disease was given to it in 1969 when the same bacterium was identified as the cause of febrile sickness in the Congo Basin. The main vector and reservoir of CCHFV are ticks of the species Hyalomma[2]. The species of Hyalomma tick that causes Crimean-Congo hemorrhagic fever (CCHF) depends on the geographic region. Hyalomma marginatum is the primary vector of CCHF in Europe and the Balkans where as Hyalomma anatolicum in Iran, Pakistan, Turkmenistan, and Tajikistan, Hyalomma asiaticum in Central Asia and China and Hyalomma rufipes in Africa.

Many non-domesticated animal species, including ostriches, buffalo, tiny rodents, hares, and rhinoceroses, may be infected with CCHFV, according to serological investigations. Important amplifying hosts for the transmission of tick-borne diseases include animals that ingest infected ticks or co-feed with them[3]. Tick bites or coming into contact with sick animals during slaughter are the most common ways for humans to get the disease. In humans, an infection usually manifests as a fever, which may later develop into a hemorrhagic condition and could be deadly. Particularly in areas without access to high-containment facilities, molecular testing have allowed for safe and quick diagnosis. Sheep, goats, and cattle are just a few of the many domestic and wild animal species that may harbour the CCHFV[4].

Although most birds can withstand infections, ostriches aren't immune and may have a high infection rate in regions where it is common. The tick-borne disease stays in an infected animal's bloodstream for around seven days after infection[5]. When another tick bites the sick animal, the tick-animal-tick cycle might resume. Hyalomma ticks are the most common vectors of the CCHFV (Figure 1), however infections may occur in other tick genera as well.

Although CCHF is common in many regions of the globe, including the Indian subcontinent, northwest China, Africa, the Balkans, and Eastern Europe, there is no vaccine that can protect people or animals against this disease at this time[6].

The four families of viruses known to cause viral hemorrhagic fevers are Arenaviridae, Bunyaviridae, Filoviridae, and Flaviviridae which cause severe systemic febrile diseases (Table 1). The arenaviridae family of viruses includes both Old World and New World Strains, and they are known to cause infections transmitted by rodents. Europe, Asia, and Africa are just a few of the numerous regions where rats are susceptible to viral infections[7]. Urine or droppings from rodents are a common vector for infection. A 50% case mortality rate has been reported in West African epidemics caused by the arenavirus Lassa. Insects and rodents may transmit viruses that belong to the Bunyaviridae family, which can cause mild to severe illness[8]. Rift Valley fever, hemorrhagic fever in the Crimean-Congo region, and hantavirus infections are among the most prominent ailments. The Ixodid tick is the vector for the virus. The family Filoviridae includes the Ebola virus and Marburg hemorrhagic sickness, both of which have been found in African bats. The danger of transmission from person to person is considerable for human infections, particularly among those in care takers[9]. In low-income nations, the case fatality rate for Marburg hemorrhagic fever may reach 82%, while in the Democratic Republic of Congo, it can reach 80% to 90%. Various forms of infections are carried by the flavivirus family, which is carried by arthropods [10]. Dengue fever affects more than a hundred nations throughout Europe, Asia, Australia, Africa, and the Pacific Islands. It is a global health crisis. The mosquitoes Aedes aegypti and Aedes albopictus are vectors for the flavivirus that causes dengue fever. The disease can progress through three stages: (1) Mild; (2) Moderate; and (3) Severe[11].

Table 1 Different types of viral haemorrhagic fevers and causative agents

Family	Causative virus	Disease	Symptoms	Treatment
Arenaviridae	Lassa virus	Lassa fever	Fever, weakness, and haemorrhage	Supportive care and ribavirin
	Junin virus	Argentine haemorrhagic fever	Fever, malaise, and haemorrhage	Supportive care
	Chapare virus	Chapare hemorrhagic fever	Fever, malaise, headache, vomiting and diarrhoea	Supportive care and early diagnosis
	Guanarito virus	Venezuelan hemorrhagic fever	Confusion, convulsions, coma, and bleeding from body orifices	No specific anti-viral treatment is available
	Lujo virus	Lujo hemorrhagic fever	Fever, headache, vomiting, diarrhea, arthralgia, and myalgia	Supportive care
	Lymphocytic choriomeningitis virus	Lymphocytic choriomeningitis	Fever (38.5 °C to 40 °C), malaise, myalgia, retro-orbital headache, photophobia, and anorexia	Supportive care and ribavirin
	Machupo virus	Bolivian hemorrhagic fever	Fever, malaise, fatigue headache, dizziness, myalgias, and severe lower back pain	Supportive care
	Sabia virus	Brazilian hemorrhagic fever	High fever, fatigue, maculopapular/petechial rash bleeding and haemorrhage	Supportive care and ribavirin antiviral drug
Bunyaviridae	Crimean-Congo haemorrhagic fever virus	Crimean-Congo haemorrhagic fever	Fever, myalgia, and haemorrhage	Supportive care and ribavirin
	Hantan virus	Hantavirus pulmonary syndrome	Fever, muscle pain, and pulmonary edema	Supportive care
	Dobrava-Belgrade virus	Hemorrhagic fever with renal syndrome	Intense headache, back and abdominal pain, fever, chills, and blurred vision	Supportive therapy, renal dialysis. Treatment with ribavirin
	Seoul virus	Hemorrhagic fever with renal syndrome	Intense headache, back and abdominal pain, fever, chills, and blurred vision	Supportive therapy, renal dialysis. Treatment with ribavirin
	Puumalavirus	Hemorrhagic fever with renal syndrome	Intense headache, back and abdominal pain, fever, chills, and blurred vision	Supportive therapy, renal dialysis. Treatment with ribavirin
	Rift Valley fever virus	Rift Valley fever	Transient fever, headache, severe muscle and joint pain, photophobia and anorexia	Drugs like Ibuprofen or Acetaminophen
	Saaremaa virus	Hemorrhagic fever with renal syndrome	Intense headache, back and abdominal pain, fever, chills, and blurred vision	Supportive therapy, renal dialysis. Treatment with ribavirin
	Sin nombre virus	Hantavirus pulmonary syndrome	Fever, muscle pain, and pulmonary edema	Intubation and oxygen therapy, fluid replacement and use of medications to support blood pressure
	Severe fever and thrombocytopenia syndrome virus	Severe fever and thrombocytopenia syndrome	Fever, vomiting, diarrhoea, multiple organ failure, thrombocytopenia, and leucopoenia elevated liver enzyme levels	Intravenous ribavirin
	Tula virus	Hemorrhagic fever with renal syndrome	Intense headache, back and abdominal pain, fever, chills, and blurred vision	Supportive therapy, renal dialysis. Treatment with ribavirin
Filoviridae	Bundibugyo ebola virus	Ebola virus disease	Fever, severe hemorrhage, and organ failure	Supportive care and experimental treatments
	Marburg marburg virus	Marburg haemorrhagic fever	Fever, severe hemorrhage, and organ failure	Supportive care and experimental treatments
	Sudan ebola virus	Ebola virus disease	Sudden onset of fever, fatigue, muscle pain, headaches, sore throat, vomiting, diarrhoea, rash, impaired kidney, and liver functions	Monoclonal antibodies like Inmazeb and Ebanga
	Tai forest ebola virus	Ebola virus disease	Sudden onset of fever, fatigue, muscle pain, headaches, sore throat, vomiting, diarrhoea, rash, impaired kidney, and liver	Monoclonal antibodies like Inmazeb and Ebanga
	Zaire ebola virus	Ebola virus disease	Sudden onset of fever, fatigue, muscle pain, headaches, sore throat, vomiting, diarrhoea, rash, impaired kidney, and liver functions	Monoclonal antibodies like Inmazeb and Ebanga
Flaviviridae	Dengue virus	Dengue fever	Fever, rash, and haemorrhage	Supportive care and fluids
	Kyasanur forest disease virus	Kyasanur forest disease	Sudden onset of chills, fever, and headache	Supportive treatment with maintenance of proper hydration and circulation by transfusion of

			IV fluids
Omsk hemorrhagic fever virus	Omsk hemorrhagic fever	Fever, headache, myalgia, cough, petechial rash or bruises	Supportive care
Yellow fever virus	Yellow fever	Fever, chills, headache, back pain, vomiting, and fatigue	Rest, hydration and seek medical advice

Transmission

Tick bites or coming into touch with infected animal blood or tissues during or just after slaughter are the main routes of transmission for the CCHF virus to humans". Direct contact with infected blood, saliva, organs, or other bodily fluids is the most typical way infectious illnesses are transmitted from one person to another (Figure 2)[12]. Additional factors that may lead to nosocomial infections include reusing needles, contaminated medical supplies, and insufficient sterilisation of medical equipment[13].

EPIDEMIOLOGY OF CCHF IN INDIA

On June 29, a male patient suffering from CCHF passed away in a private hospital in Ahmedabad. He was 51 years old and lived in the hamlet of Lakhapar in the Anjar taluka of Kutch. According to health officials, this is the first case of CCHF that has been documented in Gujarat this year. The state of Gujarat recorded five instances of confirmed CCHF in 2022. Since 2011, when the state first recorded a case of CCHF, Gujarat has been the reporting centre for the vast majority of CCHF cases in India[14]. Gujarat reported verified cases of CCHF from 2011 to 2019, with Rajasthan reporting extra cases in 2014, 2015, and 2019. A 39-year-old guy who survived after testing positive for the virus in March 2022 was a cattle rearer. One of them was a 55-year-old housewife who died of CCHF after a tick bit her while she was tending to her cattle. One case of CCHF was recorded in the Sabarkantha district in 2021 by the state[15].

EPIDEMIOLOGY OF CCHF IN AFRICA

There were 494 CCHF cases (115 fatal) recorded in Africa between January 1, 1956, and July 25, 2020. Over the last decade, nine nations Kenya, Mali, Mozambique, Nigeria, Senegal, Sierra Leone, South Sudan, Sudan, and Tunisia have reported the first cases of CCHF[16].

Within the Bunyavirales order, the Nairoviridae family, and the Orthonairo virus genus, there will be enveloped, negative-sense RNA virus known as CCHFV. One crucial component of the virus is the envelope Gn glycoprotein, which, at its C-terminus, possesses a cytoplasmic tail. The genetic code of CCHFV consists of S, M, and L segments of RNA. Segment L encodes an RNA-dependent RNA polymerase, segment M viral glycoproteins, and segment S the nucleocapsid protein (N) (Figure 3)[17].

STAGES OF CCHF

Incubation stage

The duration of incubation is usually less than a week, falling anywhere from one to nine days (depending on the way the virus was exposed to and the dosage). It lasts the shortest time after a tick bite (about 1-3 days) and the longest time after coming into contact with contaminated human or cattle blood, tissue, or secretions (5-6 days).

Pre-hemorrhagic stage

Begins suddenly with vague symptoms and lasts an average of two to four days (range: One to seven days). Signs and symptoms may manifest as a high temperature (39–41 °C), neck discomfort or stiffness, dizziness, headache, myalgia, backache, eye pain, or photophobia. Nausea, vomiting, diarrhoea, stomach ache, and a sore throat are possible side effects. Jaundice, conjunctivitis, congested sclera, and hyperaemia of the chest, neck, and face are some possible symptoms. Hepatomegaly and splenomegaly, as well as changes in mood and sensory perception (such as somnolence supplanting agitation), may be seen in extreme instances.

Hemorrhagic stage

Usually brief (around two or three days), but may last up to two weeks. One distinctive aspect of CCHF compared to other viral haemorrhagic fevers is the wide variety of hemorrhagic symptoms it may cause, which can vary from little bleeding patches (peteziae) to widespread discolouration of the skin (ecchymosis) on both the skin and mucosal membranes. Some common symptoms may include injection site bleeding, epistaxis, melena, haematuria, haemoptysis, and haematemesis.

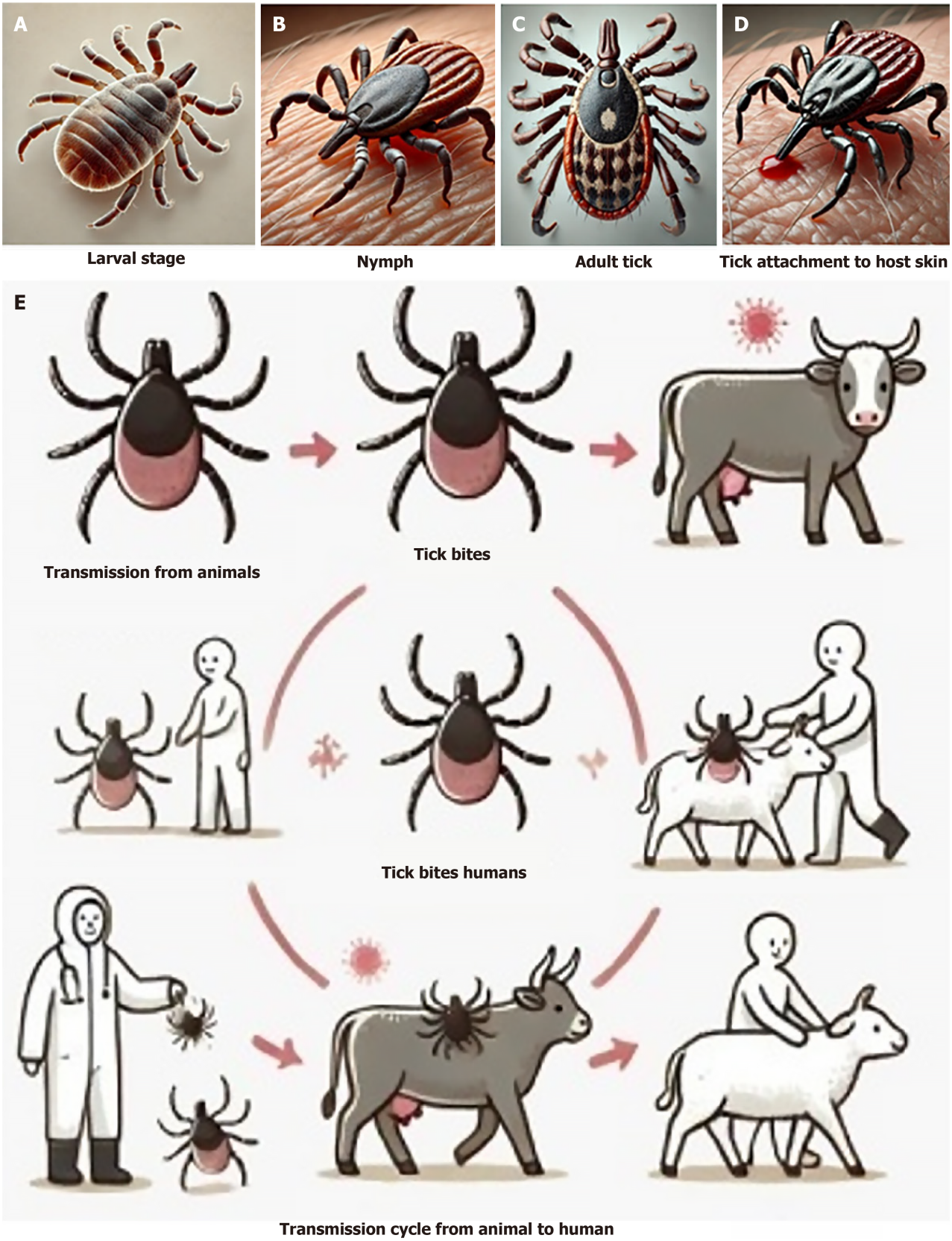


Figure 1 Lifecycle and vector role of Hyalomma ticks in Crimean-Congo hemorrhagic fever transmission. A.Larval stage of the Hyalomma tick; B: Nymphal stage attached to a host; C: Adult Hyalomma tick, the main vector for Crimean-Congo hemorrhagic fever virus; D: Close-up of the tick attachment to host skin for blood-feeding; E: Transmission cycle from animal to human through tick bites and handling infected animals.

Convalescence stage

Within 9–10 days after the beginning of the disease (within a range of 9–20 days), convalescence often occurs in survivors, in the same vein as when laboratory measurements were back to normal. Symptoms such as hypotension, bradycardia or tachycardia, polyneuritis, difficulty breathing, xerostomia, impaired vision or hearing, hair loss, memory loss, and other difficulties might manifest during this protracted period. Although long-term consequences have not been thoroughly investigated, there is no solid proof of recurrence or a biphasic progression of the illness[18-21].

Signs and symptoms

Depending on how the virus is acquired, the incubation time for CCHF might vary in duration. The incubation phase, which begins three to nine days following a tick bite, is when the disease is usually spread. Contact with infected blood or tissues may spread viruses, and the incubation period for these viruses can be anywhere from 5 days to 6 days, up to 13 days[22]. A broad variety of symptoms, including myalgia, vertigo, headache, neck pain, backache, and photophobia, all manifest suddenly. Petechiae, or red patches on the palate, red eyes, heated cheeks, and a red throat are other common early signs (Figure 4). In the beginning, you can have a sore throat, nausea, vomiting, diarrhoea, stomach ache, and confusion[23]. In the two to four days after the start of symptoms, restlessness, melancholy, and lassitude may take the place of agitation. Additionally, the stomach discomfort may shift to the upper right quadrant and be accompanied by noticeable hepatomegaly. About 30% of those with CCHF will pass away throughout the course of their disease, usually during the second week. On the ninth or tenth day after the start of symptoms, individuals who are able to recover often start to feel better. Symptoms such as extensive bruising, nosebleeds, and uncontrolled bleeding at injection sites become apparent on day four of sickness and last for around two weeks. The reported mortality rates of CCHF patients in hospitals range from 9% to 50%[24].

Diagnosis in laboratory

A numberof laboratory techniques may be used to diagnose CCHF. By integrating enzyme-linked immunosorbent assay antigen capture for viral antigen detection with RT-PCR in blood or tissues collected from a dead patient and virus isolation, it is feasible to detect CCHF in its acute phase in individuals with a proper medical history. Tissues treated with formalin may also be stained immune-histo-chemically to reveal viral antigens (Figure 5)[25].

Treatment

The main strategy is to provide general supportive care with an emphasis on symptom treatment. Both the oral and injectable forms of the antiviral medication ribavirin have shown efficacy in treating CCHF infection. Fluid balance, electrolyte imbalances, oxygenation, haemodynamic support, and secondary infection therapy should all be part of the supportive care plan[26]. At this time, neither humans nor animals have access to any immunizations that have proven successful. Without a vaccine, informing the public about the virus and its dangers and encouraging them to take precautions against exposure is the only method to lessen the likelihood of infection. Everyone who works with animals or in agriculture should wear insect repellent and stay away from potentially infectious blood and other body fluids[27]. Control and prevention The Unnoticed tick-animal-tick cycle poses a significant challenge to tick-borne disease prevention and management. Ticks are abundant, and only properly supervised livestock farms are allowed to use acaricides. There is now no widely accessible, safe, and effective vaccination against CCHF for humans, despite the development and limited use of an inactivated vaccine produced from the mouse brain in Eastern Europe[28]. Public health advice several factors should be at the centre of public health recommendations.

Reducing the risk of tick-to-human transmission: Put on protective clothing, such as long pants and sleeves. To make ticks easier to see, dress in bright colours. Approved acaricides should be applied on garments. To protect one's skin and clothes, use an authorised repellant. Keep an eye out for ticks on a frequent basis, and gently remove them if you discover any. Get out of areas where ticks are common and stay away from areas where ticks are active.

Reducing the risk of animal-to-human transmission: When dealing with animals or their tissues in endemic regions, it is important to use protective clothing, such as gloves, whether you are in an abattoir or at home. This is particularly true while butchering, culling or slaughtering. Either regularly treat animals with pesticides two weeks before slaughter or quarantine them before they reach slaughterhouses.

Reducing the risk of human-to-human transmission in the community: Avoid physical contact with someone who seems to be sick with CCHF. When caring for sick persons, always wear protective clothing, including gloves. After touching or visiting someone who is sick, be sure to wash your hands often.

Controlling infection in health-care workers: All healthcare personnel who come into contact with patients who have CCHF, whether it's suspected or proven, or who handle specimens from these patients, should follow basic procedures for infection control. Basic hand hygiene, personal protective equipment usage, safe injection procedures, and proper burial procedures are all included in this. Samples collected from patients suspected of having CCHF should only be handled by qualified personnel in labs with the proper equipment[29-31].

Health concept: A health concept, emphasizing the interconnectedness of human, animal, and environmental health in the context of CCHF. The zoonotic nature of CCHFV and the role of livestock, wildlife, and tick habitats in the transmission cycle make this approach particularly relevant. By addressing these links, the review highlights the importance of integrated efforts across human, veterinary, and environmental health sectors to effectively manage and

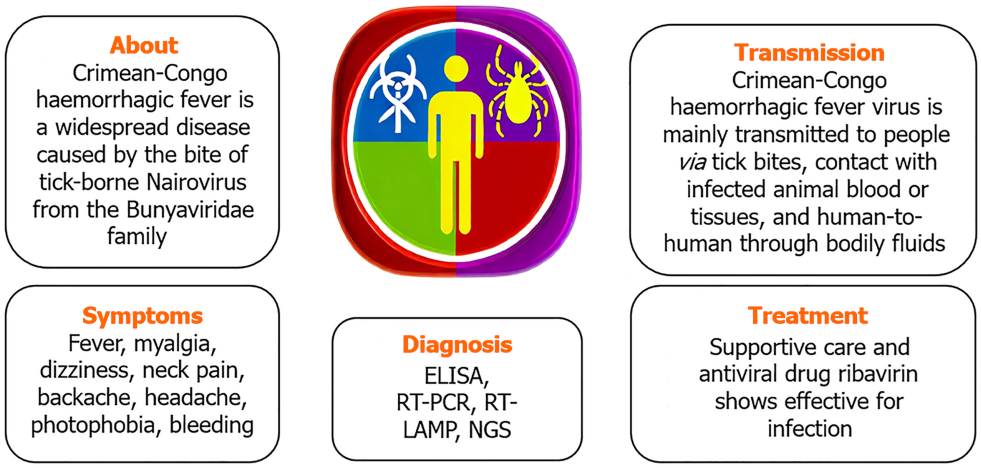


Figure 2 Overview of clinical stages and symptoms in Crimean-Congo hemorrhagic fever patients. Early stage with fever, myalgia, and fatigue. Pre-hemorrhagic stage with signs of conjunctival hemorrhage. Hemorrhagic stage showing extensive petechiae and ecchymosis. Recovery phase after supportive treatment. Post-recovery convalescence with residual symptoms. ELISA: Enzyme-linked immunosorbent assay; RT-LAMP: Reverse transcription loop-mediated isothermal amplification; NGS: Next-generation sequencing.

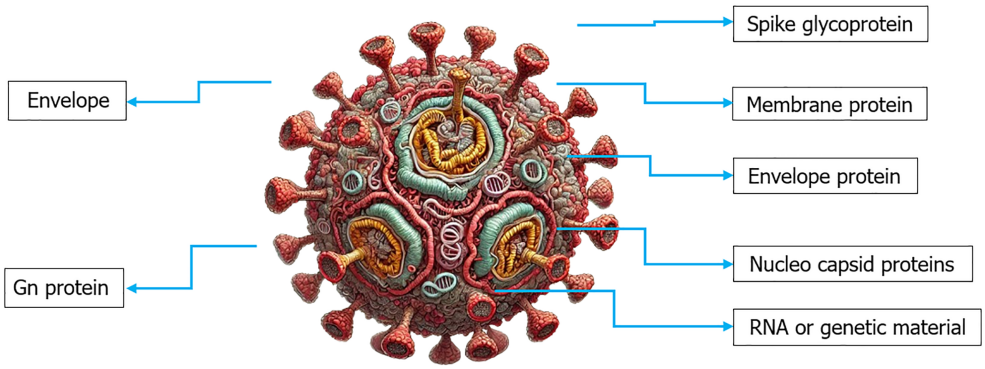


Figure 3 Structure of Crimean-Congo hemorrhagic fever virus. This includes electron microscopy image of the Crimean-Congo hemorrhagic fever virus virion, schematic of the viral genome, showing S, M, and L RNA segments, envelope structure with Gn and Gc glycoproteins, viral RNA polymerase encoded by the L segment, nucleocapsid protein (N) from the S segment.

control the disease.

CONCLUSION

Conclusion despite the vast number of individuals who could be affected and the virus's extensive circulation, much remains unclear about the viral and host variables that contribute to CCHFV pathogenesis. We will gain greater mechanistic insights into the mechanisms by which CCHFV causes illness when molecular virology techniques and better small-animal models are developed. It is probable that novel viral protein functions will yet be found. Educating the public, limiting tick contact, treating livestock to minimise infestations, quarantining animals, and safeguarding people involved in high-risk activities are all necessary preventative actions for communities at risk in endemic regions. Limiting the impact of CCHF on patients and public health systems requires effective vaccinations and antivirals, as well as prompt and accurate diagnostics. Future perspective research one hope for the future of CCHF therapy is the availability of immunoglobulin products and other alternative medicines. In order to create targeted treatments, researchers need a deeper knowledge of the CCHF pathophysiology. Developing a vaccine against CCHF is an important objective, despite the fact that it is difficult and is not nearing practical application. People in endemic locations are eagerly awaiting the development of a CCHF vaccination since it is the most effective way to decrease the mortality and morbidity caused by CCHF. This review discussed not only the development of new antiviral drugs and vaccines but also basic research aimed at better understanding the mechanisms of the virus, improving tick control strategies, and addressing the human-animal-environment interface. The review aims to serve as a foundation for future studies and interventions in these critical areas.

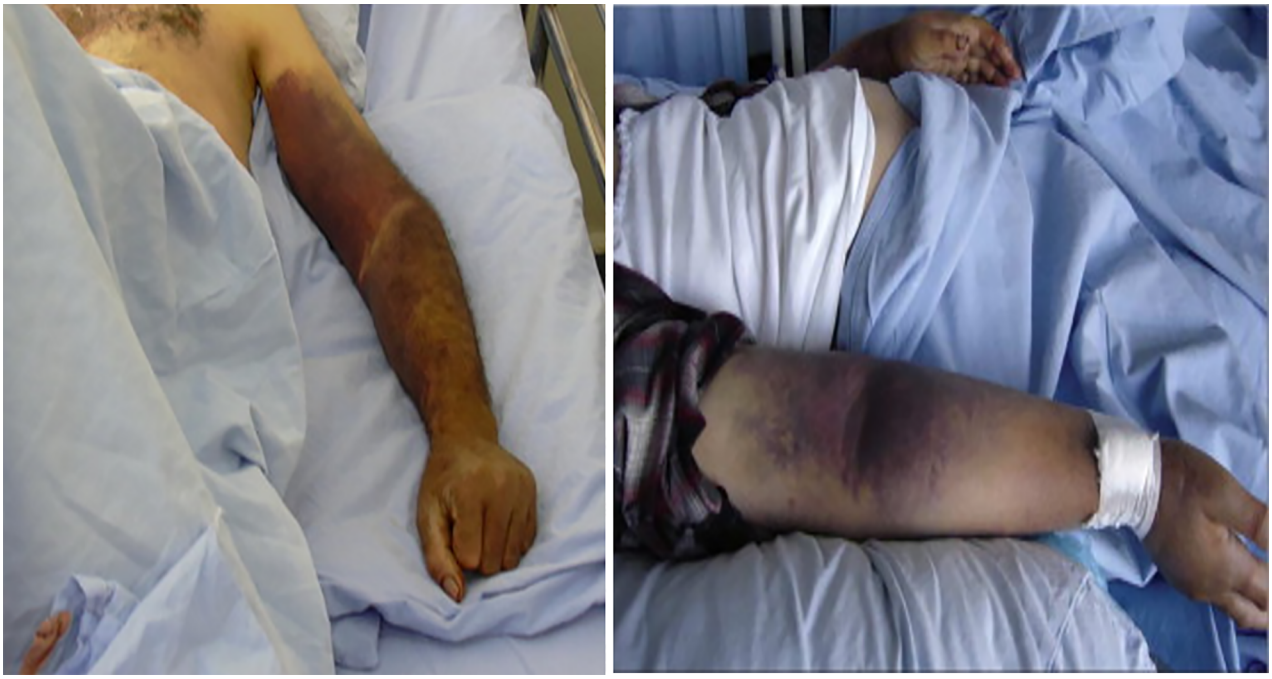


Figure 4 Petechiae on the hand of a Crimean-Congo hemorrhagic fever patient. Petechiae are small red or purple spots resulting from capillary bleeding under the skin, commonly seen in the hemorrhagic phase of Crimean-Congo hemorrhagic fever. These non-blanching spots, distributed unevenly across the skin, indicate the severity of infection and can be accompanied by additional hemorrhagic symptoms like ecchymosis and mucosal bleeding.

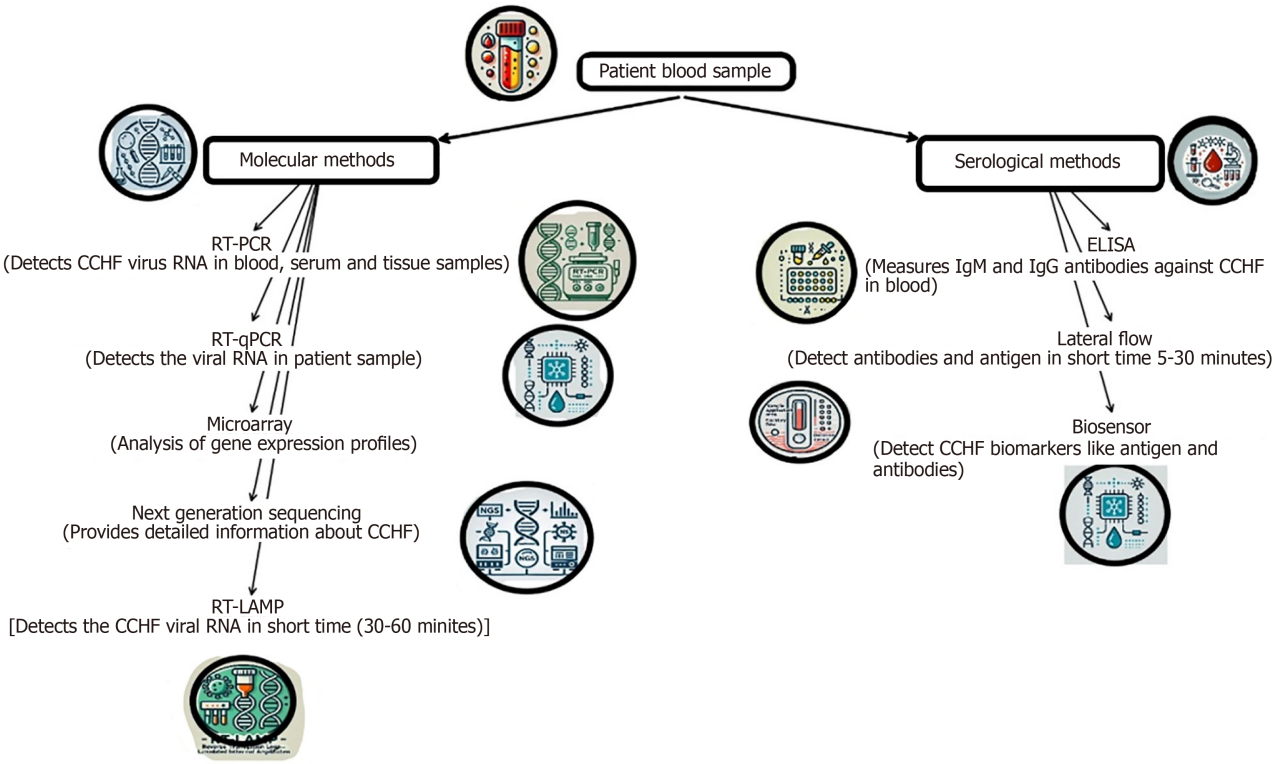


Figure 5 Laboratory diagnostic methods for Crimean-Congo hemorrhagic fever detection. PCR amplification of Crimean-Congo hemorrhagic fever virus (CCHFV) RNA in patient blood samples, enzyme-linked immunosorbent assay antigen capture method for viral antigen detection in serum samples, viral isolation technique from infected tick tissues, immunohistochemical staining of CCHFV antigens in formalin-fixed tissue, real-time PCR results showing viral load quantification. CCHF: Crimean-Congo hemorrhagic fever; RT-LAMP: Reverse transcription loop-mediated isothermal amplification.

ACKNOWLEDGEMENTS

I acknowledgement my sincere thanks to Maharajah's College of Pharmacy, Vizianagaram for continuous support and cooperation for completion of this work.

FOOTNOTES

Author contributions: Karanam SK was responsible for drafting the article or revising it critically for important intellectual content and manuscript final review; Nagvishnu K was responsible for concept, acquisition of the data, and analysis interpretation of the data; Uppala PK was responsible for design of the study, the conception, preparation of final manuscript; Edhi S was responsible for design of the study acquisition of the data, and analysis; Varri SR was responsible for literature search, acquisition of the data, and analysis; all of the authors read and approved the final version of the manuscript to be published.

Conflict-of-interest statement: The authors declare that there are no conflicts of interest regarding the publication of this manuscript. All authors have contributed to this work based solely on scientific and academic merit, without any influence from commercial, personal, political, intellectual, or religious interests.

Country of origin: India

ORCID number: Sita Kumari Karanam 0000-0003-0133-3985; Kandra Nagvishnu 0000-0002-2185-8714; Praveen Kumar Uppala 0000-0002-6524-5303; Sandhya Edhi 0009-0005-3100-1860.

S-Editor: Luo ML
L-Editor: A
P-Editor: Yu HG

REFERENCES

1 **Huang HF**, Huang I, Matschke J. Treatment strategy for venous congestion in digit replantations. *J Plast Reconstr Aesthet Surg* 2023; **83**: 80-83 [PMID: 37270999 DOI: 10.1016/j.bjps.2023.05.011]
2 **Ergonul O**, Whitehouse CA. Crimean-Congo Hemorrhagic Fever. Germany: Springer, 2007 [DOI: 10.1007/978-1-4020-6106-6]
3 **Ono K**, Kuroda H. [Pannus Formation Two Years after Bioprosthetic Aortic Valve Implantation;Report of a Case]. *Kyobu Geka* 2015; **68**: 785-787 [PMID: 26329714]
4 **Akıncı E**, Bodur H, Leblebicioglu H. Pathogenesis of Crimean-Congo hemorrhagic fever. *Vector Borne Zoonotic Dis* 2013; **13**: 429-437 [DOI: 10.1089/vbz.2012.1061]
5 **Bains RS**, Wells S, Sillito RR, Armstrong JD, Cater HL, Banks G, Nolan PM. Assessing mouse behaviour throughout the light/dark cycle using automated in-cage analysis tools. *J Neurosci Methods* 2018; **300**: 37-47 [PMID: 28456660 DOI: 10.1016/j.jneumeth.2017.04.014]
6 **Whitehouse CA**. Crimean-Congo hemorrhagic fever. *Antiviral Res* 2004; **64**: 145-160 [PMID: 15550268 DOI: 10.1016/j.antiviral.2004.08.001]
7 **Frank MG**, Weaver G, Raabe V; State of the Clinical Science Working Group of the National Emerging Pathogens Training and Education Center's Special Pathogens Research Network. Crimean Congo Hemorrhagic Fever Virus for Clinicians-Virology, Pathogenesis, and Pathology. *Emerg Infect Dis* 2024; **30**: 847-853 [PMID: 38666566 DOI: 10.3201/eid3005.231646]
8 **Vorou R**, Pierroutsakos IN, Maltezou HC. Crimean-Congo hemorrhagic fever. *Curr Opin Infect Dis* 2007; **20**: 495-500 [PMID: 17762783 DOI: 10.1097/QCO.0b013e3282a56a0a]
9 **Verreault J**, Villa RA, Gabrielsen GW, Skaare JU, Letcher RJ. Maternal transfer of organohalogen contaminants and metabolites to eggs of Arctic-breeding glaucous gulls. *Environ Pollut* 2006; **144**: 1053-1060 [PMID: 16563578 DOI: 10.1016/j.envpol.2005.10.055]
10 **Chiappelli F**, Fotovat L. Post acute CoViD-19 syndrome (PACS)-Long CoViD. *Bioinformation* 2022; **18**: 908-911 [PMID: 37654836 DOI: 10.6026/97320630018908]
11 **Haim S**, Gilhar A, Cohen A. Cutaneous manifestations associated with aminoaciduria. Report of two cases. *Dermatologica* 1978; **156**: 244-250 [PMID: 624434 DOI: 10.1159/000250922]
12 History of sports medicine in The Netherlands. *Br J Sports Med* 1989; **23**: 219-221 [PMID: 2698252 DOI: 10.1136/bjsm.23.4.219]
13 **Martin PA**, Robins HI, Dennis WH. Monitoring body site temperatures during systemic hyperthermia. *Crit Care Med* 1987; **15**: 163-164 [PMID: 3802862 DOI: 10.1097/00003246-198702000-00018]
14 **Ahmed AM**, Miyoshi SI, Shinoda S, Shimamoto T. Molecular characterization of a multidrug-resistant strain of enteroinvasive Escherichia coli O164 isolated in Japan. *J Med Microbiol* 2005; **54**: 273-278 [PMID: 15713611 DOI: 10.1099/jmm.0.45908-0]
15 **Lietard J**, Abou Assi H, Gómez-Pinto I, González C, Somoza MM, Damha MJ. Mapping the affinity landscape of Thrombin-binding aptamers on 2′F-ANA/DNA chimeric G-Quadruplex microarrays. *Nucleic Acids Res* 2017; **45**: 1619-1632 [PMID: 28100695 DOI: 10.1093/nar/gkw1357]
16 **Ergönül O**. Crimean-Congo haemorrhagic fever. *Lancet Infect Dis* 2006; **6**: 203-214 [PMID: 16554245 DOI: 10.1016/S1473-3099(06)70435-2]

17 **Yan C**, Li HR, Chen X, Zhang XQ, Cheng XB, Xu R, Huang JQ, Zhang Q. Regulating the Inner Helmholtz Plane for Stable Solid Electrolyte Interphase on Lithium Metal Anodes. *J Am Chem Soc* 2019; **141**: 9422-9429 [PMID: 31117672 DOI: 10.1021/jacs.9b05029]

18 **Marín-Burgin A**, Schinder AF. Requirement of adult-born neurons for hippocampus-dependent learning. *Behav Brain Res* 2012; **227**: 391-399 [PMID: 21763727 DOI: 10.1016/j.bbr.2011.07.001]

19 **Geier DA**, Kern JK, Geier MR. Increased risk for an atypical autism diagnosis following Thimerosal-containing vaccine exposure in the United States: A prospective longitudinal case-control study in the Vaccine Safety Datalink. *J Trace Elem Med Biol* 2017; **42**: 18-24 [PMID: 28595786 DOI: 10.1016/j.jtemb.2017.03.005]

20 **Shi L**, Li Z, Chen M, Zhu T, Wu L. Ultrasensitive and Ultraprecise Pressure Sensors for Soft Systems. *Adv Mater* 2023; **35**: e2210091 [PMID: 36625165 DOI: 10.1002/adma.202210091]

21 **Atif QAA**. An audit of operative notes in general surgery at Pakistan Institute of Medical Sciences (P.I.M.S.), Pakistan. Do we follow the Royal College of Surgeons (England) guidelines? *J Pak Med Assoc* 2020; **70**: 491-493 [PMID: 32207432 DOI: 10.5455/JPMA.8686]

22 **Travassos TC**, De Oliveira JMI, Selegatto IB, Reis LO. COVID-19 impact on bladder cancer-orientations for diagnosing, decision making, and treatment. *Am J Clin Exp Urol* 2021; **9**: 132-139 [PMID: 33816701]

23 **Xu C**, Li H, Zhang K, Binzel DW, Yin H, Chiu W, Guo P. Photo-controlled release of paclitaxel and model drugs from RNA pyramids. *Nano Res* 2019; **12**: 41-48 [PMID: 31258852 DOI: 10.1007/s12274-018-2174-x]

24 **Pasteur Institute**. Crimean-Congo Hemorrhagic Fever. Available from: https://www.pasteur.fr/en/medical-center/disease-sheets/crimean-congo-hemorrhagic-fever

25 **Leblebicioglu A**, Eroglu O, Ergonul O. Crimean-Congo Hemorrhagic Fever: A Systematic Review and Meta-Analysis of the Global Distribution of the Virus. *J Infect Dev Ctries* 2017; **11**: 359-367

26 **Keshtkar-Jahromi M**, Kuhn JH, Christova I, Bradfute SB, Jahrling PB, Bavari S. Crimean-Congo hemorrhagic fever: Current and future prospects of vaccines and therapies. *Antivir Res* 2011; **90**: 85-92 [PMID: 21362441 DOI: 10.1016/j.antiviral.2011.02.010]

27 **Al-Abri SS**, Abaidani IA, Fazlalipour M, Mostafavi E, Leblebicioglu H, Pshenichnaya N, Memish ZA, Hewson R, Petersen E, Mala P, Nhu Nguyen TM, Rahman Malik M, Formenty P, Jeffries R. Current status of Crimean-Congo haemorrhagic fever in the World Health Organization Eastern Mediterranean Region: issues, challenges, and future directions. *Int J Infect Dis* 2017; **58**: 82-89 [PMID: 28259724 DOI: 10.1016/j.ijid.2017.02.018]

28 **Tabassum S**, Naeem A, Khan MZ, Mumtaz N, Gill S, Ohadi L. Crimean-Congo hemorrhagic fever outbreak in Pakistan, 2022: A warning bell amidst unprecedented floods and COVID-19 pandemic. *Health Sci Rep* 2023; **6**: e1055 [PMID: 36655141 DOI: 10.1002/hsr2.1055]

29 **Zohaib A**, Saqib M, Athar MA, Hussain MH, Sial AU, Tayyab MH, Batool M, Sadia H, Taj Z, Tahir U, Jakhrani MY, Tayyab J, Kakar MA, Shahid MF, Yaqub T, Zhang J, Wu Q, Deng F, Corman VM, Shen S, Khan I, Shi ZL. Crimean-Congo hemorrhagic fever virus in humans and livestock, Pakistan, 2015-2017. *Emerg Infect Dis* 2020; **26**: 773-777 [PMID: 32187506 DOI: 10.3201/eid2604.191154]

30 **Fanelli A**, Buonavoglia D. Risk of Crimean Congo haemorrhagic fever virus (CCHFV) introduction and spread in CCHF-free countries in southern and Western Europe: A semi-quantitative risk assessment. *One Health* 2021; **13**: 100290 [PMID: 34307823 DOI: 10.1016/j.onehlt.2021.100290]

31 **Mazzola LT**, Kelly-Cirino C. Diagnostic tests for Crimean-Congo haemorrhagic fever: a widespread tickborne disease. *BMJ Global Health* 2019; **4**: e001114 [PMID: 30899574 DOI: 10.1136/bmjgh-2018-001114]

World Journal of Virology

Submit a Manuscript: https://www.f6publishing.com

World J Virol 2025 March 25; 14(1): 97482

DOI: 10.5501/wjv.v14.i1.97482

ISSN 2220-3249 (online)

ORIGINAL ARTICLE

Retrospective Study

Rising incidence of acute hepatitis A among adults and clinical characteristics in a tertiary care center of Pakistan

Yumna Shahid, Amna Subhan Butt, Iqra Jamali, Faisal Wasim Ismail

Specialty type: Virology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade C, Grade D, Grade D

Novelty: Grade C, Grade C, Grade C

Creativity or Innovation: Grade B, Grade C, Grade C

Scientific Significance: Grade B, Grade C, Grade C

P-Reviewer: de Melo FF; Fan YC

Received: May 31, 2024

Revised: August 29, 2024

Accepted: October 15, 2024

Published online: March 25, 2025

Processing time: 180 Days and 8.7 Hours

Yumna Shahid, Division of Gastroenterology, Department of Medicine, Aga Khan University Hospital, Karachi 74800, Pakistan

Amna Subhan Butt, Iqra Jamali, Faisal Wasim Ismail, Department of Medicine, Aga Khan University Hospital, Karachi 74800, Pakistan

Co-corresponding authors: Amna Subhan Butt and Faisal Wasim Ismail.

Corresponding author: Amna Subhan Butt, FCPS, MBBS, MSc, Associate Professor, Department of Medicine, Aga Khan University Hospital, Stadium Road, Karachi 74800, Pakistan. amna.subhan@aku.edu

Abstract

BACKGROUND

For decades, hepatitis A virus (HAV) has been a leading cause of acute hepatitis among children and was less prevalent among adults. However, recently a paradigm shift has been observed in the epidemiology of HAV, as evident by cases of acute hepatitis due to HAV among adults.

AIM

To estimate frequency of HAV in acute viral hepatitis and compare characteristics in HAV and hepatitis E virus (HEV) infection.

METHODS

This was a trend analysis conducted at Aga Khan University Hospital Karachi (Sindh, Pakistan) from February 2024 to May 2024. Individuals aged 18 years and older diagnosed with acute viral hepatitis attributed to hepatotropic viruses in 2024 were reviewed. To compare the trend patients admitted with acute hepatitis during 2019-2023 were also reviewed. Data regarding clinical and laboratory parameters were recorded. The yearly trend of acute hepatitis due to HAV and HEV was analyzed, and comparative analysis was done between HAV and HEV cases among adults.

RESULTS

A total of 396 patients were found to have acute hepatitis during our study duration. HAV was diagnosed in 234 patients (59%) while 157 patients (39.6%) were found to have acute HEV infection. Additionally, acute hepatitis B virus infection was identified in 3 patients (0.7%), whereas acute hepatitis C virus

infection was found in 2 (0.5%) cases of acute hepatitis. Yearly trends showed increasing occurrence of HAV infection among adults over last 5 years. The patients with acute HAV were younger than patients with HEV (28 years ± 8 years *vs* 30 years ± 8 years; $P < 0.01$). Higher levels of total bilirubin were seen in HEV infection, while higher levels of alanine transaminase were seen in HAV infection. However, a higher proportion of acute liver failure (ALF), coagulopathy, and mortality were observed in HEV.

CONCLUSION
An increase in acute hepatitis A cases among adults shows less severity than hepatitis E, highlighting the need for better sanitation, hygiene, and adult hepatitis A vaccination programs.

Key Words: Acute hepatitis; Hepatitis A virus; Hepatitis E virus; Acute liver failure; Hepatotropic virus; Vaccination

Core Tip: In recent years, there has been a notable shift in the prevalence of viruses causing acute hepatitis. The hepatitis A virus (HAV) is increasingly affecting adults, contrary to its previous predominance among children, while the incidence of hepatitis E virus (HEV) among adults appears to remain unchanged. This change highlights a significant epidemiological transition. Although cases of HEV infection are associated with higher mortality rates, acute liver failure, and coagulopathy compared to those of HAV, signaling distinct impacts between the two viruses in adult populations.

Citation: Shahid Y, Butt AS, Jamali I, Ismail FW. Rising incidence of acute hepatitis A among adults and clinical characteristics in a tertiary care center of Pakistan. *World J Virol* 2025; 14(1): 97482
URL: https://www.wjgnet.com/2220-3249/full/v14/i1/97482.htm
DOI: https://dx.doi.org/10.5501/wjv.v14.i1.97482

INTRODUCTION

Affecting approximately 1.4 million individuals annually worldwide, hepatitis A virus (HAV) is attributed to an increasing burden of disease especially in the developing world and individuals with underprivileged conditions[1]. HAV is a single-stranded, non-enveloped RNA virus, which belongs to the Picornaviridae family and the hepatovirus genus. HAV spreads primarily through the fecal-oral route, typically through direct person-to-person contact or consumption of contaminated food or water[2,3]. Acute hepatitis A infection typically leads to a self-limiting illness. Nonetheless, instances of fulminant liver failure have been documented, with advanced age identified as the primary risk factor for developing symptomatic disease[4]. Numerous studies have emphasized a shift in the epidemiology of HAV among adults. While children were predominantly affected by HAV in the past, there has been a noticeable increase in cases among the adult population in recent years. This trend necessitates further investigation to understand the underlying reasons for this shift. The Korea Centers for Disease Control and Prevention noted an exponential rise in symptomatic HAV infections since 2006 among adults, reflecting a shifting pattern of HAV which has also been observed in other Asian countries as well. A study conducted in Egypt also noted a significant rise in acute HAV infection rates, increasing from 2.1% in 1983 to 34% in 2002 among adult population[5,6]. Some studies have shown higher HEV prevalence compared with HAV, indicating variations in epidemiological trend[7]. HAV transmission varies globally, influenced by socioeconomic factors, with higher endemicity in resource-poor regions where early childhood infections are common and often mild, conferring lifelong immunity. By contrast, low endemicity in high-income regions results in fewer childhood exposures, leading to susceptibility in adults and the potential for severe outbreaks among high-risk groups. Improved sanitation in some low- and middle-income countries has reduced transmission but paradoxically increased morbidity and mortality by shifting the age of infection to older populations[8].

Exposure to HAV during early childhood typically leads to asymptomatic infection and confers lifelong immunity. However, with advancements in hygiene and the resulting reduction in HAV exposure, a segment of the population remains susceptible to acquiring the infection during adulthood. At this age, HAV infection carries a higher chance of morbidity and mortality. A review from the Indian subcontinent suggests that the lower prevalence of anti-HAV antibodies may be one of the reasons for the higher frequency of hepatitis A infection among adults[9]. The older studies from Pakistan reported HAV to be responsible for 50%-60% of cases of acute hepatitis among children and 3.5% among adults[10,11]. However, in Pakistan, a shift in HAV epidemiology has been observed but the data are very limited. There is one study from Pakistan where cases of HAV have been reported among adults (beyond 18 years), but this was based on a study conducted on individuals during two outbreaks of hepatitis A[12]. In 2009, the Pakistan Field Epidemiology and Laboratory Training Program collaborated with the Ministry of Health, Pakistan, and the Centers for Disease Prevention and Control, United States, to establish the Hepatitis Sentinel Surveillance System. This initiative identified five public sector tertiary care hospitals across Pakistan to collect relevant data. Analysis of the sentinel site data from June 2010 to March 2011 revealed a total of 712 cases of viral hepatitis, with acute hepatitis A accounting for 19.8% of the cases. Notably, males were more affected, comprising 69.5% of the cases. A noteworthy observation in this study was the

shift in the age distribution pattern, with the highest prevalence of HAV observed in the 20-29 age group (41.2%), followed by the 30-39 age group (16.3%), and the 6-19 age group (12.8%)[13].

HAV can lead to significant morbidity and mortality among adults if not treated in a timely manner. Very limited data were available from Pakistan on the burden of HAV among adults in Pakistan. Hence, the evaluation of HAV among adult patients with acute hepatitis will help to assess the disease burden among the adult population in Pakistan and the need for immunization against HAV in adults.

In this study, we established the increasing incidence of HAV among adults and compared the clinical characteristics, severity, and mortality rates of acute hepatitis among adults caused by HAV *vs* hepatitis E virus (HEV).

MATERIALS AND METHODS

Study population and data collection

This was a trend analysis conducted at Aga Khan University Hospital Karachi (Sindh, Pakistan). Data were collected during February 2024 to May 2024 for patients admitted with acute hepatitis during February 2024 to May 2024, as well as patients admitted with acute hepatitis during 2019 to 2023 to compare the trend. Inclusion criteria comprised individuals aged 18 years and older, who presented to the outpatient and inpatient departments and were diagnosed with acute viral hepatitis attributed to hepatotropic viruses. Exclusion criteria encompassed cases of acute hepatitis unrelated to hepatotropic viruses, drug induced, alcoholic hepatitis, and other metabolic etiologies (including hemochromatosis, autoimmune hepatitis, Wilson's disease, alpha 1 anti-trypsin deficiency, metabolic dysfunction-associated steatotic liver disease). Acute hepatitis due to hepatotropic viruses was considered if hepatitis was due to HAV, hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV) and HEV. These viruses specifically affect liver hence the term "hepatotropic." Non-hepatotropic viruses encompass all other viral agents capable of inducing acute hepatitis, such as cytomegalovirus, Epstein-Barr virus, dengue, and herpes simplex virus[14,15].

The diagnosis of acute hepatitis was established based on symptoms such as jaundice, fever, or vomiting lasting for less than 6 months recognized by elevation of liver enzymes, specifically alanine transaminase (ALT) and aspartate transaminase (AST) more than five times the upper limit of normal. Acute hepatitis A and hepatitis E were defined as patients with acute hepatitis found to have immunoglobulin M (IgM) antibody to HAV (anti-HAV) and HEV (anti-HEV), respectively[16]. Acute liver failure (ALF) was characterized by a severe, sudden liver injury lasting less than 26 weeks, accompanied by encephalopathy and impaired synthetic function (indicated by an international normalized ratio of 1.5 or higher), in individuals without preexisting liver disease or cirrhosis. Deranged coagulopathy and altered mentation are two important criteria of ALF. It is associated with high rates of complications and death, often necessitating intensive care, and in many cases, an emergency liver transplant[17].

Data pertaining to demographic characteristics, clinical and laboratory parameters, duration of hospitalization, disease severity, and mortality were retrieved by reviewing their medical charts and electronic patient medical records. The Aga Khan University hospital is a Joint Commission International accredited hospital currently using "MypatientsAku," a locally developed electronic medical record data base application with availability of data for all laboratory tests including radiology, access to medication records, diagnosis, details about hospital encounters, and discharge summaries, *etc*. This is a validated application and accessible to credentialed house staff. The records of daily follow-ups are maintained in patients' charts.

Informed consent was not required by ethic review board since methodology involved review of patient charts. Ethics approval was obtained from Ethics Review Committee Aga Khan University Pakistan (No. 2024-9479-28215).

Statistical analyses

Data were analyzed using Statistical Package for the Social Sciences version 22 (IBM, Armonk, NY, United States). Continuous variables are presented as the mean ± standard deviation or median (minimum-maximum), while categorical variables are expressed as the frequency and percentages. The normality of quantitative data was checked using histogram and Shapiro–Wilk test. In cases where the data did not meet the assumption of normality ($P < 0.05$), non-parametric alternatives were employed. Specifically, for continuous variables that were not normally distributed, the Mann-Whitney *U* test was used in place of the independent *t*-test, and the Wilcoxon signed-rank test was used instead of the paired *t*-test. Additionally, transformations of the data or bootstrapping techniques were considered when necessary to meet normality assumptions. These approaches ensured robust analysis even in the presence of deviations from normality.

Comparative analysis was performed using the independent samples *t*-test, while categorical variables were analyzed using the χ^2 or Fischer exact test wherever appropriate. $P \leq 0.05$ was considered statistically significant. The effect size estimation was done using Cohen's d test for independent samples *t*-test and Cramér's for χ^2. The Cohen's d = 0.2 d was considered a small effect, medium effect: D = 0.5 d and large effect: D = 0.8 d. For Cramér's v = 0.1, V = 0.3 and V = 0.5 were considered as small, medium, and large effect, respectively.

RESULTS

Demographics

A total of 396 patients fulfilled the eligibility criteria and were found to have acute hepatitis during our study. A total of

234 (59%) patients were diagnosed with acute HAV infection, 157 (39.6%) patients were found to have acute HEV infection. Additionally, HBV infection was identified as the cause in 3 patients (0.7%), whereas HCV infection was found in only 2 (0.5%) cases of acute hepatitis. No case of acute HDV infection was identified. The mean age of overall patients was 29 years ± 8 years. Overall frequencies of baseline characteristics are presented in Table 1.

Comparative analysis

After excluding HBV and HCV cases from dataset, comparative analysis was done between HAV and HEV cases. The mean age of patients with HAV was 28 years ± 8 years, while those with HEV were 30 years ± 8 years with a P value of 0.017 showing that older adults were affected with HEV infection. An elevated total leukocyte count of 11 ± 7 was observed among patients with HEV, whereas individuals with HAV exhibited a comparatively lower leukocyte count of 6 ± 3, with a significant P value ($P < 0.001$). Total bilirubin (TB) was noted to be raised among HEV, which is 13 ± 11 while in HAV, TB was found to be 6 ± 7 with $P < 0.001$. Increased ALT, gamma-glutamyl transferase (GGT), and AST were seen among HAV compared with HEV. Laboratory parameters of HAV and HEV are shown in detail in Table 2.

Interpretation

The effect size for the difference between patients with HAV *vs* HEV, *i.e.* Cohen's d ≥ 0.6 for white cell count, total and direct bilirubin, GGT, ALT, and AST indicating a moderate to large effect. This suggests that the observed difference between the two groups is not only statistically significant but also substantial in magnitude. Although the difference between HAV and HEV for hospital stay was statistically significant but the effect size for difference was small reflecting early recovering for both HAV and HAV. Likewise, a significantly higher proportion of patients with HEV infection develop coagulopathy, ALF with higher mortality but the effect size for this difference ranged between medium-small. However, the observed difference and higher proportion of pregnancy in HEV group as compared to HAV was moderate.

Extended hospitalization for a duration of 4 days was observed among patients diagnosed with HEV, as opposed to a 3-day stay in the HAV cohort. A total of 87 instances of coagulopathy were documented in the HEV group, while 80 cases were identified among those with HAV infection. ALF manifested in 24 patients within the HEV cohort, in contrast to a mere 8 cases observed in the HAV group. Regarding mortality, HEV resulted in 11 fatalities, contrasting with two mortalities attributed to HAV infection (Table 2). These data represent that HEV causes more severe disease and complications like coagulopathy and ALF (Figure 1).

Yearly trends revealed a notable increase in the incidence of HAV, with 30 cases reported in 2019, 27 cases in 2020, 31 cases in 2021, a substantial surge to 59 cases in 2022, and a further escalation to 63 cases in 2023. By contrast, the prevalence of HEV remained comparatively lower. HEV accounted for 33 cases in 2019, 28 cases in 2020, 40 cases in 2021, experienced a decline to 19 cases in 2022, and slightly rose to 28 cases in 2023. The cases from 2024 were not included in the bar chart because the data only cover January to May. Therefore, it does not accurately represent the yearly trend for 2024 (Figure 2).

Young adults (age 18-30 years) are the most common group affected by acute hepatitis (Figure 3). HEV was more prevalent in summers and spring while HAV was seen throughout the year (Figure 4).

DISCUSSION

In this study, we evaluated clinical spectrum of acute hepatitis, changing trends of HAV occurrence and compared the disease activity of HAV and HEV, which are the most common causes of acute hepatitis worldwide[16]. Our study revealed that HAV constituted the predominant etiology of acute hepatitis among adults, accounting for 59% of cases. Furthermore, our findings indicate a notable upward trend in HAV incidence over successive years, consistent with findings reported in recent literature. For instance, a Brazilian study has evaluated temporal trend analysis on hepatitis A cases reported from 2007 to 2018, which showed a fall in the incidence of hepatitis A among people under 20 years from 2007 to 2016, whereas after 2016 a rising trend in hepatitis A was observed among males aged 20-39 years[18]. Zakaria *et al*[6] similarly observed a parallel trend in their study, where the prevalence of HAV cases among adults increased substantially from 2.1% in 1983 to 34% in 2002. Additionally, the incidence of non-A non-B acute hepatitis demonstrated a decline from 38.7% to 31% over the same period[6]. In our study, we meticulously examined the annual patterns of HAV and HEV from 2019 to 2023 and found similar results. Our findings revealed a consistent upward trajectory in HAV cases, escalating from 30 cases in 2019 to 85 cases in 2023. Conversely, no significant fluctuation was observed in HEV cases over the same period (Figure 2).

Overall in our study, we discovered that the most common age group affected with acute hepatitis was 18-30 years, while older adults (51-60 years) were affected more in 2023 compared with previous years (Figure 3). Sun *et al*[19] studied the epidemiology of hepatitis A in three different regions of China. In 2004–2007, the median ages of HAV cases were 38 years, 29 years, and 21 years in the eastern, central, and western regions, respectively. Subsequently, during 2008–2011, the median ages rose to 40 years, 36 years, and 24 years. Lastly from 2012 to 2017, the median ages further increased to 43 years, 47 years, and 33 years in the respective regions[19]. Another study from China reported a decrease in the incidence of HAV cases among individuals aged ≤ 19 years. Their study showed a declining pattern among the younger age group (< 19 years) from 1.68 cases per 100000 individuals in 2008 to 0.22 cases per 100000 individuals in 2014. They noticed an increase in the mean age during the study period from 36.8 years in 2005 to 47.2 years in 2014[20]. Globalization has also contributed to the change in the epidemiology of hepatitis A. In low socioeconomic countries, improved sanitation has reduced the incidence of HAV but high-income countries are facing foodborne outbreaks which are mainly affecting the

Table 1 Baseline characteristics of all patients with acute hepatitis A and hepatitis E

Baseline characteristics	mean ± SD
Age in years	29 ± 8
Male	221 (55.8)
Female	175 (44.2)
Hemoglobin in g/dL	12.5 ± 2
White cell count as × 10^9/L	8 ± 5
Platelet as × 10^9/L	237 ± 127
Total bilirubin in mg/dL	9.2 ± 9
Direct bilirubin in mg/dL	7 ± 7
Gamma-glutamyl transferase in IU/L	171 ± 142
Alanine aminotransferase in IU/L	2626 ± 1925
Alkaline phosphatase	198 ± 92
Aspartate aminotransferase in IU/L	2229 ± 2036
International normalized ratio	1.6 ± 0.8
Coagulopathy	169 (42.7)
Acute liver failure	32 (8.1)
Pregnancy	39 (9.8)
Chronic liver disease	15 (3.8)
Mortality	13 (3.3)

Data are *n* (%). SD: Standard deviation.

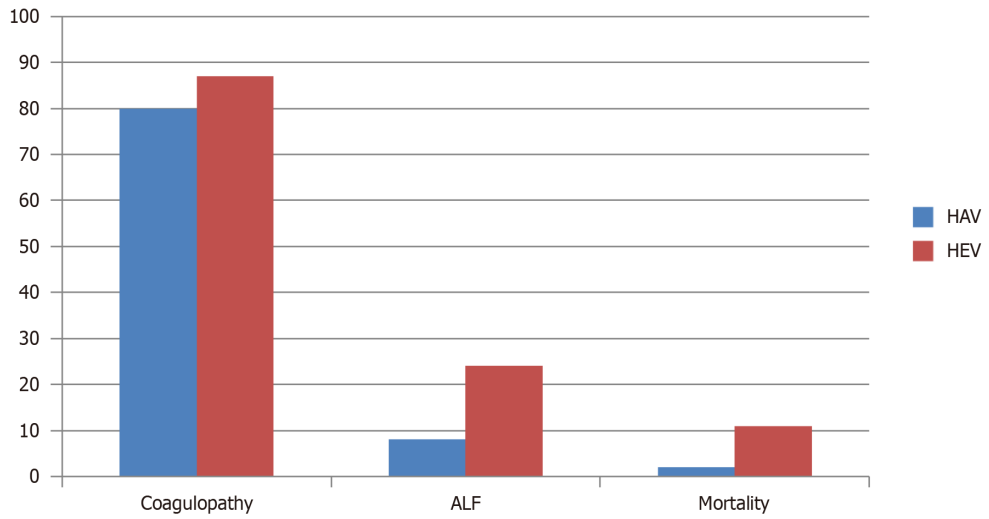


Figure 1 Rate of coagulopathy, acute liver failure, and mortality among hepatitis A virus and hepatitis E virus cases. ALF: Acute liver failure; HAV: Hepatitis A virus; HEV: Hepatitis E virus.

adult population. Rural-to-urban migration is one of the most important factors that is likely contributing to this shift in epidemiology[21]. Recent research indicates that the prevalence of anti-HAV antibodies is notably lower among individuals in their twenties and has remained relatively stable over the past decade. Conversely, the seropositivity of anti-HAV among adults in their thirties has exhibited a consistent decline, dropping from 69.6% in 2005 to 32.4% in 2014. This suggests that many young adults who have not yet encountered hepatitis A and have not received vaccination are susceptible to the infection. Consequently, there is a pressing need for effective strategies to control and prevent acute hepatitis A, particularly targeting individuals in their twenties and thirties[22].

Table 2 Comparison of patients with hepatitis A virus and hepatitis E virus

Baseline characteristics	Hepatitis A virus	Hepatitis E virus	Effect size	*P* value
Age in years	28 ± 8	30 ± 8	0.23	0.017
Sex				
Male	124 (53)	93 (59.2)	0.09	0.223
Female	110 (47)	64 (40.8)		
Hemoglobin in g/dL	12.8 ± 1	12.1 ± 2	0.32	0.002
White cell count as × 10^9/L	6 ± 3	11 ± 7	0.87	< 0.001
Platelet as × 10^9/L	236 ± 140	239 ± 106	0.03	0.80
Total bilirubin in mg/dL	6 ± 7	13 ± 11	0.76	< 0.001
Direct bilirubin in mg/dL	5 ± 5	10 ± 9	0.72	< 0.001
Gamma-glutamylcysteine in IU/L	215 ± 138	110 ± 126	0.79	< 0.001
Alanine aminotransferase in IU/L	3274 ± 1937	1760 ± 1517	0.87	< 0.001
Alkaline phosphatase	181 ± 83	224 ± 100	0.46	< 0.001
Aspartate aminotransferase in IU/L	2820 ± 2255	1445 ± 1338	0.74	< 0.001
International normalized ratio	1.5 ± 0.7	1.8 ± 1	0.36	< 0.001
Coagulopathy	80 (34.2)	87 (55.4)	0.21	< 0.001
Acute liver failure	8 (3.4)	24 (15.3)	0.22	< 0.001
Pregnancy	3 (1.3)	36 (23)	0.36	< 0.001
Chronic liver disease	1 (0.4)	13 (8.3)	0.26	< 0.001
Mortality	2 (0.9)	11 (7)	0.17	< 0.001
Hospital stay	2.8 ± 3	4 ± 3.6	0.26	0.002

Data are *n* (%).

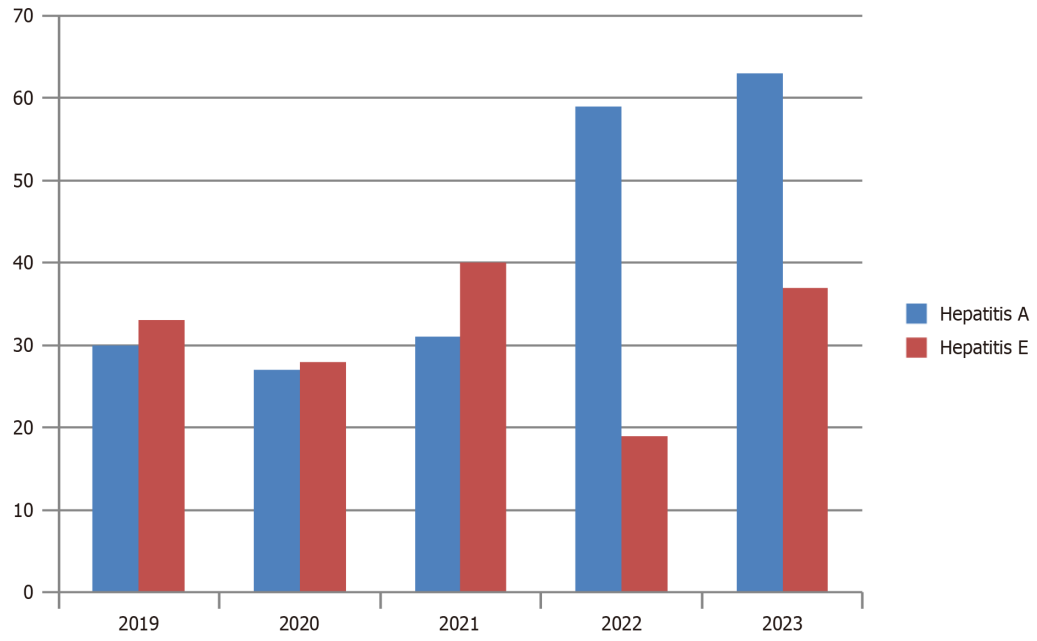


Figure 2 Yearly trends of cases of acute hepatitis from 2019 to 2023. 2024 was excluded because the entire year's worth of data were not available.

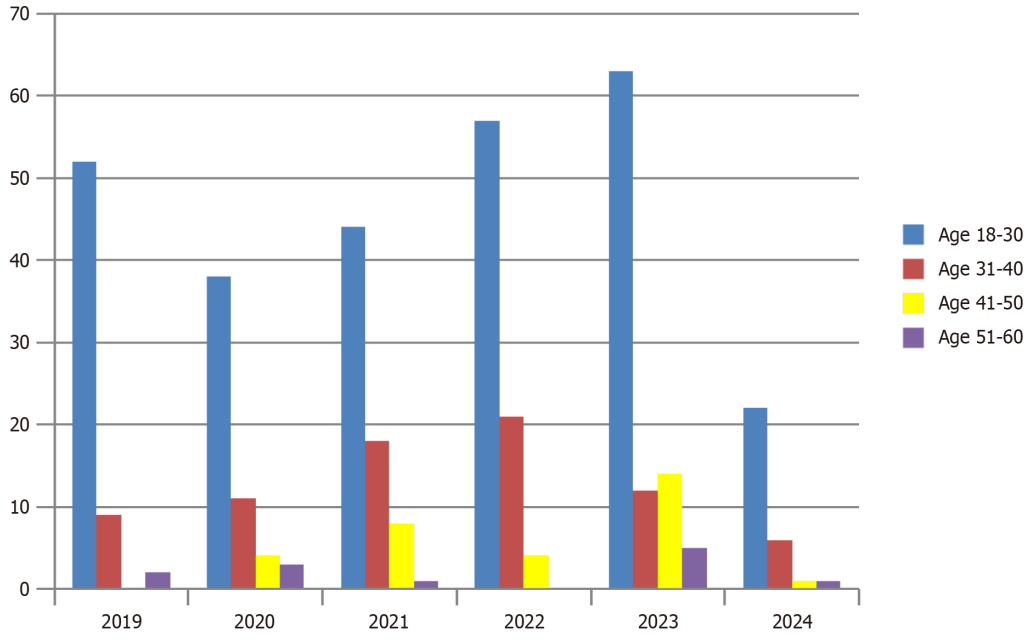


Figure 3 Common age groups affected divided in four age groups.

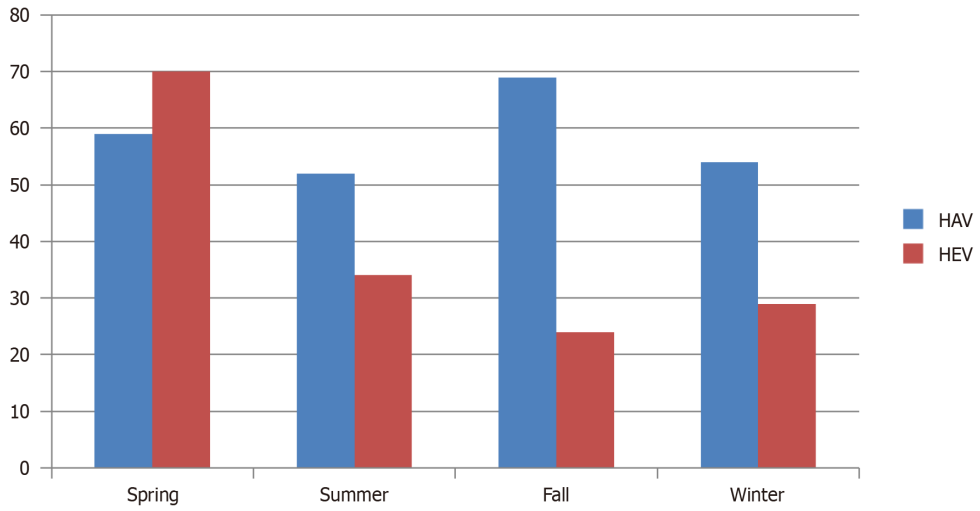


Figure 4 Seasonal trends among hepatitis A virus and hepatitis E virus. Spring: March-May; Summer: June-August; Fall: September-November; Winter: December-February; HAV: Hepatitis A virus; HEV: Hepatitis E virus.

Upon comparing patients diagnosed with HAV to those with HEV, notable differences emerged in their laboratory parameters and clinical outcomes. Specifically, individuals with HAV infection exhibited elevated aminotransferase levels (ALT = 3274 U/L; $P < 0.001$), whereas those with HEV infection demonstrated higher levels of TB (TB = 13 mg/dL ± 11 mg/dL; $P < 0.001$). Moreover, the HEV group displayed a higher incidence of mortality and ALF (Figure 2). Interestingly, these findings contrast with those reported in a study conducted in Thailand, where HAV-infected patients presented with higher aminotransferase and TB levels. Additionally, both cohorts exhibited comparable outcomes in terms of mortality, ALF, and hospitalization rates[23]. In another study, patients diagnosed with acute hepatitis E displayed markedly lower median levels of ALT (798 U/L) and TB (1.8 mg/dL) compared to those with acute hepatitis A (2326 U/L, $P < 0.001$ and 5.2 mg/dL; $P < 0.001$), indicating a relatively milder form of hepatitis. These findings diverge from our study's observations[24]. Acute-on-chronic liver failure (ACLF) represents a potentially reversible syndrome that manifests in individuals with cirrhosis or underlying chronic liver disease (CLD), marked by acute decompensation, organ failure, and elevated short-term mortality rates. Notably, HAV and HEV are prominent etiological factors contributing to ACLF[25]. Our study findings highlight the distinct impact of HEV compared to HAV on ACLF development. Among the observed cases, HEV infection was associated with a higher prevalence of underlying CLD, suggesting a greater propensity for inducing ACLF compared to HAV. Furthermore, HEV infection was characterized by a more severe disease phenotype, heightened coagulopathy, prolonged hospitalization, and a predilection for affecting pregnant individuals. Conversely, HAV infection often presented as a benign, self-limiting illness in the majority of cases.

Although more mortalities were caused by HEV (n = 11; P < 0.001) compared to HAV (n = 2; P < 0.001), a considerable number of complications such as coagulopathy and ALF were associated with HAV (Figure 2). If we compare seasonal occurrence, most of the acute hepatitis cases in our cohort occurred in summers and spring, while HAV was consistent throughout the year (Figure 4). Another study from Pakistan also showed that the majority of HAV and HEV cases were seen in June to July[26]. Although evidence indicates a tendency for increased incidence during the spring and summer months, there is no definite and consistent seasonal pattern for acute viral hepatitis[27].

Over the years, frequency of HAV has been increasing among adults. Therefore it is imperative to implement routine vaccination programs and enhance sanitation and hygiene awareness, aligning with recommendations from previous studies. HAV immunization should be made mandatory for adult population to reduce its incidence[28,29]. The HAV vaccine was first approved in 1992. Both inactivated and live attenuated vaccines are highly effective and well tolerated, providing immune protection for at least 20 years. HAV vaccination is effective for both preexposure and postexposure prophylaxis, particularly for children and young adults. Vaccination strategies for HAV differ across countries and generally include targeting high-risk populations, regional childhood vaccination programs, and universal childhood vaccination. Over the past 30 years, the incidence of hepatitis A has significantly decreased in many countries. However, outbreaks still frequently occur among high-risk groups and individuals not covered by universal vaccination programs [30]. High-risk groups for hepatitis A vaccination include travelers to or workers in areas with high or intermediate infection rates, men who have sex with men, and users of illicit drugs, whether injected or not. It is also recommended for individuals with CLD, including those with HBV or HCV infections, cirrhosis, liver fibrosis, immunocompromised or those awaiting or recovering from liver transplantation. Additionally, people with clotting factor disorders and those working with HAV-infected primates or handling HAV in research laboratories should receive the vaccine[31].

It is recommended to organize public awareness programs and provide counseling to high-risk groups, particularly in low socioeconomic countries such as Pakistan. These initiatives should encourage vaccination and promote good handwashing and hygiene practices.

The limitations of this study are important to acknowledge, but each arises from practical considerations inherent to the study design. The single-center design was chosen due to logistical and resource constraints, allowing for more controlled data collection and analysis within a specific context. While this may limit generalizability, the findings still provide valuable insights within the study population, laying the groundwork for future multicenter studies that can validate and extend these results across diverse settings. Additionally, the focus on a single ethnic group was necessary to reduce variability and enhance the internal validity of the findings, though it is recognized that broader ethnic representation would increase the external relevance of the data. Lastly, the absence of seroprevalence testing for anti-HAV immunoglobulin G antibodies was due to resource limitations, but the combination of symptoms, pattern of LFTS and reactive IgM still confirms hepatitis A diagnosis. Future studies incorporating seroprevalence testing will allow for a more accurate assessment of hepatitis A incidence and burden, addressing this gap.

CONCLUSION

A rising trend has been observed in acute hepatitis due to HAV among adults. However, cases due to HAV were less severe and had lower proportion of ALF, coagulopathy and mortality than HEV. The escalating incidence of HAV among adults highlights a concerning public health challenge, potentially stemming from reduced vaccination rates in this demographic. To address this trend, it is imperative to enhance awareness regarding the importance of vaccination and promote rigorous adherence to good hygiene practices. Mitigating the risk of foodborne outbreaks necessitates prudent choices such as minimizing dining out. Furthermore, given the global implications of this epidemiological shift, further research on a broader scale is essential to elucidate and effectively address this emerging trend.

FOOTNOTES

Author contributions: Shahid Y contributed to the design of the study, acquisition of the data, and analysis and writing of the final manuscript; Butt AS contributed to the study conception and design, data analysis and interpretation, reviewed the final manuscript, and made corrections to the manuscript; Jamali I helped retrieve patient data, entry, and analysis; Ismail FW reviewed the study and designed the manuscript; All of the authors read and approved the final version of the manuscript to be published.

Institutional review board statement: Ethics approval was obtained from Ethics Review Committee Aga Khan University Pakistan, No. 2024-9479-28215.

Informed consent statement: Informed consent was not required by the ethics review board since the methodology involved review of patient charts.

Conflict-of-interest statement: The authors have no conflicts to interest to declare.

Data sharing statement: Data files can be accessed by reasonable request to the corresponding author.

Country of origin: Pakistan

ORCID number: Yumna Shahid 0009-0006-8183-8287; Amna Subhan Butt 0000-0002-7311-4055; Faisal Wasim Ismail 0000-0003-0983-0644.

S-Editor: Luo ML
L-Editor: Filipodia
P-Editor: Zheng XM

REFERENCES

1 **The World Health Organization**. Immunization, Vaccines and Biologicals: Hepatitis A. Available from: https://www.who.int/immunization/diseases/hepatitisA/en/
2 **Coulepis AG**, Locarnini SA, Westaway EG, Tannock GA, Gust ID. Biophysical and biochemical characterization of hepatitis A virus. *Intervirology* 1982; **18**: 107-127 [PMID: 6292126 DOI: 10.1159/000149314]
3 **Coulepis AG**, Anderson BN, Gust ID. Hepatitis A. *Adv Virus Res* 1987; **32**: 129-169 [PMID: 3039812 DOI: 10.1016/s0065-3527(08)60476-5]
4 **Rezende G**, Roque-Afonso AM, Samuel D, Gigou M, Nicand E, Ferre V, Dussaix E, Bismuth H, Féray C. Viral and clinical factors associated with the fulminant course of hepatitis A infection. *Hepatology* 2003; **38**: 613-618 [PMID: 12939587 DOI: 10.1053/jhep.2003.50366]
5 **Kim YJ**, Lee HS. Increasing incidence of hepatitis A in Korean adults. *Intervirology* 2010; **53**: 10-14 [PMID: 20068335 DOI: 10.1159/000252778]
6 **Zakaria S**, Fouad R, Shaker O, Zaki S, Hashem A, El-Kamary SS, Esmat G, Zakaria S. Changing patterns of acute viral hepatitis at a major urban referral center in Egypt. *Clin Infect Dis* 2007; **44**: e30-e36 [PMID: 17243045 DOI: 10.1086/511074]
7 **Palewar MS**, Joshi S, Choudhary G, Das R, Sadafale A, Karyakarte R. Prevalence of Hepatitis A virus (HAV) and Hepatitis E virus (HEV) in patients presenting with acute viral hepatitis: A 3-year retrospective study at a tertiary care Hospital in Western India. *J Family Med Prim Care* 2022; **11**: 2437-2441 [PMID: 36119300 DOI: 10.4103/jfmpc.jfmpc_1746_21]
8 **Webb GW**, Kelly S, Dalton HR. Hepatitis A and Hepatitis E: Clinical and Epidemiological Features, Diagnosis, Treatment, and Prevention. *Clin Microbiol Newsl* 2020; **42**: 171-179 [PMID: 33110280 DOI: 10.1016/j.clinmicnews.2020.10.001]
9 **Agrawal A**, Singh S, Kolhapure S, Hoet B, Arankalle V, Mitra M. Increasing Burden of Hepatitis A in Adolescents and Adults and the Need for Long-Term Protection: A Review from the Indian Subcontinent. *Infect Dis Ther* 2019; **8**: 483-497 [PMID: 31679118 DOI: 10.1007/s40121-019-00270-9]
10 **Bosan A**, Qureshi H, Bile KM, Ahmad I, Hafiz R. A review of hepatitis viral infections in Pakistan. *J Pak Med Assoc* 2010; **60**: 1045-1058 [PMID: 21381562]
11 **Ahmed W**, Qureshi H, Arif A, Alam SE. Changing trend of viral hepatitis--"A twenty one year report from Pakistan Medical Research Council Research Centre, Jinnah Postgraduate Medical Centre, Karachi". *J Pak Med Assoc* 2010; **60**: 86-89 [PMID: 20209690]
12 **Waheed-uz-Zaman Tariq**, Hussain AB, Hussain T, Anwar M, Ghani E, Asad-ullah. Hepatitis A virus infection -- shifting epidemiology. *J Coll Physicians Surg Pak* 2006; **16**: 15-18 [PMID: 16441981]
13 **Centers for Disease Control and Prevention (CDC)**. Establishment of a viral hepatitis surveillance system-Pakistan, 2009-2011. *MMWR Morb Mortal Wkly Rep* 2021; **60**: 1385-1390 [PMID: 21993343]
14 **Lanini S**, Ustianowski A, Pisapia R, Zumla A, Ippolito G. Viral Hepatitis: Etiology, Epidemiology, Transmission, Diagnostics, Treatment, and Prevention. *Infect Dis Clin North Am* 2019; **33**: 1045-1062 [PMID: 31668190 DOI: 10.1016/j.idc.2019.08.004]
15 **Gupta E**, Ballani N, Kumar M, Sarin SK. Role of non-hepatotropic viruses in acute sporadic viral hepatitis and acute-on-chronic liver failure in adults. *Indian J Gastroenterol* 2015; **34**: 448-452 [PMID: 26589230 DOI: 10.1007/s12664-015-0613-0]
16 **Schaefer TJ**, John S. Acute Hepatitis. Updated 2022 Jul 18. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2023 [PMID: 31855397]
17 **Shah NJ**, Royer A, John S. Acute Liver Failure. Updated 2023 Apr 7. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2024 [PMID: 29493996]
18 **Grandi G**, Lopez LF, Burattini MN. Temporal Trends of Acute Hepatitis A in Brazil and Its Regions. *Viruses* 2022; **14**: 2737 [PMID: 36560740 DOI: 10.3390/v14122737]
19 **Sun XJ**, Zhang GM, Zhou RJ, Zheng H, Miao N, Yin ZD, Wang FZ. Changes in the epidemiology of hepatitis A in three socio-economic regions of China, 1990-2017. *Infect Dis Poverty* 2019; **8**: 80 [PMID: 31578150 DOI: 10.1186/s40249-019-0591-z]
20 **Wang Z**, Chen Y, Xie S, Lv H. Changing Epidemiological Characteristics of Hepatitis A in Zhejiang Province, China: Increased Susceptibility in Adults. *PLoS One* 2016; **11**: e0153804 [PMID: 27093614 DOI: 10.1371/journal.pone.0153804]
21 **Jacobsen KH**. Globalization and the Changing Epidemiology of Hepatitis A Virus. *Cold Spring Harb Perspect Med* 2018; **8**: a031716 [PMID: 29500305 DOI: 10.1101/cshperspect.a031716]
22 **Kang SH**, Kim MY, Baik SK. Perspectives on Acute Hepatitis A Control in Korea. *J Korean Med Sci* 2019; **34**: e230 [PMID: 31538417 DOI: 10.3346/jkms.2019.34.e230]
23 **Khongviwatsathien S**, Thaweerat W, Atthakitmongkol T, Chotiyaputta W, Tanwandee T. A Comparison of Clinical Manifestations and Outcomes between Acute Sporadic Hepatitis A and Hepatitis E Infections in Thailand. *Viruses* 2023; **15**: 1888 [PMID: 37766294 DOI: 10.3390/v15091888]
24 **Brehm TT**, Mazaheri O, Horvatits T, Lütgehetmann M, Schulze Zur Wiesch J, Lohse AW, Polywka S, Pischke S. Lower Levels of Transaminases but Higher Levels of Serum Creatinine in Patients with Acute Hepatitis E in Comparison to Patients with Hepatitis A. *Pathogens* 2021; **10**: 60 [PMID: 33445435 DOI: 10.3390/pathogens10010060]
25 **Bhatti TK**, Singal AK, Kwo PY. Viral Hepatitis and Acute-on-Chronic Liver Failure. *Clin Liver Dis* 2023; **27**: 617-630 [PMID: 37380286 DOI: 10.1016/j.cld.2023.03.006]
26 **Khan MA**. Comparative prevalence of different types of viral hepatitis in the district Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan. *Egypt*

Liver J 2022; **12**: 40 [DOI: 10.1186/s43066-022-00203-1]

27 **Fares A**. Seasonality of hepatitis: a review update. *J Family Med Prim Care* 2015; **4**: 96-100 [PMID: 25810997 DOI: 10.4103/2249-4863.152263]

28 **Tekin R**, Yolbas I, Dal T, Demirpençe Ö, Kaya S, Bozkurt F, Deveci Ö, Çelen MK, Tekin A. Evaluation of adults with acute viral hepatitis a and review of the literature. *Clin Ter* 2013; **164**: 537-541 [PMID: 24424220 DOI: 10.7417/CT.2013.1634]

29 **Bane A**, Sultan A, Ahmed R. Increasing Burden of Acute Hepatitis A among Ethiopian Children, Adolescents, and Young adults: A Change in Epidemiological Pattern and Need for Hepatitis A Vaccine. *Ethiop J Health Sci* 2022; **32**: 255-260 [PMID: 35693569 DOI: 10.4314/ejhs.v32i2.5]

30 **Zhang L**. Hepatitis A vaccination. *Hum Vaccin Immunother* 2020; **16**: 1565-1573 [PMID: 32649265 DOI: 10.1080/21645515.2020.1769389]

31 **Moon AM**, Lowy E, Maier MM, Chartier M, Morgan TR, Hoffman-Högg L, Beste LA. Hepatitis A Virus Prevention and Vaccination Within and Outside the Veterans Health Administration in Light of Recent Outbreaks. *Fed Pract* 2018; **35**: S32-S37 [PMID: 30766392]

World Journal of Virology

Submit a Manuscript: https://www.f6publishing.com

World J Virol 2025 March 25; 14(1): 100501

DOI: 10.5501/wjv.v14.i1.100501

ISSN 2220-3249 (online)

ORIGINAL ARTICLE

Retrospective Study

Unveiling the impact: COVID-19's influence on bacterial resistance in the Kingdom of Bahrain

Nermin K Saeed, Safiya K Almusawi, Noor A Albalooshi, Mohammed Al-Beltagi

Specialty type: Virology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade D

Novelty: Grade B, Grade B

Creativity or Innovation: Grade B, Grade C

Scientific Significance: Grade B, Grade C

P-Reviewer: Iskandar CF

Received: August 18, 2024

Revised: October 22, 2024

Accepted: November 15, 2024

Published online: March 25, 2025

Processing time: 101 Days and 16.6 Hours

Nermin K Saeed, Safiya K Almusawi, Noor A Albalooshi, Medical Microbiology Section, Department of Pathology, Salmaniya Medical Complex, Governmental Hospitals, Manama 12, Bahrain

Nermin K Saeed, Safiya K Almusawi, Medical Microbiology Section, Department of Pathology, Royal College of Surgeons in Ireland–Medical University of Bahrain, Busaiteen 15503, Muharraq, Bahrain

Mohammed Al-Beltagi, Department of Paediatrics, Faculty of Medicine, Tanta University, Tanta 31511, Alghrabia, Egypt

Mohammed Al-Beltagi, Department of Pediatric, University Medical Center, King Abdulla Medical City, Arabian Gulf University, Manama 26671, Algharbia, Bahrain

Corresponding author: Mohammed Al-Beltagi, MD, PhD, Chief Physician, Professor, Department of Paediatrics, Faculty of Medicine, Tanta University, 1 Hassan Radwan Street, Tanta 31511, Alghrabia, Egypt. mbelrem@hotmail.com

Abstract

BACKGROUND

Antibiotic resistance is a growing global health threat, and understanding local trends in bacterial isolates and their susceptibility patterns is crucial for effective infection control and antimicrobial stewardship. The coronavirus disease 2019 (COVID-19) pandemic has introduced additional complexities, potentially influencing these patterns.

AIM

To analyze trends in bacterial isolates and their antibiotic susceptibility patterns at Salmaniya Medical Complex from 2018 to 2023, with a specific focus on the impact of the COVID-19 pandemic on these trends.

METHODS

A retrospective analysis of microbiological data was conducted, covering the period from 2018 to 2023. The study included key bacterial pathogens such as *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, among others. The antibiotic susceptibility profiles of these isolates were assessed using standard laboratory methods. To contextualize the findings, the findings were compared with similar studies from

other regions, including China, India, Romania, Saudi Arabia, the United Arab Emirates, Malaysia, and United States.

RESULTS

The study revealed fluctuating trends in the prevalence of bacterial isolates, with notable changes during the COVID-19 pandemic. For example, a significant increase in the prevalence of *Staphylococcus aureus* was observed during the pandemic years, while the prevalence of *E. coli* showed a more variable pattern. Antibiotic resistance rates varied among the different pathogens, with a concerning rise in resistance to commonly used antibiotics, particularly among *Klebsiella pneumoniae* and *E. coli*. Additionally, the study identified an alarming increase in the prevalence of multidrug-resistant (MDR) strains, especially within *Klebsiella pneumoniae* and *E. coli* isolates. The impact of the COVID-19 pandemic on these trends was evident, with shifts in the frequency, resistance patterns, and the emergence of MDR bacteria among several key pathogens.

CONCLUSION

This study highlights the dynamic nature of bacterial isolates and their antibiotic susceptibility patterns at Salmaniya Medical Complex, particularly in the context of the COVID-19 pandemic. The findings underscore the need for continuous monitoring and effective anti-microbial stewardship programs to combat the evolving threat of antibiotic resistance. Further research and policy initiatives are required to address the identified challenges and improve patient outcomes in the face of these ongoing challenges.

Key Words: Multidrug-resistant organisms; Antibiotic susceptibility; COVID-19 pandemic; Antimicrobial stewardship; Bacterial isolates; Salmaniya Medical Complex; Bahrain

Core Tip: This study highlights the critical role of continuous surveillance in tracking bacterial isolates and their antibiotic susceptibility patterns, especially during the coronavirus disease 2019 pandemic. The findings underscore the need for robust antimicrobial stewardship programs to address the emergence of multidrug-resistant organisms. Regularly updated treatment protocols, informed by local epidemiological data, are essential for optimizing therapeutic strategies. The collaboration between microbiology laboratories and clinical teams is vital for timely diagnostics, which guide effective antimicrobial therapy. This study provides valuable insights that can inform healthcare practices and contribute to global efforts in combating antimicrobial resistance.

Citation: Saeed NK, Almusawi SK, Albalooshi NA, Al-Beltagi M. Unveiling the impact: COVID-19's influence on bacterial resistance in the Kingdom of Bahrain. *World J Virol* 2025; 14(1): 100501
URL: https://www.wjgnet.com/2220-3249/full/v14/i1/100501.htm
DOI: https://dx.doi.org/10.5501/wjv.v14.i1.100501

INTRODUCTION

The emergence of the coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome-coronavirus 2 virus, has profoundly impacted global healthcare systems and posed a significant global health challenge. This has led to significant shifts in medical practices, patient care protocols, and antimicrobial usage[1]. As the world grapples with the multifaceted repercussions of the COVID-19 pandemic, one critical aspect that demands attention is its effect on bacterial resistance, especially in healthcare settings[2]. The Kingdom of Bahrain, like many other nations, faced unprecedented challenges as it navigated the complexities of managing a novel infectious disease while simultaneously addressing the ongoing burden of bacterial infections. In this context, our research on the potential influence of COVID-19 on bacterial resistance patterns is of utmost significance[3].

Bacterial resistance, where bacteria develop mechanisms to resist the effects of antibiotics, is a growing public health threat, significantly complicating the treatment of infections and leading to increased morbidity, mortality, and healthcare costs[4]. The COVID-19 pandemic has introduced several factors that could potentially influence bacterial resistance patterns. Increased antibiotic use, often to treat secondary bacterial infections associated with the COVID-19 pandemic, may exert selective pressure for resistant strains[5]. Additionally, changes in healthcare-seeking behavior and potential resource limitations during the pandemic could have impacted the diagnosis and treatment of bacterial infections, leading to inappropriate antibiotic use[6]. The heightened focus on COVID-19 pandemic testing may have also diverted resources away from routine bacterial diagnostics, delaying identifying and treating susceptible bacterial infections. These pandemic-related changes have raised concerns about the acceleration of antimicrobial resistance (AMR) and the potential for fostering an environment conducive to the emergence and spread of resistant bacterial strains[7].

Moreover, the strain on healthcare resources during the pandemic may have led to inconsistent application of antimicrobial stewardship (AMS) programs, further complicating efforts to manage bacterial resistance[8]. Diagnostic delays, the reduced routine monitoring of bacterial infections, and the diversion of medical resources to focus on COVID-19 pandemic care likely impacted the timely and appropriate use of antibiotics[9]. Understanding these changes is crucial for developing effective strategies to combat AMR and ensure the continued efficacy of antibiotics[10]. By examining the interplay between pandemic-related healthcare adaptations and bacterial resistance patterns, we can inform future public health policies, optimize AMS programs, and strengthen the resilience of healthcare systems against both viral and bacterial threats.

In Bahrain, the healthcare system had to adapt rapidly to the demands of the pandemic, implementing measures such as enhanced diagnostic capabilities, increased hospitalizations, changes in infection control practices, and the deployment of broad-spectrum antibiotics[11]. While essential for managing COVID-19, these adaptations may have inadvertently influenced bacterial resistance patterns. The hospitalization surge led to higher patient densities and increased the likelihood of nosocomial infections, necessitating more frequent and broader use of antibiotics[12]. Changes in infection control practices, such as the use of personal protective equipment (PPE) and isolation protocols, altered the dynamics of bacterial transmission within healthcare settings[13]. The widespread deployment of broad-spectrum antibiotics, often used prophylactically or to treat suspected secondary bacterial infections in COVID-19 patients, may have exerted selective pressure on bacterial populations, accelerating the emergence of resistant strains[14].

This retrospective study aims to uncover the impact of COVID-19 on bacterial resistance in the Kingdom of Bahrain. We analyzed resistance rates before, during, and after the pandemic to identify significant shifts in bacterial resistance patterns associated with the COVID-19 era. This analysis provided valuable insights into the interplay between viral pandemics and bacterial resistance, which help inform future public health policies and AMS programs. Through this study, we hope to contribute to a broader understanding of the long-term implications of the COVID-19 pandemic on bacterial resistance and guide efforts to mitigate the rise of resistant infections in Bahrain and beyond. This involves informed decision-making regarding antibiotic use and stewardship practices in a post-pandemic setting.

MATERIALS AND METHODS

Study design and setting

This retrospective study was conducted at the Salmanyia Medical Complex, a major tertiary care hospital in the Kingdom of Bahrain. It lasted six years, covering two years before, two years during, and two years after the peak of the COVID-19 pandemic. This timeframe was chosen to comprehensively capture changes in bacterial resistance patterns associated with the pandemic.

Bacteriologic testing methods

The causative microorganisms were identified using standard microbiologic methods, including matrix-assisted laser desorption ionization time-of-flight (Bruker Daltonics, Bremen, Germany). Antimicrobial susceptibility testing was conducted on all isolates obtained from patients included in the study. The hospital's microbiology laboratory tested susceptibility with an automated system (Phoenix, Becton, Dickinson and Company, Franklin Lakes, NJ, United States). The minimum inhibitory concentration breakpoints were determined for 14 antimicrobial agents: (1) Amikacin; (2) Ampicillin; (3) Aztreonam; (4) Ceftazidime; (5) Ceftriaxone; (6) Cefuroxime; (7) Cefepime; (8) Imipenem; (9) Meropenem; (10) Piperacillin/tazobactam; (11) Ciprofloxacin; (12) Gentamicin; (13) Tigecycline; and (14) Trimethoprim-sulfamethoxazole. Amoxicillin-clavulanic acid was tested using the disk diffusion method. Clinical and Laboratory Standards Institute interpretive criteria were used to interpret susceptibility results and breakpoints.

Phoenix, BD, detected extended-spectrum beta-lactamases (ESBL). Atypical ESBL detected by Phoenix were confirmed by double-disk synergy testing. Colistin susceptibility was tested using the Broth microdilution method (MICRONAUT). Phenotypically similar isolates from different specimens of the same patient were considered one sample. Molecular biological studies were not performed to identify the genetic similarities or dissimilarities of the bacterial isolates.

Definitions

Bacterial resistance is defined as the ability of bacteria to survive and proliferate in the presence of antibiotics that were previously effective against them. Bacterial resistance can complicate the treatment of infections, leading to increased morbidity, mortality, and healthcare costs. This study employs a specific categorization system to assess bacterial resistance patterns within the Kingdom of Bahrain. We differentiate bacterial strains based on their susceptibility to various antibiotic classes, focusing on potential changes associated with the COVID-19 pandemic. Following the approach outlined by Falagas and Karageorgopoulos[15], we define multidrug-resistant (MDR) strains as those exhibiting resistance to at least one agent in three or more distinct antibiotic classes. This categorization reflects a significant reduction in treatment options for infections caused by these bacteria. Our focus lies on MDR strains and their prevalence within the context of COVID-19[16,17]. Certain bacteria, such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, will be assigned an MDR designation if they exhibit resistance to five or more out of the seven evaluated anti-pseudomonal antibiotic classes[18]. MDR strains of *Mycobacterium tuberculosis* fall outside the scope of this study. By employing this classification system, we aim to achieve a clear and concise understanding of bacterial resistance patterns within the context of the COVID-19 pandemic in Bahrain. For clinical isolates, the first bacterial pathogen growth from any clinical specimen for each patient was counted as a clinical isolate. Duplicate isolates, identified from the same patient with the same organism and antimicrobial profile, were not considered[19].

Data collection

Data were extracted from the electronic health records (EHR) of inpatients admitted to various departments within Salmanyia Medical Complex. The EHRs were reviewed for relevant microbiological information. The dataset included the microbiological profile: (1) Types of bacterial isolates; (2) Antimicrobial susceptibility profiles; and (3) Resistance patterns.

Inclusion criteria and exclusion criteria

Inclusion criteria: All inpatients admitted to Salmanyia Medical Complex who had documented bacterial cultures and antimicrobial susceptibility testing during the specified periods.

Exclusion criteria: Patients with incomplete records or missing key data elements were excluded from the study.

Data management

The extracted data were systematically organized and tabulated using Microsoft Excel. Each patient's record was assigned a unique identifier to ensure anonymity and confidentiality. Data were manually extracted from the EHR system by trained personnel. Inconsistent or erroneous entries were identified and corrected. A random sample of records was cross-checked against the original EHR entries to verify accuracy.

Statistical analysis

Bacterial resistance rates were calculated for each of the three time periods (pre-pandemic, pandemic, and post-pandemic). Comparative analyses were conducted to identify significant shifts in resistance patterns over time. Statistical tests, such as χ^2 tests for categorical variables and *t*-tests for continuous variables, were employed to assess the significance of observed differences.

Ethical considerations

This study was conducted according to the ethical principles of the Declaration of Helsinki. Ethical approval was obtained from the institutional review board of the Salmanyia Medical Complex. As the study was retrospective, the institutional review board granted a waiver of informed consent. Patient confidentiality was maintained throughout the study, with all data anonymized before analysis.

RESULTS

During the extensive six-year study period (2018-2023), a significant fluctuation in bacterial isolates was observed at Salmanyia Medical Complex, categorized into three crucial timeframes: (1) Pre-COVID-19 (2018-2019); (2) During COVID-19 (2020-2021); and (3) Post-COVID-19 (2022-2023).

Prevalence of bacterial isolates

Escherichia coli (*E. coli*) remained the most prevalent organism, decreasing from 2061 isolates in 2018 to 1316 in 2020 before a sharp rise to 3400 in 2023, likely due to improved detection methods or increased infections. *Klebsiella pneumoniae* declined from 1133 in 2018 to 809 in 2020, then recovered to 1354 in 2023, reflecting fluctuating infection rates or detection practices. *Pseudomonas aeruginosa* decreased from 835 isolates in 2018 to 550 in 2020, rebounding slightly to 683 in 2023, possibly due to changes in infection control. *Acinetobacter baumannii* showed variability, peaking at 353 isolates in 2023 after 336 in 2021, indicating its continued role in healthcare-associated infections (Table 1, Figure 1).

Other notable organisms include (1) *Enterobacter species*, which declined from 307 in 2018 to 173 in 2020 and recovered to 279 in 2023; (2) *Salmonella species*, which spiked from 162 isolates in 2022 to 579 in 2023, possibly indicating an outbreak; and (3) *Proteus mirabilis*, which maintained stable numbers with slight fluctuations. In addition, *Citrobacter species* remained relatively stable throughout the study period, with minor fluctuations in the number of isolates, from 116 in 2018 to 159 in 2023, indicating a consistently low prevalence. In contrast, *Serratia marcescens* showed a notable increase in isolates, rising from 84 in 2018 to a peak of 119 in 2023. This upward trend suggests that *Serratia* may be playing an increasingly important role in infections at the facility. *Stenotrophomonas maltophilia* exhibited a gradual decline, with isolates decreasing from 133 in 2018 to 112 in 2023, which could indicate improvements in infection control or changes in the patient population. *Haemophilus influenzae* (*H. influenzae*) saw a sharp decrease in isolates, from 122 in 2018 to just 40 in 2021, followed by a recovery to 116 in 2023. This pattern may be influenced by vaccination programs or other public health measures to control *H. influenzae* infections.

Gram-positive organisms also show fluctuation in their rate before, during, and after the pandemic. *Staphylococcus aureus* isolates decreased from 1631 in 2018 to 1102 in 2020, then steadily rising to 1591 in 2023. *Enterococcus species* dropped significantly from 730 isolates in 2018 to 486 in 2019, followed by a substantial increase to 856 in 2023, indicating a growing concern with *Enterococcus*-related infections. *Coagulase-negative Staphylococci* showed a sharp rise, peaking at 1734 isolates in 2021, then slightly declining to 1433 in 2023. *Streptococcus pyogenes* experienced a dramatic decline from 127 isolates in 2018 to just 16 in 2022 before recovering to 99 in 2023. Similarly, *Streptococcus pneumoniae* isolates consistently declined from 106 in 2018 to 33 in 2020, with a modest recovery to 86 in 2023.

Antibiotic susceptibility patterns

E. coli susceptibility to Amoxicillin-Clavulanate decreased slightly from 47% in 2018 to 49% in 2023, while Cefuroxime

Table 1 Comparison of bacterial isolates over three periods (before, during, and after the coronavirus disease 2019 pandemic)

Organism	Pre-pandemic			Early pandemic			Post-peak pandemic			*P* value (2018-2019 *vs* 2020-2021)	*P* value (2020-2021 *vs* 2022-2023)	*P* value (2018-2019 *vs* 2022-2023)
	Year 2018	Year 2019	Total	Year 2020	Year 2021	Total	Year 2022	Year 2023	Total			
Escherichia coli	2061	1416	3477	1316	1549	2865	1635	3400	5035	< 0.01[a]	< 0.01[a]	< 0.01[a]
Klebsiella pneumoniae	1133	908	2041	809	969	1778	892	1354	2246	0.04[a]	0.05	0.03[a]
Proteus mirabilis	204	129	333	117	131	248	144	151	295	0.08	0.12	0.15
Enterobacter spp.	307	241	548	173	196	369	201	279	480	0.03[a]	0.06	0.04[a]
Salmonella spp.	149	103	252	104	118	222	162	579	741	0.05	< 0.01[a]	< 0.01[a]
Citrobacter spp.	116	116	232	88	101	189	123	159	282	0.07	0.02[a]	0.05
Serratia marsescenes	84	97	181	77	115	192	97	119	216	0.35	0.20	0.30
Pseudomonas aeruginosa	835	577	1412	550	627	1177	668	683	1351	0.10	0.18	0.19
Acinetobacter baumannii	297	272	569	323	336	659	317	353	670	0.06	0.07	0.08
Stenotrophomonas maltophilia	133	129	262	102	139	241	128	112	240	0.20	0.45	0.35
Haemaphilus influenzae	122	99	221	44	40	84	117	116	233	< 0.01[a]	< 0.01[a]	< 0.01[a]
Staphylococcus aureus	1631	1248	2879	1102	1169	2271	1363	1591	2954	0.02[a]	0.04[a]	0.05
Enterococcus spp.	730	486	1216	511	806	1317	712	856	1568	0.05	0.04[a]	0.05
Coagulase-negative Staphylococci	1062	955	2017	1060	1734	2794	1498	1433	2931	0.10	0.12	0.15
Streptococcus pyogenes	127	83	210	42	21	63	16	99	115	< 0.01[a]	< 0.01[a]	< 0.01[a]
Streptococcus pneumoniae	106	75	181	33	47	80	90	86	176	< 0.01[a]	0.05	< 0.01[a]

[a]Means significant *P* value.

Statistically significant changes ($P < 0.05$) are observed between the three periods for several organisms, including *Escherichia coli*, *Salmonella spp.*, *Haemaphilus influenzae*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*. These organisms show significant variations in their prevalence before, during, and after the coronavirus disease 2019 pandemic. Non-significant changes ($P > 0.05$) are observed for organisms like *Serratia marsescenes*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*, indicating more stable trends.

remained stable at 50%. Ceftriaxone and Ciprofloxacin susceptibility declined from 60% and 72% in 2018 to 57% and 64%, respectively, by 2023. *Klebsiella pneumoniae* susceptibility to Meropenem dropped from 92% in 2018 to 88% in 2023, while Tigecycline remained effective (82%+ susceptibility). *Pseudomonas aeruginosa* maintained high susceptibility to Piperacillin-Tazobactam, Ceftazidime, and Cefepime (73%+), though Meropenem and Imipenem showed slight declines to 85% in 2023. *Acinetobacter baumannii* displayed persistently low susceptibility, with rates below 30% for most antibiotics and Gentamicin dropping to 31% in 2023. *Enterobacter species* showed stable susceptibility to Meropenem (97%+) but slight declines in Ceftriaxone and Ciprofloxacin. *Proteus mirabilis* maintained 100% susceptibility to Meropenem, though Ciprofloxacin decreased from 82% in 2018 to 74% in 2023 (Figure 2, Supplementary Tables 1, 2 and 3).

Staphylococcus aureus exhibited a drop in Erythromycin susceptibility (75% in 2018 to 64% in 2023), but Clindamycin improved to 90% in 2023. *Methicillin-resistant Staphylococcus aureus* (*MRSA*) rates also increased during this period. *Enterococcus species* maintained stable susceptibility to Vancomycin and Daptomycin but fluctuating susceptibility to Penicillin, Ampicillin, and Ciprofloxacin. *Streptococcus pneumoniae* remained highly susceptible to Penicillin and Ceftriaxone (95%+), while Erythromycin susceptibility decreased from 79% in 2018 to 62% in 2023.

Multidrug-resistant trends

***E. coli*:** ESBL-producing strains increased from 39% in 2018 to 47% in 2021, with a slight decrease to 42% in 2023. Carbapenem-resistant (CRE) remained low, peaking at 3% during the pandemic.

***Klebsiella pneumoniae*:** ESBL rates rose from 38% in 2018 to 49% in 2019, stabilizing post-pandemic. CRE peaked at 30% in 2021 before decreasing to 12% in 2023.

***Acinetobacter baumannii*:** MDR rates remained high (89% in 2020), dropping slightly to 80% in subsequent years.

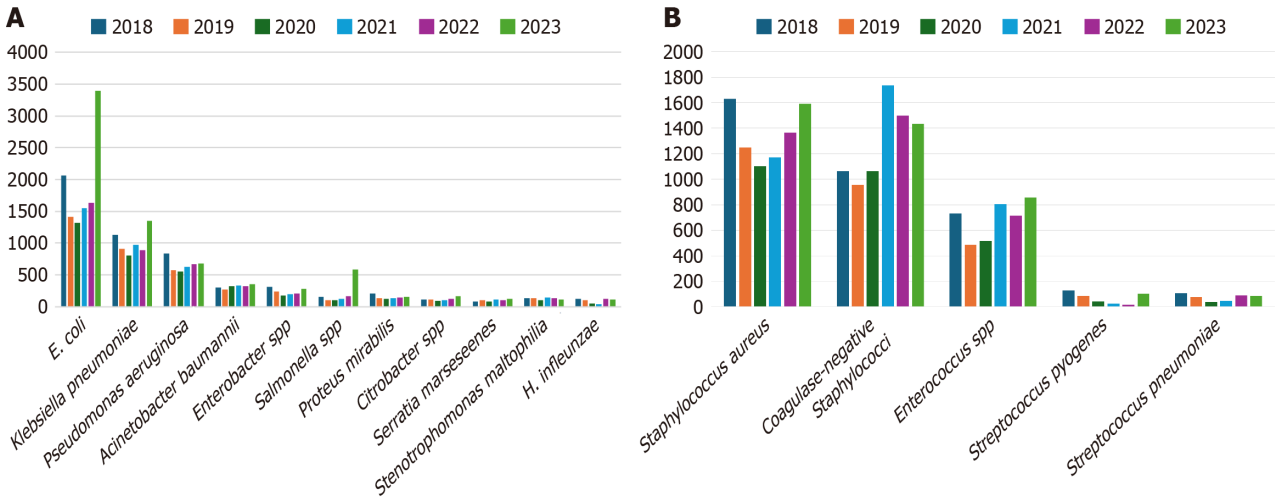


Figure 1 Most common Gram-negative and Gram-positive bacterial isolates Identified at Salmaniya Medical Complex over six years (2018-2023). A: Gram-negative bacterial isolates; B: Gram-positive bacterial isolates. *E. coil*: *Escherichia coli*; *H. influenzae*: *Haemaphilus influenzae*.

***Pseudomonas aeruginosa*:** MDR rates increased slightly during the pandemic, peaking at 17%, then declining to 9% post-pandemic.

***Staphylococcus aureus*:** *MRSA* rates rose from 36% in 2018 to 53% in 2023, particularly post-pandemic.

***Enterococcus species*:** *Vancomycin-resistant Enterococci* (*VRE*) rates increased from 9% in 2018 to 20% in 2021, stabilizing at 23% by 2023.

These trends suggest the COVID-19 pandemic had a notable impact on AMR, particularly in MDR pathogens like *Klebsiella pneumoniae, Acinetobacter baumannii*, *MRSA*, and *VRE*. While post-pandemic resistance rates decreased for some organisms, high resistance levels persist, highlighting the need for continuous AMS (Table 2, Figure 3).

DISCUSSION

We previously reported the bacterial co-infections in the very early phase of COVID-19. We observed a significant increase in bacterial and fungal co-infection in the early phase of the pandemic in the Kingdom of Bahrain[3]. Therefore, this six-year study (2018–2023) conducted at Salmaniya Medical Complex provides crucial insights into the evolving landscape of bacterial infections and AMR, particularly emphasizing the impact of the COVID-19 pandemic. The findings highlight significant changes in bacterial prevalence and resistance patterns, underscoring the ongoing challenges in managing healthcare-associated infections during and after the pandemic.

Fluctuations in bacterial isolates

Our data reveal notable fluctuations in bacterial isolate numbers across various organisms over the study period. *E. coli* consistently emerged as the most prevalent organism, with a marked increase in 2023. This spike may be attributable to rising infection rates and improved detection techniques. Similar trends were observed with *Klebsiella pneumoniae* and *Enterococcus species*, which both experienced post-pandemic surges. This resurgence suggests that the healthcare system's response to the pandemic, including altered infection control practices and the increased use of antibiotics during COVID-19 patient management.

Interestingly, *Salmonella* isolates saw a dramatic rise in 2023, which could indicate an outbreak or the result of enhanced surveillance measures, similar to trends reported in China[20]. Conversely, organisms such as *Stenotrophomonas maltophilia* and *H. influenzae* demonstrated a declining trend, likely reflecting the success of vaccination programs and improved infection control efforts. These shifts in bacterial prevalence underscore the dynamic nature of bacterial infections in the context of evolving healthcare challenges. This aligns with findings from other regions, such as India[21] and Romania[22], where similar trends in *E. coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* were noted.

Interestingly, *Salmonella* isolates saw a dramatic rise in 2023, which may suggest an outbreak or increased detection through enhanced surveillance systems. This sharp increase mirrors similar findings from China in 2023[20], where heightened awareness and targeted surveillance efforts resulted in the identification of more *Salmonella* cases than in previous years. The rise in *Salmonella* could be linked to several factors, including changes in food safety practices, increased incidence of foodborne outbreaks, or improved diagnostic capabilities in detecting bacterial pathogens[23]. Additionally, the increased use of routine microbiological screening and molecular diagnostic tools in healthcare settings may have contributed to the surge in reported cases, especially in light of the post-pandemic recovery in healthcare services. Such outbreaks often call for prompt public health interventions, including heightened surveillance, food safety regulations, and outbreak investigations to identify potential sources of infection[24].

Table 2 Percentage of multidrug-resistant among different bacterial isolates in the pre-pandemic, early, and post-peak-pandemic era

Organism	Resistance type	Pre-pandemic	Early pandemic	Post-peak pandemic	*P* value (pre-pandemic *vs* early pandemic)	*P* value (prepandemic *vs* post-peak pandemic)	*P* value (early pandemic *vs* post-peak pandemic)
Escherichia coli	ESBL	41.04	44.71	43.61	0.01[a]	0.01[a]	0.34
	CRE	1.01	2.51	1.67	< 0.0001[a]	0.01[a]	0.01[a]
Klebsiella pneumoniae	ESBL	42.92	43.98	40.20	0.50	0.07	0.01[a]
	CRE	13.82	29.58	15.14	< 0.0001[a]	0.21	< 0.0001[a]
Acinetobacter baumannii	MDR	79.09	87.86	79.40	< 0.0001[a]	0.89	< 0.0001[a]
Pseudomonas aeruginosa	MDR	12.89	13.85	8.96	0.47	0.001[a]	< 0.0001[a]
	CRE *Pseudomonas aeruginosa*	15.65	19.97	17.47	0.01[a]	0.19	0.10
Staphylococcus aureus	*Methicillin-resistant Staphylococcus aureus*	38.17	37.08	48.85	0.42	< 0.0001[a]	< 0.0001[a]
Enterococcus spp.	*Vancomycin-resistant Enterococci*	11.02	18.45	24.36	< 0.0001[a]	< 0.0001[a]	< 0.0001[a]

[a]Means significant *P* value.

CRE: Carbapenem-resistant; ESBL: Extended-spectrum beta-lactamases; Early pandemic: Years 2020 and 2021; MDR: Multidrug-resistant; Post-pandemic: Years 2022 and 2023; Pre-pandemic: Years 2018 and 2019.

Conversely, organisms such as *Stenotrophomonas maltophilia* and *H. influenzae* have a declining trend over the same period. This reduction is likely the result of improved infection control protocols and public health measures. For *Stenotrophomonas malt philia*, a pathogen often associated with immunocompromised patients and healthcare-associated infections, the decline could reflect better environmental hygiene, the increased use of isolation precautions, and more judicious use of broad-spectrum antibiotics that have been linked to its emergence. Improvements in hospital sanitation and a renewed focus on AMS during and after the pandemic may have also contributed to the reduced incidence of this pathogen[25].

The decline in *H. influenzae* isolates, on the other hand, is most likely due to the widespread success of vaccination programs, particularly the *H. influenzae* type B vaccine, which has been effective in reducing the incidence of invasive diseases caused by this pathogen[26]. The disruption of regular vaccination schedules during the pandemic was initially a concern. Still, the return to routine immunization practices post-pandemic may have contributed to the observed decline. Furthermore, the widespread use of non-pharmaceutical interventions during the pandemic, such as masking and social distancing, may have indirectly decreased the transmission of respiratory pathogens, including *H. influenzae*[27].

These shifts in bacterial prevalence underscore the dynamic nature of bacterial infections, particularly as healthcare systems adapt to evolving challenges such as the COVID-19 pandemic. Changes in infection control measures, public health policies, and diagnostic practices all shape bacterial epidemiology. The fluctuations in bacterial prevalence in Bahrain align with trends in other regions. For instance, studies from India[21] and Romania[22] have reported similar patterns in bacterial isolates, particularly with *E. coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. These organisms have shown fluctuations in response to shifting healthcare priorities, the widespread use of antimicrobials, and the influence of infection control practices. Such comparative data suggest that while the impact of the pandemic on bacterial prevalence and AMR is global, regional healthcare practices and public health strategies significantly influence the specific trends observed[28].

Staphylococcus aureus isolates decreased from 1631 in 2018 to 1102 in 2020, followed by a steady increase to 1591 in 2023. A similar trend was observed in Romania, where *Staphylococcus aureus* isolates initially rose from 2017 to 2019, then declined during 2020 and 2021, before surging again in 2022[22]. This fluctuation highlights the organism's persistent role in both community and healthcare-associated infections. The significant drop in *Enterococcus spp* isolates from 730 in 2018 to 486 in 2019, followed by a sharp increase to 856 in 2023, highlighting the growing concern regarding *Enterococcus*-related infections. This resurgence may be attributed to changes in infection control practices, increased use of antibiotics during the COVID-19 pandemic, and the pathogen's ability to acquire resistance to commonly used treatments like vancomycin[29]. *Coagulase-negative Staphylococci* also exhibited notable trends, peaking at 1734 isolates in 2021 before slightly declining to 1433 in 2023. This pattern could reflect a rise in device-related infections or shifts in clinical practices during the pandemic[30]. *Streptococcus pyogenes* experienced a dramatic decline, with isolates dropping from 127 in 2018 to just 16 in 2022, followed by a resurgence to 99 in 2023. These fluctuations may reflect variations in community-acquired infections or differences in reporting practices. Similarly, *Streptococcus pneumoniae* isolates consistently decreased from 106 in 2018 to 33 in 2020, with a modest recovery to 86 in 2023. This trend is likely influenced by vaccination efforts or other public health initiatives, as seen in the United Arab Emirates, where *Streptococcus pneumoniae* peaked in 2019, declined in 2020, and slightly increased in 2021[31].

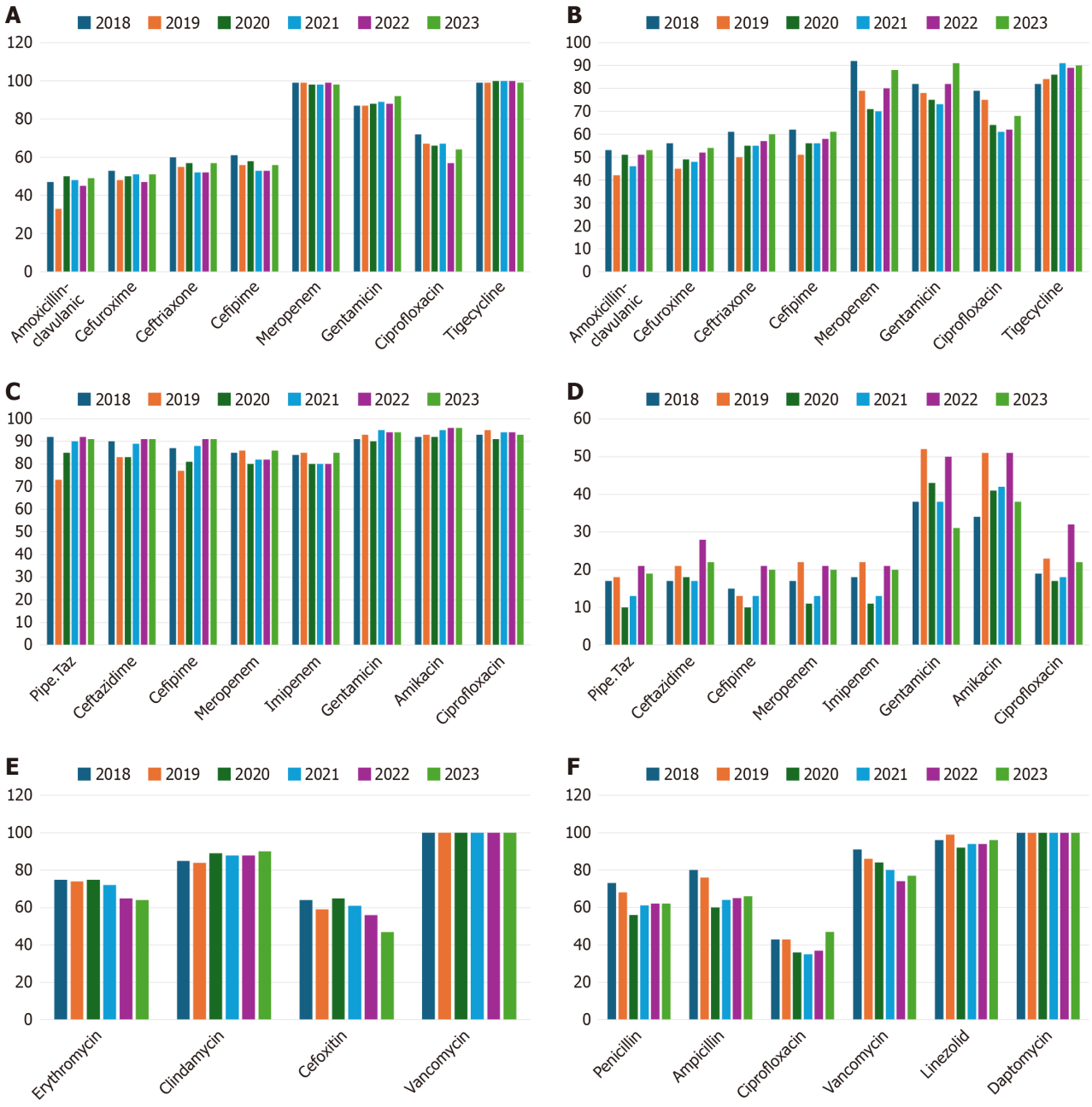


Figure 2 Percentage of antibiotic susceptibility. A: Percentage of *Escherichia coli* antibiotic susceptibility; B: Percentage of *Klebsiella pneumoniae* antibiotic susceptibility; C: Percentage of *Pseudomonas aeruginosa* antibiotic susceptibility; D: Percentage of *Acinetobacter baumannii* antibiotic susceptibility; E: Percentage of *Staphylococcus aureus* antibiotic susceptibility; F: Percentage of *Enterococcus spp.* antibiotic susceptibility. Pipe.Taz: Piperacillin-Tazobactam.

A study conducted in Malaysia identified *Acinetobacter baumannii* as the most frequently isolated organism, followed by *Klebsiella pneumoniae*, *Coagulase-negative Staphylococci*, *E. coli*, *Enterococcus faecalis*, and *Enterococcus faecium*[32]. In the United States, a multicenter study reported that *Staphylococcus aureus* and Gram-negative rods were the most commonly isolated bacterial pathogens from patients hospitalized during the pandemic[33]. These comparative analyses emphasize the broader context of bacterial isolation trends worldwide, underscoring the global nature of these challenges and the need for continued vigilance in managing bacterial infections.

Antibiotic susceptibility trends

The antibiotic susceptibility patterns highlight the growing challenge of antibiotic resistance, particularly among common pathogens like *E. coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*. *E. coli*'s decreasing susceptibility to commonly used antibiotics such as Amoxicillin-Clavulanate, Cefuroxime, and Ciprofloxacin underscores continuous monitoring and development of new therapeutic strategies. *Klebsiella pneumoniae* also showed a declining trend in susceptibility to several antibiotics, with a notable decrease in susceptibility to Meropenem, a last-resort antibiotic. This trend is concerning as it points to the increasing difficulty in treating infections caused by this pathogen. A similar finding was observed in a study from India, where Sharma *et al*[34] found an increase of antibiotic resistance among the isolated *Klebsiella pneumoniae* from patients in intensive care units (ICUs) to reach about 87.5% of the isolated strains, especially during and

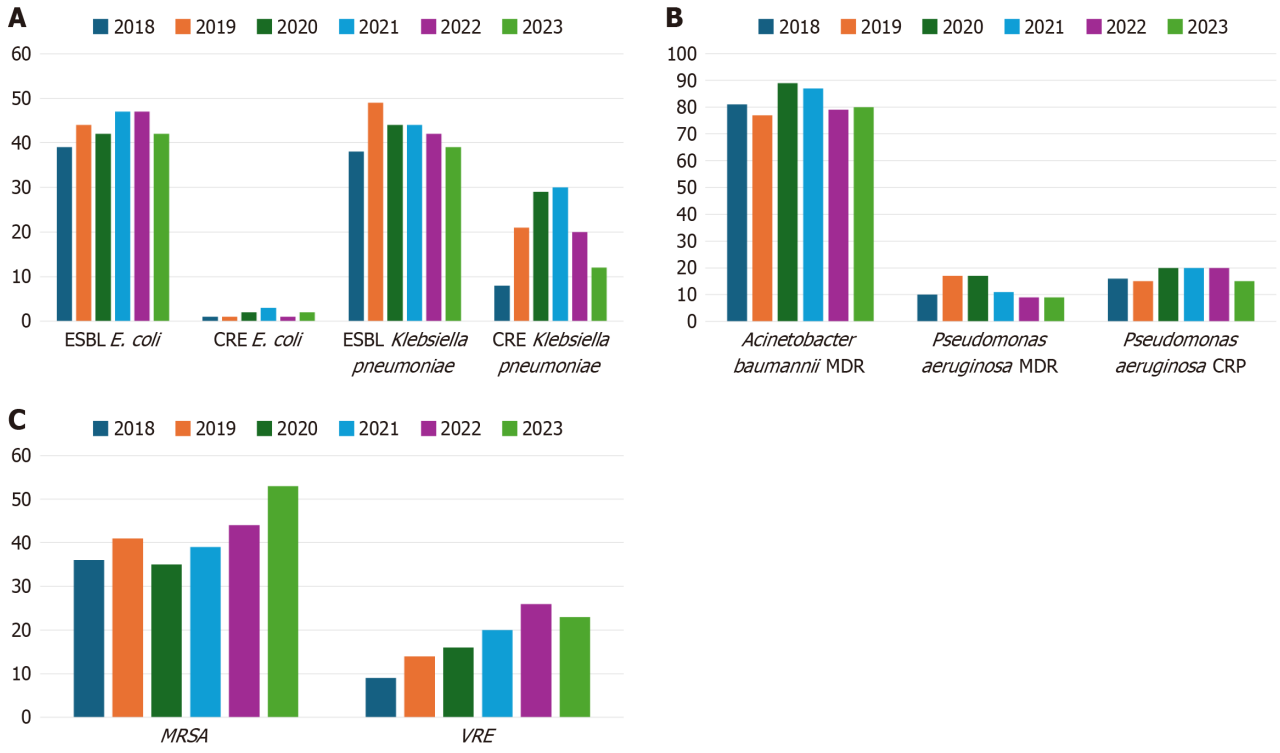


Figure 3 Percentage of multidrug-resistant. A: *Enterobacterales*: From extended-spectrum beta-lactamases to Carbapenemases; B: *Acinetobacter* and *Pseudomonas*; C: Gram-positive Bacteria. CRE: Carbapenem-resistant; CRP: Carbapenem-resistant *Pseudomonas aeruginosa*; *E. coil*: *Escherichia coli*; ESBL: Extended-spectrum beta-lactamases; MDR: Multidrug-resistant; *MRSA*: *Methicillin-resistant Staphylococcus aureus*; *VRE*: *Vancomycin-resistant Enterococci*.

after the peak of COVID-19 pandemic.

Acinetobacter baumannii, known for its role in healthcare-associated infections, displayed alarmingly low susceptibility rates to most antibiotics, with susceptibility rates for Piperacillin-Tazobactam, Ceftazidime, and Cefipime remaining below 30%. The persistently high MDR rates in *Acinetobacter baumannii* highlight the critical need for stringent infection control measures and the development of new antimicrobial agents. A systematic review by Sulayyim *et al*[35] found that *Acinetobacter baumannii* was the most commonly reported resistant gram-negative bacteria, followed by *Klebsiella pneumonia*, *E. coli*, and *Pseudomonas aeruginosa*.

MDR organisms

The rise of MDR organisms presents one of the most pressing challenges in modern healthcare. MDR organisms are defined as bacteria that are resistant to three or more antibiotics, making them particularly difficult to treat. This study highlights the increasing prevalence of MDR organisms at Salmaniya Medical Complex, particularly in pathogens like *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *MRSA*[36].

ESBL and CRE E. coli

E. coli is a common cause of both community-acquired and healthcare-associated infections, such as urinary tract infections (UTIs), bloodstream infections, and intra-abdominal infections. The emergence of ESBL-producing *E. coli* is a significant concern due to its resistance to many commonly used antibiotics, particularly cephalosporins and penicillins [37].

ESBL *E. coli*: ESBL-producing *E. coli* strains are resistant to third-generation cephalosporins like Ceftriaxone and Ceftazidime, necessitating the use of carbapenems as the primary treatment option. However, the overuse of carbapenems has led to the emergence of CRE *E. coli*, which poses an even greater therapeutic challenge[38]. The proportion of ESBL *E. coli* isolates in this study increased from 39% in 2018 to a peak of 47% in 2021 and 2022, likely influenced by the COVID-19 pandemic. A similar study in Thailand reported that 42.5% of *E. coli* isolates were ESBL-producing[39]. Conversely, a study in Finland observed a decreasing trend of ESBL *E. coli* during the pandemic compared to the pre-pandemic period[40].

CRE *E. coli*: CRE *E. coli* is resistant to nearly all available antibiotics, including carbapenems, which are often reserved for severe MDR infections. The rise in CRE *E. coli* has been associated with higher mortality rates, especially among critically ill patients in ICUs[41]. Our study found a concerning increase in CRE *E. coli* isolates during and after the pandemic, underscoring the need for strict infection control and prudent antibiotic use. Similar findings were reported in the Dominican Republic, where the CRE *E. coli* rate was about 0.15%[42], and in European countries, where CRE isolation rates rose significantly during the pandemic compared to the pre-pandemic period[43,44]. While ESBL-producing *E. coli* is more common in Asia, CRE *E. coli* and *VRE* are more prevalent in European countries.

MDR Klebsiella pneumoniae

Klebsiella pneumoniae is a major cause of healthcare-associated infections, including pneumonia, bloodstream infections, wound infections, and meningitis. During the pandemic, the prevalence of MDR *Klebsiella pneumoniae* increased significantly. A similar trend was reported in Shenzhen, China, where MDR *Klebsiella pneumoniae* isolates from hospitalized children increased during the pandemic[45]. An outbreak of hypervirulent MDR *Klebsiella pneumoniae* in COVID-19 patients in Italy was also associated with high mortality rates[46]. In Saudi Arabia, the rate of MDR *Klebsiella pneumoniae* reached 57.5% during the pandemic[47].

One of the most alarming findings in this study is the declining susceptibility of *Klebsiella pneumoniae* to carbapenems, such as Meropenem, which are often considered antibiotics of last resort for MDR infections. The emergence of CRE *Klebsiella pneumoniae* (CRKP) poses a significant threat, as it leaves few treatment options available[48]. In the Slovak Republic, the rate of CRKP increased 4.8 times during the pandemic, from 0.18% to 0.76%, with 47% of COVID-19 patients colonized with CRKP[49]. The rise in MDR *Klebsiella pneumoniae* during the pandemic may be due to the overuse of broad-spectrum antibiotics, prolonged hospital stays, and the increased use of invasive devices, all of which are known risk factors for the spread of MDR organisms[50].

MDR and CRE Pseudomonas aeruginosa

Pseudomonas aeruginosa is a common nosocomial pathogen, particularly in critically ill patients. Known for its intrinsic resistance to many antibiotics, *Pseudomonas aeruginosa* can also acquire resistance during treatment, resulting in MDR and CRE *Pseudomonas aeruginosa* (CRP)[51]. This study highlights the significant prevalence of MDR *Pseudomonas aeruginosa*, which is resistant to multiple antibiotic classes, including beta-lactams, aminoglycosides, and fluoroquinolones. The ability of *Pseudomonas aeruginosa* to form biofilms on medical devices, such as catheters and ventilators, contributes to its persistence and resistance in healthcare settings[52].

During the COVID-19 pandemic, the prevalence of MDR *Pseudomonas aeruginosa* increased by 70% compared to pre-pandemic levels, before returning to baseline after the pandemic. However, the prevalence of MDR *Pseudomonas aeruginosa* in Bahrain remained lower than in other countries[53]. CRP *Pseudomonas aeruginosa* is especially concerning due to its resistance to carbapenems, complicating treatment options. Our findings showed a significant decline in susceptibility to carbapenems like Meropenem and Imipenem, making infections caused by this pathogen more difficult to manage[54]. The use of more toxic antibiotics, such as colistin, is often required for CRP *Pseudomonas aeruginosa*, increasing morbidity and mortality[55]. Comparative studies show significantly higher rates of CRP *Pseudomonas aeruginosa* in regions outside Asia[56], with differences attributed to antibiotic prescribing practices, infection control measures, and public health policies[57].

MDR Acinetobacter baumannii

Acinetobacter baumannii is another notorious MDR pathogen, particularly in critical care settings, where it causes severe infections such as ventilator-associated pneumonia and bloodstream infections[58]. Our study showed that *Acinetobacter baumannii* had extremely low susceptibility rates to a wide range of antibiotics, with susceptibility to Piperacillin-Tazobactam, Ceftazidime, and Cefepime below 30%. The high prevalence of MDR *Acinetobacter baumannii* is concerning due to its ability to persist in hospital environments, making it a persistent threat[59]. Studies have shown a significantly increased risk of death in COVID-19 patients with MDR *Acinetobacter baumannii* co-infection[60]. In Croatia, *Acinetobacter baumannii* was the most common bloodstream infection in COVID-19 patients admitted to ICUs[61]. Similarly, a study in Mexico reported increased resistance of *Acinetobacter baumannii* to multiple antibiotics during the pandemic[62]. The emergence of CRE *Acinetobacter baumannii* further complicates treatment, with high mortality rates associated with these infections[63]. The persistence of MDR *Acinetobacter baumannii* during and after the pandemic highlights the urgent need for aggressive infection control measures and the development of alternative therapeutic strategies[64].

MRSA

MRSA has long been recognized as a significant cause of hospital-acquired infections. Our study observed an increase in *MRSA* isolates during the pandemic, likely due to the increased strain on healthcare systems, which may have led to lapses in infection control practices[65]. *MRSA* is resistant to all beta-lactam antibiotics, including penicillins and cephalosporins, limiting treatment options. The rise in *MRSA* during the pandemic aligns with global trends, which have reported an increase in healthcare-associated infections due to the pandemic's impact on infection control practices[66].

The fluctuations in *MRSA* rates in Bahrain during the COVID-19 pandemic, with an initial decrease followed by a rise to levels exceeding pre-pandemic rates, can be attributed to multiple factors. Early in the pandemic, strict infection control measures were implemented to prevent COVID-19 transmission in healthcare settings, likely reducing *MRSA* spread. These measures included enhanced hygiene protocols, reduced elective surgeries, and minimized hospital admissions for non-COVID-19 conditions[67]. However, as the pandemic progressed, the focus on managing COVID-19 cases may have disrupted routine infection control practices for other pathogens like *MRSA*[68]. Additionally, the widespread use of broad-spectrum antibiotics to treat suspected bacterial infections in COVID-19 patients may have promoted the emergence and spread of resistant strains, including *MRSA*[3,69]. As healthcare facilities resumed normal operations, there was likely an increase in hospital admissions and surgeries, along with weakened infection control measures, creating conditions conducive to *MRSA* transmission[70]. Prolonged strain on healthcare systems, resource limitations, and staff fatigue during the pandemic may have also contributed to the resurgence of *MRSA* in the later stages[71].

VRE

Enterococcus species, particularly *Enterococcus faecium*, are important causes of healthcare-associated infections, including bacteremia, endocarditis, and UTIs[72]. *VRE* is a significant challenge in healthcare settings due to its resistance to vancomycin, one of the key antibiotics for serious *Enterococcus* infections[73]. The progressive increase in *VRE* during the pandemic, particularly in Bahrain in 2022 and 2023, can be attributed to several factors. The pandemic disrupted routine healthcare practices and infection control measures as the focus shifted toward managing COVID-19 patients. The increased use of broad-spectrum antibiotics, such as vancomycin, likely exerted selective pressure, favoring the proliferation of *VRE*[74]. The strain on healthcare systems, lapses in infection prevention, and the resumption of routine hospital operations likely contributed to rising *VRE* cases in the later years of the pandemic[75]. *VRE* is particularly problematic in immunocompromised patients and those with underlying chronic conditions, where infections are more severe and difficult to treat[76]. The rise in *VRE* underscores the importance of strict infection control practices, including hand hygiene, environmental cleaning, and appropriate antibiotic use[77].

Implications of MDR organisms

The rise of MDR organisms has profound implications for clinical practice, patient outcomes, and public health. Infections caused by MDR organisms are associated with higher morbidity and mortality rates, prolonged hospital stays, and increased healthcare costs[78]. The limited treatment options for these infections often necessitate the use of more toxic or less effective antibiotics, which can lead to adverse patient outcomes[79]. The findings of this study underscore the urgent need for robust AMS programs to curb the overuse and misuse of antibiotics. AMS involves optimizing the selection, dosage, and duration of antimicrobial treatment to maximize clinical outcomes while minimizing the risk of resistance [80]. Infection control measures are equally crucial in preventing the spread of MDR organisms within healthcare facilities. These measures include hand hygiene, environmental cleaning, PPE, and the appropriate isolation of infected patients[81]. Additionally, ongoing surveillance of antibiotic resistance patterns is essential for guiding empirical therapy and developing targeted interventions to combat the spread of MDR organisms[82]. The study's data provide a critical foundation for informing local antibiotic prescribing practices and tailoring infection control policies to the specific challenges faced at Salmaniya Medical Complex.

Impact of the COVID-19 pandemic

The COVID-19 pandemic appears to have significantly influenced various bacterial pathogens' prevalence and resistance patterns. The increase in MDR organisms during the pandemic years, particularly in Klebsiella pneumoniae, *Acinetobacter baumannii*, and *MRSA*, suggests a potential correlation between the pandemic and the emergence of resistance[83]. The pandemic's impact on healthcare practices, including the increased use of antibiotics, changes in infection control practices, and the strain on healthcare resources may have contributed to this trend[84]. Interestingly, the post-pandemic period showed reduced resistance rates for some pathogens, possibly due to a renewed focus on infection control and AMS[85]. However, the persistently high levels of resistance in several key pathogens underscore the ongoing challenges in managing bacterial infections in the post-pandemic era.

Implications for clinical practice and public health

The findings of this study have important implications for clinical practice and public health. The rising prevalence of MDR organisms, particularly post-pandemic, highlights the need for continued vigilance in antibiotic stewardship and infection control practices[86]. The data also underscore the importance of ongoing surveillance of bacterial infections and resistance patterns to inform treatment guidelines and public health interventions. In conclusion, the study highlights the dynamic nature of bacterial infections and resistance patterns at Salmaniya Medical Complex. The fluctuations in bacterial isolates and the increasing resistance to commonly used antibiotics underscore the need for continuous monitoring, effective infection control measures, and the development of new antimicrobial agents[87]. The impact of the COVID-19 pandemic on resistance patterns further emphasizes the need for a comprehensive approach to managing bacterial infections in the healthcare setting.

Limitation of the study

This study has several limitations that should be considered when interpreting its findings. Firstly, the retrospective design, relying on historical data from 2018-2023, may limit the ability to establish causality and may be subject to inaccuracies or incomplete records. Additionally, as a single-center study conducted at Salmaniya Medical Complex, the findings may not be generalizable to other healthcare settings within Bahrain or other regions, as local practices, patient populations, and healthcare infrastructures could differ significantly. Over the six-year study period, changes in laboratory practices, diagnostic criteria, and data recording methods may have introduced variability and bias, affecting the analysis of trends. Furthermore, the study did not control for potential confounding factors such as changes in hospital admission policies, antibiotic prescribing practices, or infection control measures, which could have influenced the observed trends in bacterial isolates and antibiotic resistance patterns.

The lack of detailed clinical correlation with patient outcomes, such as mortality, morbidity, or length of hospital stay, also limits the ability to assess the direct impact of these trends on patient care. Moreover, the focus on selected bacterial pathogens may have overlooked trends in less frequently isolated organisms or emerging pathogens, potentially missing significant developments in the microbial landscape. The reliance on standard antibiotic susceptibility testing methods may not fully capture more nuanced resistance mechanisms, and variations in testing protocols over time could affect the comparability of results. While the study highlights the impact of the COVID-19 pandemic, it does not fully explore the complex interplay between COVID-19 treatment protocols and the observed trends in bacterial infections and antibiotic

resistance. Lastly, the absence of molecular data, such as genotyping or whole-genome sequencing, limits the ability to identify specific resistance genes or track the spread of particular clones within the hospital. These limitations underscore the need for caution in interpreting the results and point to areas for further research.

Recommendations

Based on the study's findings and limitations, several recommendations are suggested to enhance the understanding and management of bacterial infections and antibiotic resistance at Salmaniya Medical Complex and similar healthcare settings. Continuous and comprehensive surveillance of bacterial isolates and their antibiotic susceptibility patterns is crucial, with standardized methods to ensure data consistency over time, allowing for accurate trend analysis and early detection of emerging resistance patterns. Expanding surveillance to include a broader range of pathogens and integrating molecular techniques, such as genotyping or whole-genome sequencing, into routine practices would provide deeper insights into resistance mechanisms and the spread of specific clones. The study also underscores the importance of ongoing education and training of healthcare professionals in AMS practices, focusing on appropriate antibiotic use and infection control measures. Strengthening collaboration between microbiology laboratories, clinicians, and infection control teams is essential for translating surveillance data into effective clinical decision-making and policy development. Given the impact of the COVID-19 pandemic on bacterial infection trends, further investigation into the interplay between viral pandemics, treatment protocols, and bacterial resistance patterns is recommended to inform future healthcare responses. Additionally, improving data recording and reporting practices will enhance the accuracy of retrospective analyses. Lastly, conducting similar research in other healthcare settings within Bahrain and the wider region is advised to validate the findings and assess their generalizability, with comparative studies providing valuable insights into global trends in bacterial infections and resistance, aiding in developing coordinated public health strategies.

CONCLUSION

This study offers crucial insights into the evolving trends of bacterial isolates and their antibiotic susceptibility patterns at Salmaniya Medical Complex from 2018 to 2023, highlighting the significant impact of the COVID-19 pandemic on these dynamics. The fluctuating prevalence of key pathogens, including *E. coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*, alongside varying levels of antibiotic resistance, underscores the persistent challenge of AMR in healthcare settings. The results emphasize the importance of continuous monitoring through robust AMS programs to mitigate the rising threat of antibiotic resistance. Moreover, the study calls for implementing standardized surveillance systems and enhanced data collection methods to generate accurate, real-time insights that can inform clinical decisions and guide health policy development. The observed shifts in bacterial infection patterns during the pandemic further stress the need for a holistic approach to infectious disease management, recognizing the complex interplay between viral and bacterial pathogens. By advancing our understanding of bacterial resistance trends in Bahrain, this study lays a foundation for future research and policy initiatives to combat AMR and improve patient outcomes. Addressing the identified limitations and applying the recommended strategies can equip healthcare facilities with the tools to better manage bacterial infections and optimize antibiotic therapies amidst evolving resistance challenges.

FOOTNOTES

Author contributions: Saeed NK conceived the study and supervised the entire research process, including the study's design and the interpretation of the results, and contributed to revising the manuscript critically for important intellectual content; Almusawi SK was involved in acquiring data and played a key role in analyzing the laboratory results and organizing the data collection process, and contributed to revising the manuscript; Albalooshi NA contributed to the interpretation of the findings and ensured the accuracy of the clinical context in the manuscript, revised the manuscript and provided feedback on the final draft; Al-Beltagi M wrote the manuscript, performed the statistical analyses, and was responsible for revising it, communicated with the authors and journal reviewers, ensuring the manuscript met submission requirements; all of the authors read and approved the final version of the manuscript to be published.

Institutional review board statement: This study was conducted in accordance with the ethical standards of the Declaration of Helsinki. It was approved by the Institutional Review Board (IRB) of Salmaniya Medical Complex, Kingdom of Bahrain, on April 2024. Given the retrospective nature of the study and the use of de-identified patient data, the requirement for informed consent was waived by the IRB.

Informed consent statement: This retrospective study did not involve the direct collection of patient data or the identification of individual patients. As such, the Institutional Review Board of Salmaniya Medical Complex, Kingdom of Bahrain, waived informed consent in accordance with institutional guidelines and ethical standards.

Conflict-of-interest statement: The authors declare no conflict of interest. No financial, personal, or professional interests influenced this manuscript's research, authorship, or publication.

Data sharing statement: The data supporting this study's findings are available upon reasonable request from the corresponding author, Al-Beltagi M. However, the data are not publicly available due to privacy and ethical restrictions.

Country of origin: Bahrain

ORCID number: Nermin K Saeed 0000-0001-7875-8207; Safiya K Almusawi 0000-0003-0884-9907; Mohammed Al-Beltagi 0000-0002-7761-9536.

S-Editor: Luo ML
L-Editor: A
P-Editor: Zheng XM

REFERENCES

1 **Haileamlak A**. The impact of COVID-19 on health and health systems. *Ethiop J Health Sci* 2021; **31**: 1073-1074 [PMID: 35392335 DOI: 10.4314/ejhs.v31i6.1]
2 **Founou RC**, Blocker AJ, Noubom M, Tsayem C, Choukem SP, Dongen MV, Founou LL. The COVID-19 pandemic: a threat to antimicrobial resistance containment. *Future Sci OA* 2021; **7**: FSO736 [PMID: 34290883 DOI: 10.2144/fsoa-2021-0012]
3 **Saeed NK**, Al-Khawaja S, Alsalman J, Almusawi S, Albalooshi NA, Al-Biltagi M. Bacterial co-infection in patients with SARS-CoV-2 in the Kingdom of Bahrain. *World J Virol* 2021; **10**: 168-181 [PMID: 34367932 DOI: 10.5501/wjv.v10.i4.168]
4 **Ventola CL**. The antibiotic resistance crisis: part 1: causes and threats. *P T* 2015; **40**: 277-283 [PMID: 25859123]
5 **Knight GM**, Glover RE, McQuaid CF, Olaru ID, Gallandat K, Leclerc QJ, Fuller NM, Willcocks SJ, Hasan R, van Kleef E, Chandler CI. Antimicrobial resistance and COVID-19: Intersections and implications. *Elife* 2021; **10**: e64139 [PMID: 33588991 DOI: 10.7554/eLife.64139]
6 **Toro-Alzate L**, Hofstraat K, de Vries DH. The Pandemic beyond the Pandemic: A Scoping Review on the Social Relationships between COVID-19 and Antimicrobial Resistance. *Int J Environ Res Public Health* 2021; **18**: 8766 [PMID: 34444511 DOI: 10.3390/ijerph18168766]
7 **Arshad AR**, Ijaz F, Siddiqui MS, Khalid S, Fatima A, Aftab RK. COVID-19 pandemic and antimicrobial resistance in developing countries. *Discoveries (Craiova)* 2021; **9**: e127 [PMID: 34754900 DOI: 10.15190/d.2021.6]
8 **Subramanya SH**, Czyż DM, Acharya KP, Humphreys H. The potential impact of the COVID-19 pandemic on antimicrobial resistance and antibiotic stewardship. *Virusdisease* 2021; **32**: 330-337 [PMID: 34056051 DOI: 10.1007/s13337-021-00695-2]
9 **Petrakis V**, Panopoulou M, Rafailidis P, Lemonakis N, Lazaridis G, Terzi I, Papazoglou D, Panagopoulos P. The Impact of the COVID-19 Pandemic on Antimicrobial Resistance and Management of Bloodstream Infections. *Pathogens* 2023; **12**: 780 [PMID: 37375470 DOI: 10.3390/pathogens12060780]
10 **Uchil RR**, Kohli GS, Katekhaye VM, Swami OC. Strategies to combat antimicrobial resistance. *J Clin Diagn Res* 2014; **8**: ME01-ME04 [PMID: 25177596 DOI: 10.7860/JCDR/2014/8925.4529]
11 **Mukhaimer J**, Mihdawi MO, Al-ghatam R, Alhourani F, Opinion F. Assessment of the healthcare workers' physical, educational and operational needs during the COVID-19 pandemic in Bahrain. *AGJSR* 2024; **42**: 730-743 [DOI: 10.1108/agjsr-03-2023-0096]
12 **Gavi F**, Fiori B, Gandi C, Campetella M, Bientinesi R, Marino F, Fettucciari D, Rossi F, Moretto S, Murri R, Pierconti F, Racioppi M, Sacco E. Prevalence and Antimicrobial Resistance Patterns of Hospital Acquired Infections through the COVID-19 Pandemic: Real-Word Data from a Tertiary Urological Centre. *J Clin Med* 2023; **12**: 7278 [PMID: 38068329 DOI: 10.3390/jcm12237278]
13 **Garcia R**, Barnes S, Boukidjian R, Goss LK, Spencer M, Septimus EJ, Wright MO, Munro S, Reese SM, Fakih MG, Edmiston CE, Levesque M. Recommendations for change in infection prevention programs and practice. *Am J Infect Control* 2022; **50**: 1281-1295 [PMID: 35525498 DOI: 10.1016/j.ajic.2022.04.007]
14 **Granata G**, Schiavone F, Pipitone G, Taglietti F, Petrosillo N. Antibiotics Use in COVID-19 Patients: A Systematic Literature Review. *J Clin Med* 2022; **11**: 7207 [PMID: 36498781 DOI: 10.3390/jcm11237207]
15 **Falagas ME**, Karageorgopoulos DE. Extended-spectrum beta-lactamase-producing organisms. *J Hosp Infect* 2009; **73**: 345-354 [PMID: 19596491 DOI: 10.1016/j.jhin.2009.02.021]
16 **Magiorakos AP**, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; **18**: 268-281 [PMID: 21793988 DOI: 10.1111/j.1469-0691.2011.03570.x]
17 **Kalın G**, Alp E, Chouaikhi A, Roger C. Antimicrobial Multidrug Resistance: Clinical Implications for Infection Management in Critically Ill Patients. *Microorganisms* 2023; **11**: 2575 [PMID: 37894233 DOI: 10.3390/microorganisms11102575]
18 **Karaiskos I**, Giamarellou H. Multidrug-resistant and extensively drug-resistant Gram-negative pathogens: current and emerging therapeutic approaches. *Expert Opin Pharmacother* 2014; **15**: 1351-1370 [PMID: 24766095 DOI: 10.1517/14656566.2014.914172]
19 **Saeed NK**, Kambal AM, El-Khizzi NA. Antimicrobial-resistant bacteria in a general intensive care unit in Saudi Arabia. *Saudi Med J* 2010; **31**: 1341-1349 [PMID: 21135998]
20 **Peng S**, Xiong H, Lu J, Luo F, Liu C, Zhou H, Tong W, Xia Z, Liu D. Epidemiological and Whole Genome Sequencing Analysis of Restaurant Salmonella Enteritidis Outbreak Associated with an Infected Food Handler in Jiangxi Province, China, 2023. *Foodborne Pathog Dis* 2024; **21**: 316-322 [PMID: 38354216 DOI: 10.1089/fpd.2023.0123]
21 **Arun N**, Saurabh K, Muni S, Kumari N, Dev A. A Comparative Study of Bacterial Infections Between COVID-19 and Non-COVID-19 Patients With Respect to Different Isolates and Their Antibiotic Sensitivity Pattern. *Cureus* 2023; **15**: e40387 [PMID: 37456417 DOI: 10.7759/cureus.40387]
22 **Tălăpan D**, Sandu AM, Rafila A. Antimicrobial Resistance of Staphylococcus aureus Isolated between 2017 and 2022 from Infections at a Tertiary Care Hospital in Romania. *Antibiotics (Basel)* 2023; **12**: 974 [PMID: 37370293 DOI: 10.3390/antibiotics12060974]
23 **Ehuwa O**, Jaiswal AK, Jaiswal S. Salmonella, Food Safety and Food Handling Practices. *Foods* 2021; **10**: 907 [PMID: 33919142 DOI: 10.3390/foods10050907]
24 **Filip R**, Gheorghita Puscaselu R, Anchidin-Norocel L, Dimian M, Savage WK. Global Challenges to Public Health Care Systems during the

COVID-19 Pandemic: A Review of Pandemic Measures and Problems. *J Pers Med* 2022; **12**: 1295 [PMID: 36013244 DOI: 10.3390/jpm12081295]

25 **Ishikawa K**, Nakamura T, Kawai F, Uehara Y, Mori N. Stenotrophomonas maltophilia Infection Associated with COVID-19: A Case Series and Literature Review. *Am J Case Rep* 2022; **23**: e936889 [PMID: 35852985 DOI: 10.12659/AJCR.936889]

26 **Ai L**, Fang L, Liu B, Zhou C, Gong F. Impact of the COVID-19 pandemic on Haemophilus influenzae infections in pediatric patients hospitalized with community acquired pneumonia. *Sci Rep* 2024; **14**: 12737 [PMID: 38830922 DOI: 10.1038/s41598-024-62728-2]

27 **Kırca F**, Aydoğan S, Gozalan A, Güler E, Uyan Erten AZ, Özşen Uygur AS, Doğan A, Dinc B. Impact of non-pharmaceutical interventions on circulating respiratory viruses during the COVID-19 pandemic in Turkey. *Ann Saudi Med* 2023; **43**: 143-153 [PMID: 37270680 DOI: 10.5144/0256-4947.2023.143]

28 **Hirabayashi A**, Kajihara T, Yahara K, Shibayama K, Sugai M. Impact of the COVID-19 pandemic on the surveillance of antimicrobial resistance. *J Hosp Infect* 2021; **117**: 147-156 [PMID: 34562548 DOI: 10.1016/j.jhin.2021.09.011]

29 **Kraszewska Z**, Skowron K, Kwiecińska-Piróg J, Grudlewska-Buda K, Przekwas J, Wiktorczyk-Kapischke N, Wałecka-Zacharska E, Gospodarek-Komkowska E. Antibiotic Resistance of Enterococcus spp. Isolated from the Urine of Patients Hospitalized in the University Hospital in North-Central Poland, 2016-2021. *Antibiotics (Basel)* 2022; **11**: 1749 [PMID: 36551406 DOI: 10.3390/antibiotics11121749]

30 **Paranthaman K**, Wilson A, Verlander N, Rooney G, Macdonald N, Nsonwu O, Hope R, Fleming P, Hatcher J, Ogundipe E, Ratnaraja N, Wan Y, Pichon B, Westrop SJ, Brown CS, Demirjian A. Trends in coagulase-negative staphylococci (CoNS), England, 2010-2021. *Access Microbiol* 2023; **5**: acmi000491.v3 [PMID: 37424540 DOI: 10.1099/acmi.0.000491.v3]

31 **Senok A**, Thomsen J, Abdulrazzaq NM; UAE AMR Surveillance Consortium, Menezes GA, Ayoub Moubareck C, Everett D. Antimicrobial resistance in Streptococcus pneumoniae: a retrospective analysis of emerging trends in the United Arab Emirates from 2010 to 2021. *Front Public Health* 2023; **11**: 1244357 [PMID: 38074724 DOI: 10.3389/fpubh.2023.1244357]

32 **Rahman NA**, Soh TT, Sekawi Z, Zakariah S. Risk Factors Influencing Bacterial Infection in COVID-19 Patients in Hospital Sungai Buloh. *IJID* 2023; **130**: S107-S108 [DOI: 10.1016/j.ijid.2023.04.267]

33 **Shah MM**, Patel K, Milucky J, Taylor CA, Reingold A, Armistead I, Meek J, Anderson EJ, Weigel A, Reeg L, Como-Sabetti K, Ropp SL, Muse A, Bushey S, Shiltz E, Sutton M, Talbot HK, Chatelain R, Havers FP; CDC COVID-NET Surveillance Team. Bacterial and viral infections among adults hospitalized with COVID-19, COVID-NET, 14 states, March 2020-April 2022. *Influenza Other Respir Viruses* 2023; **17**: e13107 [PMID: 36875205 DOI: 10.1111/irv.13107]

34 **Sharma A**, Thakur A, Thakur N, Kumar V, Chauhan A, Bhardwaj N. Changing Trend in the Antibiotic Resistance Pattern of Klebsiella Pneumonia Isolated From Endotracheal Aspirate Samples of ICU Patients of a Tertiary Care Hospital in North India. *Cureus* 2023; **15**: e36317 [PMID: 37077586 DOI: 10.7759/cureus.36317]

35 **Sulayyim HJA**, Ismail R, Hamid AA, Ghafar NA. Antibiotic Resistance during COVID-19: A Systematic Review. *Int J Environ Res Public Health* 2022; **19**: 11931 [PMID: 36231256 DOI: 10.3390/ijerph191911931]

36 **van Duin D**, Paterson DL. Multidrug-Resistant Bacteria in the Community: Trends and Lessons Learned. *Infect Dis Clin North Am* 2016; **30**: 377-390 [PMID: 27208764 DOI: 10.1016/j.idc.2016.02.004]

37 **Kang CI**, Wi YM, Lee MY, Ko KS, Chung DR, Peck KR, Lee NY, Song JH. Epidemiology and risk factors of community onset infections caused by extended-spectrum β-lactamase-producing Escherichia coli strains. *J Clin Microbiol* 2012; **50**: 312-317 [PMID: 22162561 DOI: 10.1128/JCM.06002-11]

38 **Shaikh S**, Fatima J, Shakil S, Rizvi SM, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi J Biol Sci* 2015; **22**: 90-101 [PMID: 25561890 DOI: 10.1016/j.sjbs.2014.08.002]

39 **Siriphap A**, Kitti T, Khuekankaew A, Boonlao C, Thephinlap C, Thepmalee C, Suwannasom N, Khoothiam K. High prevalence of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolates: A 5-year retrospective study at a Tertiary Hospital in Northern Thailand. *Front Cell Infect Microbiol* 2022; **12**: 955774 [PMID: 36004324 DOI: 10.3389/fcimb.2022.955774]

40 **Ilmavirta H**, Ollgren J, Räisänen K, Kinnunen T, Hakanen AJ, Rantakokko-Jalava K, Jalava J, Lyytikäinen O. Impact of the COVID-19 pandemic on extended-spectrum β-lactamase producing Escherichia coli in urinary tract and blood stream infections: results from a nationwide surveillance network, Finland, 2018 to 2022. *Antimicrob Resist Infect Control* 2024; **13**: 72 [PMID: 38971782 DOI: 10.1186/s13756-024-01427-z]

41 **Adesanya OA**, Igwe HA. Carbapenem-resistant Enterobacteriaceae (CRE) and gram-negative bacterial infections in south-west Nigeria: a retrospective epidemiological surveillance study. *AIMS Public Health* 2020; **7**: 804-815 [PMID: 33294483 DOI: 10.3934/publichealth.2020062]

42 **Mena Lora AJ**, Sorondo C, Billini B, Gonzalez P, Bleasdale SC. Antimicrobial resistance in Escherichia coli and Pseudomonas aeruginosa before and after the coronavirus disease 2019 (COVID-19) pandemic in the Dominican Republic. *Antimicrob Steward Healthc Epidemiol* 2022; **2**: e191 [PMID: 36505946 DOI: 10.1017/ash.2022.347]

43 **Fasciana T**, Antonelli A, Bianco G, Lombardo D, Codda G, Roscetto E, Perez M, Lipari D, Arrigo I, Galia E, Tricoli MR, Calvo M, Niccolai C, Morecchiato F, Errico G, Stefani S, Cavallo R, Marchese A, Catania MR, Ambretti S, Rossolini GM, Pantosti A, Palamara AT, Sabbatucci M, Serra N, Giammanco A. Multicenter study on the prevalence of colonization due to carbapenem-resistant Enterobacterales strains before and during the first year of COVID-19, Italy 2018-2020. *Front Public Health* 2023; **11**: 1270924 [PMID: 38186699 DOI: 10.3389/fpubh.2023.1270924]

44 **Langford BJ**, Soucy JR, Leung V, So M, Kwan ATH, Portnoff JS, Bertagnolio S, Raybardhan S, MacFadden DR, Daneman N. Antibiotic resistance associated with the COVID-19 pandemic: a systematic review and meta-analysis. *Clin Microbiol Infect* 2023; **29**: 302-309 [PMID: 36509377 DOI: 10.1016/j.cmi.2022.12.006]

45 **Chang D**, Sharma L, Dela Cruz CS, Zhang D. Clinical Epidemiology, Risk Factors, and Control Strategies of Klebsiella pneumoniae Infection. *Front Microbiol* 2021; **12**: 750662 [PMID: 34992583 DOI: 10.3389/fmicb.2021.750662]

46 **Falcone M**, Tiseo G, Arcari G, Leonildi A, Giordano C, Tempini S, Bibbolino G, Mozzo R, Barnini S, Carattoli A, Menichetti F. Spread of hypervirulent multidrug-resistant ST147 Klebsiella pneumoniae in patients with severe COVID-19: an observational study from Italy, 2020-21. *J Antimicrob Chemother* 2022; **77**: 1140-1145 [PMID: 35040981 DOI: 10.1093/jac/dkab495]

47 **Ahmed OB**, Asghar AH, Bamaga M, Bahwerth FS, Ibrahim ME. Characterization of aminoglycoside resistance genes in multidrug-resistant Klebsiella pneumoniae collected from tertiary hospitals during the COVID-19 pandemic. *PLoS One* 2023; **18**: e0289359 [PMID: 37506109 DOI: 10.1371/journal.pone.0289359]

48 **Sheu CC**, Chang YT, Lin SY, Chen YH, Hsueh PR. Infections Caused by Carbapenem-Resistant Enterobacteriaceae: An Update on Therapeutic Options. *Front Microbiol* 2019; **10**: 80 [PMID: 30761114 DOI: 10.3389/fmicb.2019.00080]

49 **Ficik J**, Andrezál M, Drahovská H, Böhmer M, Szemes T, Liptáková A, Slobodníková L. Carbapenem-Resistant Klebsiella pneumoniae in COVID-19 Era-Challenges and Solutions. *Antibiotics (Basel)* 2023; **12**: 1285 [PMID: 37627705 DOI: 10.3390/antibiotics12081285]

50 **Ibrahim ME**. Risk factors in acquiring multidrug-resistant Klebsiella pneumoniae infections in a hospital setting in Saudi Arabia. *Sci Rep* 2023; **13**: 11626 [PMID: 37468757 DOI: 10.1038/s41598-023-38871-7]

51 **Pachori P**, Gothalwal R, Gandhi P. Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review. *Genes Dis* 2019; **6**: 109-119 [PMID: 31194018 DOI: 10.1016/j.gendis.2019.04.001]

52 **Elfadadny A**, Ragab RF, AlHarbi M, Badshah F, Ibáñez-Arancibia E, Farag A, Hendawy AO, De Los Ríos-Escalante PR, Aboubakr M, Zakai SA, Nageeb WM. Antimicrobial resistance of Pseudomonas aeruginosa: navigating clinical impacts, current resistance trends, and innovations in breaking therapies. *Front Microbiol* 2024; **15**: 1374466 [PMID: 38646632 DOI: 10.3389/fmicb.2024.1374466]

53 **Li Y**, Roberts JA, Walker MM, Aslan AT, Harris PNA, Sime FB. The global epidemiology of ventilator-associated pneumonia caused by multi-drug resistant Pseudomonas aeruginosa: A systematic review and meta-analysis. *Int J Infect Dis* 2024; **139**: 78-85 [PMID: 38013153 DOI: 10.1016/j.ijid.2023.11.023]

54 **Souza GHA**, Rossato L, Brito GT, Bet GMDS, Simionatto S. Carbapenem-resistant Pseudomonas aeruginosa strains: a worrying health problem in intensive care units. *Rev Inst Med Trop Sao Paulo* 2021; **63**: e71 [PMID: 34586305 DOI: 10.1590/S1678-9946202163071]

55 **Tenover FC**, Nicolau DP, Gill CM. Carbapenemase-producing Pseudomonas aeruginosa -an emerging challenge. *Emerg Microbes Infect* 2022; **11**: 811-814 [PMID: 35240944 DOI: 10.1080/22221751.2022.2048972]

56 **Lee YL**, Ko WC, Hsueh PR. Geographic Patterns of Carbapenem-Resistant Pseudomonas aeruginosa in the Asia-Pacific Region: Results from the Antimicrobial Testing Leadership and Surveillance (ATLAS) Program, 2015-2019. *Antimicrob Agents Chemother* 2022; **66**: e0200021 [PMID: 34807753 DOI: 10.1128/AAC.02000-21]

57 **Karruli A**, Catalini C, D'Amore C, Foglia F, Mari F, Harxhi A, Galdiero M, Durante-Mangoni E. Evidence-Based Treatment of Pseudomonas aeruginosa Infections: A Critical Reappraisal. *Antibiotics (Basel)* 2023; **12**: 399 [PMID: 36830309 DOI: 10.3390/antibiotics12020399]

58 **Howard A**, O'Donoghue M, Feeney A, Sleator RD. Acinetobacter baumannii: an emerging opportunistic pathogen. *Virulence* 2012; **3**: 243-250 [PMID: 22546906 DOI: 10.4161/viru.19700]

59 **Elbehiry A**, Marzouk E, Moussa I, Mushayt Y, Algarni AA, Alrashed OA, Alghamdi KS, Almutairi NA, Anagreyyah SA, Alzahrani A, Almuzaini AM, Alzaben F, Alotaibi MA, Anjiria SA, Abu-Okail A, Abalkhail A. The Prevalence of Multidrug-Resistant Acinetobacter baumannii and Its Vaccination Status among Healthcare Providers. *Vaccines (Basel)* 2023; **11**: 1171 [PMID: 37514987 DOI: 10.3390/vaccines11071171]

60 **Alenazi TA**, Shaman MSB, Suliman DM, Alanazi TA, Altawalbeh SM, Alshareef H, Lahreche DI, Al-Azzam S, Araydah M, Karasneh R, Rebahi F, Alharbi MH, Aldeyab MA. The Impact of Multidrug-Resistant Acinetobacter baumannii Infection in Critically Ill Patients with or without COVID-19 Infection. *Healthcare (Basel)* 2023; **11**: 487 [PMID: 36833021 DOI: 10.3390/healthcare11040487]

61 **Dobrović K**, Škrobo T, Selec K, Jelić M, Čivljak R, Peršec J, Sakan S, Bušić N, Mihelčić A, Hleb S, Andrašević AT. Healthcare-Associated Bloodstream Infections Due to Multidrug-Resistant Acinetobacter baumannii in COVID-19 Intensive Care Unit: A Single-Center Retrospective Study. *Microorganisms* 2023; **11**: 774 [PMID: 36985347 DOI: 10.3390/microorganisms11030774]

62 **Cureño-Díaz MA**, Plascencia-Nieto ES, Loyola-Cruz MÁ, Cruz-Cruz C, Nolasco-Rojas AE, Durán-Manuel EM, Ibáñez-Cervantes G, Gómez-Zamora E, Tamayo-Ordóñez MC, Tamayo-Ordóñez YJ, Calzada-Mendoza CC, Bello-López JM. Gram-Negative ESKAPE Bacteria Surveillance in COVID-19 Pandemic Exposes High-Risk Sequence Types of Acinetobacter baumannii MDR in a Tertiary Care Hospital. *Pathogens* 2024; **13**: 50 [PMID: 38251357 DOI: 10.3390/pathogens13010050]

63 **Vahhabi A**, Hasani A, Rezaee MA, Baradaran B, Hasani A, Kafil HS, Soltani E. Carbapenem resistance in Acinetobacter baumannii clinical isolates from northwest Iran: high prevalence of OXA genes in sync. *Iran J Microbiol* 2021; **13**: 282-293 [PMID: 34540166 DOI: 10.18502/ijm.v13i3.6388]

64 **Vrancianu CO**, Cristian R, Dobre E, Zenoaga-barbarosie C, Chirea E, Crunteanu I, Dionisie M. The Impact of Acinetobacter baumannii Infections in COVID-19 Patients Admitted in Hospital Intensive Care Units. *ECM* 2023; **28**: 1 [DOI: 10.3390/ecm2023-16479]

65 **Kavanagh KT**, Cormier LE. Success and failures in MRSA infection control during the COVID-19 pandemic. *Antimicrob Resist Infect Control* 2022; **11**: 118 [PMID: 36153597 DOI: 10.1186/s13756-022-01158-z]

66 **Ali Alghamdi B**, Al-Johani I, Al-Shamrani JM, Musamed Alshamrani H, Al-Otaibi BG, Almazmomi K, Yusnoraini Yusof N. Antimicrobial resistance in methicillin-resistant staphylococcus aureus. *Saudi J Biol Sci* 2023; **30**: 103604 [PMID: 36936699 DOI: 10.1016/j.sjbs.2023.103604]

67 **Dancer SJ**. Reducing the risk of COVID-19 transmission in hospitals: focus on additional infection control strategies. *Surgery (Oxf)* 2021; **39**: 752-758 [PMID: 34658477 DOI: 10.1016/j.mpsur.2021.10.003]

68 **Abubakar U**, Al-Anazi M, Alanazi Z, Rodríguez-Baño J. Impact of COVID-19 pandemic on multidrug resistant gram positive and gram negative pathogens: A systematic review. *J Infect Public Health* 2023; **16**: 320-331 [PMID: 36657243 DOI: 10.1016/j.jiph.2022.12.022]

69 **Ghosh S**, Bornman C, Zafer MM. Antimicrobial Resistance Threats in the emerging COVID-19 pandemic: Where do we stand? *J Infect Public Health* 2021; **14**: 555-560 [PMID: 33848884 DOI: 10.1016/j.jiph.2021.02.011]

70 **Coimbra R**, Edwards S, Coimbra BC, Tabuenca A. Resuming elective surgical services in times of COVID-19 infection. *Trauma Surg Acute Care Open* 2020; **5**: e000511 [PMID: 34192158 DOI: 10.1136/tsaco-2020-000511]

71 **Ismaeil R**, Nahas ARF, Kamarudin NB, Abubakar U, Mat-Nor MB, Mohamed MHN. Evaluation of the impact of COVID-19 pandemic on hospital-acquired infections in a tertiary hospital in Malaysia. *BMC Infect Dis* 2023; **23**: 779 [PMID: 37946158 DOI: 10.1186/s12879-023-08770-3]

72 **Sangiorgio G**, Calvo M, Migliorisi G, Campanile F, Stefani S. The Impact of Enterococcus spp. in the Immunocompromised Host: A Comprehensive Review. *Pathogens* 2024; **13**: 409 [PMID: 38787261 DOI: 10.3390/pathogens13050409]

73 **Cimen C**, Berends MS, Bathoorn E, Lokate M, Voss A, Friedrich AW, Glasner C, Hamprecht A. Vancomycin-resistant enterococci (VRE) in hospital settings across European borders: a scoping review comparing the epidemiology in the Netherlands and Germany. *Antimicrob Resist Infect Control* 2023; **12**: 78 [PMID: 37568229 DOI: 10.1186/s13756-023-01278-0]

74 **Micheli G**, Sangiorgi F, Catania F, Chiuchiarelli M, Frondizi F, Taddei E, Murri R. The Hidden Cost of COVID-19: Focus on Antimicrobial Resistance in Bloodstream Infections. *Microorganisms* 2023; **11**: 1299 [PMID: 37317274 DOI: 10.3390/microorganisms11051299]

75 **Wang YC**, Wang LS, Hsieh TC, Chung HC. Factors affecting vancomycin-resistant Enterococcus faecium colonization of in-hospital patients in different wards. *Tzu Chi Med J* 2024; **36**: 83-91 [PMID: 38406568 DOI: 10.4103/tcmj.tcmj_117_23]

76 **Cetinkaya Y**, Falk P, Mayhall CG. Vancomycin-resistant enterococci. *Clin Microbiol Rev* 2000; **13**: 686-707 [PMID: 11023964 DOI: 10.1128/CMR.13.4.686]

77 **Fukushige M**, Syue LS, Morikawa K, Lin WL, Lee NY, Chen PL, Ko WC. Trend in healthcare-associated infections due to vancomycin-resistant Enterococcus at a hospital in the era of COVID-19: More than hand hygiene is needed. *J Microbiol Immunol Infect* 2022; **55**: 1211-1218 [PMID: 35989164 DOI: 10.1016/j.jmii.2022.08.003]

78 **Poudel AN**, Zhu S, Cooper N, Little P, Tarrant C, Hickman M, Yao G. The economic burden of antibiotic resistance: A systematic review and meta-analysis. *PLoS One* 2023; **18**: e0285170 [PMID: 37155660 DOI: 10.1371/journal.pone.0285170]

79 **Izadpanah M**, Khalili H. Antibiotic regimens for treatment of infections due to multidrug-resistant Gram-negative pathogens: An evidence-based literature review. *J Res Pharm Pract* 2015; **4**: 105-114 [PMID: 26312249 DOI: 10.4103/2279-042X.162360]

80 **Doron S**, Davidson LE. Antimicrobial stewardship. *Mayo Clin Proc* 2011; **86**: 1113-1123 [PMID: 22033257 DOI: 10.4065/mcp.2011.0358]

81 **Fregonese L**, Currie K, Elliott L. Hospital patient experiences of contact isolation for antimicrobial resistant organisms in relation to health care-associated infections: A systematic review and narrative synthesis of the evidence. *Am J Infect Control* 2023; **51**: 1263-1271 [PMID: 37061166 DOI: 10.1016/j.ajic.2023.04.011]

82 **Morel CM**, de Kraker MEA, Harbarth S; Enhanced Surveillance Expert Consensus Group (CANSORT-SCI). Surveillance of Resistance to New Antibiotics in an Era of Limited Treatment Options. *Front Med (Lausanne)* 2021; **8**: 652638 [PMID: 33954161 DOI: 10.3389/fmed.2021.652638]

83 **Witt LS**, Howard-Anderson JR, Jacob JT, Gottlieb LB. The impact of COVID-19 on multidrug-resistant organisms causing healthcare-associated infections: a narrative review. *JAC Antimicrob Resist* 2023; **5**: dlac130 [PMID: 36601548 DOI: 10.1093/jacamr/dlac130]

84 **Rodríguez-Baño J**, Rossolini GM, Schultsz C, Tacconelli E, Murthy S, Ohmagari N, Holmes A, Bachmann T, Goossens H, Canton R, Roberts AP, Henriques-Normark B, Clancy CJ, Huttner B, Fagerstedt P, Lahiri S, Kaushic C, Hoffman SJ, Warren M, Zoubiane G, Essack S, Laxminarayan R, Plant L. Key considerations on the potential impacts of the COVID-19 pandemic on antimicrobial resistance research and surveillance. *Trans R Soc Trop Med Hyg* 2021; **115**: 1122-1129 [PMID: 33772597 DOI: 10.1093/trstmh/trab048]

85 **Mahida N**, Winzor G, Wilkinson M, Jumaa P, Gray J. Antimicrobial stewardship in the post COVID-19 pandemic era: an opportunity for renewed focus on controlling the threat of antimicrobial resistance. *J Hosp Infect* 2022; **129**: 121-123 [PMID: 36280374 DOI: 10.1016/j.jhin.2022.10.001]

86 **Adebisi YA**, Alaran AJ, Okereke M, Oke GI, Amos OA, Olaoye OC, Oladunjoye I, Olanrewaju AY, Ukor NA, Lucero-Prisno DE 3rd. COVID-19 and Antimicrobial Resistance: A Review. *Infect Dis (Auckl)* 2021; **14**: 11786337211033870 [PMID: 34376994 DOI: 10.1177/11786337211033870]

87 **Jansen WT**, van der Bruggen JT, Verhoef J, Fluit AC. Bacterial resistance: a sensitive issue complexity of the challenge and containment strategy in Europe. *Drug Resist Updat* 2006; **9**: 123-133 [PMID: 16807066 DOI: 10.1016/j.drup.2006.06.002]

World Journal of Virology

Submit a Manuscript: https://www.f6publishing.com

World J Virol 2025 March 25; 14(1): 100338

DOI: 10.5501/wjv.v14.i1.100338

ISSN 2220-3249 (online)

ORIGINAL ARTICLE

Observational Study

Serological surveillance for SARS-CoV-2 antibodies among students, faculty and staff within a large university system during the pandemic

Marcos G Pinheiro, Gabriela G O Alves, Maria Eduarda R Conde, Sofia L Costa, Regina C S Sant'Anna, Isa M F Antunes, Mônica C Carneiro, Fabio S Ronzei, Julia C Scaffo, Felipe R Pinheiro, Lialyz S Andre, Helvecio C Povoa, Valéria T Baltar, Fabíola Giordani, Eduarda S Hemerly, Gisele C Alexandre, Karla C de Paula, Márcio Watanabe, Antonio Claudio L da Nóbrega, Jackeline Christiane P Lobato, Fabio Aguiar-Alves

Specialty type: Virology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification
Scientific Quality: Grade A, Grade C
Novelty: Grade A, Grade B
Creativity or Innovation: Grade A, Grade C
Scientific Significance: Grade A, Grade B

P-Reviewer: Majeed HM; Rotondo JC

Received: August 20, 2024
Revised: September 30, 2024
Accepted: November 1, 2024
Published online: March 25, 2025
Processing time: 99 Days and 6 Hours

Marcos G Pinheiro, Gabriela G O Alves, Maria Eduarda R Conde, Sofia L Costa, Regina C S Sant'Anna, Isa M F Antunes, Julia C Scaffo, Felipe R Pinheiro, Lialyz S Andre, Laboratory of Molecular Epidemiology and Biotechnology, Rodolpho Albino University Laboratory, Fluminense Federal University, Niterói 24241000, Rio de Janeiro, Brazil

Marcos G Pinheiro, Mônica C Carneiro, Fabio S Ronzei, Estácio de Sá University, Nova Friburgo 28611135, Rio de Janeiro, Brazil

Marcos G Pinheiro, Felipe R Pinheiro, Lialyz S Andre, Fabio Aguiar-Alves, Pathology Program, Medicine school, Fluminense Federal University, Niterói 24070090, Rio de Janeiro, Brazil

Gabriela G O Alves, Fabio Aguiar-Alves, Department of Pharmaceutical Sciences, Lloyd L. Gregory School of Pharmacy, Palm Beach Atlantic University, West Palm Beach, FL 33401, United States

Helvecio C Povoa, Fabíola Giordani, Health Institute of Nova Friburgo, Fluminense Federal University, Nova Friburgo 28613001, Rio de Janeiro, Brazil

Valéria T Baltar, Eduarda S Hemerly, Gisele C Alexandre, Jackeline Christiane P Lobato, Department of Epidemiology and Biostatistics, Institute of Collective Health (ISC), Fluminense Federal University, Niterói 24033900, Rio de Janeiro, Brazil

Karla C de Paula, Institute of Physical Education, Fluminense Federal University, Niterói 24020005, Rio de Janeiro, Brazil

Márcio Watanabe, Department of Statistics, Fluminense Federal University, Niterói 24210200, Rio de Janeiro, Brazil

Antonio Claudio L da Nóbrega, Department of Physiology and Pharmacology, Fluminense Federal University, Niterói 24210130, Rio de Janeiro, Brazil

Co-corresponding authors: Jackeline Christiane P Lobato and Fabio Aguiar-Alves.

Corresponding author: Fabio Aguiar-Alves, DSc, MSc, PharmD, Associate Professor, Depart-

ment of Pharmaceutical Sciences, Lloyd L. Gregory School of Pharmacy, Palm Beach Atlantic University, 901 S Flagler Drive, West Palm Beach, FL 33401, United States. fabio_aguiaralves@pba.edu

Abstract

BACKGROUND

At the end of December 2019, the world faced severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), which led to the outbreak of coronavirus disease 2019 (COVID-19), associated with respiratory issues. This virus has shown significant challenges, especially for senior citizens, patients with other underlying illnesses, or those with a sedentary lifestyle. Serological tests conducted early on have helped identify how the virus is transmitted and how to curb its spread. The study hypothesis was that the rapid serological test for SARS-CoV-2 antibodies could indicate the immunoreactive profile during the COVID-19 pandemic in a university population.

AIM

To conduct active surveillance for serological expression of anti-SARS-CoV-2 antibodies in individuals within a university setting during the COVID-19 pandemic.

METHODS

This sectional study by convenience sampling was conducted in a large university in Niteroi-RJ, Brazil, from March 2021 to July 2021. The study population consisted of students, faculty, and administrative staff employed by the university. A total of 3433 faculty members, 60703 students, and 3812 administrative staff were invited to participate. Data were gathered through rapid serological tests to detect immunoglobulin (Ig) M and IgG against SARS-CoV-2. The χ^2 or Fisher's exact test was used to conduct statistical analysis. A 0.20 significance level was adopted for variable selection in a multiple logistic regression model to evaluate associations.

RESULTS

A total of 1648 individuals were enrolled in the study. The proportion of COVID-19 positivity was 164/1648 (9.8%). The adjusted logistic model indicate a positive association between the expression of IgM or IgG and age [odds ratio (OR) = 1.16, 95%CI: 1.02-1.31] ($P < 0.0024$), individuals who had been in contact with a COVID-19-positive case (OR = 3.49, 95%CI: 2.34-5.37) ($P < 0.001$), those who had received the COVID-19 vaccine (OR = 2.33, 95%CI: 1.61-3.35) ($P < 0.001$) and social isolation (OR = 0.59, 95%CI: 0.41-0.84) ($P < 0.004$). The likelihood of showing a positive result increased by 16% with every ten-year increment. Conversely, adherence to social distancing measures decreased the likelihood by 41%.

CONCLUSION

These findings evidenced that the population became more exposed to the virus as individuals discontinued social distancing practices, thereby increasing the risk of infection for themselves.

Key Words: Serological surveillance; SARS-CoV-2 antibodies; COVID-19; Serological rapid test; Risk factors for COVID-19

Core Tip: This study highlights the significance of serological surveillance in a university population during the coronavirus disease 2019 (COVID-19) pandemic. The findings show that age, contact with COVID-19-positive individuals, and vaccination status are positively associated with the manifestation of severe acute respiratory syndrome-coronavirus 2 antibodies. Additionally, adherence to social distancing measures significantly reduces the likelihood of infection. The prevalence of infection increased with relaxed social distancing practices, emphasizing the continued importance of preventive measures in controlling viral transmission.

Citation: Pinheiro MG, Alves GGO, Conde MER, Costa SL, Sant'Anna RCS, Antunes IMF, Carneiro MC, Ronzei FS, Scaffo JC, Pinheiro FR, Andre LS, Povoa HC, Baltar VT, Giordani F, Hemerly ES, Alexandre GC, de Paula KC, Watanabe M, Nóbrega ACLD, Lobato JCP, Aguiar-Alves F. Serological surveillance for SARS-CoV-2 antibodies among students, faculty and staff within a large university system during the pandemic. *World J Virol* 2025; 14(1): 100338

URL: https://www.wjgnet.com/2220-3249/full/v14/i1/100338.htm

DOI: https://dx.doi.org/10.5501/wjv.v14.i1.100338

INTRODUCTION

In late 2019, the global landscape was disrupted by the emergence of a novel coronavirus-induced acute respiratory syndrome known as coronavirus disease 2019 (COVID-19), caused by a newly identified severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2). Coronaviruses, belonging to the *Coronaviridae family*, can cause respiratory infections in various mammals and avian species[1]. In recent years, they have acquired the ability to adapt to humans *via* zoonotic transmission in a mechanism analogous to the one observed in the Zika virus outbreak[2].

The virus was highly contagious and was transmitted human-to-human *via* respiratory droplets expelled by infected individuals, which led to respiratory difficulties, particularly impacting individuals with pre-existing conditions, such as chronic obstructive pulmonary disease (COPD), asthma, cystic fibrosis, and lung cancer, including mesothelioma[3-5]. It also severely affected individuals over 60 and those with a sedentary lifestyle[6].

Many studies have established a connection between adipose and remote tissue inflammation among overweight and obese individuals[7]. Severe cases of coronavirus disease would dwell in the inflammation of the lungs. Therefore, a body naturally more prone to inflammation will experience the consequences of viral attacks on pulmonary alveoli more intensely[8].

Although significant advances in understanding the virus molecular and pathogenic characteristics remain limited, several vaccines have been developed in record time[9]. The first variant emerged in the population in February 2020 and was more infectious; after that, more variants were identified[10]. The evolution of SARS-CoV-2 is related to a virus changing behavior over pandemic stages[11].

Diagnostic strategies were used to detect viral infections, including immunoassays based on human anti-SARS-CoV-2 antigens or antibodies and even serological methods[12].

The production and release of specific immunoglobulin (Ig) M antibodies in the bloodstream start a few days after exposure to SARS-CoV-2. IgG antibodies become detectable 7-10 days after infection and can persist in the circulation for up to 12 weeks post-infection[13]. However, in specific individuals, traces of antibodies may still be detected several days after the initial infection. These antibodies are identified in the patient's blood and specifically target the spike glycoprotein[14].

Serology testing at the pandemic onset could help us understand SARS-CoV-2 distribution and infection patterns. Furthermore, rapid diagnostic methodologies in population testing can potentially mitigate disease progression[6,15]. Positive IgG results could also correlate with age and profession, with an understanding that healthcare workers were the most likely to be exposed and develop infection[16].

A significant challenge in accurately collecting data on infected individuals is regarding those who recently displayed symptoms and showed positive results in rapid serological tests. If these people are COVID-19-negative, their absence could unnecessarily decrease the workforce and disrupt the return to normal activities. However, if employees continue their activities while unknowingly COVID-19 positive, they may transmit the virus to other professionals, thereby increasing the disease's spread[17].

This study aimed to conduct active surveillance for serological expression of anti-SARS-CoV-2 antibodies in individuals within a university setting during the COVID-19 pandemic.

MATERIALS AND METHODS

Study design and participants

This sectional study comprised active surveillance for anti-SARS-CoV-2 antibodies by convenience sampling and was conducted at several Fluminense Federal University campuses (Niteroi, Campos dos Goytacazes, Macae, Nova Friburgo, Rio das Ostras, Santo Antonio de Padua, and Volta Redonda) from March 2021 to July 2021.

Study population

The study population consisted of students, faculty, and administrative staff employed by the Fluminense Federal University. Each participant provided a blood sample from their fingertip pulp and was invited to complete a survey regarding potential associated factors associated with SARS-CoV-2 infection.

Sample and sampling procedure

All 3433 faculty members, 60703 students (distance education, sequential, and in-person), and 3812 administrative staff were invited to participate [data available at (https://app.uff.br/transparencia/), accessed on 02/24/2021]. The study included students, faculty, and administrative staff with active enrollment during data collection. There were no exclusion criteria.

Data collection

The tests were performed per the manufacturer's guidelines (FIOCRUZ-Brazil)[18-20]. A single drop of blood from the individual's fingertip pulp was collected using a plastic capillary tube and used for an assay on a test cartridge.

A positive test result for COVID-19 was defined as a positive test result for the presence of IgG or IgM antibodies. The following variables were evaluated through a questionnaire: (1) Gender (male/female); (2) Age (adolescents-under 20 years/adults-20-59 years/older adults-60 years and over); (3) Affiliation (student/faculty/administrative staff); (4) Smoking (yes/no/used to smoke); (5) COVID-19 vaccination (no/yes); (6) Adherence to distancing measures (no/yes);

(7) Contact with a positive case (no/yes/don't know); (8) Having a healthcare professional in the family (no/yes); (9) Symptoms (no/yes); (10) Whether symptoms led to missing a day of work (no/yes); (11) Need for medical care (no/yes); and (12) Need for hospitalization (no/yes), comorbidities (no/yes) for diabetes, Alzheimer's disease, hypertension, asthma, human immunodeficiency virus/acquired immunodeficiency syndrome, cancer, depression, stroke, Parkinson's, and COPD. Data collection was performed by trained students.

Statistical analysis

Data was described by calculating the relative frequencies of COVID-19 positive results in every qualitative variable grouping. The χ^2 or Fisher's exact test was used to verify further the observed associations between the qualitative variables and positive results[21]. A 0.20 significance level was adopted for variable selection in a multiple logistic regression model to evaluate associations between sociodemographic, behavioral, and clinical factors and positive COVID-19 results[22]. A 0.05 significance level was utilized in the multiple models. A diagnostics analysis for the model was performed through the standard deviation of residuals, influential points (Cook's distance), and leverage. All statistical analyses were performed through R4.2.1.

Ethics information

All participants provided informed consent. This study was approved by the Research Ethics Committee (No. 43947221.6.0000.5243) and has been conducted under the code of Ethics of the World Medical Association.

RESULTS

A total of 1648 individuals were enrolled in the study. Most were students (763/1545, 49.4%), adults (1499/1648, 91%), Caucasian (710/1648, 43.1%), and female (962/1648, 58.4%). The proportion of COVID-19 positivity was 164/1648 (9.8%). A total of 1114/1601 (69%) of the studied population adhered to social distancing measures, and 884/1644 (53.8%) reported contact with a positive case, 1203/1648 (73%) individuals had symptoms, and 27/1648 (1.6%) required hospitalization. Only 374 individuals (23%) had been vaccinated, and 435 out of 1648 (26.4%) had some chronic disease (Table 1).

The variables associated with COVID-19 positivity included age (P = 0.015), having a family member who is a health professional (P = 0.003), frequent close contact with a confirmed COVID-19 case (P < 0.001), need for medical care (P < 0.001), severity of symptoms (P < 0.001), adherence to social distancing measures (P < 0.001), and COVID-19 vaccination status (P < 0.001).

In an adjusted logistic model (Table 2), individuals in contact with a confirmed COVID-19-positive case were more likely [odds ratio (OR) = 3.49, 95%CI: 2.34-5.37] to test positive for COVID-19 (IgM or IgG) than those who were vaccinated against COVID-19 (OR = 2.33, 95%CI: 1.61-3.35). The likelihood of a positive result increased by 16% with each ten-year age increment. On the other hand, adhering to social distancing measures decreased the likelihood by 41%.

DISCUSSION

This study examined the immunoreactive profile for serological assessment of anti-SARS-CoV-2 antibodies within a university environment during the COVID-19 pandemic. The serological rapid test may reveal the presence of COVID-19 antibodies in individuals.

The COVID-19 positivity rate was 162/1648 (9.8%). A commercial rapid test detected SARS-CoV-2 antibodies in the blood, distinguishing between positive and negative results for SARS-CoV-2 specific IgM and IgG antibodies. However, this test does not confirm the presence of the virus itself, a molecular test would be required for that confirmation[23]. Throughout the study, it was impossible to ascertain whether IgG/IgM-positivity resulted from prior exposure to the virus or vaccination. Participants without symptoms who tested positive for IgM or IgG were subjected to a real-time PCR test to confirm the presence of SARS-CoV-2, following recommendations by Hoffman *et al*[24].

However, this test does not confirm the presence of the virus itself; a molecular test would be required for that confirmation

According to Shahbazi *et al*[25], individuals exposed to COVID-19-positive patients were at higher risk of acquiring the virus. Moreover, there was also an association between positive results on rapid tests and exposure to COVID-19-positive individuals, representing a risk factor for infections. Although the virus detection was not confirmed among these participants, the detection of circulating antibodies indicated this association.

Gujski *et al*[26] described the detection of antigens in 15 (3.5%) of the subjects in a study of 423 medical students through the rapid antigen test in Warsaw, Poland, from November 15 to December 10, 2021. In the present study, 68/763 (8.9%) undergraduate students were positive for the rapid COVID-19 antibody test. These findings could be associated with an immunological memory from previous infections or a current acute phase of COVID-19.

Notably, we observed the prevalence of negative results on antibody rapid tests among subjects who adhered to social distancing measures, accounting for 1114/1601 (69%). Sims *et al*[27], in a meta-analysis study, discussed distancing's efficacy in reducing the transmission of infectious diseases, including COVID-19, as discussed across several papers.

Social distancing includes measures to limit interactions in a community that may include infected subjects who have not yet been identified and are, therefore, not isolated. Given that diseases transmitted by respiratory droplets require some physical proximity for contagion to occur, social distancing allows limiting transmission[28]. This study also

Table 1 Prevalence of coronavirus disease 2019 (immunoglobulin M or immunoglobulin G) according to sociodemographic, behavioral, and clinical characteristics on coronavirus disease 2019 among Brazilian students, professors, and technical employees from a public university, Niteroi, Brazil, 2021, *n* (%)

Characteristics	Total	Coronavirus disease 2019		*P* value[1]
		No (*n* = 1484)	Yes (*n* = 164)	
Tie to the university	1545			0.3
Students	763 (49.4)	695 (91)	68 (8.9)	
Professors	438 (28.3)	391 (89)	47 (11)	
Technical employees	344 (22.3)	303 (88)	41 (12)	
Gender	1648			> 0.9
Female	962 (58.4)	867 (90)	95 (9.9)	
Male	686 (41.6)	617 (90)	69 (10)	
Age	1648			0.015
Adolescents (younger than 20 years of age)	48 (2.9)	44 (92)	4 (8.3)	
Adults (20–59 years of age)	1499 (91)	1358 (91)	141 (9.4)	
Older adults (60 years of age or more)	101 (6.1)	82 (81)	19 (19)	
Ethnicity	1648			0.4
Asian	12 (0.7)	12 (100)	0 (0)	
Caucasian	710 (43.1)	646 (91)	64 (9.0)	
Mixed race	273 (16.6)	242 (89)	31 (11)	
Afro-Brazilian	144 (8.7)	133 (92)	11 (7.6)	
Non-declared	509 (30.9)	451 (89)	58 (11)	
Has a family member in healthcare	1642			0.003
No	1211 (73.7)	1106 (91.3)	105 (8.7)	
Yes	431 (26.3)	372 (86)	59 (14)	
Adhered to social distancing measures	1601			< 0.001
No	487 (30.4)	419 (86)	68 (14)	
Yes	1114 (69.6)	1021 (91.7)	93 (8.3)	
Was in contact with a positive result patient	1644			< 0.001
No	700 (42.6)	667 (95.3)	33 (4.7)	
Does not know	60 (3.6)	57 (95)	3 (5)	
Yes	884 (53.8)	756 (86)	128 (14)	
Presented symptoms	1648			
Yes	1203 (73)	1064 (88)	139 (12)	
Healthcare was needed	1648			< 0.001
No	1216 (73.8)	1126 (93)	90 (7.4)	
Does not know	1 (0.1)	1 (100)	0 (0)	
Yes	431 (26.1)	357 (83)	74 (17)	
Did symptoms make one miss a day of work	1648			< 0.001
No	1410 (85.5)	1299 (92.1)	111 (7.9)	
Does not know	1 (0.1)	1 (100)	0 (0)	
Yes	237 (14.4)	184 (78)	53 (22)	
Was the individual hospitalized	1648			

Yes	27 (1.6)	19 (70)	8 (30)	
Presence of chronic disease	1648			0.5
Negative	1213 (73.6)	1089 (90)	124 (10)	
Positive	435 (26.4)	395 (90.8)	40 (9.2)	
Smoker	1643			0.5
Not currently	175 (10.7)	159 (91)	16 (9.1)	
Non-smokers	1333 (81.1)	1202 (90)	131 (9.8)	
Current smokers	135 (8.2)	118 (87)	17 (13)	
The patient is vaccinated against severe acute respiratory syndrome-coronavirus 2	1605			< 0.001
No	1231 (76.7)	1135 (92.2)	96 (7.8)	
Yes	374 (23.3)	309 (83)	65 (17)	

[1]Pearson's χ^2 test, Fisher's exact test.

Table 2 Multiple logistic regression analysis of the effect of sociodemographic, behavioral, and clinical characteristics on coronavirus disease 2019 among Brazilian students, professors, and technical employees from a public university, Niteroi, Brazil, 2021, (*n* = 1648)

Characteristic	Odds ratio	95%CI	*P* value
Gender			
Female	1	-	
Male	0.97	0.68-1.37	0.9
Age (10 years)	1.16	1.02-1.31	0.024
Social distancing			
No	1	-	
Yes	0.59	0.41-0.84	0.004
Case positive contact			
No	1	-	
Yes	3.49	2.34-5.37	< 0.001
Get vaccinated			
No	1	-	
Yes	2.33	1.61-3.35	< 0.001

observed a relationship between participants who adhered to social distancing measures with negative results for COVID-19 IgG and IgM-specific rapid test ($P < 0.001$).

In this study, a history of prior hospitalization was associated with COVID-19-specific IgG and IgM antibodies, suggesting exposure to infected patients or increased virus circulation within the hospital setting. Additionally, Ko *et al* [29] found that higher COVID-19 hospitalization rates might be associated with age and gender.

Whitaker *et al*[30] outlined an association between age and positive outcomes for COVID-19 IgG and IgM-specific rapid tests. This study also observed a 16% rise in the likelihood of testing positive for COVID-19 IgG and IgM-specific rapid tests with every ten-year age increase (Table 2), which can be attributed to the immune response observed in older adults, as shown by Grifoni *et al*[31].

The literature supports the statement that reported symptoms are associated with COVID-19 infections[32]. Although it is acknowledged that data collection methods used in this study could not definitively confirm COVID-19 infection, we observed an association between previous symptoms and positivity on COVID-19 antibody rapid tests. Although rapid test can indicate the presence of the antibodies, we could not confirm the COVID-19 infection without molecular characterization. These findings were justified because most individuals 1203/1648 (73%) who reported COVID-19-specific symptoms tested positive for detecting circulating antibodies. Although COVID-specific antibody rapid tests are no longer in use, these results still offer insights into understanding the spread of COVID-19 during that period and that symptomatic subjects play a crucial role in disease transmission.

Although specific factors indicating immune protection against SARS-CoV-2 through vaccination are not clearly outlined, there is wide consensus that elevated levels of antibodies are beneficial[33]. Therefore, our study revealed an association between participants vaccinated against SARS-CoV-2 and positivity for IgG and IgM antibodies in the COVID-19 rapid test. This result may reflect the potential immunity conferred by the vaccination.

As a limitation of this study, the proportion of positivity in the rapid test might be underestimated due to possible test errors[23]. Convenience sampling may reflect a study population more exposed to SARS-CoV-2, that had been vaccinated, or was more concerned about their immunity status, not representing the total university population. The lack of molecular characterization of clinical samples may interfere with the confirmation of rapid test results.

CONCLUSION

The immunoreactive profile observed in the rapid test was associated with prior contact with people positive for COVID-19-positive individuals, COVID-19 vaccination status, age group, and having a family member who is a healthcare professional. On the other hand, negativity on the rapid test was linked to individuals adhering to social distancing measures. This study suggests that examining the immunoreactive profile could help us understand the presence of COVID-19 antibodies in individuals and identify associated factors.

FOOTNOTES

Author contributions: Pinheiro MG, Nóbrega ACLD, Lobato JCP, and Aguiar-Alves F conceptualized and supervised the study; Nóbrega ACLD, Lobato JCP, and Aguiar-Alves F were involved in funding acquisition, resources and project administration; Baltar VT and Giordani F were involved in statistical analysis; Pinheiro MG, Alves GGO, Povoa HC, Hemerly ES, Alexandre GC, de Paula KC, Watanabe M, Lobato JCP, and Aguiar-Alves F were involved in data curation and formal analysis in this study; Pinheiro MG, Alves GGO, Conde MER, Costa SL, Sant'Anna RCS, Antunes IMF, Carneiro MC, Ronzei FS, Scaffo JC, Pinheiro FR, Andre LS, Povoa HC, Baltar VT, Giordani F, Hemerly ES, Alexandre GC, de Paula KC, Watanabe M, Nóbrega ACLD, Lobato JCP, and Aguiar-Alves F were involved developing the methodology, writing, validation, investigation and visualization of the study; all of the authors read and approved the final version of the manuscript to be published.

Supported by The Fluminense Federal University.

Institutional review board statement: The study protocol received approval from the Appropriate Ethics Committee of the Medicine University–Fluminense Federal University. Prior to participating, all individuals provided informed consent. Throughout the study, patient information was handled with strict confidentiality and privacy.

Informed consent statement: Informed written consent was obtained from all participants before they were enrolled in the study.

Conflict-of-interest statement: The authors have no conflicts of interest to disclose.

Data sharing statement: Consent was obtained from all participants prior to their inclusion in the study. Patient information was handled with strict confidentiality and privacy throughout the entire process. The data are not publicly available due to privacy or ethical restrictions.

STROBE statement: The authors have read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to the STROBE Statement-checklist of items.

Country of origin: Brazil

ORCID number: Marcos G Pinheiro 0000-0002-7460-4830; Fabíola Giordani 0000-0003-2919-856X; Fabio Aguiar-Alves 0000-0001-6235-9367.

S-Editor: Luo ML
L-Editor: A
P-Editor: Guo X

REFERENCES

1 **Sharma A**, Ahmad Farouk I, Lal SK. COVID-19: A Review on the Novel Coronavirus Disease Evolution, Transmission, Detection, Control and Prevention. *Viruses* 2021; **13**: 202 [PMID: 33572857 DOI: 10.3390/v13020202]

2 **Azhar EI**, El-Kafrawy SA, Farraj SA, Hassan AM, Al-Saeed MS, Hashem AM, Madani TA. Evidence for camel-to-human transmission of

MERS coronavirus. *N Engl J Med* 2014; **370**: 2499-2505 [PMID: 24896817 DOI: 10.1056/NEJMoa1401505]

3 **Brackenborough K**, Ellis H, Flight WG. Respiratory Viruses and Cystic Fibrosis. *Semin Respir Crit Care Med* 2023; **44**: 196-208 [PMID: 36535663 DOI: 10.1055/s-0042-1758728]

4 **Çakır Edis E**. Chronic Pulmonary Diseases and COVID-19. *Turk Thorac J* 2020; **21**: 345-349 [PMID: 33031727 DOI: 10.5152/TurkThoracJ.2020.20091]

5 **Mangone L**, Mancuso P, Bisceglia I, Giorgi Rossi P, Chellini E, Negro C, Benfatto L, Migliore E, Casotto V, Mensi C, Romanelli A, Tumino R, Grappasonni I, Cavone D, Mazzoleni G, Tallarigo F, Marinaccio A; ReNaM Working Group. The impact of COVID-19 on new mesothelioma diagnoses in Italy. *Thorac Cancer* 2022; **13**: 702-707 [PMID: 35076994 DOI: 10.1111/1759-7714.14296]

6 **Castro R**, Luz PM, Wakimoto MD, Veloso VG, Grinsztejn B, Perazzo H. COVID-19: a meta-analysis of diagnostic test accuracy of commercial assays registered in Brazil. *Braz J Infect Dis* 2020; **24**: 180-187 [PMID: 32330437 DOI: 10.1016/j.bjid.2020.04.003]

7 **Kawai T**, Autieri MV, Scalia R. Adipose tissue inflammation and metabolic dysfunction in obesity. *Am J Physiol Cell Physiol* 2021; **320**: C375-C391 [PMID: 33356944 DOI: 10.1152/ajpcell.00379.2020]

8 **Hu B**, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. *Nat Rev Microbiol* 2021; **19**: 141-154 [PMID: 33024307 DOI: 10.1038/s41579-020-00459-7]

9 **Rotondo JC**, Martini F, Maritati M, Mazziotta C, Di Mauro G, Lanzillotti C, Barp N, Gallerani A, Tognon M, Contini C. SARS-CoV-2 Infection: New Molecular, Phylogenetic, and Pathogenetic Insights. Efficacy of Current Vaccines and the Potential Risk of Variants. *Viruses* 2021; **13**: 1687 [PMID: 34578269 DOI: 10.3390/v13091687]

10 **Roederer AL**, Cao Y, Denis KS, Sheehan ML, Li CJ, Lam EC, Gregory DJ, Poznansky MC, Iafrate AJ, Canaday DH, Gravenstein S, Garcia-Beltran WF, Balazs AB. Ongoing evolution of SARS-CoV-2 drives escape from mRNA vaccine-induced humoral immunity. *medRxiv* 2024 [PMID: 38496628 DOI: 10.1101/2024.03.05.24303815]

11 **Xiao B**, Wu L, Sun Q, Shu C, Hu S. Dynamic analysis of SARS-CoV-2 evolution based on different countries. *Gene* 2024; **916**: 148426 [PMID: 38575101 DOI: 10.1016/j.gene.2024.148426]

12 **Contini C**, Rotondo JC, Perna B, Guarino M, De Giorgio R. Special Issue: Advances in SARS-CoV-2 Infection. *Microorganisms* 2023; **11**: 1048 [PMID: 37110471 DOI: 10.3390/microorganisms11041048]

13 **Rotondo JC**, Martini F, Maritati M, Caselli E, Gallenga CE, Guarino M, De Giorgio R, Mazziotta C, Tramarin ML, Badiale G, Tognon M, Contini C. Advanced Molecular and Immunological Diagnostic Methods to Detect SARS-CoV-2 Infection. *Microorganisms* 2022; **10**: 1193 [PMID: 35744711 DOI: 10.3390/microorganisms10061193]

14 **Pavlova IP**, Nair SS, Kyprianou N, Tewari AK. The Rapid Coronavirus Antibody Test: Can We Improve Accuracy? *Front Med (Lausanne)* 2020; **7**: 569 [PMID: 32984390 DOI: 10.3389/fmed.2020.00569]

15 **Vena A**, Berruti M, Adessi A, Blumetti P, Brignole M, Colognato R, Gaggioli G, Giacobbe DR, Bracci-Laudiero L, Magnasco L, Signori A, Taramasso L, Varelli M, Vendola N, Ball L, Robba C, Battaglini D, Brunetti I, Pelosi P, Bassetti M. Prevalence of Antibodies to SARS-CoV-2 in Italian Adults and Associated Risk Factors. *J Clin Med* 2020; **9**: 2780 [PMID: 32867328 DOI: 10.3390/jcm9092780]

16 **Vimercati L**, Stefanizzi P, De Maria L, Caputi A, Cavone D, Quarato M, Gesualdo L, Lopalco PL, Migliore G, Sponselli S, Graziano G, Larocca AMV, Tafuri S. Large-scale IgM and IgG SARS-CoV-2 serological screening among healthcare workers with a low infection prevalence based on nasopharyngeal swab tests in an Italian university hospital: Perspectives for public health. *Environ Res* 2021; **195**: 110793 [PMID: 33508260 DOI: 10.1016/j.envres.2021.110793]

17 **Ji T**, Liu Z, Wang G, Guo X, Akbar Khan S, Lai C, Chen H, Huang S, Xia S, Chen B, Jia H, Chen Y, Zhou Q. Detection of COVID-19: A review of the current literature and future perspectives. *Biosens Bioelectron* 2020; **166**: 112455 [PMID: 32739797 DOI: 10.1016/j.bios.2020.112455]

18 **Castro N**, Lopes T, Cassaro B, Fonseca F, Fernandes A, Teixeira S, Gazzinelli R. Fragment production of the spike protein from SARS-CoV-2 in insect cells for the development of serological diagnosis. In: International Symposium on Immunobiological. Rio de Janeiro: Bio-Manguinhos; 2021: 89 [DOI: 10.35259/isi.2021_46672]

19 **Lopes T**, Coelho F, Santos A, Castro N, Bagno F, Gazzinelli R, Fernandes A. Development of a rapid test for detection of anti-COVID-19 IgG and IgM antibodies. In: International Symposium on Immunobiological. Rio de Janeiro: Bio-Manguinhos; 2021: 84 [DOI: 10.35259/isi.2021_46669]

20 **Stutz C**, Alcantara DMC, Dos Santos CM, Torres JM, Rodrigues R, Marcon GEB, Ferreira EC, Sarti ECFB, de Oliveira TF, Mendes FML, Lemos EF, Demarchi LHF, Lichs GGC, Zardin MCSU, Gonçalves CCM, Guilhermino JF, Perdomo RT, Fernandez ZDC. Seroprevalence of antibodies against SARS-CoV-2 in the school community in Campo Grande, state of Mato Grosso do Sul, Brazil, October 2021-November 2022. *Front Immunol* 2024; **15**: 1354786 [PMID: 38596680 DOI: 10.3389/fimmu.2024.1354786]

21 **Kim HY**. Statistical notes for clinical researchers: Chi-squared test and Fisher's exact test. *Restor Dent Endod* 2017; **42**: 152-155 [PMID: 28503482 DOI: 10.5395/rde.2017.42.2.152]

22 **Schober P**, Vetter TR. Logistic Regression in Medical Research. *Anesth Analg* 2021; **132**: 365-366 [PMID: 33449558 DOI: 10.1213/ANE.0000000000005247]

23 **Dinnes J**, Deeks JJ, Adriano A, Berhane S, Davenport C, Dittrich S, Emperador D, Takwoingi Y, Cunningham J, Beese S, Dretzke J, Ferrante di Ruffano L, Harris IM, Price MJ, Taylor-Phillips S, Hooft L, Leeflang MM, Spijker R, Van den Bruel A; Cochrane COVID-19 Diagnostic Test Accuracy Group. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. *Cochrane Database Syst Rev* 2020; **8**: CD013705 [PMID: 32845525 DOI: 10.1002/14651858.CD013705]

24 **Hoffman T**, Nissen K, Krambrich J, Rönnberg B, Akaberi D, Esmaeilzadeh M, Salaneck E, Lindahl J, Lundkvist Å. Evaluation of a COVID-19 IgM and IgG rapid test; an efficient tool for assessment of past exposure to SARS-CoV-2. *Infect Ecol Epidemiol* 2020; **10**: 1754538 [PMID: 32363011 DOI: 10.1080/20008686.2020.1754538]

25 **Shahbazi R**, Yasavoli-Sharahi H, Alsadi N, Ismail N, Matar C. Probiotics in Treatment of Viral Respiratory Infections and Neuroinflammatory Disorders. *Molecules* 2020; **25**: 4891 [PMID: 33105830 DOI: 10.3390/molecules25214891]

26 **Gujski M**, Mularczyk-Tomczewska P, Raciborski F, Samel-Kowalik P, Samoliński Ł, Olczak-Kowalczyk D, Jankowski M. Screening for SARS-CoV-2 Infection in Students at the Medical University of Warsaw, Poland Between November 15 and December 10, 2021 Using a Single Lateral Flow Test, the Panbio™ COVID-19 Ag Rapid Test. *Med Sci Monit* 2022; **28**: e936962 [PMID: 35665746 DOI: 10.12659/MSM.936962]

27 **Sims S**, Harris R, Hussein S, Rafferty AM, Desai A, Palmer S, Brearley S, Adams R, Rees L, Fitzpatrick JM. Social Distancing and Isolation Strategies to Prevent and Control the Transmission of COVID-19 and Other Infectious Diseases in Care Homes for Older People: An International Review. *Int J Environ Res Public Health* 2022; **19**: 3450 [PMID: 35329137 DOI: 10.3390/ijerph19063450]

28 **Wilder-Smith A**, Freedman DO. Isolation, quarantine, social distancing and community containment: pivotal role for old-style public health measures in the novel coronavirus (2019-nCoV) outbreak. *J Travel Med* 2020; **27**: taaa020 [PMID: 32052841 DOI: 10.1093/jtm/taaa020]

29 **Ko JY**, Danielson ML, Town M, Derado G, Greenlund KJ, Kirley PD, Alden NB, Yousey-Hindes K, Anderson EJ, Ryan PA, Kim S, Lynfield R, Torres SM, Barney GR, Bennett NM, Sutton M, Talbot HK, Hill M, Hall AJ, Fry AM, Garg S, Kim L; COVID-NET Surveillance Team. Risk Factors for Coronavirus Disease 2019 (COVID-19)-Associated Hospitalization: COVID-19-Associated Hospitalization Surveillance Network and Behavioral Risk Factor Surveillance System. *Clin Infect Dis* 2021; **72**: e695-e703 [PMID: 32945846 DOI: 10.1093/cid/ciaa1419]

30 **Whitaker M**, Davies B, Atchison C, Barclay W, Ashby D, Darzi A, Riley S, Cooke G, Donnelly CA, Chadeau-Hyam M, Elliott P, Ward H. SARS-CoV-2 rapid antibody test results and subsequent risk of hospitalisation and death in 361,801 people. *Nat Commun* 2023; **14**: 4957 [PMID: 37587102 DOI: 10.1038/s41467-023-40643-w]

31 **Grifoni A**, Alonzi T, Alter G, Noonan DM, Landay AL, Albini A, Goletti D. Impact of aging on immunity in the context of COVID-19, HIV, and tuberculosis. *Front Immunol* 2023; **14**: 1146704 [PMID: 37292210 DOI: 10.3389/fimmu.2023.1146704]

32 **Subramanian A**, Nirantharakumar K, Hughes S, Myles P, Williams T, Gokhale KM, Taverner T, Chandan JS, Brown K, Simms-Williams N, Shah AD, Singh M, Kidy F, Okoth K, Hotham R, Bashir N, Cockburn N, Lee SI, Turner GM, Gkoutos GV, Aiyegbusi OL, McMullan C, Denniston AK, Sapey E, Lord JM, Wraith DC, Leggett E, Iles C, Marshall T, Price MJ, Marwaha S, Davies EH, Jackson LJ, Matthews KL, Camaradou J, Calvert M, Haroon S. Symptoms and risk factors for long COVID in non-hospitalized adults. *Nat Med* 2022; **28**: 1706-1714 [PMID: 35879616 DOI: 10.1038/s41591-022-01909-w]

33 **Barnes E**, Goodyear CS, Willicombe M, Gaskell C, Siebert S, I de Silva T, Murray SM, Rea D, Snowden JA, Carroll M, Pirrie S, Bowden SJ, Dunachie SJ, Richter A, Lim Z, Satsangi J, Cook G, Pope A, Hughes A, Harrison M, Lim SH, Miller P, Klenerman P; PITCH consortium, Basu N, Gilmour A, Irwin S, Meacham G, Marjot T, Dimitriadis S, Kelleher P, Prendecki M, Clarke C, Mortimer P, McIntyre S, Selby R, Meardon N, Nguyen D, Tipton T, Longet S, Laidlaw S, Orchard K, Ireland G; CONSENSUS, Thomas D, Kearns P, Kirkham A, McInnes IB; OCTAVE Collaborative Group. SARS-CoV-2-specific immune responses and clinical outcomes after COVID-19 vaccination in patients with immune-suppressive disease. *Nat Med* 2023; **29**: 1760-1774 [PMID: 37414897 DOI: 10.1038/s41591-023-02414-4]

World Journal of Virology

Submit a Manuscript: https://www.f6publishing.com

World J Virol 2025 March 25; 14(1): 96098

DOI: 10.5501/wjv.v14.i1.96098

ISSN 2220-3249 (online)

ORIGINAL ARTICLE

Basic Study

Prevalence of transfusion transmissible infections among various donor groups: A comparative analysis

Sanjay K Thakur, Anil K Sinha, Santosh K Sharma, Aarzoo Jahan, Dinesh K Negi, Ruchika Gupta, Sompal Singh

Specialty type: Virology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification
Scientific Quality: Grade C
Novelty: Grade B
Creativity or Innovation: Grade B
Scientific Significance: Grade B

P-Reviewer: Kumar R

Received: April 26, 2024
Revised: October 16, 2024
Accepted: November 13, 2024
Published online: March 25, 2025
Processing time: 215 Days and 9 Hours

Sanjay K Thakur, Dinesh K Negi, Regional Blood Transfusion Centre, Hindu Rao Hospital and NDMC Medical College, North Delhi 110007, Delhi, India

Sanjay K Thakur, Anil K Sinha, Department of Zoology, Veer Kunwar Singh University, Ara 802301, Bihar, India

Santosh K Sharma, Department of Pathology, Hindu Rao Hospital and NDMC Medical College, North Delhi 110007, Delhi, India

Aarzoo Jahan, Sompal Singh, Regional Blood Transfusion Centre and Pathology, Hindu Rao Hospital and NDMC Medical College, North Delhi 110007, Delhi, India

Ruchika Gupta, Department of Cytopathology, ICMR-National Institute of Cancer Prevention and Research, Noida 201301, Uttar Pradesh, India

Co-first authors: Sanjay K Thakur and Anil K Sinha.

Co-corresponding authors: Ruchika Gupta and Sompal Singh.

Corresponding author: Sompal Singh, MD, Regional Blood Transfusion Centre and Pathology, Hindu Rao Hospital and NDMC Medical College, Malkaganj, North Delhi 110007, Delhi, India. sompalsingh@mcd.nic.in

Abstract

BACKGROUND

Transfusion transmissible infections (TTIs) are illnesses spread through contaminated blood or blood products. In India, screening for TTIs such as hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV)-I/II, malaria, and syphilis is mandatory before blood transfusions. Worldwide, HCV, HBV, and HIV are the leading viruses causing mortality, affecting millions of people globally, including those with co-infections of HIV/HCV and HIV/HBV. Studies highlight the impact of TTIs on life expectancy and health risks, such as liver cirrhosis, cancer, and other diseases in individuals with chronic HBV. Globally, millions of blood donations take place annually, emphasizing the importance of maintaining blood safety.

AIM

To study the prevalence of TTIs, viz., HBV, HCV, HIV I/II, syphilis, and malaria parasite (MP), among different blood donor groups.

METHODS

The study assessed the prevalence of TTIs among different blood donor groups in Delhi, India. Groups included total donors, in-house donors, total camp donors, institutional camp donors, and community camp donors. Tests for HIV, HBV, and HCV were done using enzyme-linked immunosorbent assay, while syphilis was tested with rapid plasma reagins and MP rapid card methods. The prevalence of HBV, HCV, HIV, and syphilis, expressed as percentages. Differences in infection rates between the groups were analyzed using χ^2 tests and *P*-values (less than 0.05).

RESULTS

The study evaluated TTIs among 42158 blood donors in Delhi. The overall cumulative frequency of TTIs in total blood donors was 2.071%, and the frequencies of HBV, HCV, HIV-I/II, venereal disease research laboratory, and MP were 1.048%, 0.425%, 0.221%, 0.377%, and 0.0024%, respectively. In-house donors, representing 37656 donors, had the highest transfusion transmissible infection (TTI) prevalence at 2.167%. Among total camp donors (4502 donors), TTIs were identified in 1.266% of donors, while community camp donors (2439 donors) exhibited a prevalence of 1.558%. Institutional camp donors (2063 donors) had the lowest TTI prevalence at 0.921%. Statistical analysis revealed significant differences in overall TTI prevalence, with total and in-house donors exhibiting higher rates compared to camp donors.

CONCLUSION

Ongoing monitoring and effective screening programs are essential for minimizing TTIs. Customizing blood safety measures for different donor groups and studying socio-economic-health factors is essential to improving blood safety.

Key Words: Blood donors; Transfusion transmissible infections; Hepatitis B virus; Human immunodeficiency virus; Hepatitis C virus; Malaria parasite; Syphilis

Core Tip: The study examined transfusion transmissible infection (TTI) among blood donors, assessing differences across groups: (1) In-house donors; (2) Total camp donors; (3) Institutional camp donors; and (4) Community camp donors. Findings revealed higher TTI prevalence in in-house donors (2.17%), followed by total donors (2.07%). Community camp donors and total camp donors had lower TTI rates at 1.56% and 1.27%, respectively, while institutional camp donors had the lowest rate at 0.92%. Statistical comparisons indicated significant differences in TTI prevalence between various groups.

Citation: Thakur SK, Sinha AK, Sharma SK, Jahan A, Negi DK, Gupta R, Singh S. Prevalence of transfusion transmissible infections among various donor groups: A comparative analysis. *World J Virol* 2025; 14(1): 96098
URL: https://www.wjgnet.com/2220-3249/full/v14/i1/96098.htm
DOI: https://dx.doi.org/10.5501/wjv.v14.i1.96098

INTRODUCTION

Transfusion transmissible infections (TTIs) include a range of illnesses that can be transmitted through the use of contaminated blood or blood products. Key TTIs include hepatitis C virus (HCV), hepatitis B virus (HBV), human immunodeficiency virus (HIV)-I/II, and syphilis. In India, screening for TTIs such as HIV-I/II, HBV, HCV, malaria parasite (MP), and syphilis is mandatory before any blood transfusion[1,2].

Globally, the three most prevalent viruses causing mortality are HCV, HBV, and HIV[3]. Epidemiological data indicate that there are approximately 71.0 million individuals living with HCV, 25.70 million with HBV, and 36.70 million with HIV worldwide. Additionally, according to an estimate, 2.30 million and 2.70 million patients suffer from co-infections of HIV/HCV and HIV/HBV, respectively, due to similar transmission routes[4]. A study in Tuscany, Italy, revealed an increase in life expectancy at birth of 2.9 years for men and 2.6 years for women, while an increase in infectious disease mortality resulted in a decrease in life expectancy by 0.11 years for men and 0.16 years for women[5]. Research in Pakistan found that HBV and HCV prevalence rates were 6.7% and 14.3%, respectively, with 80% of co-morbidities consisting of HIV/HCV and 20% consisting of HBV/HCV[6,7]. Chronic HBV infection heightens the risk for liver cirrhosis, cancer, and other diseases, posing a high level of contagion[8]. HCV and HBV infections are also prevalent among individuals living with HIV due to shared transmission pathways. Co-morbidities such as liver complications due to HCV or HBV infection are significant concerns for those infected with HIV[9]. Yearly, about 93 million blood donations

take place worldwide. In India, about 30 million blood components are transfused annually[10].

TTIs are a major health concern due to their potential to be transmitted through blood transfusions, which necessitates careful screening and monitoring. Their prevalence varies across different populations, making it crucial to understand the distribution of TTIs in specific regions. On study of English literature, we found an occasional study[11] highlighting the different prevalence of TTIs among various donor groups. Consequently, this study focuses on assessing the prevalence of TTIs among various groups of blood donors in Delhi.

MATERIALS AND METHODS

Blood donation

All necessary biosafety measures and infection control protocols were strictly adhered to during the collection and testing of blood samples. Volunteer and replacement blood donors were counseled and evaluated according to standard operating procedures before being permitted to donate blood at the blood bank.

Inclusion criteria

The study selected blood donors who met specific health and safety standards and did not have a risk of transmission of TTIs. Eligible donors had no recent, past, or current history of hepatitis, chronic diseases, sexually transmitted diseases, surgery, asthma, high-risk activities (such as unprotected intercourse), or pregnancy. Participants were required to be in good physical health, aged 18 years to 65 years, weighing over 45 kg, and with hemoglobin levels greater than 12.5 gm/dL[12].

Exclusion criteria

Donors who did not meet the qualifications for blood donation were excluded from this study.

Serological testing

After donation, the donor's blood was tested for TTIs. Commercially available testing kits were used according to the manufacturer's guidelines. The blood samples underwent testing for HIV-I/II, HBV, and HCV using standard enzyme-linked immunosorbent assay test kits. The detection of hepatitis B surface antigen (HBsAg) utilized Monolisa™ HBsAg ULTRA (BIO-RAD, Marnes-la-Coquette, France) with 100% sensitivity and 99.94% specificity. Combined screening for anti-HCV antibodies and HCV antigens in serum/plasma was conducted with Monolisa™ HCV Ag-Ab ULTRA V2 (BIO-RAD, Marnes-la-Coquette, France), which had 100% sensitivity and 99.94% specificity. Screening for the detection of HIV P24 antigen and antibodies to HIV-I and II in human plasma/serum was carried out using Genscreen™ ULTRA HIV Ag-Ab (BIO-RAD, Marnes-la-Coquette, France), with 100% sensitivity and 99.95% specificity. Antibodies for Treponema pallidum were tested using rapid plasma reagins carbon antigen test (RECKON DIAGNOSTICS P. LTD., Gorwa, Vadodara, India). Screening tests for malaria parasite antigens, including *Plasmodium falciparum* (Pf) and *Plasmodium vivax* (Pv), were detected by utilizing the Malaria *Pf*/*Pv* Ag Rapid Test kit, a lateral flow chromatographic immunoassay (BIOGENIX INC. PVT. LTD., Lucknow, India).

Statistical analysis

This study provides a comparative analysis of the prevalence of TTIs among various groups of blood donors. Groups included in the study are defined as follows: (1) In-house donors encompass all blood donation units, including in-house donors (both voluntary and replacement donors who donated blood at the blood center for the transfusion needs of their relatives or friends); (2) Total camp donors refer to blood units collected from voluntary blood donation camps, encompassing both institutional and community camp donors; (3) Institutional camp donors are voluntary donors who donated blood within institutional settings; (4) Community camp donors consist of voluntary donors who donated blood at community gatherings; and (5) Total donors include total camp donors and all in-house donors.

Data were collected from blood bank inventory registers [blood donation and transfusion transmissible infection (TTI) testing registers] and entered into Microsoft Excel spreadsheets for analysis. The study examined the prevalence of HBV, HCV, HIV, and syphilis, expressed as percentages. Statistical comparisons were made using χ^2 and *P*-values to assess the differences in infection rates between these groups. Analyses were performed using the open-source statistical software R version 4.0.0. A *P*-value less than 0.05 were considered statistically significant.

RESULTS

In the present study, data from 42158 blood donors (total donors) was included (Table 1). There were 37656 voluntary and replacement blood donors who had donated blood in-house in the blood center for the transfusion needs of their relatives or friends (in-house donors). A total of 4502 units were collected from voluntary blood donation camps that include institutional camp donors and community camp donors (total camp donors). There were 2063 voluntary blood donors who donated blood in institutional settings (institutional camp donors); this group of voluntary blood donors were either employed in the institutions or were students studying in the education institutions. There were 2439 voluntary blood donors who had donated blood at a community gathering (community camp donors).

Table 1 Prevalence of transfusion transmissible infections in donor groups, *n* (%)

		Total donors	In-house donors	Total camp donors	Institutional camp donors	Community camp donors
	Donors	42158 (100)	37656 (100)	4502 (100)	2063 (100)	2439 (100)
Hepatitis B virus	Reactive	442 (1.048)	416 (1.105)	26 (0.578)	11 (0.533)	15 (0.615)
	Non reactive	41716 (98.95)	37240 (98.9)	4476 (99.42)	2052 (99.47)	2424 (99.38)
Hepatitis C virus	Reactive	179 (0.425)	168 (0.446)	11 (0.244)	4 (0.194)	7 (0.287)
	Non reactive	41979 (99.58)	37488 (99.55)	4491 (99.76)	2059 (99.81)	2432 (99.71)
Human immunodeficiency virus	Reactive	93 (0.221)	85 (0.226)	8 (0.178)	2 (0.097)	6 (0.246)
	Non reactive	42065 (99.78)	37571 (99.77)	4494 (99.82)	2061 (99.9)	2433 (99.75)
Syphilis	Reactive	159 (0.377)	147 (0.39)	12 (0.267)	2 (0.097)	10 (0.41)
	Non reactive	41999 (99.62)	37509 (99.61)	4490 (99.73)	2061 (99.9)	2429 (99.59)
Transfusion transmissible infection	Reactive	873 (2.071)	816 (2.167)	57 (1.266)	19 (0.921)	38 (1.558)
	Non reactive	41285 (97.93)	36840 (97.83)	4445 (98.73)	2044 (99.08)	2401 (98.44)

Among the total donors (total donors) the proportion of TTI was as follows (Table 1, Figure 1): One or more TTI was identified in a total of 873 (2.071%) donors. Of these, HBV reactivity was seen in 442 (1.048%), HCV reactivity was seen in 179 (0.425%), HIV reactivity was seen in 93 (0.221%), and syphilis reactivity was seen in 159 (0.377%) donors. Among total blood donors, only one (0.0023%) was positive for MP.

Among the in-house donor (in-house donors) the proportion of TTI was as follows (Table 1, Figure 1): One or more TTI was identified in a total of 816 (2.167%) donors. Of these, HBV reactivity was seen in 416 (1.105%), HCV reactivity was seen in 168 (0.446%), HIV reactivity was seen in 85 (0.226%), and syphilis reactivity was seen in 147 (0.39%) donors.

Among the total blood donation in camp (total camp donors), the proportion of TTI was as follows (Table 1, Figure 1): One or more TTI was identified in a total of 57 (1.266%) donors. Of these, HBV reactivity was seen in 26 (0.578%), HCV reactivity was seen in 11 (0.244%), HIV reactivity was seen in 8 (0.178%), and syphilis reactivity was seen in 12 (0.267%) donors.

Among the blood donation camps organized in institutions (institutional camp donors), the proportion of TTI was as follows (Table 1, Figure 1): One or more TTI was identified in a total of 19 (0.921%) donors. Of these, HBV reactivity was seen in 11 (0.533%), HCV reactivity was seen in 4 (0.194%), HIV reactivity was seen in 2 (0.097%), and syphilis reactivity was seen in 2 (0.097%) donors.

Among the blood donation camps organized in the community (community camp donors), the proportion of TTI was as follows (Table 1, Figure 1): One or more TTI was identified in a total of 38 (1.558%) donors. Of these, HBV reactivity was seen in 15 (0.615%), HCV reactivity was seen in 7 (0.287%), HIV reactivity was seen in 6 (0.246%), and syphilis reactivity was seen in 10 (0.41%) donors.

Overall, the distribution of infections varied across donor groups, with in-house donors showing the highest rates of TTIs and related infections.

Statistical comparisons for differences in TTIs between different donor groups

Across the various donor groups, the prevalence of TTIs varied, with the highest incidence observed among in-house donors (2.167%), followed by total donors (2.0708%), community camp donors (1.558%), total camp donors (1.2661%), and lowest in institutional camp donors (0.921%). Across all groups (Tables 1 and 2, Figure 1), the majority of donations were non-reactive for each infection, with non-reactivity rates ranging from 98.44% to 99.90%. The overall prevalence of TTI was significantly higher in total donors compared to total camp donors (P = 0.0002) and institutional camp donors (P = 0.0003). The overall prevalence of TTI was significantly higher in in-house donors compared to total camp donors (P = 0.0001) and institutional camp donors (P = 0.0001) as well as community camp donors (P = 0.043).

Across the various donor groups (Tables 1 and 2, Figure 1), the prevalence of HBV varied, with the highest incidence observed among in-house donors (1.105%), followed by total donors (1.048%), community camp donors (0.615%), total camp donors (0.578%), and institutional camp donors (0.533%). There was a statistically significant difference in HBV prevalence between total donors and total camp donors (χ^2 = 9.0846, P = 0.0026), as well as between total donors and institutional camp donors (χ^2 = 5.1494, P = 0.0233), and between total donors and community camp donors (χ^2 = 4.2706, P = 0.0388). In contrast, no significant differences were observed in HBV prevalence between total donors and in-house donors.

Table 2 Statistical differences (χ^2 and *P* value) in prevalence of transfusion transmissible infections in donor groups

	Hepatitis B virus		Hepatitis C virus		Human immunodeficiency virus		Syphilis		Transfusion transmissible infections	
	χ^2	*P* value	χ^2	*P* value	χ^2	*P* value	χ^2	*P* value	χ^2	*P* value
Total donors and in-house donors	0.5929	0.4413	0.2134	0.6441	0.0235	0.8781	0.0911	0.7628	0.8887	0.3458
Total donors and total camp donors	9.0846	0.0026	3.259	0.071	0.3466	0.556	1.3628	0.2431	13.483	0.0002
Total donors and institutional camp donors	5.1494	0.0233	2.54	0.111	1.4028	0.2363	4.2569	0.0391	13.155	0.0003
Total donors and community camp donors	4.2706	0.0388	1.0509	0.3053	0.0672	0.7955	0.0659	0.7974	3.0295	0.0818
In-house donors and total camp donors	10.774	0.001	3.8736	0.0491	0.4214	0.5162	1.641	0.2002	16.093	0.0001
In-house donors and institutional camp donors	6.0074	0.0142	2.8864	0.0893	1.4841	0.2231	4.506	0.0338	14.754	0.0001
In-house donors and community camp donors	5.1663	0.023	1.335	0.2479	0.0416	0.8384	0.0226	0.8804	4.075	0.0435
Total camp donors and institutional camp donors	0.0496	0.8238	0.1579	0.6911	0.6066	0.4361	1.9123	0.1667	1.4726	0.2249
Total camp donors and community camp donors	0.0379	0.8457	0.1113	0.7386	0.3667	0.5448	1.0304	0.3101	0.9986	0.3177
Institutional camp donors and community camp donor	0.1303	0.7182	0.3975	0.5284	1.3999	0.2367	4.1204	0.0424	3.6281	0.0568

Across the various donor groups (Tables 1 and 2, Figure 1), the prevalence of HCV varied, with the highest incidence observed among in-house donors (0.446%), followed by total donors (0.425%), community camp donors (0.87%), total camp donors (0.244%), and institutional camp donors (0.194%). Regarding HCV, there were significant differences between in-house donors and total camp donors (χ^2 = 3.8736, *P* = 0.0491), but comparisons between total donors and other groups did not show significant differences.

Across the various donor groups (Tables 1 and 2, Figure 1), the prevalence of HIV varied, with the highest incidence observed among community camp donors (0.246%), followed by in-house donors (0.226%), total donors (0.221%), total camp donors (0.178%), and institutional camp donors (0.097%). The comparisons for HIV prevalence did not reveal statistically significant differences across different donor groups.

Across the various donor groups (Tables 1 and 2, Figure 1), the prevalence of syphilis varied, with the highest incidence observed among community camp donors (0.41%), followed by in-house donors (0.39%), total donors (0.377%), total camp donors (0.267%), and institutional camp donors (0.097%). In terms of syphilis, a significant difference was noted between total donors and institutional camp donors (χ^2 = 4.2569, *P* = 0.0391), but not between total donors and other groups.

DISCUSSION

The prevalence of TTIs varies significantly across different regions within India and in other countries worldwide. The current study focused on the prevalence of TTIs among blood donors in Delhi, India, and found values consistent with figures observed in other parts of the country and worldwide (Table 3)[10,12-27].

Our study results show, overall cumulative frequency of TTIs in blood donors was 2.071%, and frequency of HBV, HCV, HIV-I/II, venereal disease research laboratory (VDRL) and MP were 1.048%, 0.425%, 0.221%, 0.377% and 0.0024% respectively. Our study results show the overall cumulative frequency of TTIs in blood donors as 2.071%, and the frequencies of HBV, HIV-I/II, HCV, syphilis, and MP were 0.425%, 1.048%, 0.221%, 0.377%, and 0.0024%, respectively. The overall prevalence of TTIs in the present study was similar to the findings of Thakur *et al*[12], who have reported an

Table 3 Prevalence of transfusion transmissible infections in Indian states and other countries in the world

No.	States of India	Transfusion transmissible infections	Hepatitis B virus	Hepatitis C virus	Human immunodeficiency virus	Syphilis	Malaria parasite
1	Present study (Delhi, India)	2.071	1.048	0.425	0.221	0.377	0.002
2	India, 2016, ABBI[13]	1.58	0.87	0.34	0.14	0.17	0.06
	Northern zone						
3	Delhi, 2023, Thakur *et al*[12]	2.04	1.11	0.43	0.20	0.29	0.01
4	Delhi, 2016, ABBI[13]	2.03	1.06	0.54	0.2	0.22	0.01
5	Jammu and Kashmir, 2016, ABBI [13]	0.86	0.32	0.26	0.04	0.23	0.01
6	Himachal Pradesh, 2016, ABBI [13]	0.69	0.38	0.1	0.03	0.17	0.01
7	Punjab, 2016, ABBI[13]	2.64	0.65	1.35	0.14	0.49	0.01
8	Chandigarh, 2016, ABBI[13]	1.21	0.52	0.56	0.06	0.07	0.00
9	Haryana, 2016, ABBI[13]	1.97	0.87	0.8	0.12	0.16	0.02
10	Rajasthan, 2016, ABBI[13]	1.75	1.21	0.12	0.09	0.31	0.02
	Central zone						
11	Uttarakhand, 2016, ABBI[13]	1.79	0.76	0.67	0.1	0.13	0.13
12	Uttar Pradesh, 2016, ABBI[13]	1.7	0.9	0.49	0.1	0.17	0.04
13	Madhya Pradesh, 2016, ABBI[13]	2.24	1.14	0.1	0.08	0.36	0.56
14	Bhopal, 2023, Shrivastava *et al*[10]	2.7	1.8	0.42	0.2	0.31	0.02
15	Chhattisgarh, 2016, ABBI[13]	1.32	0.68	0.17	0.13	0.3	0.04
16	Central India, 2019, Varma *et al* [14]	1.43	1.29	0.07	0.08	-	-
	Eastern zone						
17	Sikkim, 2016, ABBI[13]	1.1	0.59	0.26	0.06	0.19	0.00
18	West Bengal, 2016, ABBI[13]	2.05	0.9	0.52	0.26	0.35	0.02
19	Bihar, 2016, ABBI[13]	1.84	1.42	0.14	0.16	0.05	0.07
20	Odisha, 2016, ABBI[13]	1.29	0.8	0.17	0.11	0.13	0.08
21	Odisha, 2020, Prakash *et al*[15]	1.89	0.97	0.41	0.35	-	-
22	Jharkhand, 2016, ABBI[13]	0.98	0.61	0.1	0.08	0.11	0.08
23	Ranchi, India, 2015, Sunderam *et al*[16]		1.01	0.14	0.08	0.03	0.33
	Western zone						
24	Maharashtra, 2016, ABBI[13]	1.66	1.02	0.31	0.21	0.06	0.06
25	Gujarat, 2016, ABBI[13]	1.03	0.59	0.13	0.1	0.2	0.01
26	Ahmadabad, 2022, Patel *et al*[17]	0.58	0.31	0.04	0.05	0.19	-
27	Gujarat, 2016, Bharadva *et al*[18]		0.57	0.05	0.10	0.05	-
28	Dadra and Nagar Haveli, 2016, ABBI[13]	2.18	1.79	0.03	0.08	0.28	0.00
29	Daman and Diu, 2016, ABBI[13]	0.59	-	0.35	0.12	0.06	0.06
	Southern zone						
30	Telangana, 2016, ABBI[13]	1.31	0.67	0.24	0.14	0.04	0.22
31	Telangana, 2016, Fatima *et al*[19]	0.96	0.69	0.01	0.20	0.03	

32	Andhra Pradesh, 2016, ABBI[13]	1.91	1.39	0.23	0.18	0.07	0.04
33	Karnataka, 2016, ABBI[13]	1.36	0.94	0.22	0.13	0.07	0.00
34	Tamil Nadu, 2016, ABBI[13]	0.92	0.68	0.11	0.05	0.07	0.01
35	Kerala, 2016, ABBI[13]	0.56	0.28	0.17	0.05	0.04	0.02
36	Puducherry, 2016, ABBI[13]	3.13	2.12	0.55	0.37	0.09	0.00
37	Andaman and Nicobar, 2016, ABBI[13]	2	0.85	0.27	0.00	0.12	0.76
38	Goa, 2016, ABBI[13]	0.69	0.44	0.13	0.1	0.01	0.01
	North East zone						
39	Arunachal Pradesh, 2016, ABBI [13]	2.19	0.74	0.08	0.04	0.97	0.36
40	Meghalaya, 2016, ABBI[13]	2.18	0.78	0.47	0.16	0.73	0.04
41	Mizoram, 2016, ABBI[13]	2.48	0.94	1.24	0.3	0.00	0.00
42	Manipur, 2016, ABBI[13]	1.62	0.59	0.83	0.15	0.04	0.01
43	Tripura[13]	1.5	1.25	0.08	0.08	0.08	0.01
44	Assam[13]	1.23	0.54	0.24	0.12	0.3	0.03
45	Nagaland[13]	1.01	0.34	0.27	0.26	0.14	0.00
	Country (worldwide)						
46	Tehran, Iran, 2014, Mohammadali and Pourfathollah *et al*[20]		0.39	0.11	0.01	0.010	-
47	Peshawar, Pakistan, 2017, Batool *et al*[21]	5.33	2.30	1.30	0.07	0.90	0.76
48	Islamabad, Pakistan, 2022, Bhatti *et al*[22]	4.41	0.01	1.5	0.1	0.8	0.004
49	WR Saudi Arabia, 2022, Altayar *et al*[23]	7.93	3.97	-	0.02	-	2.21
50	NW, Ethiopia, 2022, Legese *et al* [24]	5.43	2.8	0.3	0.8	1.5	-
51	Eastern Ethiopia, 2018, Ataro *et al* [25]	7.06	4.67	0.96	1.24	0.44	-
52	Brazil, 2019, Pessoni *et al*[26]	4.04	1.63	0.46	0.21	0.87	-
53	Malawi, Kenya and Ghana, 2023, Singogo *et al*[27]	10.7	3.4	2.4	2.4	3.3	-

ABBI: Assessment of Blood Banks in India.

overall TTI prevalence of 2.038% (1.111% for HBV, 0.431% for HCV, 0.201% for HIV, 0.29% for syphilis, and 0.006% for MP). This aligns closely with the figures reported by Assessment of Blood Banks in 2016 for Delhi (2.03% TTI prevalence) and other nearby Northern Indian states like Punjab (2.64% TTI prevalence) and Haryana (1.97% TTI prevalence)[13].

The variation in TTI prevalence is notable across different states of India. For instance, the highest TTI prevalence rates in India were observed in Puducherry (3.13%) and Arunachal Pradesh (2.19%). In contrast, Kerala had one of the lowest rates at 0.56%[13]. This variability suggests that local factors such as healthcare infrastructure, blood donor screening processes, and population health may influence TTI prevalence rates[13].

On an international scale, the TTI prevalence also varies widely across different countries. Notable high prevalence rates were observed in Malawi, Kenya, and Ghana, with a combined rate of 10.7%, including 3.4% for HBV, 2.4% for HCV, and 2.4% for HIV[27]. Similarly, countries like Pakistan[21,22], Saudi Arabia[23], Ethiopia[24,25], and Brazil[26] also reported relatively high TTI prevalence rates compared to India and other nations (Table 3)[10,12-27].

In the present study, blood donors were stratified into in-house donors and camp donors. Camp donors were further divided into institutional camp donors and community camp donors. We found the lowest prevalence of TTIs among institutional camp donors, whereas the highest prevalence was noted among in-house donors. The institutions in the present study were mostly educational institutes (example, colleges and various government offices); there was a lower prevalence of TTIs among the students and employees of these institutes. Shrivastava *et al*[10] in their study found that seroprevalence rate of TTI was significantly higher (3.8%) in replacement donations compared to voluntary blood donors (2.3%). Similarly, Jain *et al*[11] found a higher prevalence of TTIs in family donors in comparison to voluntary donors.

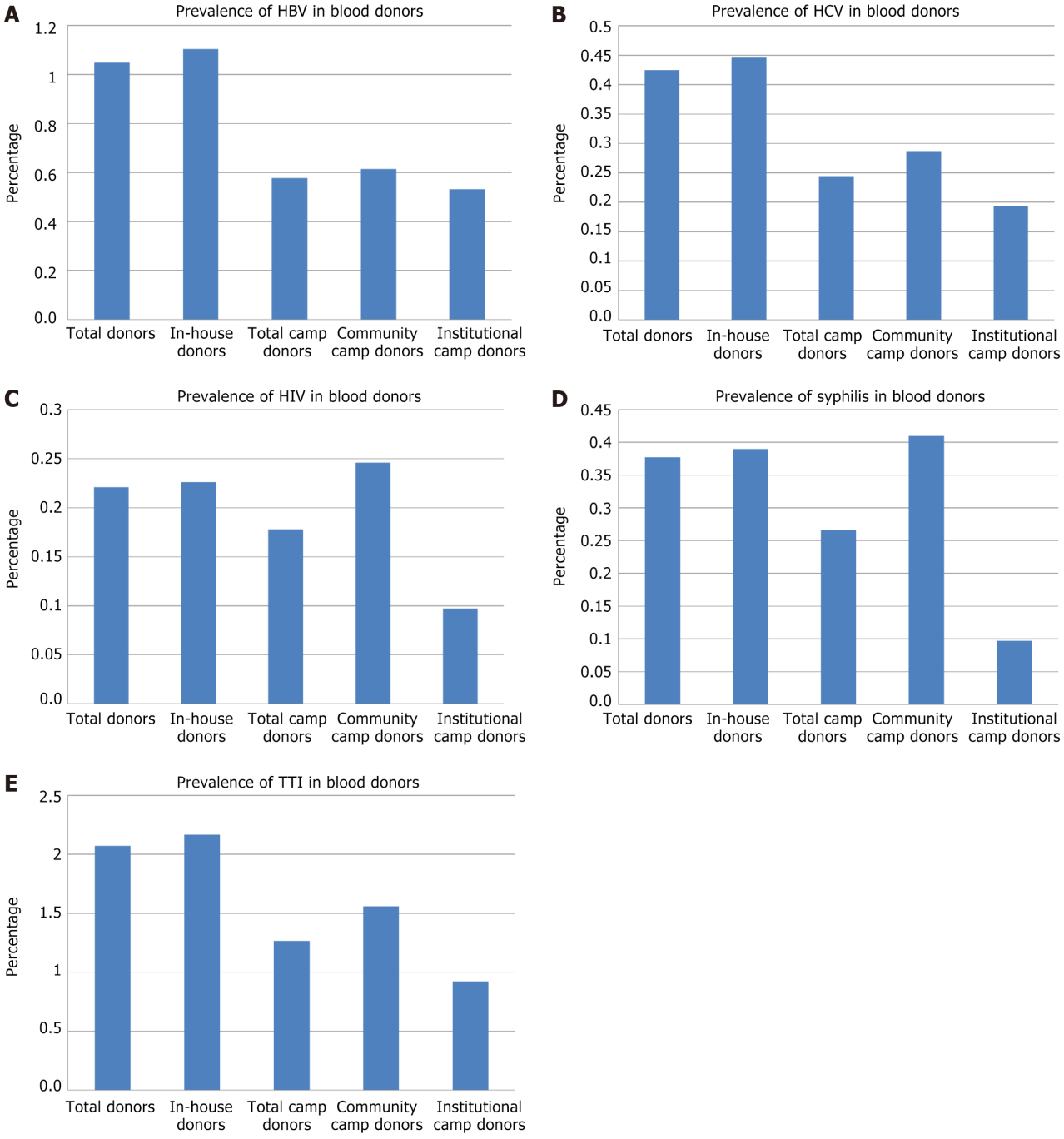


Figure 1 Prevalence. A: Prevalence of hepatitis B virus among different groups of blood donors; B: Prevalence of hepatitis C virus in different groups of blood donors; C: Prevalence of human immunodeficiency virus among different groups of blood donors; D: Prevalence of syphilis among different groups of blood donors; E: Prevalence of transfusion transmissible infections among different groups of blood donors. HCV: Hepatitis C virus; TTI: Transfusion transmissible infection.

In-house donors, being a heterogeneous group, include relatives of admitted patients. These donors, although highly motivated, had the highest prevalence of TTIs.

Among the camp donors, the prevalence of VDRL reactivity was significantly higher in community donors as compared to institutional donors.

The impact of education levels and socio-economic conditions of blood donors on the prevalence of TTI was not the focus of the present study. Moreover, the present study does not discuss strategies to enhance current screening technologies or donor counseling methods to reduce the risk of infected donations. These are few limitations of the present study. Future studies should explore these variables to gain a deeper understanding of their role in reducing TTIs among blood donors. Research into advanced screening techniques and more effective pre-donation counseling could lead to better outcomes in blood safety.

CONCLUSION

The data underscores the importance of continuous monitoring and effective screening programs for blood transfusions to minimize the transmission of TTIs. The differences in TTI prevalence rates across various donor groups and regions suggest the need for tailored approaches to blood safety measures and targeted interventions to address the health challenges. Further research of social–economic–health factors is essential to improve blood safety and reduce the risk of transfusion-related infections across diverse populations.

ACKNOWLEDGEMENTS

The work bears a sense of gratitude to Kumar N, Singh S, Anwar MF, Kumar N, Kumawat S, Kumar L, Kumar V, Kumar S, Singh K, Kumar S, Ali, Kumar M, and other staff of Hindu Rao Hospital for their support.

FOOTNOTES

Author contributions: Thakur SK carried out the literature review, data collection, data analysis, and manuscript preparation; Thakur SK, Sinha AK, Gupta R and Singh S performed statistical analysis; Thakur SK, Sinha AK, Sharma SK, Negi DK, Gupta R and Singh S were responsible for the research design; all authors equally contributed to data processing, interpretation, writing, and revising the final text; all of the authors read and approved the final version of the manuscript to be published.

Institutional review board statement: The present study received approval from the Institutional Ethical Review Committee of Hindu Rao Hospital and NDMC Medical College, Delhi, No: IEC/NDMC/2021/69. All participant blood donors provided informed consent for blood donation. Data used in the study included routine blood grouping and TTI screening test results from the blood bank inventory registers. As no additional blood samples were collected from donors for the study, separate informed consent was not required.

Conflict-of-interest statement: The authors declare no conflicts of interest.

Data sharing statement: All study datasets are available from the corresponding author at sompalsingh@mcd.nic.in and co-corresponding author ruchika.gupta79@gov.in.

Country of origin: India

ORCID number: Sanjay K Thakur 0000-0001-8358-2517; Anil K Sinha 0000-0002-9866-220X; Santosh K Sharma 0000-0001-8556-4611; Aarzoo Jahan 0009-0002-4910-6263; Dinesh K Negi 0000-0002-5197-9082; Ruchika Gupta 0000-0003-0235-063X; Sompal Singh 0000-0002-7766-4708.

S-Editor: Luo ML
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Bihl F**, Castelli D, Marincola F, Dodd RY, Brander C. Transfusion-transmitted infections. *J Transl Med* 2007; **5**: 25 [PMID: 17553144 DOI: 10.1186/1479-5876-5-25]
2 **Das S**, Kumar M. Association of blood group types to hepatitis B and hepatitis C virus infection among blood donors: A five years institutional based study. *Int J Basic Appl* 2012; **2**: 191-195
3 **Dong Y**, Qiu C, Xia X, Wang J, Zhang H, Zhang X, Xu J. Hepatitis B virus and hepatitis C virus infection among HIV-1-infected injection drug users in Dali, China: prevalence and infection status in a cross-sectional study. *Arch Virol* 2015; **160**: 929-936 [PMID: 25616842 DOI: 10.1007/s00705-014-2311-0]
4 **Weldemhret L**. Epidemiology and Challenges of HBV/HIV Co-Infection Amongst HIV-Infected Patients in Endemic Areas: Review. *HIV AIDS (Auckl)* 2021; **13**: 485-490 [PMID: 33981165 DOI: 10.2147/HIV.S273649]
5 **Kundisova L**, Nante N, Martini A, Battisti F, Giovannetti L, Messina G, Chellini E. The impact of mortality for infectious diseases on life expectancy at birth in Tuscany, Italy. *EJPH* 2020; **30** [DOI: 10.1093/eurpub/ckaa166.832]
6 **Wali A**, Khan D, Safdar N, Shawani Z, Fatima R, Yaqoob A, Qadir A, Ahmed S, Rashid H, Ahmed B, Khan S. Prevalence of tuberculosis, HIV/AIDS, and hepatitis; in a prison of Balochistan: a cross-sectional survey. *BMC Public Health* 2019; **19**: 1631 [PMID: 31801496 DOI: 10.1186/s12889-019-8011-7]
7 **Samo AA**, Laghari ZA, Baig NM, Khoso GM. Prevalence and Risk Factors Associated with Hepatitis B and C in Nawabshah, Sindh, Pakistan. *Am J Trop Med Hyg* 2020; **104**: 1101-1105 [PMID: 33350368 DOI: 10.4269/ajtmh.20-1228]
8 **Alqahtani SA**, Colombo M. Viral hepatitis as a risk factor for the development of hepatocellular carcinoma. *Hepatoma Res* 2020; **6**: 58 [DOI:

10.20517/2394-5079.2020.49]
9 **Khan MY**, Sijjeel F, Khalid A, Khurshid R, Habiba UE, Majid H, Naeem M, Nawaz B, Nawaz B, Abbas S, Malik NUA. Prevalence of ABO and Rh blood groups and their association with Transfusion-Transmissible Infections (TTIs) among Blood Donors in Islamabad, Pakistan. *BioSci Rev* 2021; **3** [DOI: 10.32350/bsr.0304.02]
10 **Shrivastava M**, Mishra S, Navaid S. Time Trend and Prevalence Analysis of Transfusion Transmitted Infections among Blood Donors: A Retrospective Study from 2001 to 2016. *Indian J Community Med* 2023; **48**: 274-280 [PMID: 37323745 DOI: 10.4103/ijcm.ijcm_231_22]
11 **Jain R**, Gupta G. Family/friend donors are not true voluntary donors. *Asian J Transfus Sci* 2012; **6**: 29-31 [PMID: 22623839 DOI: 10.4103/0973-6247.95047]
12 **Thakur SK**, Singh S, Negi DK, Sinha AK. Prevalence of TTI among Indian blood donors. *Bioinformation* 2023; **19**: 582-589 [PMID: 37886140 DOI: 10.6026/97320630019582]
13 A Report on the Assessment of Blood Banks in India. Delhi: NACO, NBTC, Ministry of Health, Welfare F, Government of India 2016
14 **Varma AV**, Malpani G, Kosta S, Malukani K, Sarda B, Raghuvanshi A. Seroprevalence of transfusion transmissible infections among blood donors at a tertiary care teaching hospital in Central India. *Int J Contemp Med Res* 2019; **6**: L1-L4
15 **Prakash S**, Sahoo D, Mishra D, Routray S, Ray GK, Das PK, Mukherjee S. Association of transfusion transmitted infections with ABO and Rh D blood group system in healthy blood donors: a retrospective analysis. *Int J Community Med Public Health* 2020; **7**: 4444-8
16 **Sunderam S**, Karir S, Haider S, Singh S, Kiran A. Sero-Prevalence of Transfusion Transmitted Infections among Blood Donors at Blood Bank of Rajendra Institute of Medical Sciences, Ranchi. *Heal J of Indian Assoc Prev Soc Med* 2015; **6**: 36-40
17 **Patel D**, Shah RJ. Correlation of ABO-Rh blood group and transfusion transmitted infections (TTI) among blood donors. *ACHR* 2022; **7**: 229-232 [DOI: 10.18231/j.achr.2022.051]
18 **Bharadva S**, Vachhani J, Dholakiya S. ABO and Rh association to transfusion transmitted infections among healthy blood donors in Jamnagar, Gujarat, India. *J Res Med Den Sci* 2016; **4**: 58 [DOI: 10.5455/jrmds.20164113]
19 **Fatima A**, Begum F, Kumar KM. Seroprevalence of Transfusion Transmissible Infections among Blood Donors in Nizamabad District of Telangana State-A Six Years Study. *Int Arch Integ Med***3**: 73-78
20 **Mohammadali F**, Pourfathollah A. Association of ABO and Rh Blood Groups to Blood-Borne Infections among Blood Donors in Tehran-Iran. *Iran J Public Health* 2014; **43**: 981-989 [PMID: 25909065]
21 **Batool Z**, Durrani SH, Tariq S. Association Of Abo And Rh Blood Group Types To Hepatitis B, Hepatitis C, Hiv And Syphilis Infection, A Five Year' Experience In Healthy Blood Donors In A Tertiary Care Hospital. *J Ayub Med Coll Abbottabad* 2017; **29**: 90-92 [PMID: 28712183]
22 **Bhatti MM**, Junaid A, Sadiq F. The Prevalence of Transfusion Transmitted Infections among Blood Donors in Pakistan: A Retrospective Study. *Oman Med J* 2022; **37**: e386 [PMID: 35712377 DOI: 10.5001/omj.2022.65]
23 **Altayar MA**, Jalal MM, Kabrah A, Qashqari FSI, Jalal NA, Faidah H, Baghdadi MA, Kabrah S. Prevalence and Association of Transfusion Transmitted Infections with ABO and Rh Blood Groups among Blood Donors in the Western Region of Saudi Arabia: A 7-Year Retrospective Analysis. *Medicina (Kaunas)* 2022; **58**: 857 [PMID: 35888577 DOI: 10.3390/medicina58070857]
24 **Legese B**, Shiferaw M, Tamir W, Eyayu T, Damtie S, Berhan A, Getie B, Abebaw A, Solomon Y. Association of ABO and Rhesus Blood Types with Transfusion-Transmitted Infections (TTIs) Among Apparently Healthy Blood Donors at Bahir Dar Blood Bank, Bahir Dar, North West, Ethiopia: A Retrospective Cross-Sectional Study. *J Blood Med* 2022; **13**: 581-587 [PMID: 36238231 DOI: 10.2147/JBM.S374851]
25 **Ataro Z**, Urgessa F, Wasihun T. Prevalence and Trends of Major Transfusion Transmissible Infections among Blood Donors in Dire Dawa Blood bank, Eastern Ethiopia: Retrospective Study. *Ethiop J Health Sci* 2018; **28**: 701-710 [PMID: 30607086 DOI: 10.4314/ejhs.v28i6.4]
26 **Pessoni LL**, Aquino ÉC, Alcântara KC. Prevalence and trends in transfusion-transmissible infections among blood donors in Brazil from 2010 to 2016. *Hematol Transfus Cell Ther* 2019; **41**: 310-315 [PMID: 31409582 DOI: 10.1016/j.htct.2019.03.009]
27 **Singogo E**, Chagomerana M, Van Ryn C, M'bwana R, Likaka A, M'baya B, Puerto-Meredith S, Chipeta E, Mwapasa V, Muula A, Reilly C, Hosseinipour MC; Malawi BLOODSAFE Program. Prevalence and incidence of transfusion-transmissible infections among blood donors in Malawi: A population-level study. *Transfus Med* 2023; **33**: 483-496 [PMID: 37828838 DOI: 10.1111/tme.13006]

World Journal of Virology

Submit a Manuscript: https://www.f6publishing.com

World J Virol 2025 March 25; 14(1): 99923

DOI: 10.5501/wjv.v14.i1.99923

ISSN 2220-3249 (online)

CASE REPORT

Septic shock due to cytomegalovirus colitis associated with rituximab use: A case report

Siddharth Patel, Jordan Jay, Prutha Pathak, Mc Anto Antony, Mrudula Thiriveedi

Specialty type: Virology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade C
Novelty: Grade B
Creativity or Innovation: Grade C
Scientific Significance: Grade C

P-Reviewer: Alshimerry AF

Received: August 3, 2024
Revised: September 27, 2024
Accepted: October 29, 2024
Published online: March 25, 2025
Processing time: 116 Days and 6 Hours

Siddharth Patel, Mrudula Thiriveedi, Department of Internal Medicine, Decatur Morgan Hospital, Decatur, AL 35601, United States

Jordan Jay, Department of Medicine, Alabama College of Osteopathic Medicine, Dothan, AL 36303, United States

Prutha Pathak, Department of Internal Medicine, North Alabama Medical Center, Florence, AL 35630, United States

Mc Anto Antony, Department of Endocrinology, Diabetes and Metabolism, Medical University of South Carolina/AnMed Campus, Anderson, SC 29621, United States

Corresponding author: Siddharth Patel, MBBS, Attending Doctor, Director, Department of Internal Medicine, Decatur Morgan Hospital, 1201 7th Street SE, Decatur, AL 35601, United States. sidd8801@gmail.com

Abstract

BACKGROUND

Cytomegalovirus (CMV) infections can cause significant morbidity and mortality in immunocompromised individuals. CMV targets dysfunctional lymphocytes. Chronic rituximab (RTX) therapy can cause B-lymphocyte dysfunction, increasing CMV risk. Rarely, CMV infections present with critical illness such as septic shock.

CASE SUMMARY

A 64-year-old African American woman presented with generalized weakness and non-bloody watery diarrhea of 4-6 weeks duration. She did not have nausea, vomiting or, abdominal pain. She had been on monthly RTX infusions for neuromyelitis optica. She was admitted for septic shock due to pancolitis. Blood investigations suggested pancytopenia and serology detected significantly elevated CMV DNA. Valganciclovir treatment led to disease resolution.

CONCLUSION

This case illustrates an extremely rare case of CMV colitis associated with RTX use presenting with septic shock. High suspicion for rare opportunistic infections is imperative in individuals with long-term RTX use.

Key Words: Cytomegalovirus colitis; Rituximab use; Immunocompromised status; Septic shock; Pancytopenia; Case report

Core Tip: With the increasing use of biologics in medicine, there has been an emergence of opportunistic infections. Cytomegalovirus (CMV) infections remain asymptomatic or cause only mild symptoms in most immunocompetent adults. However, it can cause infectious complications in immunocompromised individuals-especially those with defective T-lymphocyte function. Rituximab (RTX) use is associated with B-lymphocyte depletion and increased risk of infections. However, CMV infections after long-term RTX are uncommon. Our case describes septic shock due to CMV colitis associated with RTX use which is an extremely rare entity. It highlights the need for consideration of rare opportunistic infections in all patients on biologics.

Citation: Patel S, Jay J, Pathak P, Antony MA, Thiriveedi M. Septic shock due to cytomegalovirus colitis associated with rituximab use: A case report. *World J Virol* 2025; 14(1): 99923
URL: https://www.wjgnet.com/2220-3249/full/v14/i1/99923.htm
DOI: https://dx.doi.org/10.5501/wjv.v14.i1.99923

INTRODUCTION

Most adults (40%-100%) have been infected and are carriers of cytomegalovirus (CMV)[1-3]. In the immunocompetent, CMV's acute presentation is asymptomatic. Once infected, CMV remains latent in the host's cells indefinitely[1-3]. If an infected person becomes immunocompromised, their latent CMV infection can reactivate and present itself in a variety of manifestations including pneumonia, esophagitis, retinitis, hepatitis, and colitis[1,2].

Rituximab (RTX)- an anti-CD20 antibody, is used to treat patients with various autoimmune conditions[3]. Its use can lead to adverse effects, the most notable being increased infection risk due to its effect on humoral immunity[3-5]. A rare complication of long-term RTX use can be the development of opportunistic infections, like in our case, CMV. Some studies have found an association between RTX use and the development of low levels of immunoglobulins, increasing susceptibility for opportunistic infections[2,3,6].

CASE PRESENTATION

Chief complaints

A 64-year-old African American woman presented to the emergency room with generalized weakness for 24 hours. She complained of non-bloody, watery diarrhea of 4-6 times per day, for 4-6 weeks duration.

History of present illness

She denied nausea, vomiting, abdominal pain, fever, chills, or rash, as well as recent travel, consumption of contaminated food, or sick contacts.

History of past illness

She had a history of neuromyelitis optica (on RTX), hyperlipidemia (on atorvastatin), and diabetes mellitus type II (on metformin and glipizide). Colonoscopy 10 years ago was unremarkable.

Personal and family history

Past and family history were negative for neoplasm or autoimmune disease. She denied tobacco, alcohol, or illicit drug use.

Physical examination

On initial assessment, the patient was tachycardic (119/min) and hypotensive (81/47 mmHg) without fever or hypoxia on room air. Physical examination was remarkable for lethargy, dry oral cavity, and conjunctival pallor. Abdomen was soft, non-tender, and non-distended with bowel sounds in all four quadrants.

Laboratory examinations

Laboratory investigations were significant for pancytopenia, hypoglycemia, acute kidney injury, and lactic acidosis (Table 1).

Imaging examinations

Computed tomography scan of the abdomen and pelvis with contrast detected diffuse thickening of the colonic wall with peri-colonic stranding consistent with pancolitis (Figure 1).

Table 1 Lab investigations on admission and during hospital stay

	Patient values	Normal values
White blood cell count (× 10^3 cells/mL)	1.35	4.8-10.8
Red blood cell count (million cells/mL)	2.59	4.2-5.4
Hemoglobin (mmol/L)	4.96	8.7-11.2
Hematocrit (%)	25.6	37.0-47.0
Mean corpuscular volume (FL)	98.8	81-99
Mean corpuscular hemoglobin (PG)	30.9	27-31
Mean corpuscular hemoglobin concentration (g/dL)	31.3	33-37
Red cell distribution width (%)	14.9	11.5-14.5
Platelet count (× 1000 cells/mL)	16	130-400
Neutrophils (%)	37.1	42.2-75.2
Lymphocytes (%)	57.0	20.5-51.5
Monocytes (%)	3.7	1.7-9.3
Percentage reticulocyte count (%)	0.57	0.8-2.1
Sodium (mmol/L)	137	135-145
Potassium (mmol/L)	4.7	3.5-5
Chloride (mmol/L)	98	98-105
Blood urea nitrogen	5.36	3.6-7.1
Serum creatinine (μmol/L)	97.26	44-97
Aspartate transaminase (U/L)	74	10-30
Alanine transaminase (U/L)	18	10-36
Alkaline phosphatase (U/L)	53	32-104
Erythrocyte sedimentation rate (mm/hour)	56	0-20
C- reactive protein (mg/L)	160.98	0-5
Cytomegalovirus DNA by polymerase chain reaction	14800 IU/mL	Undetected
Immunoglobulin M (g/L)	10	40-230
Immunoglobulin A (g/L)	239	70-400
Immunoglobulin G (g/L)	1200	700-1600
Blood culture	Negative	Negative
Stool tests: *Clostridioides difficile*, *Escherichia coli*, *Shiga toxin*, *Cryptosporidium*, *Cyclospora*, *Adenovirus*, *Norovirus*, *Rotavirus*, *Giardia*, *Entamoeba*	Negative	Negative
Peripheral smear	Normocytic normochromic anemia, thrombocytopenia	No anemia or thrombocytopenia
Bone marrow and flow cytometry	Hypocellular (10%) bone marrow with mild erythroid hyperplasia. Negative for monoclonal plasma cell or blast cell population	Normal cellularity without any monoclonal or blast cell population

FINAL DIAGNOSIS

She was diagnosed with septic shock due to acute colitis and admitted to the critical care unit with intravenous fluids, vasopressor, and antibiotics.

TREATMENT

Stool studies ruled out *Clostridioides difficile*, *Escherichia coli*, *Shigella*, and *Salmonella* among other infections (Table 1). Blood cultures and hepatitis panel were negative. Filgrastim injections were started to address pancytopenia. CMV

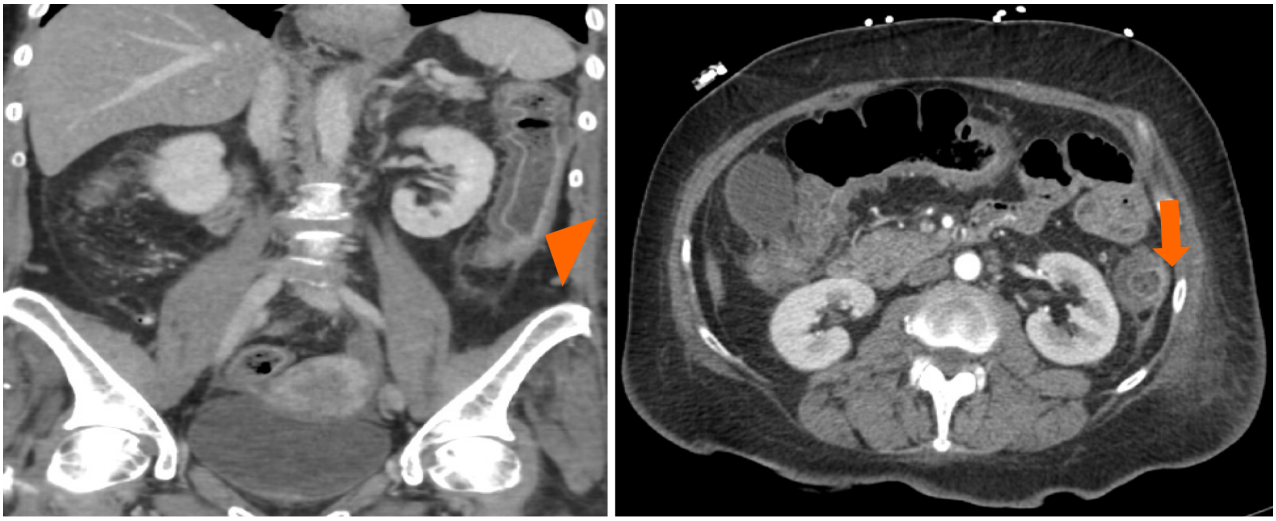


Figure 1 Coronal and transverse sections of a computerized tomographic scan of abdomen and pelvis without contrast showing pan-colitis with thickening of the intestinal wall and peri-colonic stranding (arrowhead and arrow).

serology demonstrated elevated CMV DNA. The patient could not undergo colonoscopy for tissue biopsy due to thrombocytopenia. Hence, the diagnosis of CMV colitis due to RTX was made based on clinical presentation and serology.

OUTCOME AND FOLLOW-UP

Valganciclovir was started. Her diarrhea and pancytopenia resolved over the next 7 days. She was discharged home in stable condition.

DISCUSSION

CMV infects about 40%-100% of the population by the adult life[1,2]. In the immunocompetent, it is mostly asymptomatic or presents as a mild mononucleosis-like syndrome (Figure 2)[1-3]. Once the primary infection resolves, CMV remains latent in its host indefinitely[1]. Most CMV disease manifestations are due to viral reactivation which commonly occurs when an infected individual becomes immunocompromised[1,2]. For example in solid organ and bone marrow transplant recipients, and HIV patients, reactivation of CMV can manifest as pneumonia, esophagitis, retinitis, hepatitis, and colitis [1,3].

CMV disease in immunocompromised individuals occurs through one of three different pathways: Acute infection, latent viral reactivation, or reinfection with a new viral strain[1]. Most commonly, reactivation is due to dysfunctional or inadequate numbers of T lymphocytes and natural killer cells[1,2]. Disease severity is closely related to the patient's degree of immunodeficiency[1,2].

RTX is an anti-CD20 antibody used for the treatment of various conditions including non-Hodgkin's lymphoma, rheumatoid arthritis, and neuromyelitis optica[3-6]. The most notable adverse effect of its use is an increased risk of infection[3-6] which is primarily related to B lymphocyte depletion[1,2]. One study found that 30% of patients reported side effects were infectious in nature[3]. Of these, 63% were bacterial infections, 33% viral and 3.3% fungal[3]. Despite an increased risk of infection, opportunistic infections are uncommon, highlighting the uniqueness of this case.

A rare complication of RTX is CMV reactivation. Although CMV primarily affects T lymphocytes, humoral immunity plays a critical role in suppressing CMV[2,3,6]. A literature review explored the association between CMV and RTX.

We queried PubMed and Google Scholar for articles related to CMV colitis induced by chronic RTX use. Key words included: Cytomegalovirus, CMV, colitis and rituximab. Articles were case reports or series written in English involving adult patients. The articles needed to include RTX use and definitive CMV diagnosis *via* biopsy or serology. Only one case of CMV colitis associated with RTX use was found[3]. All other cases demonstrated either non-colitis CMV manifestations- encephalitis, pneumonitis, retinitis, gastritis, and death[7,8]; or were precipitated by concomitant use of multiple immunosuppressing drugs such as bendamustine or fludarabine[8-10]. Unfortunately, these multi-drug regimens complicated the cases and made it impossible to determine if RTX or another medication induced the CMV infection.

Only Vallet *et al*[3] case report demonstrated RTX induced CMV-colitis. Like our report, their patient was a middle-aged woman who developed CMV colitis after 2 cycles of RTX, although our patient was on RTX for > 3 years. The patient in our case had neuromyelitis optica while theirs suffered from rheumatoid arthritis. Both experienced hypogammaglobulinemia, hypo-IgM in our patient while hypo-IgG in theirs. There was no description of the severity of the symptoms in Vallet *et al*'s report whereas our case had septic shock requiring critical care management. Both patients

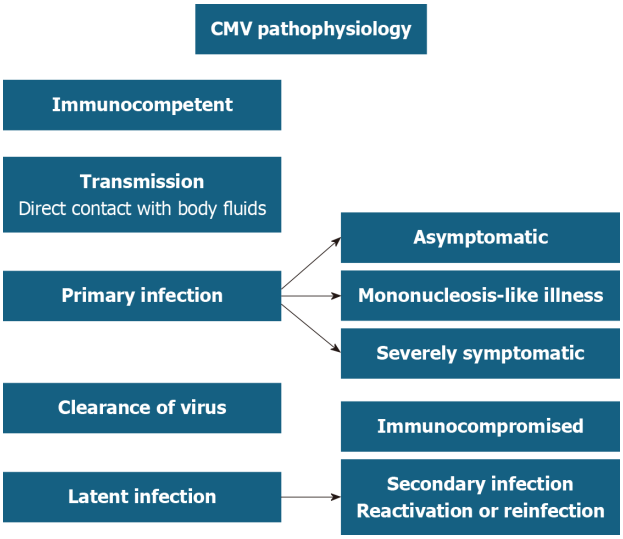


Figure 2 Pathophysiology of cytomegalovirus infections in immunocompetent and immunocompromised individuals. CMV: Cytomegalovirus.

eventually improved with valganciclovir[3].

Other reports have found that hypogammaglobulinemia increases the risk of CMV reactivation[3-6]. One paper reported CMV in five patients with hypo-IgG[3]. Repeated RTX treatments increase patients' risk of developing hypo-IgG [4]. Our case is unique because our patient had hypo-IgM and normal IgG. Studies have found an association between RTX use and hypo-IgM leading to an increased risk of infections; however, there are no cases of RTX induced hypo-IgM leading to CMV colitis[4,5]. Low IgM levels were strongly associated with sepsis for those on RTX[3-5]. Similarly, our patient suffered from colitis and sepsis.

CONCLUSION

Overall, this case illustrates a rare case of CMV colitis associated with RTX use presenting with septic shock. High suspicion for rare opportunistic infections is imperative in individuals with RTX use. Immunocompromised patients with gastrointestinal signs and symptoms should be evaluated for possible opportunistic infections such as CMV.

FOOTNOTES

Author contributions: Patel S and Jay J contributed to manuscript writing, editing, and data collection; Pathak P, Antony MA, and Thiriveedi M contributed to conceptualization and supervision; all authors have read and approved the final manuscript.

Informed consent statement: Informed verbal consent was obtained from the patient for publication of this report and any accompanying images.

Conflict-of-interest statement: The authors declare that they have no conflict of interest to disclose.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

Country of origin: United States

ORCID number: Siddharth Patel 0000-0001-9306-0878.

Corresponding Author's Membership in Professional Societies: American College of Physicians, No. 03124089; American Association of Physicians of Indian Origin; Medical Association of State of Alabama.

S-Editor: Lin C
L-Editor: A

P-Editor: Wang WB

REFERENCES

1 **Goodgame RW**. Gastrointestinal cytomegalovirus disease. *Ann Intern Med* 1993; **119**: 924-935 [PMID: 8215005 DOI: 10.7326/0003-4819-119-9-199311010-00010]

2 **Ho M**. Epidemiology of cytomegalovirus infections. *Rev Infect Dis* 1990; **12** Suppl 7: S701-S710 [PMID: 2173099 DOI: 10.1093/clinids/12.supplement_7.s701]

3 **Vallet H**, Houitte R, Azria A, Mariette X. Cytomegalovirus colitis and hypo-IgG after rituximab therapy for rheumatoid arthritis. *J Rheumatol* 2011; **38**: 965-966 [PMID: 21532065 DOI: 10.3899/jrheum.100818]

4 **Rutherford AI**, Patarata E, Subesinghe S, Hyrich KL, Galloway JB. Opportunistic infections in rheumatoid arthritis patients exposed to biologic therapy: results from the British Society for Rheumatology Biologics Register for Rheumatoid Arthritis. *Rheumatology (Oxford)* 2018; **57**: 997-1001 [PMID: 29529307 DOI: 10.1093/rheumatology/key023]

5 **Ezhuthachan I**, Shapero M, Fishbein J, Kaplan B. Lower IgM levels after rituximab use is associated with sepsis. *J Allergy Clin Immunol* 2021; **147**: AB73 [DOI: 10.1016/j.jaci.2020.12.286]

6 **Aggarwal A**, Levoy E, Desimone M, Bhattacharya K. Rituximab-Associated Colitis: An Unusual Case Report. *Am J Gastroenterol* 2018; **113**: S849 [DOI: 10.14309/00000434-201810001-01479]

7 **Eckmann JD**, Chedid V, Quinn KP, Bonthu N, Nehra V, Raffals LE. De Novo Colitis Associated With Rituximab in 21 Patients at a Tertiary Center. *Clin Gastroenterol Hepatol* 2020; **18**: 252-253 [PMID: 30905719 DOI: 10.1016/j.cgh.2019.03.027]

8 **Libby E**, Movva S, Quintana D, Abdul-Jaleel M, Das A. Cytomegalovirus retinitis during chemotherapy with rituximab plus hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone. *J Clin Oncol* 2010; **28**: e661-e662 [PMID: 20855824 DOI: 10.1200/JCO.2010.29.6467]

9 **Magliano G**, Cuccaro A, d'Alo' F, Maiolo E, Bellesi S, Hohaus S, Bacigalupo A, Pagano L. Severe CMV Infection after Chemo-Immunotherapy with Dose-Reduced Bendamustine and Rituximab in a Mantle Cell Lymphoma Old Patient. *Mediterr J Hematol Infect Dis* 2021; **13**: e2021054 [PMID: 34527206 DOI: 10.4084/MJHID.2021.054]

10 **Chan TS**, Cheung CY, Yeung IY, Hwang YY, Gill H, Wong IY, Kwong YL. Cytomegalovirus retinitis complicating combination therapy with rituximab and fludarabine. *Ann Hematol* 2015; **94**: 1043-1047 [PMID: 25572171 DOI: 10.1007/s00277-014-2296-5]

World Journal of Virology

Submit a Manuscript: https://www.f6publishing.com

World J Virol 2025 March 25; 14(1): 98359

DOI: 10.5501/wjv.v14.i1.98359

ISSN 2220-3249 (online)

LETTER TO THE EDITOR

Revisiting dexamethasone dosage in COVID-19 management

Abhishet Varama

Specialty type: Virology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification
Scientific Quality: Grade B
Novelty: Grade B
Creativity or Innovation: Grade B
Scientific Significance: Grade B

P-Reviewer: Hassan FE

Received: June 25, 2024
Revised: November 18, 2024
Accepted: November 26, 2024
Published online: March 25, 2025
Processing time: 154 Days and 17.5 Hours

Abhishet Varama, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Krung Thep Maha Nakhon, Thailand

Corresponding author: Abhishet Varama, BSc, Master's Student, Faculty of Medicine, Chulalongkorn University, 1873 Rama IV Road, Pathum Wan, Bangkok 10330, Krung Thep Maha Nakhon, Thailand. abhishet.varama@docchula.com

Abstract

The ongoing coronavirus disease 2019 (COVID-19) pandemic has necessitated rapid advancements in therapeutic strategies, with dexamethasone emerging as a key treatment for severe cases. This editorial discusses the systematic review conducted by Sethi *et al*, published in the *World Journal of Virology*. The review critically examines the efficacy and safety of varying dosages of dexamethasone in severe COVID-19 patients, providing a comprehensive meta-analysis that underscores the current clinical recommendations favoring a low-dose regimen. Despite these findings, the review highlights the potential benefits of tailored dosages for specific patient subgroups, suggesting a need for personalized treatment approaches. This editorial expands on the implications of these findings, advocating for the integration of evolving clinical data into treatment protocols and calling for further research into patient-specific responses to therapy. It emphasizes the importance of adaptability and precision in pandemic response, urging the medical community to consider both the robustness of existing evidence and the potential for innovative approaches to enhance patient outcomes in the face of global health challenges.

Key Words: COVID-19 treatment; Dexamethasone dosage; Personalized medicine; Editorial; Clinical adaptability

Core Tip: This editorial delves into the critical analysis provided by Sethi *et al* on dexamethasone dosing in severe coronavirus disease 2019 (COVID-19) cases. Highlighting the review's challenge to the one-size-fits-all approach, it emphasizes the need for personalized medicine and calls for further research to refine treatment protocols based on emerging evidence and patient-specific factors. The editorial advocates for the integration of nuanced clinical data to enhance therapeutic strategies against COVID-19, fostering a broader discussion on the adaptability of clinical practice in pandemic responses and the potential for tailored patient care.

Citation: Varama A. Revisiting dexamethasone dosage in COVID-19 management. *World J Virol* 2025; 14(1): 98359
URL: https://www.wjgnet.com/2220-3249/full/v14/i1/98359.htm
DOI: https://dx.doi.org/10.5501/wjv.v14.i1.98359

TO THE EDITOR

The coronavirus disease 2019 (COVID-19) pandemic has relentlessly challenged the global medical community to adapt and innovate under pressure. Among the therapeutic arsenal deployed against severe manifestations of COVID-19, dexamethasone, a synthetic glucocorticoid, has emerged as a cornerstone treatment, especially for patients experiencing severe respiratory complications[1]. In this issue of the *World Journal of Virology*, Sethi *et al*[2] present a systematic review titled "Dosage and utilization of dexamethasone in the management of COVID-19: A critical review" which provides a meticulous analysis of the effectiveness and safety of various dosages of dexamethasone. This article not only enriches our understanding but also ignites a crucial discussion on the optimization of therapeutic strategies in the face of a global health crisis.

Context and Importance of the study

From the early days of the pandemic, the Recovery Collaborative group's findings highlighted the mortality reduction benefits of dexamethasone among severely affected patients requiring respiratory support[3]. However, the optimal dosage-whether a standard low dose or a higher therapeutic dose-remains a subject of contention within the medical community. The significance of Sethi *et al*'s review lies in its timely challenge to the one-size-fits-all approach, suggesting a more nuanced application of dexamethasone based on emerging evidence and patient-specific factors[2].

Study synopsis and findings

The authors embarked on a comprehensive review, adhering to PRISMA guidelines, which involved a detailed literature search across multiple databases up to March 2024. Their methodological rigor included the use of the Cochrane Risk of Bias Tool and the Newcastle-Ottawa scale, ensuring the robustness of the evidence evaluated. The meta-analysis, employing a random-effects model, revealed no significant differences in 28-day and 60-day all-cause mortality, mean length of hospital stays, or adverse events between high and low dosages of dexamethasone.

Despite these findings, the study is pivotal not for the answers it provides but for the questions it raises. It underscores an essential aspect of clinical practice in pandemic response: The adaptability of treatment protocols to incorporate patient-specific variables and evolving clinical data.

Implications for clinical practice

The core implication of Sethi *et al*'s findings advocate for maintaining the current low-dose regimen as a safe and effective treatment for the majority of severe COVID-19 cases[2]. However, the subtle variations in data suggest potential benefits of higher doses in specific patient subsets, particularly those with certain clinical or demographic characteristics. This insight is crucial as it prompts a re-evaluation of our therapeutic strategies and supports the call for personalized medicine in the management of complex diseases like COVID-19.

Future directions and research

The discussion section of the paper brilliantly sets the stage for future research. It suggests that further trials should be designed to stratify patients more clearly by various demographic and clinical factors. Such stratification could potentially reveal critical insights into how different patient groups respond to varying dosages of dexamethasone, thus enabling more tailored and potentially more effective treatment protocols.

Moreover, the study highlights the necessity for ongoing research into the long-term impacts of different dexamethasone dosing regimens on outcomes like long COVID-19, a growing concern as the pandemic evolves.

Conclusion and call to action

In conclusion, Sethi *et al*'s systematic review does more than just contribute to the academic discourse[2]. It acts as a catalyst for rethinking how we manage severe COVID-19. By providing a thorough, evidence-based analysis, the authors encourage not only adherence to proven therapies but also the exploration of new, potentially more effective ways to manage patient care under pandemic conditions.

This editorial board commends the authors for their rigorous and insightful contribution. We encourage our readers to consider not just the conclusions of this review, but also the broader implications for clinical practice and future research. As we continue to navigate the challenges posed by COVID-19, let us remain committed to enhancing our therapeutic approaches through precision, evidence, and a deep commitment to patient-specific care.

This editorial and the article it discusses serve as vital instruments for expanding our collective knowledge and improving our responses to a virus that has affected millions worldwide. They remind us of the power of well-conducted research to influence not just clinical outcomes but also global health policy and practice.

FOOTNOTES

Author contributions: Abhishet V drafted, wrote and edited the editorial. No external help was used in the making of this manuscript.

Conflict-of-interest statement: The author declares no conflict of interest associated with this manuscript.

Country of origin: Thailand

ORCID number: Abhishet Varama 0009-0002-2671-2711.

S-Editor: Liu H
L-Editor: A
P-Editor: Zhang L

REFERENCES

1 **Hassan ME**, Hasan HM, Sridharan K, Elkady A, ElSeirafi MM. Dexamethasone in severe COVID-19 infection: A case series. *Respir Med Case Rep* 2020; **31**: 101205 [PMID: 32874905 DOI: 10.1016/j.rmcr.2020.101205]

2 **Sethi I**, Shaikh A, Sethi M, Chohan HK, Younus S, Khan SA, Surani S. Dosage and utilization of dexamethasone in the management of COVID-19: A critical review. *World J Virol* 2024; **13**: 95709 [PMID: 39323444 DOI: 10.5501/wjv.v13.i3.95709]

3 **Horby P**, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, Staplin N, Brightling C, Ustianowski A, Elmahi E, Prudon B, Green C, Felton T, Chadwick D, Rege K, Fegan C, Chappell LC, Faust SN, Jaki T, Jeffery K, Montgomery A, Rowan K, Juszczak E, Baillie JK, Haynes R, Landray MJ; RECOVERY Collaborative Group. Dexamethasone in Hospitalized Patients with Covid-19. *N Engl J Med* 2021; **384**: 693-704 [PMID: 32678530 DOI: 10.1056/NEJMoa2021436]

World Journal of Virology

Submit a Manuscript: https://www.f6publishing.com

World J Virol 2025 March 25; 14(1): 100160

DOI: 10.5501/wjv.v14.i1.100160

ISSN 2220-3249 (online)

LETTER TO THE EDITOR

Rhabdomyolysis-related acute kidney injury in COVID-19: A critical concern

Md Safiullah Sarker

Specialty type: Virology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade A
Novelty: Grade A
Creativity or Innovation: Grade A
Scientific Significance: Grade A

P-Reviewer: Jin H

Received: August 8, 2024
Revised: September 24, 2024
Accepted: October 15, 2024
Published online: March 25, 2025
Processing time: 111 Days and 16.1 Hours

Md Safiullah Sarker, Virology Laboratory, Infectious Diseases Division, Dhaka 1212, Bangladesh

Corresponding author: Md Safiullah Sarker, PhD, Research Scientist, Virology Laboratory, Infectious Diseases Division, 68 Shaheed Tajuddin Ahmed Sarani, Dhaka 1212, Bangladesh. ssarker@icddrb.org

Abstract

Rhabdomyolysis is a severe condition characterized by the breakdown of muscle tissue leading to the release of intracellular components into the bloodstream. This condition, when associated with acute kidney injury (AKI), can result in significant morbidity and mortality, particularly in the context of coronavirus disease 2019 (COVID-19). This editorial discusses a retrospective study on patients with COVID-19 who developed rhabdomyolysis-related AKI. The study highlights that patients with rhabdomyolysis exhibited higher inflammatory markers, such as C-reactive protein, ferritin, and procalcitonin, and experienced worse clinical outcomes compared to those with other causes of AKI. The findings underscore the importance of early recognition and management of rhabdomyolysis in COVID-19 patients to improve prognosis and reduce mortality rates.

Key Words: Rhabdomyolysis; Acute kidney injury; COVID-19; SARS-CoV-2; Creatine kinase; Inflammation; Prognosis; Mortality

Core Tip: Rhabdomyolysis is a significant complication in coronavirus disease 2019 (COVID-19) patients, leading to severe acute kidney injury (AKI) with high mortality rates. This editorial highlight a study that found higher inflammatory markers and worse outcomes in patients with rhabdomyolysis-related AKI compared to other causes of AKI. Early detection and appropriate management are crucial to mitigate the adverse effects of this condition in the context of COVID-19.

Citation: Sarker MS. Rhabdomyolysis-related acute kidney injury in COVID-19: A critical concern. *World J Virol* 2025; 14(1): 100160
URL: https://www.wjgnet.com/2220-3249/full/v14/i1/100160.htm
DOI: https://dx.doi.org/10.5501/wjv.v14.i1.100160

TO THE EDITOR

Rhabdomyolysis, a syndrome resulting from the breakdown of muscle fibers with the release of muscle cell contents into the bloodstream, can cause life-threatening complications such as acute kidney injury (AKI). In the context of coronavirus disease 2019 (COVID-19), rhabdomyolysis has emerged as a critical concern due to its potential to exacerbate the already complex clinical presentations associated with the virus. This editorial explores the findings of a study conducted on COVID-19 patients who developed rhabdomyolysis-related AKI and discusses the implications for clinical practice[1-3].

Rhabdomyolysis and COVID-19

The study[1] in question involved 115 COVID-19 patients who developed AKI, 15 of whom were diagnosed with rhabdomyolysis. These patients were found to have significantly higher levels of inflammatory markers, including C-reactive protein (CRP), procalcitonin, and ferritin, compared to those with AKI from other causes. The elevated inflammatory response likely reflects the severity of rhabdomyolysis in the setting of COVID-19 and its contribution to the overall disease burden[4].

Clinical outcomes

Patients with rhabdomyolysis-related AKI had markedly worse clinical outcomes, with a mortality rate of 73.3%, significantly higher than the 18.1% observed in patients with AKI due to other causes[1]. This stark contrast underscores the need for heightened clinical awareness and proactive management strategies for rhabdomyolysis in COVID-19 patients[5].

Implications for practice

The findings from this study[1] suggest that early identification and aggressive management of rhabdomyolysis in COVID-19 patients could be pivotal in improving outcomes. Monitoring markers such as creatine kinase, CRP, and ferritin should be an integral part of the management protocol for COVID-19 patients at risk of rhabdomyolysis[6].

Rhabdomyolysis complicates the clinical course of COVID-19 and significantly increases the risk of mortality in patients who develop AKI. This editorial emphasizes the need for clinicians to be vigilant in recognizing and managing this condition to mitigate its impact on patient outcomes[7].

FOOTNOTES

Author contributions: Sarker MS designed and conceptualized the study, performed data acquisition, analysis, and interpretation, drafted the first version of the manuscript, commented on consecutive versions, and approved the final version.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country of origin: Bangladesh

ORCID number: Md Safiullah Sarker 0000-0002-5320-1274.

S-Editor: Liu H
L-Editor: A
P-Editor: Zheng XM

REFERENCES

1 **Murt A**, Altiparmak MR. Rhabdomyolysis-related acute kidney injury in patients with COVID-19. *World J Virol* 2024; **13**: 91107 [PMID: 39323452 DOI: 10.5501/wjv.v13.i3.91107]
2 **Giannoglou GD**, Chatzizisis YS, Misirli G. The syndrome of rhabdomyolysis: Pathophysiology and diagnosis. *Eur J Intern Med* 2007; **18**: 90-100 [PMID: 17338959 DOI: 10.1016/j.ejim.2006.09.020]

3 **Bagley WH**, Yang H, Shah KH. Rhabdomyolysis. *Intern Emerg Med* 2007; **2**: 210-218 [PMID: 17909702 DOI: 10.1007/s11739-007-0060-8]

4 **Haroun MW**, Dieiev V, Kang J, Barbi M, Marashi Nia SF, Gabr M, Eman G, Kajita G, Swedish K. Rhabdomyolysis in COVID-19 Patients: A Retrospective Observational Study. *Cureus* 2021; **13**: e12552 [PMID: 33575135 DOI: 10.7759/cureus.12552]

5 **Nadim MK**, Forni LG, Mehta RL, Connor MJ Jr, Liu KD, Ostermann M, Rimmelé T, Zarbock A, Bell S, Bihorac A, Cantaluppi V, Hoste E, Husain-Syed F, Germain MJ, Goldstein SL, Gupta S, Joannidis M, Kashani K, Koyner JL, Legrand M, Lumlertgul N, Mohan S, Pannu N, Peng Z, Perez-Fernandez XL, Pickkers P, Prowle J, Reis T, Srisawat N, Tolwani A, Vijayan A, Villa G, Yang L, Ronco C, Kellum JA. COVID-19-associated acute kidney injury: consensus report of the 25th Acute Disease Quality Initiative (ADQI) Workgroup. *Nat Rev Nephrol* 2020; **16**: 747-764 [PMID: 33060844 DOI: 10.1038/s41581-020-00356-5]

6 **Mohamed MMB**, Lukitsch I, Torres-Ortiz AE, Walker JB, Varghese V, Hernandez-Arroyo CF, Alqudsi M, LeDoux JR, Velez JCQ. Acute Kidney Injury Associated with Coronavirus Disease 2019 in Urban New Orleans. *Kidney360* 2020; **1**: 614-622 [PMID: 35372932 DOI: 10.34067/KID.0002652020]

7 **Hirsch JS**, Ng JH, Ross DW, Sharma P, Shah HH, Barnett RL, Hazzan AD, Fishbane S, Jhaveri KD; Northwell COVID-19 Research Consortium; Northwell Nephrology COVID-19 Research Consortium. Acute kidney injury in patients hospitalized with COVID-19. *Kidney Int* 2020; **98**: 209-218 [PMID: 32416116 DOI: 10.1016/j.kint.2020.05.006]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: office@baishideng.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com