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MINIREVIEWS

COVID-19 compared to other epidemic coronavirus diseases and the flu

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Abstract

Coronaviruses are among the largest group of known positive - sense RNA viruses with a wide range of animal hosts as reservoir. In the last two decades, newly evolved coronaviruses such as the severe acute respiratory syndrome coronavirus (SARS-CoV) which caused the infamous 2002 outbreak, the Middle East respiratory syndrome coronavirus (MERS-CoV) which caused an outbreak in 2012, and now the SARS-CoV-2 [responsible for the current coronavirus disease 2019 (COVID-19)] have all posed notable threats to global public health. But, how does the current COVID-19 outbreak compare with previous coronaviruses diseases? In this review, we look at the key differences between SARS-CoV, MERS-CoV, and SARS-CoV-2, and examine challenges in determining accurate estimates of the severity of COVID-19. We discuss coronavirus outbreaks in light of key outbreak severity indicators including, disease fatality, pathogen novelty, ease of transmission, geographical range, and outbreak preparedness. Finally, we review clinical trials of emerging treatment modalities and provide recommendations on the control of COVID-19 based on the mode of transmission of the coronaviruses. We also recommend the development and use of a standardized predictive epidemic severity models to inform future epidemic response.

Key words: Severe acute respiratory syndrome, SARS; Middle East respiratory syndrome, MERS; COVID-19; SARS-CoV2; Coronaviruses; Influenza, Flu; Respiratory viruses

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Core tip: In this review, we look at differences and similarities between severe acute respiratory syndrome coronavirus, Middle East respiratory syndrome coronavirus, and severe acute respiratory syndrome coronavirus 2 and we discuss the challenges in the determination of case fatality rates in pandemics like the current and propose the need for standardization of predictive epidemic severity models that considers critical factors that can influence the severity of outbreaks.

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INTRODUCTION

In December 2019, a cluster of pneumonia was reported in Wuhan, China. Nucleotide sequencing of samples from patients revealed a novel beta coronavirus that was designated novel coronavirus and subsequently as severe acute respiratory syndrome coronavirus 2 (SARS-CoV2)[1,2,3]. The disease caused by this novel coronavirus has been designated by the World Health Organization (WHO) as coronavirus disease 2019 (COVID-19) meaning coronavirus disease of the year 2019. Initial cases of this disease were epidemiologically linked to the Huanan Seafood and Wet Animal Wholesale Market in Wuhan, Hubei Province, China, suggesting a possible zoonotic spill over from wildlife to humans. The disease subsequently spread and the global expansion was facilitated by human to human transmission[1].

On March 11, the WHO declared the current COVID-19 outbreak, a global pandemic. This is not the first time the world is experiencing a coronavirus epidemic in recent times[4]. Severe acute respiratory syndrome coronavirus (SARSCoV) occurred in 2002, which reportedly infected 8098 people and caused 774 deaths worldwide. Ten years later, the Middle East respiratory syndrome coronavirus (MERS-CoV) emerged causing a total of 2494 infections, and 858 fatalities[5]. SARS-CoV-2 is the third coronavirus epidemic to emerge in the human population in the past two decades. Preliminary laboratory investigations suggest that the virus grows in the same cell lines that are used for growing SARS-CoV and MERS-CoV, however, SARS-CoV-2 grows better in primary human airway epithelial cells than in standard tissue culture cells unlike SARS-CoV or MERS-CoV[6].

But how exactly is COVID-19 different from the SARS or MERS? Initial reports have suggested that the SARS-CoV and MERS-CoV may be more severe than SARS-CoV-2 while the later may be more infectious[7]. These observations are based on case fatality rates (CFRs), which to the opinion of the authors of this review, is yet to be definitively established for SARS-CoV-2 as we discuss later in this paper. Given how new SARS-CoV-2 is, there are still a lot of unknowns regarding its morbidity and mortality. Since the onset of the disease in late 2019, several important questions regarding the virus and its disease are still being investigated and studied, *e.g.*, what is the shape of the disease pyramid? What proportion of infected people develop the disease? What proportion of infected persons are asymptomatic? And, what proportion of those with the disease die? Furthermore, indices other than fatality and transmissibility are necessary to establish a comprehensive estimate of disease severity. For example, the psychosocial severity of COVID-19 is yet to be determined. Also, because COVID-19 and the flu share commonalities in initial signs and symptoms, questions have been raised on the difference between the two in the context of comparing which epidemic or disease is most serious. However, understanding the differences in seriousness between the COVID-19 pandemic and the seasonal flu needs a comprehensive estimate of epidemic/outbreak severity. Below, we discuss key concepts of epidemic severity including fatality, disease severity, pathogen novelty, preparedness, geographic range, and ease of transmission.

CORONAVIRUS DIVERSITY AND RESERVIORS

Coronaviruses belong to the family *Coronaviridae* which are enveloped, positive-sense, single-stranded RNA viruses of about 80-120nm diameter and 31 kb in size[8]. There

are at least 7 types of human coronaviruses grouped into either alpha or beta coronaviruses. The alpha coronaviruses include 229E and NL63, and the beta include OC43, HKU1, MERS-CoV, SARS-CoV, and the novel SARS-CoV-2. Acute respiratory infections caused by 229E, NL63, OC43, and HKU1 are often mild while SARS-CoV, MERS-CoV, and SARS-CoV-2 cause both mild and severe disease and have been responsible for global epidemics that began in 2002, 2012, and 2019 respectively[5]. Coronaviruses are ecologically diverse with the greatest variety seen in bats, which are known to be a reservoir for many emerging viruses. Peri-domestic animals may also serve as intermediate hosts, facilitating transmission to humans. Given the diversity of coronaviruses that infect animals and increasing human–animal interfaces, novel coronaviruses are likely to emerge periodically in humans through cross-species infections and occasional spillover events.

SYMPTOMS AND FATALITY OF SARS-COV-2 AND OTHER EPIDEMIC CORONAVIRUSES

Fatality is the most commonly used indicator to measure disease and outbreak severity. While the CFR is a well-known metric, standardized symptom-scoring metrics for coronaviruses are scarce. Nonetheless, the route of transmission, pathologies, and clinical manifestation of SARS-CoV-2 show resemblance to SARS-CoV and MERS-CoV[5]. Symptoms of SARS-CoV include fever, cough, dyspnea, and occasionally watery diarrhea. During the epidemic in 2002 - 2003, the virus infected about 8098 individuals resulting in 774 fatalities, placing the CFR at 9.6%[8]. MERS-CoV on the other hand caused explosive nosocomial transmission events, in some cases linked to a single super spreader. According to the WHO, as of November 2019, a total of 2494 persons had been infected with the MERS-CoV resulting in 858 deaths (CFR of 34.4%) with the majority in Saudi Arabia (Table 1). The clinical features of MERS share many similarities with SARS and COVID-19 such as severe atypical pneumonia, gastrointestinal symptoms, and acute kidney failure[9]. With regards to the CFR for COVID-19, a recent study by researchers from China's Center for Disease Control and Prevention revealed some interesting clinical features on 44672 confirmed cases that were associated with 1023 fatalities (CFR of 2.3%) (Figure 1). The fatality was significantly higher in older patients (up to 14.8% in patients over 80). In critically ill patients, the death rate was over 49%. Interestingly, the majority of the cases, 81%, were classified as mild, meaning they did not result in pneumonia or resulted in only mild pneumonia, 14% were severe and 5% were critical. More than 87% of cases were aged 30 to 79 years and 2% less than 19 years of age, and 3.8% healthcare personnel were infected.

Finally, it is worth noting that most secondary transmission of SARS and MERS occurred in the hospital settings through super spreaders. Although, the transmission of COVID-19 is occurring in this context too, it appears that considerable transmission is occurring in communities[10]. Caution should be applied when interpreting these head-to-head CFR comparisons as they might be impacted by confounding independent variables such as time and place.

GLOBAL SPREAD OF SARS-COV, MERS-COV, AND SARS-COV-2

The extent to which an outbreak spreads is dependent on human and environmental factors. Figure 2 shows the geographical distribution of cases of SARS, MERS, and COVID-19 (SARS in 29 countries, MERS in 27 countries, and COVID-19 in 185 countries/regions as of April 12, 2020). Of interest is that, COVID-19 has the largest geographic range. However, it has mostly impacted countries within Asia, Europe, and North America. Africa and South America have experienced the least impact of the coronavirus epidemics in general. Although COVID-19 pandemic has now expanded to these regions (Figure 2), the disease reproduction number is still relatively low. It is not entirely clear why there is limited impact of the disease in Sub Sahara Africa and Latin America, especially considering that transmission may be facilitated by sub-optimal health infrastructure and crowded communities in these regions. On the other hand, it may be construed that the low report of cases may be due to limited testing and surveillance mechanisms. Together, whether environmental factors contribute to the transmission of SARS-COV-2 is obviously an area that requires further research as we learn more about the transmissibility of epidemic coronaviruses. It was recently proposed that high temperature and high relative

Table 1 Comparison of severe acute respiratory syndrome coronavirus, Middle East respiratory syndrome coronavirus and severe acute respiratory syndrome coronavirus2

	Severe acute respiratory syndrome (SARS-CoV)	Middle East respiratory syndrome (MERS-CoV)	Severe acute respiratory syndrome (SARS-CoV2)
Classification[5]	Beta coronavirus	Beta coronavirus	Beta coronavirus
Country of onset[4,5,7]	First reported in November 2002 in the Guangdong province, China	First reported in April 2012 in Saudi Arabia	First reported in December 2019 in Wuhan, China
Origin[2,4]	From bats, which infected civets and then humans	From dromedary camels to humans	Believed to have spread from contact with bats
Global spread[5,8]	29 countries worldwide	27 countries worldwide	185 countries and territories worldwide as of April 12, 2020 (ongoing)
Timeline[4,5,7]	Last case in 2004	Last case in 2019	Ongoing (as of May 7, 2020)
Cases and fatalities[5,8]	It infected 8098 persons and resulted in 774 deaths	It infected 2494 persons and resulted in 858 deaths	About than 3,836,215 cases and 268,999 deaths as of May 7, 2020
Transmission[5]	Droplets/contact	Droplets/contact	Droplets/contact
	In addition, it is possible that these viruses might be spread more broadly through the air (airborne spread) especially during an aerosol generating medical procedures.		
Incubation period[5]	Typically, 2-7 d or up to 10-14 d in some cases	2-14 d	1-14 d
Symptoms[3]	Fever, non-productive cough, sore throat, headache, myalgia, malaise, shortness of breath, chest pain, vomiting, and pneumonia	Fever, severe acute respiratory illness, cough, and shortness of breath, and pneumonia	Fever, cough, headache, body weakness and myalgia (fatigue), shortness of breath, and breathing difficulties. In severe cases, individuals may show symptoms of pneumonia
CFR[4,5,8]	9.6%	34.4%	2.2% (initial reports)
Treatment[4]	There are no antiviral drugs effective against coronaviruses. Supportive treatment using corticosteroids (methylprednisolone) to reduce lung injury induced by inflammation has been used to reduced acute respiratory distress		
Vaccines[4]	There is no approved and marketed vaccine against SARS-CoV, MERS-CoV, or SARS-CoV-2		

SARS-CoV: Severe acute respiratory syndrome coronavirus; MERS-CoV: Middle East respiratory syndrome coronavirus; CFR: Case fatality Rate; The ratio of deaths from a disease to the total number of people diagnosed with this disease for a certain period of time.

humidity significantly reduce the transmission of COVID-19. The authors suggested that one-degree Celsius increase in temperature and one percent increase in relative humidity lower R_0 (basic reproductive number) by 0.0383 and 0.0224, respectively[11]. It is still unclear if this could be a reason for the low transmission in tropical regions. However, this hypothesis may suggest that the arrival of summer and rainy season in the northern hemisphere may affect the transmissibility of the virus. Sociocultural differences in human interactions in different parts of the world may also explain differences in transmission and epidemic expansion; *e.g.*, in contemporary Europe, salutation of friends and close acquaintances is often accompanied by hugging and a kiss on both cheeks. Such close and direct contact with infected persons who may be asymptomatic or unaware of their infections (given the long incubation period of the virus) may facilitate the spread of the virus. Similarly, the coincidence of the onset of COVID-19 outbreak, just prior to China's annual Lunar New Year holiday, was an important factor that had serious impact on the global spread of the disease. Because this is the largest and most important holiday of the year in China, millions of domestic and international trips are made by residents and visitors in often crowded planes, trains, buses, and local transit systems. Therefore, each infected person could have numerous close contacts over a protracted time and across long distances thereby, impacting the global expansion of the disease and complicating response efforts.

PATHOGEN NOVELTY, REPRODUCTION NUMBER, AND THE IMPACT ON TRANSMISSION

The extent to which a pathogen is novel can impact outbreak response, and consequently severity. Factors that determine the extent of pathogen novelty include, knowledge on the pathogen's primary and secondary reservoirs, transmission modes,

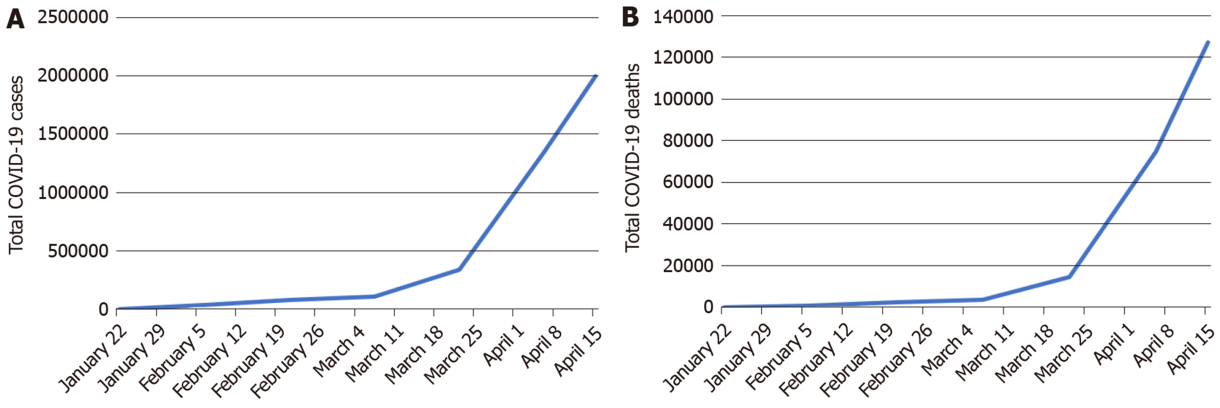

Figure 1 Number of cases of coronavirus disease 2019 and number of deaths due to coronavirus disease 2019 by April 15, 2020. A: Number of cases of coronavirus disease 2019 by April 15, 2020; B: Number of deaths due to coronavirus disease 2019 by April 15, 2020. COVID-19: Coronavirus disease 2019.

control measures, incubation time, diagnostic procedures and treatments, *etc*[14]. In the case of SARS, the causative agent was only isolated and named after about 5 months into the outbreak, it was absolutely novel at the time[15]. Unlike this first SARS outbreak, SARS-CoV-2's sequenced genome was already published less than a month after the first case in humans was reported[15]. Similarly, MERS-CoV was identified at the onset of the outbreak[14]. Hence it is fair to imply that, SARS was more novel than COVID-19 and MERS when it emerged.

Compared to the MERS outbreak, the SARS and COVID-19 outbreaks showed higher basic reproductive numbers (R_0; the expected number of cases directly generated by one case in a population where all individuals are susceptible to infection). The R_0 for SARS and COVID-19 is similar (3 and 3.2 respectively) and MERS is < 1[16-18]. The higher R_0 for SARS and COVID-19 may support the reason why their global spread is higher than MERS.

HOW DOES COVID-19 COMPARE TO THE SEASONAL FLU?

Human coronaviruses such as 229E, NL63, OC43, and HKU1 have long been considered inconsequential pathogens, causing the "common cold" and other mild respiratory symptoms in healthy people[5,6]. However, in the last two decades highly pathogenic coronaviruses have emerged including the current SARS-CoV-2 causing widespread morbidity and mortality. Although the initial symptoms of both COVID-19 and the flu are associated with acute respiratory infection (Table 2), the global morbidity and mortality of the current COVID-19 pandemic is expected to surpass that of the seasonal flu. So far, the novel SARS-CoV-2 has led to about 3836215 illnesses and 268999 deaths as of May 7, 2020. This fatality is likely to increase before the pandemic resolves. The flu on the other hand sickens about 5 million people worldwide, killing up to 650000 people every year according to the WHO[19]. Despite these figures, caution should be applied when interpreting global disease burden of these diseases. It is important to note that the burden of infections differ by place (country or region) and time (when). Hence, comparing the CFRs of COVID-19 and the seasonal flu without considering these differences is inappropriate. For example, this season (October 2019 to May 2020) the Centre for Disease Control estimates that as of March 28, 2020, about 24000-63000 of the 39-55 million people who contracted influenza in the United States have died. Historically, the CFR of influenza in the United States has always been < 0.1%. As of May 7, about 76512 of the 1.29M confirmed cases of COVID-19 in the United States had died (CFR = 5.9%). Comparing the CFR of COVID-19 to the CFR of the seasonal flu from earlier years is inappropriate as place and time are independent variables that may influence disease transmission. Finally, it is essential to note that the occurrence of COVID-19 and the flu are not mutually exclusive. COVID-19 could potentially exacerbate the disease burden of the flu and vice versa. Despite the burden of the flu, a lot is known about the virus and the seasonal expectations and projections. In contrast, very little is known about SARS-CoV-2 (which obviously is not a flu), and the outbreak is yet to peak in several countries and jurisdictions. However, so far COVID-19 seems to have spread much faster than the flu causing severe illnesses and leading to a shutdown of the socio-economic activities worldwide. This seems to be a severe disease and its real burden will only be accurately reflected and evaluated post resolution of the

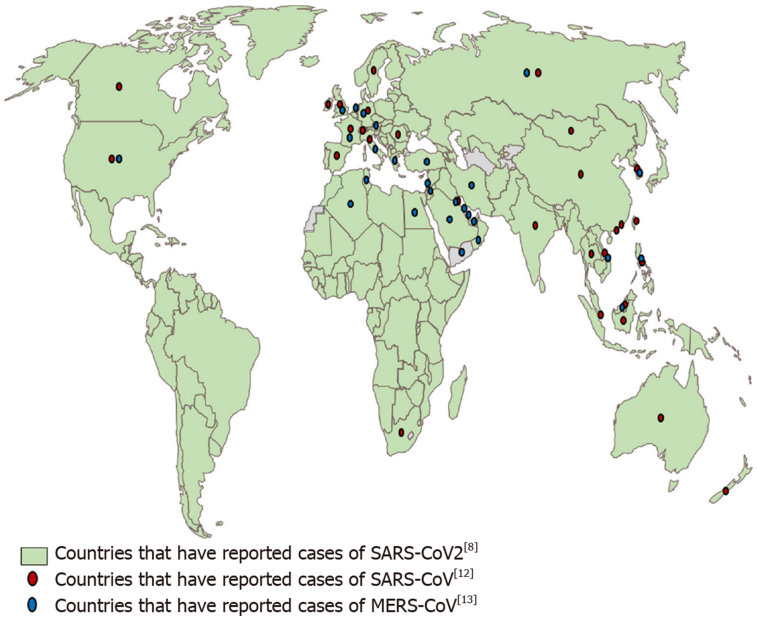

Figure 2 The global spread of severe acute respiratory syndrome coronavirus, Middle East respiratory syndrome coronavirus and severe acute respiratory syndrome coronavirus2 as of April 15, 2020. SARS: Several acute respiratory syndrome; MERS: Middle East respiratory syndrome; CoV: Coronavirus.

pandemic.

It should be noted that the above comparisons are made against endemic flu. However, the CDC estimates that 151700-575400 people worldwide died from the 2009 H1N1 flu pandemic during the first year the virus circulated. Strangely, > 80% of related deaths were estimated to have occurred in people younger than 65 years of age. This differs greatly from typical seasonal influenza epidemics about 70%-90% of deaths are estimated to occur in people 65 years and older.

THE CASE FATALITY RATIO OF COVID-19, AN UNRESOLVED DILEMMA

The CFR is the ratio of the number of deaths from a disease to the total number of people diagnosed with the disease for a certain period of time. For an emerging infectious disease like the COVID-19, CFR is a vital indicator to assess clinical severity. Initial reports from China and other global health agencies have reported that the CFR of COVID-19 is about 2.2%, relatively lower than SARS or MERS. In Canada and United States, the CFR as of April 6 is 2.2% and 3.4% respectively. However, in Spain and Italy during the same time, the CFR is 10% and 12.6%, respectively. For a disease that is in its nascent stage, we think it is far too early to definitively establish the crude fatality rate of COVID-19. We believe, these initial estimates are based on the intuitive calculation of dividing the death toll by the number of confirmed cases. For example, if we consider the estimate as of April 12, 2020 from Table 1, 113296 deaths divided by 1836338 confirmed cases (No. of deaths + recovered) multiplied by 100, we get a CFR of 6.1% while the CFR as of February 12 was 2.1%. As simplistic as this may be, biases in the estimate of population fatality rates during outbreaks may occur if critical cofounding factors are not considered. It has been suggested that CFRs calculated from individual outcome data are likely to be more reliable than estimates calculated from population level data[23]. If estimates from population-based data are used, they must include the lag time between reporting cases and reporting deaths in order to account for reported cases for whom the disease outcome is yet unknown. This is particularly important if there is a delay from symptom onset to case report or delay from death to fatality report[24]. Moreover, from previous experience, equal reporting of cases and deaths is unlikely in an emergency pandemic situation, and even less likely to be consistent across multiple countries. This is because different reporting systems may be used by different countries to record confirmed cases and deaths, leading to inaccuracies in estimating the CFR. Simply dividing the total reported deaths by the total reported cases over multiple countries neglects such variability across countries and this may skew the calculation

Table 2 Comparison of coronavirus disease 2019 and the flu

Factors	COVID-19	Flu
Incubation period	2-14 d	1-4 d
Symptoms[3]	The most common symptoms are cough, sore throat, headache, body weakness and myalgia (fatigue) due to severe respiratory illnesses associated with shortness of breath and breathing difficulties. In severe cases, individuals may show symptoms of pneumonia	Typical flu symptom is characterized by a sudden onset of fever, cough (usually dry), headache, muscle and joint pain, severe malaise (feeling unwell), sore throat and a runny nose
Case fatality rate[8,20]	Initial reports from China suggest the case fatality rate is at least 2.2% (unresolved), and United States 3.9% as of April 12, 2020	The case fatality rate for the flu in the United States is < 0.1%
Virus transmission[17,21]	The basic reproduction number, R_0 is about 3.2	The production number of the flu is about 1.28
Characteristic	Pandemic	Endemic, potential for epidemic or pandemic
Prevention[19,22]	There is no approved vaccine for COVID-19	There is an annual flu vaccine

COVID-19: Coronavirus disease 2019; R_0: the average number of people who catch the virus from a single infected person.

of the CFR[25]. Countries specific variation in CFR is very prominent. As of April 12, 2020, the CFR of Italy was like ten times higher than that of most countries in Africa. Furthermore, the preferential reporting of apparently severe cases or symptomatic infections may neglect mild or asymptomatic infections and this bias can lead to faulty CFR calculation. Unfortunately, monitoring asymptomatic infections in an outbreak situation like the current one is not a public health recommendation and is not an area to prioritize resources during an active pandemic.

Together, as the pandemic spreads rapidly through countries, and as country specific surveillance significantly differ, the CFR estimates may fluctuate substantially. Therefore, without adequate knowledge of the relative reporting of cases to deaths, estimates of CFR calculated from population level data should be interpreted with caution. A retrospective study that will assess the serostatus of close contacts of patients irrespective of symptoms would help to determine the proportion of asymptomatic and mild infections and help guide the calculation of the near true CFR. Until then, the exact reproduction number (R_0) estimate or CFR of COVID-19 still remains an issue to be thoroughly investigated.

OUTBREAK PREPAREDNESS AND THE IMPACT ON DISEASE CONTROL

There is an old adage that says, "luck favours the prepared mind". Slow and ineffective responses can prolong an outbreak and consequently increase severity. In this section we use the 2019 Global Health Security (GHS) index domains, an outbreak preparedness metric, to explore the outbreak severity of coronavirus outbreaks. GHS is an index that contains 34 indicators organized across 6 domains that measure, (1) Prevention of the emergence or release of pathogens; (2) Early detection and reporting of epidemics of potential international concern; (3) Rapid response to and mitigation of the spread of an epidemic; (4) Sufficiency and robustness of health systems to treat the sick and protect health workers; (5) Commitments to improving national capacity, financing plans to address gaps and adhering to global norms; and (6) Overall risk environment and country vulnerability to biological threats[26].

Although the GHS index was only recently developed and can't be used for the inferences of prior health events, it is important to note that the frequent emergence and re-emergence of epidemics with pandemic potential has increased outbreak awareness and preparedness in the global community. Between 2011 and 2018, the WHO tracked 1483 epidemics in 172 countries[26]. The frequency of these outbreaks has led to improved outbreak preparedness globally. A testament to increased outbreak preparedness in the global community is the implementation and monitoring of International Health Regulations (2005), which aim to prevent and control the international spread of disease through committed national leadership, health system strengthening, financing to address gaps, and international collaboration.

The GHS Agenda through its partners in over 64 countries is helping to build capacities to prevent or respond to infectious disease threats. This initiative focuses on 11 areas of action (action packages). Prevent 1: Antimicrobial Resistance; Prevent 2:

Zoonotic Disease; Prevent 3: Biosafety and Biosecurity; Prevent 4: Immunization; Detect 1: National Laboratory System; Detect 2: Real-Time Surveillance; Detect 3: Reporting; Detect 5: Workforce Development; Respond 1: Emergency Operations Centers; Respond 2: Linking Public Health with Law and Multisectoral Rapid Response; Respond 3: Medical Countermeasures and Personnel Deployment Action Package.

Although some countries have achieved significant progress in capacity level improvement in areas like immunization, biosafety, and biosecurity, there is a limited focus on surveillance of zoonotic diseases, infection prevention and control, and early detection capacity of emerging pathogens.

It is also important to mention that the severity of the outbreak can differ significantly by country's readiness. For example, countries with low GHS index are likely to be highly impacted (*e.g.*, the GHS score of the Democratic Republic of Congo is 26.5). If a country such as this is badly hit while already struggling to contain re-emerging Ebola outbreaks, the consequences might be dire. Hence, caution must be applied when inferring the overall severity and impact of the COVID-19 outbreak, as country readiness, capacity level, resources, and context are essential independent variables that should not be neglected in the assessment.

TREATMENT OPTIONS AND CLINICAL TRIALS FOR COVID-19

The emergence of SARS-COV-2 and COVID-19 has left the scientific community searching for potential therapeutics to manage the disease. There is no known effective antiviral against SARS-COV-2, however previously used antivirals and pharmacologics are currently being investigated and in some cases used in the clinical setting on an off-label basis to treat patients suffering from COVID-19. Chloroquine and its hydroxyderivative - hydroxychloroquine, are currently being used to treat COVID-19 patients in some countries across world (*e.g.*, China, France and United States). Chloroquine and hydroxychloroquine have been used for decades for the effective treatment of malaria with a well-known tolerability and safety record. Based on its known *in vitro* antiviral activities against diverse human viruses (reviewed in Devaux *et al*[27], 2020) and SARS coronaviruses[27-29], and the recent reports of its *in vitro* efficacy against SARS-COV-2[30-32], a non-randomized trial to evaluate the clinical efficacy and safety was carried out in small cohort of hospitalized patients with COVID-19 pneumonia in China[33,34]. Compared to control treatment (Lopinavir/Rotinavir), chloroquine demonstrated superior efficacy in the inhibition of the exacerbation of pneumonia both clinically and based on improved lung imaging findings, shortened disease course and promoted complete viral clearance. In these patients, 500 mg of chloroquine was administered orally twice daily for 10 d. Chloroquine has now been included in the Guidelines for the Prevention, Diagnosis and Treatment of Pneumonia Caused by COVID-19 by the National Health Commission of China[34,35]. A non-randomized open label trial carried out in France treated hospitalized COVID-19 patients with variable disease severity with a combination of hydroxychloroquine (600 mg/d for 10 d) and azithromycin (500 mg on day one followed by 250 mg/d for four d) or no treatment[36,37]. Results from this study indicated that hydroxychloroquine and azithromycin were effective treatments for COVID-19 patients resulting in faster clinical improvement and discharge; and complete viral clearance (based on a negative polymerase chain reaction test results or viral culture). Despite the encouraging findings from these studies, it is important to note that the trials were non-randomized, had design flaws with relatively few participants (less than a few hundred participants in each study). It is, therefore, prudent for the scientific community to carry out more well-designed clinical trials to assess the efficacy and safety of chloroquine for the treatment and management of COVID-19 patients prior to making a final recommendation for its use. This will allow for the development of appropriate treatment guidelines including dosage, patient monitoring, duration of treatment and expected outcomes. The United States Food and Drug Administration has since issued an authorization to permit the emergency use of chloroquine phosphate to treat adult and adolescent hospitalized COVID-19 patients for whom a clinical trial is not available, or participation is not feasible[38]. More than 30 clinical trials are ongoing in different parts of the world on the use of chloroquine for COVID-19 treatment[39-41]. While chloroquine may be well tolerated, safe and cheap, the drug has a narrow therapeutic index and long-term use may be associated with cardiomyopathy and retinopathy[42,43]. Toxic concentrations can be lethal as such self-prescription is not recommended and administration should be done only in a hospital setting.

Another drug in clinical trials used for treatment of COVID-19 patients is remdesivir (GS-5734). It is a broad-spectrum antiviral nucleotide analogue with reported efficacy against SARS-COV1 and MERS-COV coronaviruses in cell culture and animal models that was used to treat a COVID-19 patient in the United States who showed significant improvement and tolerability one day after intravenous administration of the drug[32,43,44]. Apart from chloroquine and remdesivir, several drugs both new and old being repurposed for the treatment of COVID-19 are now under clinical trials with the hope that they may be available at patients bed-side in the near future.

The most effective strategy to control the spread, eradicate and minimize the burden of infectious diseases is through mass immunization. Unfortunately, given the novelty of SARS-COV-2 and COVID-19 and the speed with which the virus spread around the world, scientists have had little time to develop any vaccine candidates. As such there is no known effective vaccine against SARS-COV-2 at this time, however emerging epidemiological data suggests that the Bacillus Calmette-Guérin (BCG) vaccine (the vaccine for tuberculosis) may be effective in decreasing spread of infection, disease severity and mortality from COVID-19[45-48]. These reports suggest that there is a correlation between either universal or mandated BCG vaccination and morbidity and mortality from COVID-19. The evidence comes from historical vaccination data review and the current morbidity and mortality rates due to COVID-19 in different countries. Countries without historical universal policies of BCG vaccination at birth such as Italy, Netherlands, United States have been severely afflicted compared to countries with compulsory and long-standing BCG policies consistent with a possible protective role of the BCG vaccine against COVID-19[45,47]. As promising and hopeful as these data may be, these are epidemiology studies and not controlled trials thus it is imperative for large scale randomized control trials be carried out to test this theory. The BRACE (Australia) and BCG-CORONA (Netherlands) randomized-controlled trials are currently in progress to assess the effectiveness of the BCG vaccine to enhance the immune systems of healthcare workers against COVID-19[49,50]. Results from these studies will provide empirical data to support the epidemiological reports above and offer some hope to the world. As the pandemic escalates globally, basic infection prevention and control guidelines appear to be the best option to mitigate the spread of the disease.

INFECTION PREVENTION AND CONTROL FOR COVID-19

The route of transmission, pathologies, and manifestation of SARS-CoV-2 clearly show some similarities to SARS-CoV and MERS-CoV. Both SARS-CoV and MERS-CoV infect intrapulmonary epithelial cells better than cells of the upper airways making transmission to occur primarily from patients with recognized illness and not from patients with mild, nonspecific signs[51]. The incubation period of SARS-CoV-2 is between 1-14 d and patients present with fever associated with flu-like symptoms including cough, sore throat, headache, body weakness and myalgia (fatigue) to severe respiratory illnesses associated with shortness of breath and breathing difficulties[52]. In critical cases, individuals may show symptoms of pneumonia associated with complications of severe acute respiratory and cardiac distress, and kidney failure, which can eventually lead to death. The long incubation period facilitates the spread of the infection to others through contact and exposure to infected droplets.

It has been suggested that SARS-CoV-2 uses the same cellular receptor (human angiotensin-converting enzyme 2) as SARS-CoV, making transmission to occur mainly after signs of lower respiratory tract disease has developed[53]. Similar to SARS-CoV and MERS-CoV, the transmission of SARS-CoV-2 occurs by means of droplets and contact with infected persons. Therefore, public health measures and strict adherence to standard precautions in health care settings, are critical in controlling the pandemic[54]. Together, breaking the chain of transmission of a pandemic like COVID-19 is a shared responsibility; the population and the state have unique roles to play.

Population

Individuals must practice physical distancing (staying 2 metres apart from other people at all times). Anyone who is ill, including mild respiratory symptoms, must stay home and monitor their health for fever, cough or difficulty breathing and based on national legislation, report their symptoms to the public health authorities for tracing and eventual testing. All returning international travellers must stay home for 14 d. The population must be encouraged to practice good hand hygiene and cough etiquette. For example, washing of hands often with soap and warm running water,

or alcohol-based hand sanitizers and covering mouth and nose with the arm when coughing or sneezing to avoid the expulsion of droplets to others. People should avoid touching their eyes, nose, and mouth unless they have just washed their hands. Unnecessary movements should be restricted and if someone should go out for essential visits, he or she should wear a mask that covers the nose and mouth and care should be observed when handling the mask.

Health care establishments

All healthcare establishments should perform active and passive screening. Persons conducting screening should ideally be behind an impermeable barrier to protect them from droplet from sneezing/coughing patients. If a patient screens positive, he or she should immediately be asked to don a surgical mask and be isolated. From this time onwards, healthcare workers should apply standard and transmission-based precautions including the appropriate use of personal protective equipment such as gloves, gown, surgical/procedure masks and eye protection (goggles or face shields) for patient care[54]. As a general rule, health care workers should use a risk assessment approach before and during each patient interaction to evaluate the likelihood of exposure. In the event that an aerosol generating medical procedure has to be done, droplet, contact and airborne precautions should be observed, and the procedure should be done in an airborne infection isolation room that is under negative pressure. These precautions include wearing the following personal protective equipment - gloves, gown, N95 fit-tested respirators and eye protection (goggles/face shields)[55,56]. Patients who test positive for SARS-CoV-2 should not be cohorted with non-COVID-19 patients, but may be cohorted with other patients confirmed to have COVID-19. It is essential to routinely clean and disinfect care equipment, surfaces and environment using approved hospital-grade disinfectants.

Governments and public health authorities

It is the responsibility of every nation to protect the lives of its citizens. Once an outbreak of a disease with pandemic potential is determined, there should be declaration of a state of emergency to help contain the spread and protect the public. Consequently, the following establishments are required to closed to prevent congregation of persons; bars and restaurants (except to the extent that such facilities provide takeout and deliveries), indoor recreational centers, public libraries, churches, schools, child care centres, movie cinemas, theatres, concert venues and other communal or shared public or private centres. Additionally, all organized public events of over 5 people (or when a 2 m separation cannot be maintained) should be prohibited, including parades, funeral, weddings, and other social gatherings. As much as possible employees should be encouraged to work from home if feasible. Travel restrictions should be put in place to discourage the population from international travels especially to highly impacted countries. Returning travellers must self-isolate and monitor for symptoms for 14 d.

Also, it is absolutely necessary that the right information is given to the population to avoid the dissemination of false and inaccurate information and all rumours and conspiracies should be debunked with scientific evidence. The population through community leaders should be involved in decision making as an inclusive approach will results in better compliance and positive outcomes.

CONCLUSION

From 2002, there has been a pattern of coronaviruses emerging and causing epidemics every 8-10 years. The SARS-CoV, MERS-CoV, and now SARS-CoV-2 that have been responsible for global epidemics starting in 2002, 2012, and 2019 respectively[57]. It is known that coronaviruses reside in animal reservoirs but the spillover mechanism into human population is not fully understood. In our opinion, coronaviruses will continue to emerge periodically and unpredictably, spreading and inducing serious infectious diseases of huge global health impact.

Although the first vaccine against COVID-19 is being developed and a chain of therapeutic clinical trials are underway, there are no approved drugs or vaccine for the treatment or prevention of coronavirus infections[58]. Furthermore, the range of animal reservoirs for coronaviruses makes the threat to the human population worse. A starting point in the prevention of future coronavirus outbreak is the regulation of wildlife meat trades in order to reduce the risk of animal to human spillover of the virus, surveillance and development of laboratory capacities for early detection.

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ORIGINAL ARTICLE

Basic Study

GadE regulates *fliC* gene transcription and motility in *Escherichia coli*

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Abstract

BACKGROUND

Escherichia coli (*E. coli*) express flagella to ascend human urinary tracts. To survive in the acidic pH of human urine, *E. coli* uses the glutamate decarboxylase acid response system, which is regulated by the GadE protein.

AIM

To determine if growth in an acidic pH environment affected *fliC* transcription and whether GadE regulated that transcription.

METHODS

A *fliC-lacZ* reporter fusion was created on a single copy number plasmid to assess the effects of acidic pH on *fliC* transcription. Further, a Δ*gadE* mutant strain of a uropathogenic *E. coli* was created and tested for motility compared to the wild-type strain.

RESULTS

Escherichia coli cells carrying the *fliC-lacZ* fusion displayed significantly less *fliC* transcription when grown in an acidic pH medium compared to when grown in a neutral pH medium. Transcription of *fliC* fell further when the *E. coli* was grown in an acidic pH/high osmolarity environment. Since GadE is a critical regulator of one acid response system, *fliC* transcription was tested in a *gadE* mutant strain grown under acidic conditions. Expression of *fliC* was derepressed in the *E. coli gadE* mutant strain grown under acidic conditions compared to that in wild-type bacteria under the same conditions. Furthermore, a *gadE* mutation in a uropathogenic *E. coli* background exhibited significantly greater motility than the wild-type strain following growth in an acidic medium.

CONCLUSION

Together, our results suggest that GadE may down-regulate *fliC* transcription and motility in *E. coli* grown under acidic conditions.

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Core tip: *Escherichia coli* (*E. coli*) is the number one cause of urinary tract infections in women. The infections are the result of the *E. coli* cells ascending the urinary tract via flagella presented on the outside of the cells. In this study, we have shown that *E. coli* grown in a low pH/high-osmolarity environment display transcriptional repression of the *fliC* flagellin subunit gene. Furthermore, we demonstrate that GadE may regulate *fliC* transcription and subsequent motility of the *E. coli* cells.

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INTRODUCTION

In the United States, approximately 10.5 million women suffer from a urinary tract infection each year. Around 80% of urinary tract infection are caused by uropathogenic *Escherichia coli* (UPEC), resulting in over 100000 hospitalizations and an approximate cost of $ 3.5 billion per year[1-3]. UPEC sometimes ascend all of the way to the kidneys, causing life-threatening pyelonephritis in some of the women[2,3]. The ability of *Escherichia coli* (*E. coli*) to move up the human urinary tract is due to the presence of flagella expressed by the bacteria[4-7].

Bacterial flagella allow the directional movement of *E. coli* based upon a chemotactic response[8,9]. Several genes are involved in the expression of flagella, although *fliC* encodes the flagellin subunits that comprise the bulk of a flagellum structure[10]. Several studies have shown the importance of flagella in UPEC pathogenesis[4-7,11]. For instance, several studies have examined the prevalence of the *fliC* gene in UPEC strains. One study showed the prevalence of the *fliC* gene in UPEC strains varied from 84% (community-acquired) to 95% (nosocomial-acquired)[12], whereas another study reported that only 16% of the UPEC strains had the *fliC* gene[13]. Part of the disparity in the frequency of *fliC* gene prevalence could be due to the respective primers used in each study. Certainly, UPEC flagella are critical for ascension out of the bladder into the kidneys of an animal host. Within a mouse or human urinary tract, UPEC are continuously bathed in urine. Typically, human and murine urine will have a slightly acidic pH and variations in osmolality[14-16], although the osmolality within murine urine is usually higher than human urine[15]. Hence, pH is one critical environmental factor found in the urinary tract.

Within *E. coli*, homeostasis in an acidic environment is mediated by at least five acid response (AR) systems[17-21]. System two (AR2) is induced in stationary phase and requires a glutamate decarboxylase and a glutamate: γ-aminobutyric acid antiporter. AR2 is the predominant and best characterized of the five AR system pathways[22-25]. The AR2 requires the antiporter GadC and two inducible glutamate decarboxylases: GadA and GadB. The antiporter is responsible for transporting glutamate into the cell while transporting the product of glutamate decarboxylation, glutamate: γ-aminobutyric acid, out of the cell[22,24-30]. GadE, belonging to the LuxR family of regulatory proteins[31], has been identified as the central transcriptional activator of *gadA/BC*, and provides the primary means of *gadA/BC* activation[32,33]. Microarray studies done under acidic conditions originally identified the *yhiE* gene (renamed *gadE*), which was found to encode for this transcriptional regulator protein[31]. GadE binds to a 20-bp sequence (GAD box: 5′-TTAGGATTTTGTTATTTAAA-3′) located -63 bp from the transcriptional start site of both *gadA* and the *gadBC* operon and is necessary for expression of these genes under all conditions[28,34,35].

In this study, we have studied the role GadE may play in *E. coli* flagella expression. Through the use of a *gadE* mutant, a *fliC-lacZ* reporter system, and a motility assay; we demonstrate that GadE regulates transcription of *fliC* in *E. coli*, which in turn affects bacterial motility.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media

All of the bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* strain NU149 is a clinical isolate obtained from a patient with cystitis[36]. The *E. coli* strain DH5α was used to construct the *fliC-lacZ* reporter system. *E. coli* strains MC4100 (supplied by Linda Kenney) and EK227 (supplied by John Foster) were subsequently tested under various pH and osmotic conditions with the *fliC-lacZ* reporter system. The Δ*gadE* strain EF1007 and Δ*gadE*/pPCRScript Amp *gadE* strain EF1083 were also supplied by John Foster. Multicopy plasmid pUJ9[37] and single copy plasmid pPP2-6[38] were used for cloning. The pUJ9 plasmid contains a promoterless *lacZ* gene and an ampicillin antibiotic resistance gene. Plasmid pPP2-6 is a single copy plasmid with a multiple cloning site that possesses a chloramphenicol resistance gene[38]. The pPCRScript Amp *gadE* plasmid had the *gadE* gene cloned into the multicopy plasmid pPCRScript Amp[33]. Luria Agar (LA) supplemented with 12.5 μg/mL chloramphenicol was used to grow the recombinant *E. coli* cells containing the reporter system. Luria Bertani (LB) broth containing 1% glycerol at pH's ranging from 5.5 to 8.0 was used to test pH ranges, and LB broth (pH 5.5 and pH 7.0, 1% glycerol, 0.1 mol/L Na_3PO_4 buffering) coupled with osmotic variation of 0 to 400 mmol/L NaCl was used to gauge pH plus osmotic changes[38]. Under these growth conditions, the recombinant *E. coli* strains were assayed for β-galactosidase activity.

Construction of the fliC-lacZ fusion

Oligonucleotide primers FliC1 (5'-GAGAGAATTCGATGAAATACTTGCCATGC-3') and FliC2 (5'-AGAAGGATCCAGACGCTGGATAGAACTC-3') specific for a 397-bp segment of the *E. coli* strain NU149 *fliC* promoter were amplified with the *BamH*I and *Eco*RI restriction endonuclease sites flanking the DNA promoter sequence. Polymerase chain reaction (PCR) amplification using these primers was set up as follows: An initial denaturation of five minutes, then 35 cycles 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C, finishing with a 7 min elongation at 72 °C after the 35th cycle. Chromosomal DNA from *E. coli* strain NU149 extracted with a PurElute Bacterial Genomic kit (Edge Biosystems, Mountain View, CA, United States) served as the template in the PCR. The 397-bp product was visualized on a 0.8% agarose gel containing ethidium bromide with a 1 kb ladder (New England Biolabs, Ipswich, MA, United States) served as the molecular weight standard.

The PCR amplified 397-bp *fliC* promoter DNA fragment was passed through a Microcon 30 filter (MilliporeSigma, Burlington, VT, United States) to concentrate the DNA. Subsequently, the DNA was digested with the restriction endonucleases *Eco*RI and *BamH*I (New England Biolabs). The digested DNA fragment was ligated to *Eco*RI/*BamH*I digested pUJ9 plasmid DNA and transformed into competent DH5α cells. The resulting transformants were selected on LA containing 100 μg/mL ampicillin and X-Gal (Promega, Madison, WI, United States). Blue colonies were screened for β-galactosidase[39] and the plasmid DNA was extracted with a QIAPrep kit (Qiagen, Valencia, CA, United States) to verify the appropriate size. One recombinant plasmid, pNK1-1, was carried further in the process. This plasmid DNA was digested with the restriction endonuclease *Not*I (New England Biolabs) and ligated to *Not*I cut pPP2-6 DNA. Following ligation, the DNA was transformed into DH5α and clones were selected on LA containing 12.5 μg/mL chloramphenicol and X-Gal. One clone, pNK2-29, was selected for *in vitro* analysis.

Galactosidase assays

Galactosidase assays were performed on DH5α/pNK2-29 and MC4100/pPP2-6 cells grown in LB media at various pH and in the presence and absence of NaCl at pH 5.5 and 7.0[39]. Bacteria were grown mid-logarithmically and β-galactosidase activity on the sodium dodecyl sulfate and $CHCl_3$ permeabilized cells. The mean values + standard deviation was calculated from at least three separate experiments for each bacterial strain.

Creation of a ΔgadE mutation in uropathogenic E. coli strain NU149

To create a deletion mutation of the *gadE* gene, the red recombinase system described by Datsenko and Wanner[40] was used. Briefly, the primer pair GadE1 (5'GATGACATATTCGAAACGATAACGGCTAAGGAGCAAGTTTGTGTAGGCTGG AGCTGCTTCG-3') and GadE2 (5'TCGTCATGCCAGCCATGAATTTCA-GTTGCTTATGTCCTGACATATGAATATCCTCCTTAG-3') was used to create a PCR product, using pKD4 plasmid DNA as a template. The PCR conditions that were used were an initial denaturation at 95 °C for 5 min followed by 35 cycles of 95 °C, 1 min; 57 °C, 1 min; and 72 °C, 2 min. The resulting PCR product was concentrated and separated on a 0.8% agarose gel, cut out, and the DNA extracted from the agarose gel.

Table 1 Bacterial strains and plasmids used in the study

Strain/plasmid	Description	Source
Strain		
DH5a MCR	Transformation efficient strain	Gibco/BRL
MC4100	*E. coli* K-12 strain	Linda Kenney
EK227	*E. coli* K-12 strain	[53]
EF1007	*gadE*::Km in EK227	[54]
EF1083	*gadE*::Km/pPCRScript Amp *gadE*	[33]
NU149	Clinical isolate	[36]
NU149 *gadE*	Δ*gadE* mutation in NU149	This study
NU149 LacZ1	Δ*lacZ* mutation in NU149	This study
Plasmid		
pUJ9	Promoterless *lacZ* gene, Ap^R	[37]
pPP2-6	Single copy plasmid, Cm^R	[38]
pKD4	Flp recombinase sites, Km^R	[40]
pKD46	Red recombinase, ApR	[40]
pCP20	Flp recombinase, Ap^R	[40]
pNK2-29	*fliC*::*lacZ* on pPP2-6, Ap^R	This study
pPCRScript Amp *gadE*	*gadE* on pPCRScript Amp	[33]

With this purified PCR product, an electroporation was performed on strain NU149/pKD46 cells as described previously[40], selecting for transformants on LA with 40 μg/mL kanamycin. One transformant, NU149 *gadE*, was chosen for further analysis. To remove the kanamycin resistance gene, plasmid pCP20 was introduced into NU149 *gadE* by electroporation. The resulting strain was processed as noted previously[6]. To confirm the *gadE* deletion, a PCR-based assay was used with the GadE5 (5′-ACAGGGCTTTTGGCAGTTGAA-3′) and GadE6 (5′-AAATATTAG-CGTCGACGTGA-3′) primers. The PCR conditions that were used were an initial denaturation at 95 °C for 5 min followed by 30 cycles of 95 °C, 1 min; 57 °C, 1 min; and 72 °C, 2 min. This Δ*gadE* mutation was complemented by electroporating the pPCRScript Amp *gadE* plasmid into NU149 *gadE* and selecting for transformants on LA with 100 μg/mL ampicillin. The wild-type NU149 strain was used a positive control and *Staphylococcus aureus* genomic DNA was used as a negative control.

Construction of ΔlacZ mutation in uropathogenic E. coli strain NU149

To construct the Δ*lacZ* mutation in UPEC strain NU149, the procedure described above was used. The LacZ1 (5′-CCTTACGCGAAATACGGGCAGACATGG-CCTGCCCGGTTAT

TACATATGAATATCCTCCTTAG-3′) and LacZ2 (5′-TGGAATTGTGAGCGG-ATAACAA

TTTCACACAGGAAACAGCTTGTGTAGGCTGGAGCTGCTTCG-3′) primer pair were used to create the PCR product using the amplification conditions noted above. To confirm the Δ*lacZ* mutation, the LacZ3 (5′-ATGAAACGCCGAGTTAACGC-3′) and LacZ4 (5′-AGCTGGCGTAATAGCGAAGA-3′) primers were used in the PCR amplification conditions described above. Plasmid pNK2-29 was electroporated into strain NU149 and colonies were selected on MacConkey containing 12.5 mg/mL chloramphenicol.

Soft agar assay for motility

A soft agar motility test was performed as previously described[41] for the wild-type *vs* *gadE* mutant and complemented mutant analysis. Each strain was inoculated into the center of the agar plate and the amount of bacterial spread measured after 24 h post-inoculation. The motility assays were repeated two more times on separate days.

Statistical analyses

A two-tailed Student's *t*-test was used to calculate statistical variation with a $P < 0.05$ considered significant.

RESULTS

Examination of the fliC::lacZ fusion at different pHs

To assess whether pH affected the transcription of our *fliC-lacZ* fusion plasmid, the pH of buffered LB medium was adjusted to 5.5 to 8.0 by using 0.1 M Na_3PO_4 buffering and glycerol to maintain the pH[38]. The resulting media were inoculated with MC4100/pNK2-29 and theβ-galactosidase activities of mid-logarithmic-phase cells were determined. The optimal pH for *fliC* expression was found to be at pH 7.0 (1111 Miller units; Table 2). As the pH shifted to the acidic range, *fliC* transcription declined until there was a significant 3.9-fold difference observed comparing *fliC* transcription at pH 7.0 compared to pH 5.5 (288 Miller units, $P < 0.01$). When the pH of the buffered LB was raised into the alkaline range, there was a slight decline in *fliC* transcription that was 1.5-fold lower at pH 8.0 (738 Miller units, $P < 0.05$) *vs* growth in pH 7.0 medium. These results indicate that pH alone affects *fliC* transcription.

Effects of pH and osmotic conditions together on fliC::lacZ transcription

In an environment such as the the human or murine urinary tract, fluctuations in both pH and osmolarity can occur[14-16]. To determine if the combination of acidic pH and high osmolarity affect *fliC* transcription, MC4100/pNK2-29 was grown in buffered pH with variation in both the pH (5.5 and 7.0) and the osmolarity (0 to 400 mmol/L NaCl). When MC4100/pNK2-29 was grown in pH 7.0/low-osmolarity (0 mmol/L NaCl) LB, *fliC* transcription was the highest (1,132 Miller units, Table 3). An increase in the osmolarity to 400 mmol/L NaCl in the pH 7.0 LB caused *fliC* transcription to significantly fall by 2.5-fold (454 Miller units, $P < 0.01$) compared to growth in the pH 7.0 low-osmolarity LB. *E. coli* with the pNK2-29 plasmid grown in pH 5.5/low-osmolarity conditions displayed *fliC* transcription of 308 Miller units (Table 3); however, *fliC* transcription dropped almost 5-fold to 62 Miller Units ($P < 0.01$) as the osmolarity increased to 400 mmol/L NaCl. A comparison of *fliC* transcription in *E. coli* grown in pH 7.0/low-osmolarity LB to the *E. coli* population grown in pH 5.5/high-osmolarity LB showed a highly significant 18.2-fold change ($P < 0.001$). Thus, a growth environment possessing both an acidic pH and high osmolarity substantially repressed *fliC* transcription in the *E. coli* K-12 strain.

To determine if the same *fliC* transcriptional changes occurred in a UPEC strain, a Δ*lacZ* mutation was created in UPEC strain NU149. The pNK2-29 plasmid containing the *fliC-lacZ* fusion was moved into *E. coli* strain NU149 LacZ1 and the same environmental conditions tested for the *E. coli* K-12 strain were used. Growth of NU149 LacZ1/pNK2-29 in pH 7.0 with no added NaCl displayed the highest *fliC* transcription (1353 Miller Units, Table 3), whereas *fliC* transcription significantly fell 3.06-fold when the strain was grown in pH 5.5 LB (442 Miller Units, $P < 0.01$). An increase in the osmolarity to 400 mM NaCl in pH 7.0 LB caused *fliC* transcription to fall 2.77-fold (489 Miller Units, $P < 0.01$). Moreover, the growth of NU149 LacZ1/pNK2-29 in pH 5.5 LB with 400 mM added NaCl showed the lowest level of fliC transcription (147 Miller Units) that was 9.2-fold lower than when grown in pH 7.0 no added salt medium ($P < 0.01$). Overall, the *fliC* transcription results in the UPEC strain mirrored the *E. coli* K-12 strain's results.

Transcription of fliC was affected by the gadE mutation in E. coli grown in acidic pH media

As shown above, acidic pH growth conditions led to lower *fliC* transcription compared to transcription in neutral pH growth conditions. Previous work has shown that the glutamate decarboxylase system is critical for acid resistance in *E. coli* and GadE is an important regulator of this AR system[31-33]. We then asked whether GadE might also regulate *fliC* transcription under acidic growth conditions. We examined an *E. coli* K-12 wild-type strain, a *gadE* mutant strain as well as a complemented *gadE* mutant strain all of which contained the *fliC-lacZ* pNK2-29 plasmid. The strains were grown in buffered LB set at pH 5.5 or 7.0 with (400 mmol/L) or without (0 mmol/L) added NaCl and monitored for galactosidase activity. Derepression of *fliC* transcription occurred in the *gadE* mutant grown in acidic pH LB (Table 4). After growth in pH 5.5/low-osmolarity medium, the *gadE* mutant strain (1742 Miller units) exhibited a 3.2-fold increase in *fliC* transcription, compared to the wild-type strain (540 Miller units, $P < 0.001$), which indicated that GadE repressed *fliC* under acidic conditions. Complementation with an intact *gadE* gene reduced the activity below the wild-type levels to 295 Miller units, below even wild-type levels, confirming the repressive effect of GadE on *fliC* expression. The repressive effect of GadE on fliC expression was reduced in pH 7.0/low-osmolarity medium with the *gadE* mutant strain showing only slightly higher *fliC* transcription (2196 Miller units) *vs* the *gadE*+ wild-type strain (1520 Miller units, $P < 0.01$). However, when the growth conditions were changed to a high osmolarity environment (400 mmol/L NaCl), the *gadE*

Table 2 Effect of pH on *fliC::lacZ* gene transcription in *Escherichia coli* strain MC4100/pNK2-29 grown in buffered Luria Bertani media

pH	Gal activity[1]
5.5	288 ± 81.5
6	528 ± 82.5
6.5	629 ± 114
7	1111 ± 110
7.5	932 ± 190
8	738 ± 125

[1]Galactosidase activity measured as Miller units.

mutation had no significant effect on *fliC* transcription (540 Miller units). A change to a pH 5.5/ high-osmolarity environment caused a further repression of *fliC* transcription (165 Miller units, $P < 0.05$) that was significant.

A gadE mutation affects uropathogenic E. coli motility

The data above suggested that GadE may repress *fliC* transcription when *E. coli* is grown under acidic pH conditions. Since transcriptional differences do not always translate into protein level differences or functional differences, the effects of a *gadE* mutation on *E. coli* motility was next tested. First, motility was tested using the *E. coli* K-12 strain EF227 (wild-type), EK1007 (*gadE* mutation), and EF1083 (*gadE* mutation complemented with the pPCRScript Amp *gadE* plasmid). All strains were grown in pH 5.5 buffered LB and spotted onto motility agar plates. Wild-type *E. coli* strain EF227 displayed an 8.33 mm spread diameter, whereas strain EF1007 showed a significantly larger spread diameter of 45 mm ($P < 0.001$, Table 5). When the *gadE* mutation was complemented in strain 1083, the spread diameter dropped below the wild-type level (6.67 mm diameter).

A *gadE* mutation was also created in the uropathogenic *E. coli* clinical isolate NU149 using a λred recombinase system. The NU149, NU149 *gadE*, and NU149 *gadE*/pPCRScript Amp *gadE* strains were grown in pH 5.5 buffered LB and spotted onto motility agar plates. Wild-type *E. coli* strain NU149 had a 10.67 mm spread diameter, whereas strain NU149 *gadE* had a 57.34 mm spread diameter that was significantly wider ($P < 0.05$). Complementation of the *gadE* mutation brought the spread diameter back down to a wild-type level (7.00 mm). These results indicate that GadE also affects UPEC motility.

DISCUSSION

The production of flagella in UPEC is vital for their pathogenesis in a human host, enabling the bacteria to ascend the urinary tract[4-7,11]. A transcriptome study of a UPEC strain in the murine urinary tract over time demonstrated that several genes that are involved in flagella biosynthesis and chemotaxis, including the *fliC* structural gene, had their transcription down-regulated in this environment[42]. Within the urinary tract, the *E. coli* encounter an environment that typically has a slightly acidic pH and osmotic changes that increase as the bacteria move into the kidneys of the host[14-16]. *E. coli* is able to survive in acidic pH environments that include the human and murine urinary tracts because of AR systems that include the glutamate decarboxylase system[15-18]. GadE is an important protein that regulates this AR system[31-33]. Since GadE is important for regulating genes in one AR system, could the GadE regulator of the glutamate decarboxylase AR system also be involved in the down-regulation of *fliC* in uropathogenic *E. coli* growing in the murine urinary tract?

To answer the question above, we designed a *fliC-lacZ* reporter system on a single copy number plasmid to measure *fliC* transcription within *E. coli* growing in various environments that might be encountered in the urinary tract. Our results showed *fliC* transcription fell in both *E. coli* strains grown in a pH 5.5 environment compared to a neutral pH environment, suggesting one or more proteins produced by *E. coli* growing in an acidic pH environment represses *fliC* transcription. A previous study revealed a substantial drop in motility by *E. coli* grown in an acidic environment *vs* a neutral pH environment[43] that correlates with our experimental observations in this study. Moreover, *E. coli* growth in a high salt concentration medium also caused repression of *fliC* transcription. Li *et al*[44] observed that *E. coli* grown in a high-

Table 3 Effect of osmolarity on *fliC::lacZ* gene transcription in *Escherichia coli* grown in buffered pH 5.5 and 7.0 Luria Bertani media with different osmolarities

E. coli strain	NaCl (mmol/L)	Gal activity[1]	
		pH 5.5	pH 7.0
MC4100/pNK2-29	0	308 ± 104[2]	1132 ± 130
MC4100/pNK2-29	100	338 ± 128	806 ± 41
MC4100/pNK2-29	200	251 ± 68.5	689 ± 173
MC4100/pNK2-29	400	62 ± 22.0	454 ± 71
NU149 LacZ1/pNK2-29	0	442 ± 72	1353 ± 98
NU149 LacZ1/pNK2-29	100	418 ± 61	976 ± 52
NU149 LacZ1/pNK2-29	200	293 ± 43	811 ± 75
NU149 LacZ1/pNK2-29	400	147 ± 39	489 ± 61

[1]Galactosidase activity measured as Miller units.
[2]Data represents the mean ± standard deviation from three separate runs.

osmolarity medium were less motile compared to *E. coli* grown in a low-osmolarity medium.

A combination of pH changes and osmolarity changes was also examined using our *fliC-lacZ* system. In a low pH/high-osmolarity medium, the growing *E. coli* exhibited an additive level of repression of *fliC* transcription that is in line with the previous transcriptome study[42].

Two environmental variables are at play in a low pH/high-osmolarity environment. To adapt to acidic pH conditions, *E. coli* rely on AR systems and their corresponding regulators, such as GadE. On the other hand, the OmpR-EnvZ two-component system is the main osmotic stress regulatory system in *E. coli*[45]. OmpR has been shown to regulate flagella expression[46,47] and is likely partially responsible for repressing *fliC* transcription in the high-osmolarity environment that we tested. Furthermore, OmpR-regulated genes are tied to the acid response in *E. coli* and *Salmonella enterica*[48,49].

Since GadE is a central player in AR system regulation, we examined *fliC* transcription and motility in *gadE* mutant strains *vs* the wild-type strains. By deleting the *gadE* gene, *E. coli fliC* transcription was derepressed, particularly in *E. coli* growing in an acidic pH environment. Complementation of the *gadE* mutation with the *gadE* gene on a multicopy plasmid caused additional suppression of *fliC* transcription that was below wild-type levels. Furthermore, a Δ*gadE* mutation in K-12 and UPEC strains led to significantly greater motility compared to the wild-type strain. Together, these data suggest that GadE represses *fliC* transcription either by directly binding to the *fliC* promoter to repress transcription or acting in an indirect manner by influencing expression of FlhD that in turn regulates *fliC*[50,51]. However, GadE does not appear to affect osmotic control of *fliC* transcription.

What would be the advantage of a loss of flagella expression in *E. coli* growing in the human kidney? Flagella protruding from the surface of *E. coli* cells represent a target of the host's immune system. Flagellated *E. coli* cells are more likely to be phagocytized than no-flagellated cells[52]. *E. coli* that have reached the kidneys would be in a low pH/high-osmolarity environment where the flagella are no longer needed and may in fact be a detriment to their survival. Through the regulatory effects of the GadE and OmpR proteins, *fliC* transcription may be shut down, causing the bacterial cells to lose their flagella and be able to hide behind their anti-phagocytic capsules.

Table 4 Assessing a *gadE* and mutation and complementation on *fliC::lacZ* gene transcription in *Escherichia coli* grown in buffered pH 5.5 and 7.0 Luria Bertani media with different osmolarities

E. coli strain	Gal activity[1]			
	pH 5.5	pH 5.5[2]	pH 7.0	pH 7.0
EK227/pNK2-29	540 ± 51[3]	165 ± 59	1520 ± 144	540 ± 66
EF1007/pNK2-29[4]	1742 ± 109	470 ± 106	2196 ± 173	681 ± 135
EF1083/pNK2-29	295 ± 93	131 ± 20	794 ± 145	404 ± 41

[1]Galactosidase activity measured as Miller units.
[2]400 mmol/L added NaCl.
[3]Data represents the mean ± standard deviation from three separate runs.
[4]EF1007 is *gadE* and EF1083 is *gadE*/pGadE+.

Table 5 Motility of *Escherichia coli* strains NU149 and EK227, their *gadE* mutants, and complemented *gadE* mutants grown in pH 5.5 Luria Bertani

Strain	Motility (mm)[1]
NU149	10.67 ± 1.25[2]
NU149 *gadE*	57.34 ± 10.21
NU149 *gadE*/pPCRScript *gadE*	7.00 ± 0.82
EK227	8.33 ± 1.52
EF1007 (*gadE*)	45.00 ± 2.00
EF1083 (*gadE*/pPCRScript *gadE*)	6.67 ± 1.53

[1]Spread diameter after 24 h on a motility plate measured in mm.
[2]Data represents the mean ± standard deviation from three separate runs.

ARTICLE HIGHLIGHTS

Research background
Uropathogenic *Escherichia coli* (UPEC) is the number one cause of urinary tract infection in women. Motility driven by the action of flagella is critical for UPEC pathogenesis. How *Escherichia coli* (*E. coli*) adapts to a low pH/high osmolarity environment is essential for the species survival. Acid tolerance systems, such as the System two system, are important for UPEC survival in a low pH environment.

Research motivation
Our key problem to be solved was whether GadE, a part of the acid response two system, regulates transcription of the *fliC* gene, and in turn, UPEC motility.

Research objectives
Determine whether GadE regulated *fliC* transcription and subsequent motility of the *E. coli*.

Research methods
We created a *fliC-lacZ* reporter system on a single-copy number plasmid and measured b-galactosidase levels in both a K-12 and UPEC clinical isolate. Furthermore, motility was assessed in both *E. coli* strains by inoculating wild-type, *gadE* mutant, and complemented *gadE* mutant strains onto motility agar.

Research results
Transcription of *fliC* was significantly lower in *E. coli* grown in pH 5.5 Luria Bertani compared to pH 7.0 Luria Bertani. A mutation in the *gadE* gene led to higher *fliC* expression in that strain *vs* wild-type bacteria. Motility was significantly higher in the *gadE* mutant strain compared to the wild-type strain.

Research conclusions
We confirmed that *fliC* transcription was down-regulated in *E.* coli grown in a low pH/high osmolarity environment compared to a neutral pH/low osmolarity environment. GadE appears to either directly or indirectly regulate *fliC* transcription in *E. coli*.

Research perspectives
Future work could be done to affirm the GadE regulation of flagella expression in *E. coli*.

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SYSTEMATIC REVIEWS

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SYSTEMATIC REVIEWS

Predictors of severe and critical COVID-19: A systematic review

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Abstract

BACKGROUND

Coronavirus disease 2019 (COVID-19) has emerged as a public health crisis that was declared as a global pandemic by the World Health Organization. Although most cases have no or mild symptoms, around 10% of patients develop severe or critical illness that necessitates hospitalization and intensive care unit admission.

AIM

To assess the literature for the predictive factors that can identify patients having severe/critical COVID-19 disease.

METHODS

A Preferred Reporting Items for Systematic Reviews and Meta-Analyses-compliant systematic search of the literature was conducted. Electronic databases including PubMed/MEDLINE, Scopus, and Cochrane Library were queried. The main outcome measures were the predictors of severe/critical COVID-19 and mortality.

RESULTS

Five studies including 583 patients of a median age of 50.5 years were reviewed. Patients were 346 (59.4%) male and 237 (40.6%) female. Of 583 hospitalized patients, 242 (41.5%) had critical illness. Acute respiratory distress disease occurred in 291 patients, accounting for 46.7% of total complications. One-hundred (17.1%) mortalities were recorded. The most commonly reported predictors of severe COVID-19 were older age, medical comorbidities, lymphopenia, elevated C-reactive protein, increased D-dimer, and increased neutrophil ratio. Findings on computed tomography (CT) scanning predictive of severe disease were bronchial wall thickening, CT score > 7, linear opacities, consolidation, right upper lobe affection, and crazy paving pattern.

CONCLUSION

Several demographic, clinical, laboratory, and radiologic factors can help predict

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severe and critical COVID-19 along with the potential need for mechanical ventilation. Factors that were more commonly reported were older age, medical comorbidities, lymphopenia, increased neutrophil ratio, elevated C-reactive protein, and increased D-dimer.

Key words: COVID-19; SARS-CoV-2; Predictors; Severe; Critical; Systematic review

Core tip: After systematic literature search, several demographic, clinical, laboratory, and radiologic factors were found to be predictive of severe and critical coronavirus disease 2019 along with the potential need for mechanical ventilation. Factors that were more commonly reported were older age, medical comorbidities, lymphopenia, increased neutrophil ratio, elevated C-reactive protein, and increased D-dimer. Findings on computed tomography (CT) scanning predictive of severe disease were bronchial wall thickening, CT score > 7, linear opacities, consolidation, right upper lobe affection, and crazy paving pattern.

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INTRODUCTION

Coronaviruses are enveloped non-segmented positive-sense RNA viruses belonging to the family Coronaviridae[1]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative organism of coronavirus disease 2019 (COVID-19) belongs to the family of beta coronavirus, akin to the Middle Eastern respiratory distress syndrome coronavirus and SARS-CoV[2].

By the end of 2019, several cases of pneumonia of unknown cause emerged in Wuhan, China[2]. The World Health Organization (WHO) announced that the official name of the disease caused by the virus as COVID-19[3]. As of March 1st 2020, according to WHO situation report worldwide there are 87137 confirmed cases of COVID-19[4].

COVID-19 has a wide spectrum of disease severity, ranging from mild disease to severe acute respiratory distress disease (ARDS). According to a study by Wang *et al*[5], 44.9% of patients with COVID-19 developed complications, with ARDS occurring in 61% of patients. According to a study by Lai *et al*[6] fever was the most common symptom, followed by a cough, dyspnea, myalgia, and headache.

Timely identification of patients who are having a severe disease can play a pivotal role in improving outcomes. Basic disease treatment, secondary infection prevention, and timely organ function support are needed for these patients. Therefore, it is crucial to evaluate the prognostic markers of COVID-19.

Several studies had pointed towards various risk factors of severe disease. Comorbidities such as diabetes mellitus, cardiovascular disease, chronic lung disease and advanced age have been linked with more severe disease[7]. Other studies have shown that laboratory parameters including d-dimer level and leucocyte count can have a prognostic significance. An interesting study by Huang *et al*[8] showed that COVID-19 patients can have exaggerated cytokine storm with effected patients having high amounts of IL1B, IFNγ, IP10, and MCP1, probably leading to activated T-helper-1 (Th1) cell responses. The authors also showed that this exaggerated immune response is linked to disease severity. During our literature search we did not find previous reviews that could uniformly address these risk factors, therefore we aimed to evaluate different risk factors that can identify patients having severe/critical COVID-19 disease.

MATERIALS AND METHODS

Literature search strategy

The guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRSIMA) have been followed when reporting this systematic review[9] (Figure 1). An organized, systematic literature search was conducted querying electronic databases. Two authors searched PubMed, Scopus and Cochrane Library for all relevant published and ahead-of-publication studies dating from December 2019 through March 2020.

There were no restrictions to study design and population and language. Using the "related articles" PubMed function further publications were retrieved and screened. The reference section of the retrieved studies was screened for other potentially eligible studies.

The keywords used for the literature search were: ("novel coronavirus" OR "severe acute respiratory syndrome-coronavirus 2" OR "SARS-CoV-2" OR "COVID-19" OR "coronavirus disease 2019") AND (risk factors OR predictive factors OR predictors OR age OR comorbidities OR laboratory tests OR d-dimer OR WBC count) AND (outcomes OR mortality OR ARDS OR prognosis) were used in the search process. The medial subject headings terms "coronavirus", "COVID-19" and "Outcome" were also used in the search process.

Two authors screened the articles first by the title and abstract and then full text screening was conducted. In the case of disagreement about the inclusion of an article, the final decision was made after mutual discussion and agreement.

Inclusion and exclusion criteria

After removing duplicates, the articles retrieved were screened on the basis of pre-defined inclusion and exclusion criteria. We included studies that reported predictive factors for severe/critical COVID-19 as odds ratio (OR) and 95% confidence interval (95%CI). Severe/critical COVID-19 was defined as COVID-19 that warranted mechanical ventilation, whether was associated with mortality or not.

We excluded case reports, editorial, letters to the editor, previous reviews and meta analyses, and articles that did not report the main parameters of the review clearly and completely.

Quality appraisal

Two authors independently appraised the selected articles for risk of bias (validity) and applicability. Judgments were discussed then a consensus was reached. The methodological index for non-randomized studies (MINORS) was used to assess the quality of the studies reviewed[10]. MINORS score consists of 12 items, the first eight being specifically for non-comparative studies and the last four items pertain to comparative studies. The maximum score for non-comparative studies is 16 and for comparative studies is 24. Non-comparative studies that score greater than 12 and comparative studies that score greater than 20 are considered of low risk of bias.

Data collected

One author (Emile SH) extracted the following data: (1) Study design, duration, and country; (2) Total number of patients, male to female ratio, and age in years; (3) Clinical symptoms including fever, cough, dyspnea, myalgia and headache; (4) Number of patients in critical condition, number of complications of the disease, and mortality; and (5) The risk factors for severe COVID-19 expressed as odds OR and 95%CI.

Outcomes of the review

The primary outcome of the review was the predictive factors of severe/critical COVID-19. Secondary outcomes comprised prevalence of clinical symptoms, number of complications and mortalities of COVID-19.

Statistical analysis

Data were analyzed using SPSS™ version 25 (IBM corp, Chicago, USA). Continuous variables were expressed as mean ± SD, or median and normal range. Categorical variables were expressed as numbers and percentages. *P* value less than 0.05 was considered significant.

RESULTS

Patients and study characteristics

Five studies[11-15] were included to this systematic review. All studies were retrospective series, were conducted in China, and were published in 2020. Although all studies have been conducted in China, no overlap of the study participants was noted. The studies included a total of 583 patients, who were 346 (59.4%) male and

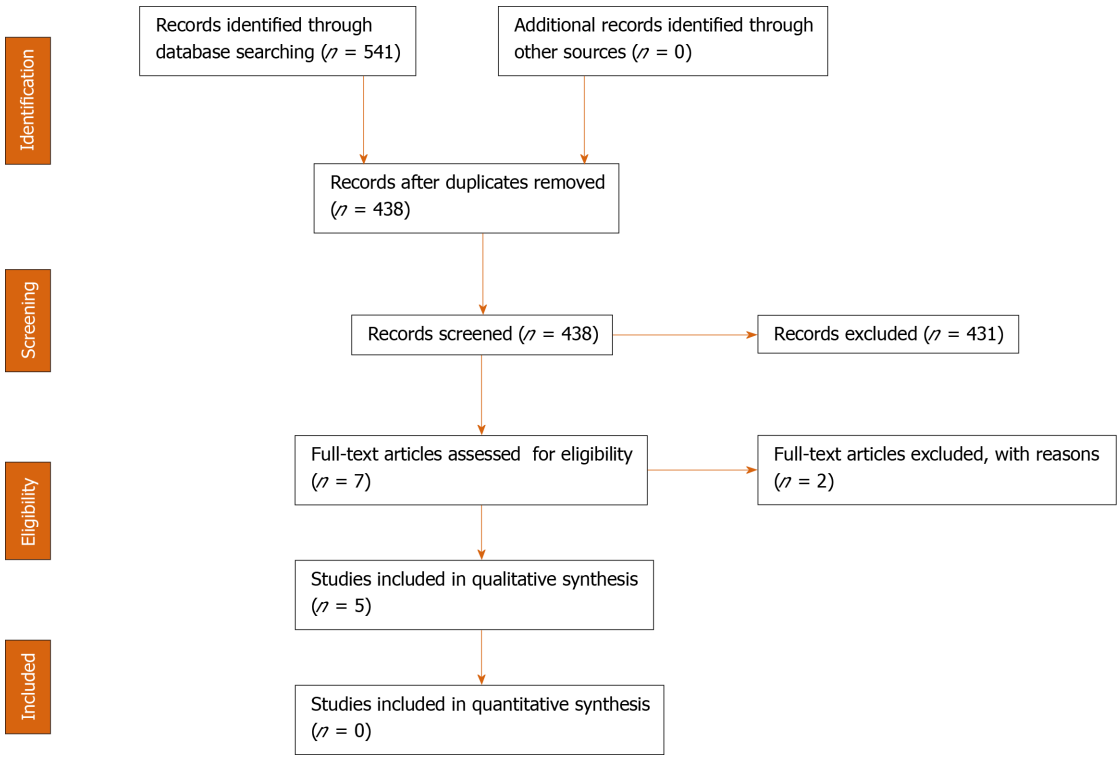

Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow chart for study selection and exclusion.

237 (40.6%) female of a median age of 50.5 (range, 38-56) years (Table 1).

Clinical presentations

A total of 440 (75.5%) patients presented with fever, 413 (70.8%) with cough, 109 (18.7%) with myalgia, 89 (15.3%) with dyspnea, 16 (2.7%) with abdominal pain, and 9 (1.5%) with headache. Of 583 hospitalized patients, 242 (41.5%) had critical illness (Table 2).

Complications and mortality

There were 622 recorded complications. ARDS occurred in 291 patients, accounting for 46.7% of total complications. Other complications of COVID-19 included: Heart failure (n = 44), septic shock (n = 38), coagulopathy (n = 37), acute cardiac injury (n = 33), acute kidney injury (n = 28), secondary infection (n = 28), hypoproteinemia (n = 22). One-hundred (17.1%) mortalities were recorded across the studies (Table 3).

Predictors of poor outcome

Demographic and clinical predictors: (1) Age. Four studies[11-13,15] reported older age as a predictor for poor outcome. The odds ratios of having critical/severe COVID-19 with older age were as follows: (a) Age > 50: OR = 7.596 (2.664-21.659); (b) Age > 60: OR = 1.10 (1.03-1.17); (c) Age > 60: OR = 8.546 (1.628-44.864); and (d) Age > 65: OR = 3.26 (2.08-5.11); (2) Presence of comorbidities. Four studies[11-13,15] reported medical comorbidities as predictor for poor outcome. The odds ratios of having critical/severe COVID-19 with medical comorbidities were as follows: (a) Comorbidities of any type: OR = 10.607 (2.930-38.399); (b) Smoking: OR = 14.285 (1.577-25.000); (c) Coronary heart disease: OR = 2.14 (0.26-17.79); (d) Diabetes mellitus: OR = 2.34 (1.35-4.05); and (e) Hypertension: OR = 1.82 (1.13-2.95); and (3) Clinical symptoms. Three studies[11,13,15] reported clinical symptoms as predictor for poor outcome. The odds ratios of having poor outcome with clinical symptoms were as follows: (a) Dyspnea: OR = 10.899 (2.073-57.198); (b) Chest pain: OR = 10.85 (1.14-102.77); (c) Cough: OR = 9.95 (1.24-79.55); (d) Expectoration: OR = 4.87 (1.5-15.78); (e) Temp > 37.3: OR = 8.999 (1.036-78.147); (f) Temp > 39: OR = 1.77 (1.11-2.84); and (g) Respiratory failure: OR = 8.772 (1.942-40.000).

Laboratory parameters: (1) Reported by more than two studies. Lymphopenia was reported by three studies[11,12,15] as poor prognosticator of COVID-19 with the following odds ratio: OR = 12 (3.21-44.81), OR = 0.19 (0.02-1.62), and OR = 0.37 (0.21-0.63); (2) Reported by two studies: (a) Elevated D-dimer levels > 1: OR = 18.42 (2.64-128.55) and OR = 1.03 (1.01-1.04); (b) Neutrophilia: OR = 9.67 (3.27-28.57) and OR = 1.14 (1.09-

Table 1 Characteristics of the studies included

Study	Duration	Design	Country	Number	Male	Age	MINORs score
Li *et al*[11], 2020	Jan 2020- Feb 2020	Retrospective	China	83	44	45.5	14 (Low)
Zhou *et al*[12], 2020	Dec 2019- Jan 2020	Retrospective	China	191	119	56	13 (High)
Liu *et al*[13], 2020	Dec 2019- Jan 2020	Retrospective	China	78	39	38	12 (High)
Qu *et al*[14], 2020	Dec 2019- Jan 2020	Retrospective	China	30	16	50.5	13 (High)
Wu *et al*[15], 2020	Dec 2019- Jan 2020	Retrospective	China	201	128	51	12 (High)

MINORs: Methodological index for non-randomized studies.

1.19); and (c) Elevated C-reactive protein (CRP): OR = 13.2 (2.84-61.23) and OR = 4.81 (1.52-15.27); and (3) Reported by one study: (a) Decreased monocyte ratio: OR = 18 (2.03-159.1); (b) Decreased lymphocyte ratio: OR = 7.6 (2.48-23.28); (c) Increased procalcitonin: OR = 7.989 (2.426-26.305); (d) Decreased oxyhemoglobin saturation: OR = 8.329 (2.483-27.933); (e) Platelet lymphocyte ratio: OR = 0.993 (0.983-1.003); (f) Reduced CD3: OR = 0.83 (0.72-0.96); (g) Reduced CD4: OR = 0.74 (0.59-0.93); (h) Increased bilirubin: OR = 1.05 (1.02-1.08); (i) Elevated AST: OR = 1.02 (1.01-1.03); (j) Hypoalbuminemia: OR = 0.49 (0.37-0.66); (k) Hyperglobulinemia: OR = 2.32 (1.45-3.71); (l) Decreased prealbumin: OR = 0.99 (0.98-0.99); (m) Increased urea: OR = 1.13 (1.09-1.18); (n) Increased creatinine: OR = 1.05 (1.01-1.10); (o) Hypoglycemia: OR = 1.13 (1.08-1.19); (p) Increased cholinesterase: OR = 1.13 (1.08-1.19); (q) Increased cystatin: OR = 1.69 (1.31-2.19); (r) Increased LDH: OR = 1.61 (1.44-1.79); (s) Increased alpha HBDH: OR = 1.74 (1.52-1.99); (t) Increased LDL: OR = 0.63 (0.44-0.88); and (u) Increased serum ferritin: OR = 3.53 (1.52-8.16).

Radiologic parameters in CT scanning: (1) Bronchial wall thickening: OR = 32.593 (7.876-134.880); (2) CT score > 7: OR = 19.200 (5.820-63.336); (3) Linear opacities: OR = 10.016 (2.160-46.454); (4) Consolidation: OR = 6.387 (1.720-23.719); (5) Right upper lobe affection: OR = 5.603 (1.195-26.277); and (6) Crazy paving pattern: OR = 3.341 (1.257-8.878).

Development of a prognostic scoring system

The odds ratios of all predictive factors of severe COVID-19 found after literature search were reviewed and the factors that had the highest odds (OR > 10) were selected to construct a prognostic scoring system. Each predictive factor was given points according to its relative weight and OR. The prognostic score ranged from 0 to 16. According to the probability to develop severe COVID-19, the outcome of the score was summarized as: Low probability (0-5 points), moderate probability (6-10 points), and high probability (11-16 points, Table 4).

DISCUSSION

As the COVID-19 pandemic is one the rise, the numbers of people with critical illness increase and so does the pressure on hospitals and intensive care units[16]. Since the disease tends to progress rapidly once pulmonary affection has occurred, there is a pressing need to predict which patients are more vulnerable to succumb into critical illness and may require mechanical ventilation.

The present systematic review aimed to explore the available literature on COVID-19 on the risk factors for developing critical illness that may result in fatality. Several predictive factors were found and were classified into clinical, laboratory, and radiologic parameters.

Among the important demographic and clinical factors, older age was reported by almost all the studies reviewed. The cut-off point for age varied between 50, 60, and 65 years among the studies[11-13,15]. This observation can be attributed to the effect of aging on the respiratory system. This effect includes chest wall and thoracic spine deformities that tends to impair the total respiratory system compliance, loss of supporting structure of the lung causing dilation of air spaces, weakness of respiratory muscles that impairs effective cough and airway clearance, and diminished ventilatory response to hypoxia and hypercapnia, making elderly more prone to respiratory failure during high demand states[17]. In addition, impaired immune functions in individuals > 65 years, known as immunosenescence, is associated with increased susceptibility to diseases, infections and poor response to treatments[18].

Table 2 Clinical symptoms of the patients, *n* (%)

Study	Fever	Cough	Dyspnea	Abdominal	Myalgia	Headache	Critical cases
Li *et al*[11], 2020	72 (86.7)	65 (78.3)	9 (10.8)	7 (8.4)	15 (18.1)	9 (10.8)	25 (30.1)
Zhou *et al*[12], 2020	180 (94.2)	151 (79)	NA	9 (4.7%)	29 (15.1)	NA	119 (62.3)
Liu *et al*[13], 2020	NA	34 (34.6)	NA	NA	NA	NA	11 (14.1)
Qu *et al*[14], 2020	NA	NA	NA	NA	NA	NA	3 (10)
Wu *et al*[15], 2020	188 (93.5)	163 (81.1)	80 (39.8)	NA	65 (32.3)	NA	84 (41.8)

NA: Not applicable.

Medical comorbidities are strongly linked to poor outcome with COVID-19 as reported by several investigators[11,12,15]. Patients with poor state of health may have weakened immunity against the SARS-COV2 as compared to other healthy individuals. A recent analysis demonstrated the impact of comorbidity on COVID-19 patients in China and reported that patients with comorbidities such as diabetes mellitus, hypertension, COPD, and malignancy had greater disease severity compared with those without comorbid conditions and the greater the number of comorbidities the greater the severity of COVID-19[19].

Smoking appeared to increase the odds of progression to critical illness 14 times as compared to non-smokers[13]. Smoking predisposes to COPD and small airway disease and has well-documented effects on the pulmonary functions that include decreased forced vital capacity (FVC), forced expiratory volume in one second (FEV1), FEV1/FVC, and forced expiratory flow[20]. Therefore, rapid progression of respiratory infection in active smokers into critical lung disease with impaired ventilation may be reasonable.

Some clinical symptoms including dyspnea, chest pain, and fever were also associated with more severe COVID-19. Chest pain is usually caused by inflammation of the pleural membrane. Dyspnea can be attributed to damage of the alveoli in severe illness and elevated temperature may indicate high activation of the immune system towards the intrusive pathogen[11].

Laboratory parameters can help inform the clinical about the progression of COVID-19 towards critical illness. Parameters reported in more than one study were lymphopenia, increased D-dimer and elevated CRP. Lymphopenia can be an important prognosticator of COVID-19 as it reflects poor immune response to the infection. The primary target cells of viral infection in general are lymphocytes and hence when viral infection starts to induce damage to the immune system it usually presents as a decrease in the absolute number of lymphocytes[21]. One study[11] concluded that COVID-19 patients with lymphopenia are 12 times more likely to develop critical illness as compared to people with normal lymphocyte count. Decreased lymphocyte count could be used as an important index of evaluation of severity of COVID-19[22].

Elevation of C-reactive protein along with increased neutrophil ratio and procalcitonin were associated with higher odds of developing more severe disease. These parameters may be related to the cytokine storm syndrome induced by viral infection[23]. Increased D-Dimer implies a coagulation dysfunction that is related to the development of ARDS and progression from ARDS to death. This may suggest that disseminated intravascular coagulation is a step on the pathway to death in some patients[15].

Findings in CT scanning of the lungs can greatly help clinicians understand the current disease state and possible outcome. Li *et al*[11] found that patients with severe disease have lung consolidation secondary to complete filling of the alveoli with inflammatory exudate. Extrapulmonary lesions including pleural and pericardial effusion and enlarged lymph nodes may indicate more severe inflammation. Moreover, the overall CT scores of patients with severe/critical illness were significantly higher than the patients with mild disease.

Limitation of the present review includes the small number and retrospective nature of the studies included. Owing to the statistical heterogeneity and lack of essential data, the conduction of formal meta-analysis was not possible. Given the limitations with regards to study execution and article selection, no solid conclusions can be reached.

In conclusion, several demographic, clinical, laboratory, and radiologic factors may help predict severe and critical COVID-19 along with the potential need for mechanical ventilation. Factors that were more commonly reported were older age, medical comorbidities, lymphopenia, increased neutrophil ratio, elevated C-reactive

Table 3 Complications and mortality of coronavirus disease 2019 in the studies

Study	Complications	ARDS	Mortality
Li *et al*[11], 2020	25	25	NA
Zhou *et al*[12], 2020	493	162	54
Liu *et al*[13], 2020	20	20	2
Qu *et al*[14], 2020	NA	NA	NA
Wu *et al*[15], 2020	84	84	44

ARDS: Acute respiratory distress syndrome.

protein, and increased D-dimer. As CT scanning has paramount importance in the making the diagnosis and assessment of COVID-19, it may also have a role in predicting more severe course of COVID-19. Nonetheless, as more studies on the COVID-19 pandemic are being conducted, more data on the predictors assessed in this review in addition to other predictors may be obtained.

Table 4 Prognostic scoring system to predict severe coronavirus disease 2019

Predictive factor	Odds ratio	Score points
Bronchial wall thickening in CT scan	32.593	3
CT score > 7	19.200	2
Elevated D-dimer	18.42	2
Decreased monocyte ratio	18	2
Smoking	14.285	2
Elevated C-reactive protein	13.2	1
Lymphopenia	12	1
Dyspnea	10.899	1
Chest pain	10.85	1
Medical comorbidities	10.607	1

CT: Computed tomography.Probability of severe COVID-19: Low (0-5 points), moderate (6-10 points), high (11-16 points).

ARTICLE HIGHLIGHTS

Research background

Coronavirus disease 2019 (COVID-19) has been declared by the World Health Organization as a global pandemic. Although the majority of patients have mild or no symptoms, about 10% of patients may present with severe or critical disease that necessitates mechanical ventilation and may progress to death.

Research motivation

Patients who develop severe/critical COVID-19 disease have higher morbidity and mortality rates. Predicting which patients who are more likely to develop severe COVID-19 is highly required in order to implement more aggressive treatment measures to prevent potential deterioration.

Research objectives

The main objectives of the study were the incidence of severe COVID-19, mortality rate, and predictive factors of severe/critical disease.

Research methods

A Preferred Reporting Items for Systematic Reviews and Meta-Analyses-compliant systematic review of the existing literature was conducted. Three databases were searched and the articles reporting the predictors of severe/critical COVID-19 were retrieved. The quality of the articles was assessed with the methodological index for non-randomized studies index. Outcomes were summarized in a qualitative form.

Research results

Five studies including 583 patients of a median age of 50.5 years were included. 242 (41.5%) of 583 hospitalized patients had critical illness. Acute respiratory distress disease occurred in 291 patients, accounting for 46.7% of total complications. The most commonly reported predictors of severe COVID-19 were older age, medical comorbidities, lymphopenia, elevated C-reactive protein, increased D-dimer, and increased neutrophil ratio. Findings on computed tomography (CT) scanning predictive of severe disease were bronchial wall thickening, CT score > 7, linear opacities, consolidation, right upper lobe affection, and crazy paving pattern.

Research conclusions

Several factors may help predict severe/critical COVID-19. Factors that were more commonly reported were older age, medical comorbidities, lymphopenia, increased neutrophil ratio, elevated C-reactive protein, and increased D-dimer. As CT scanning has paramount importance in the making the diagnosis and assessment of COVID-19, it may also have a role in predicting more severe course of COVID-19.

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SYSTEMATIC REVIEWS

Antibiotics for complicated urinary tract infection and acute pyelonephritis: A systematic review

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Abstract

BACKGROUND

The increasing rates of antibiotic-resistance in recent years have supported emergence of multiple drug-resistant bacteria. Therefore, antibiotics that are recommended by the current clinical guidelines may not be effective for the treatment of complicated urinary tract infection (UTI) and acute pyelonephritis.

AIM

To determine the clinical efficacy and safety of antibiotics for the treatment of complicated UTI and acute pyelonephritis.

METHOD

A search of PubMed, EMBASE, and Google Scholar was conducted for eligible articles describing the use of antibiotics in managing complicated UTI and acute pyelonephritis. The following keywords were used to perform the literature search: "urinary tract infection", "complicated UTI", "pyelonephritis", "treatment", and "antibiotics". Additional articles of interest were retrieved from the reference lists of selected papers. Eligibility criteria for this systematic review were diagnosis of either complicated UTI or acute pyelonephritis and use of antibiotics in management. Clinical trials and observational studies were included, while case reports and reviews were excluded. The methodological quality of clinical trials and observational studies was assessed. A descriptive approach was adopted to analyze the data, due to the variation of methodology and interventions.

RESULT

A total of 183 studies were screened, and 8 matched all the eligibility criteria and were included in this review. The antibiotics used included ceftazidime-avibactam, doripenem, levofloxacin, meropenem-vaborbactam, piperacillin-tazobactam, plazomicin, tazobactam-ceftolozane, and gentamicin. Two clinical trials reported that shorter-duration levofloxacin or non-fluoroquinolone antibiotic treatment was as effective as the duration of antibiotic therapy recommended by the current guidelines in treating complicated UTI and pyelonephritis. Besides that, ceftazidime-avibactam, piperacillin-tazobactam and

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tazobactam-ceftolozane can be used as alternatives to carbapenem in treating extended-spectrum -lactamase-producing *Escherichia coli*. The cure rates of complicated UTI and pyelonephritis by meropenem-vaborbactam, piperacillin-tazobactam and tazobactam-ceftolozane was comparable (95.6%-98.4%). Furthermore, levofloxacin had a relatively high rate of adverse events (33.1% and 47.7% in two clinical trials respectively), while tazobactam-ceftolozane had a relatively low rate of adverse events (17.5%). All studies have limitations and a potential for bias.

Key Words: Antibiotics; Urinary tract infections; Pyelonephritis; Therapeutics; Drug resistance

Core Tip: There is an increasing resistance rate to the antibiotics recommended by current guidelines for the treatment of complicated urinary tract infection (UTI) and acute pyelonephritis. Therefore, alternative antibiotics need to be explored to increase the cure rate and improve the outcomes of patients. The aim of this systematic review is to investigate the efficacy and safety of different antibiotic therapy in treating complicated UTI and acute pyelonephritis. The use of novel antibiotics and combination antibiotic therapy can be considered in treating complicated UTI and acute pyelonephritis when resistance to recommended antibiotics occurs.

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INTRODUCTION

A complicated urinary tract infection (UTI) is associated with structural or functional abnormalities of the genitourinary tract or presence of any underlying disease[1]. Patients who have complicated UTI may experience relapse with an organism similar to the pretherapy isolate or reinfection with a new organism[1]. Complicated UTI may be associated with severe morbidity, such as septic shock, renal failure or even death[1]. Acute pyelonephritis is a bacterial infection causing inflammation of the kidney and renal pelvis, which occurs due to the spread of bacteria from the bladder to the kidneys in ascending UTI[2]. The rates of acute pyelonephritis in the United States are about 15 to 17 cases per 10000 females and 3 to 4 cases per 10000 males annually[2].

Current guidelines (Infectious Diseases Society of America and European Society of Clinical Microbiology and Infectious Diseases) recommend the use of oral fluoroquinolones for treatment of acute pyelonephritis and complicated UTI as an outpatient, because fluoroquinolones are absorbed well from the gastrointestinal tract and can penetrate the kidney[3]. Oral amoxicillin-clavulanate potassium, a cephalosporin, and trimethoprim-sulfamethoxazole can be used as alternatives[3]. One of the following three intravenous therapies is recommended by the Infectious Diseases Society of America for patients hospitalized for acute pyelonephritis: (1) A fluoroquinolone; (2) An aminoglycoside (with or without ampicillin); or (3) An extended-spectrum cephalosporin (with or without an aminoglycoside)[3].

However, there are limitations of the antibiotics currently recommended, such as adverse events associated with the antibiotics, presence of antibiotic-resistant bacteria, or compliance of medication. Therefore, alternative antibiotics must be considered to improve the prognosis and outcome of the patients. Alternative antibiotics, such as novel antibiotics or combination therapy, may be more effective than the antibiotics suggested by the guidelines in treating complicated UTI or acute pyelonephritis.

The aim of this review was to investigate the clinical efficacy and safety of antibiotics for the treatment of complicated UTI and acute pyelonephritis based on the current literature.

MATERIALS AND METHODS

Search strategy

A systematic search was conducted to identify studies involving the treatment of complicated UTI or pyelonephritis with antibiotics. Search terms included the following keywords and word combinations: "urinary tract infection", "complicated UTI", "pyelonephritis", "treatment", and "antibiotics". The search was conducted using the three major literature databases of PubMed, EMBASE and Google Scholar. Relevant articles published in English from 2010 to 2019 were identified. Additional articles of interest were retrieved from the reference list of selected papers.

Eligibility criteria

Only adults diagnosed with complicated UTI or acute pyelonephritis were included in this review. The eligibility criteria included diagnosis of the complicated UTI or acute pyelonephritis based on clinical or microbiological evaluation and the use of antibiotics in management. Both oral and intravenous antibiotic therapies were included in this review. Case reports, articles without original data, and review articles were excluded from this study.

Selection of studies and analyses

The titles and abstracts of all studies were screened for their eligibility for inclusion. The full-text manuscript was used to assess eligibility when a decision could not be made based on title and abstract solely. Data on population, study design, intervention, clinical outcomes, and adverse events were collected using a standardized electronic database within Microsoft Word. Outcome of the patients was defined as one of the following: Clinical failure rate; microbiological eradication; cure rate; duration of treatment; or length of hospital stay. Due to variation among the interventions and study designs, a descriptive approach was used to report the data (instead of a meta-analysis). The methodological quality of the studies was assessed using Cochrane risk of bias assessment for randomized control trials (RCTs)[4], The Newcastle-Ottawa scale for non-randomized control trial[5] and Downs and Black Checklist for Study Quality for observational studies[6] (author LTO) were used. PRISMA guidelines were used as a basis for reporting the results of this systematic review.

RESULTS

A total of 331 articles were retrieved by the search strategy, of which 183 studies were screened and 12 studies were assessed for eligibility based on the full manuscript. After exclusion, 8 studies matched the eligibility criteria and were included in the review for analyses[7-14]. Among them, 5 studies were RCTs, 2 studies were observational studies, and 1 study was a non-randomized trial (Figure 1). A total of 2531 participants were enrolled in all the studies identified. The antibiotics included in the studies were ceftazidime-avibactam, doripenem, levofloxacin, meropenem-vaborbactam, piperacillin-tazobactam, plazomicin, tazobactam-ceftolozane and gentamicin. *Escherichia coli* (*E. coli*) was the most common causative pathogen of the cases of complicated UTI and pyelonephritis, but other Gram-negative and Gram-positive species had been isolated from patients.

Therapy and outcomes

Two observational studies included in this review were retrospective cohort studies. Park *et al*[7] compared the efficacy of carbapenem and non-carbapenem antibiotics in treating patients with acute pyelonephritis due to extended-spectrum -lactamase (ESBL)-producing *E. coli*[7]. The non-carbapenem antibiotics used in the treatment were aminoglycosides, -lactam/-lactamase inhibitors, fluoroquinolones, and trimethoprim/sulfamethoxazole. The risk of microbiological failure (weighted hazard ratio: 0.99) and clinical failure rate (weighted hazard ratio: 1.05) were similar for the two groups. The aim of the study was to determine if the initial dosing of gentamicin improved patient's outcomes in pyelonephritis[8]. Initial dosing of gentamicin decreased the intravenous (IV) antibiotic treatment length and length of hospital stay. Patients who were given gentamicin, in general, showed an association with better outcomes.

Based on the RCTs and a non-randomized trial, 1 study used oral antibiotic

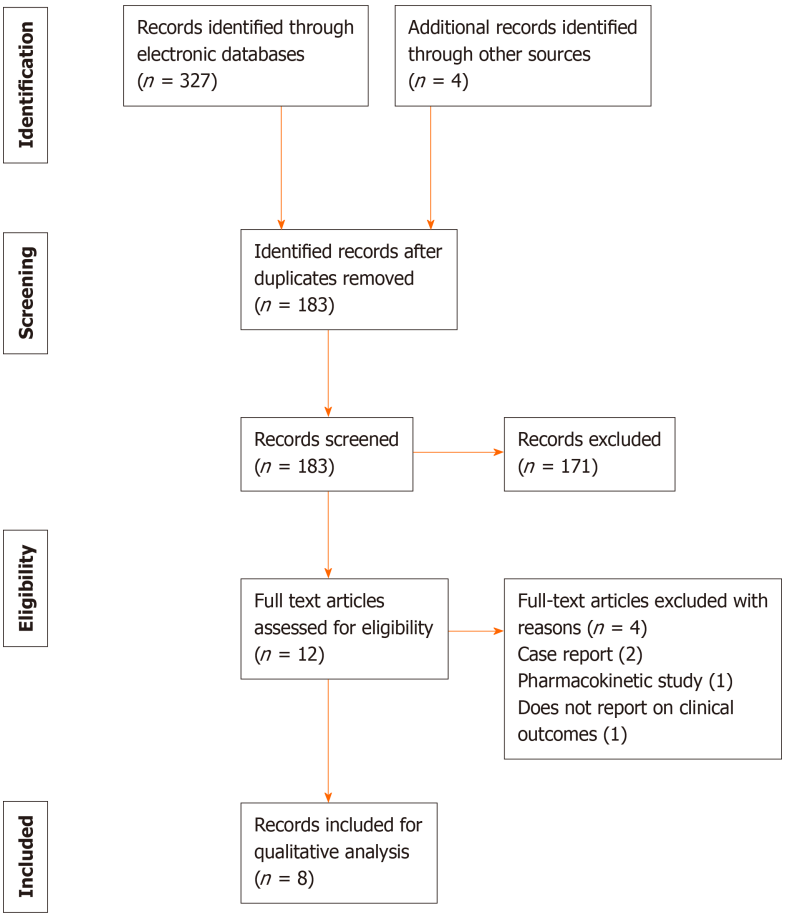

Figure 1 Flow diagram of the study selection process.

therapy[8], 6 studies used IV antibiotic therapy[8-13], and 2 studies used a combination of oral and IV antibiotic therapy[9,10]. Two RCTs involved studying the efficacy of antibiotics used in different doses and duration, while three RCTs involved studying the efficacy of different antibiotic therapies. The outcome was most commonly assessed at 5-9 d post-treatment and 1-2 mo post-treatment[8-13]. Most of the patients showed improvement in clinical symptoms, such as fever, dysuria, urinary frequency, and suprapubic pain, after 5-9 d of initiation of antibiotic therapy[8-13]. All of the antibiotic therapy used in the studies had cure rates greater than 60%[8-13]. All the studies described the microbiological etiology in their cases. The infections were caused primarily by *E. coli*, and *Klebsiella pneumoniae* was the second most common bacteria identified[8-13]. All of the clinical findings of the studies are shown in Table 1.

Adverse events

The rates of adverse events associated with the antibiotic therapy in the trials were mostly around 30% to 50%[8-11]. Levofloxacin, in the Connolly *et al*[11] trial, had a relatively high rate of adverse events (47.7%)[11]. However, this could be due to the small population of patients (n = 7) taking levofloxacin therapy in that trial. The most common adverse effects reported in the trials was headache, which was reported in the use of ceftazidime-avibactam, doripenem, levofloxacin, meropenem-vaborbactam. piperacillin-tazobactam, and plazomicin[8-11]. Both levofloxacin and tazobactam-ceftolozane were frequently associated with gastrointestinal illness and abnormal laboratory findings, which were reduced leukocyte count and increased aminotransferase respectively[11,13]. Tazobactam-ceftolozane had a low rate (at 17.5%) of adverse events reported[13]. All the adverse events associated with antibiotic therapy are shown in Table 2.

Quality assessment

The clinical studies included in this review varied in study design, eligibility, time to follow-up, and outcomes. The most common diagnostic criteria used in the studies were pyuria, presence of 1-2 uropathogens, or presence of clinical symptoms, such as

Table 1 Key studies of antibiotic therapy for complicated urinary tract infections and pyelonephritis

Ref.	Study design	Population	Therapy	Findings
Park *et al*[7], 2014	Observational study	152 patients with pyelonephritis caused by ESBL-producing *Escherichia coli*	Carbapenems for median 12 d *vs* non-carbapenems for median 8 d	Clinical failure was similar between the two groups (weighted HR: 1.05)
Wagenlehner *et al*[8], 2016	RCT	1033 with suspected or confirmed cUTI/APN, randomized 1:1 to each arm	Ceftazidime-avibactam *vs* doripenem up to 10 d or 14 d for patients with bacteremia	Microbiological eradication rate: 77.4% ceftazidime-avibactam; 71.0% doripenem
Ren *et al*[9], 2017	TCT	330 patients diagnosed with cUTI or APN, randomized 1:1 to each arm	IV levofloxacin 750 mg for 5 d *vs* IV levofloxacin 500 mg and shift to oral levofloxacin 500 mg for 7-14 d	Clinical success rate: 89.87% in IV levofloxacin 750 mg *vs* 89.31% in IV/oral levofloxacin 50 *vs* 0 mg
Kaye *et al*[10], 2018	RCT	550 patients with cUTI or APN, randomized 1:1 to each arm	Meropenem-vaborbactam *vs* piperacillin-tazobactam for 10 d	Clinical success rate: 98.4% in the meropenem-vaborbactam group *vs* 95.6% in the piperacillin-tazobactam group
Connolly *et al*[11], 2018	RCT	145 patients diagnosed with cUTI and APN, randomized at 22, 76 and 47 in each arm	Plazomicin at 10 mg/kg *vs* plazomicin at 15 mg/kg *vs* levofloxacin 750 mg for 5 d	Microbiological eradication rate in MITT and MIE population: 50.0% and 85.7% (plazomicin at 10 mg/kg) *vs* 60.8% and 88.6% (plazomicin at 15 mg/kg) *vs* 58.6% and 81.0% (levofloxacin)
Rudrabhatla *et al*[12], 2018	RCT	54 patients diagnosed with APN, randomized 1:1 to each arm	Non-fluoroquinolone antibiotics for 7 d *vs* 14 d	Patients who received antibiotics for 7 d had shorter hospital stay (8 d *vs* 14 d) and less antibiotic consumption (8.4 DDs *vs* 17.4 DDs) No patients required retreatment
Arakawa *et al*[13], 2018	Non-randomized, trial	115 patients diagnosed with pyelonephritis or complicated cystitis	IV tazobactam-ceftolozane every 8 h for 7 d	Clinical response rate was 96.6%
Ryanto *et al*[14], 2019	Observational study	152 patients diagnosed with severe pyelonephritis/urosepsis	Gentamicin was prescribed for 43.4% patients; 32% of patients were given initial dosing of gentamicin	Duration of IV, time of resolution, and length of stay is short in patients given gentamicin; initial dose of IV gentamicin improved the outcome of patients

APN: Acute pyelonephritis; cUTI: Complicated urinary tract infection; DD: Daily dose; ESBL: Extended-spectrum -lactamase; HR: Hazard ratio; IV: Intravenous; ME: Microbiologically evaluable; MITT: Modified intent-to-treat; RCT: Randomized control trial.

dysuria, urinary frequency, flank tenderness, or fever. Biases were identified in the RCTs, including selection bias, performance bias, and response bias. Overall, the methodological quality of the studies was moderate. One RCT had good quality and four RCTs had fair quality, based on the thresholds for converting the Cochrane risk of bias tool to agency for healthcare research and quality standards[4]. The total score for methodological quality for the two observational studies based on the Downs and Black Checklist for Study Quality[6] was 12 and 15.

DISCUSSION

Antibiotic resistance is one of the major reasons for exploration of other antibiotics to manage complicated UTI and acute pyelonephritis[3]. Rates of quinolone resistance among *Enterobacteriaceae* were 1% in the mid-to-late 1900s and 1% to 3% as late as 2008 but the quinolone resistance rates have increased to 10%-30% in recent years[15]. Besides that, some of the antibiotics recommended by the current clinical guidelines may cause serious adverse drug reactions. For example, cephalosporin may result in rashes, diarrhea, anaphylaxis and haemolytic anaemia, and has shown a frequent association with morbidity from *Clostridium difficile* infection[16]. Besides that, trimethoprim-sulfamethoxazole therapy has been associated with neurological defect, reduced oxygen-carrying capacity, gastrointestinal illness and drug hypersensitivity, while aminoglycosides have been associated with nephrotoxicity, such as acute tubular necrosis and ototoxicity[17,18].

ESBL-producing *E. coli* is one of the causative bacteria for acute pyelonephritis and carbapenems are considered first-choice treatment for ESBL producers[19]. However, due to the increasing carbapenem resistance rate in *Enterobacteriaceae*, carbapenems should be used judiciously[7]. The study by Park *et al*[7] suggested non-carbapenem antibiotics had the same efficacy against ESBL-producing *E. coli* as carbapenems;

Table 2 Adverse events associated with antibiotic therapy reported in the studies

Antibiotics	Ref.	Adverse events reported	Most common adverse effects	Frequency, *n* /total (%)
Ceftazidime-avibactam	Wagenlehner *et al*[8]	Headache, nausea, diarrhea, constipation	Headache	185/511 (36.2)
Doripenem	Wagenlehner *et al*[8]	Headache, nausea, diarrhea, constipation	Headache	158/509 (31.0)
Levofloxacin	Ren *et al*[9]	Reduction in leukocyte count, reduction in neutrophil count, increased ALT, increased AST, increased platelet count, increased blood pressure, gastrointestinal, reaction at injection site, cutaneous/subcutaneous, nervous system/mental, immune, infection, hepatobiliary, metabolic/nutritional, musculoskeletal/connective tissue	Reduction in leukocyte count and gastrointestinal	109/329 (33.1)
	Connolly *et al*[11]	Headache, diarrhea, vomiting, nausea, dizziness	Headache	21/44 (47.7)
Meropenem-vaborbactam	Kaye *et al*[10]	Headache, diarrhea, nausea, asymptomatic bacteriuria, catheter site phlebitis, infusion site phlebitis, urinary tract infection, hypokalemia, vaginal infection, ALT increased, anemia, AST increased, pyrexia	Headache	106/272 (39.0)
Piperacillin-tazobactam	Kaye *et al*[10]	Headache, diarrhea, nausea, asymptomatic bacteriuria, catheter site phlebitis, infusion site phlebitis, urinary tract infection, hypokalemia, vaginal infection, ALT increased, anemia, AST increased, pyrexia, dyspnea	Headache	97/273 (35.5)
Plazomicin	Connolly *et al*[11]	Headache, diarrhea, vomiting, nausea, dizziness	Headache	33/96 (34.4)
Tazobactam-ceftolozane	Arakawa *et al*[13]	Diarrhea, ALT increased, constipation, AST increased, insomnia, headache, pyelonephritis, pyelonephritis acute, contusion, viral upper respiratory tract infection	Diarrhea and ALT increased	20/114 (17.5)

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

however, insufficient research data and conflicting study results have discouraged the use of non-carbapenem antibiotics[7]. Besides that, amikacin was suggested as an alternative due to low resistance rate but there are insufficient data about the therapeutic efficacy and association of amikacin with nephrotoxicity[7,20].

An RCT showed that ceftazidime-avibactam and doripenem have the same efficacy in treating hospitalized patients with complicated UTI and acute pyelonephritis[8]. Moreover, the clinical cure rate of ceftazidime-avibactam was found to be similar for patients with ceftazidime-nonsusceptible and ceftazidime-susceptible pathogens[8]. Therefore, ceftazidime-avibactam can be used as an alternative to carbapenem to reduce the spread of carbapenem-resistant bacteria.

Dosing of antibiotics is also an important factor in reducing antibiotic resistance; therefore, it is essential to optimize the current regimens. An RCT showed that levofloxacin at 750 mg/d for 5 d is as effective as 500 mg/d plus oral regimen of levofloxacin for 7-14 d in treating complicated UTI and acute pyelonephritis in terms of clinical efficacy, microbiological efficacy, and tolerance[9]. High-dose levofloxacin can have prolonged bactericidal activity against *E. coli* with minimum inhibitory concentration up to 32 mg/mL, due to increased concentration of the antibiotic in the urine[21]. Therefore, levofloxacin at 750 mg/d is preferred because the duration of treatment is shorter and the total drug dose was 23% less[9]. Another RCT involved patients stopping non-fluoroquinolone antibiotics at day 7 or continuing treatment until day 14[12]. Truncating non-fluoroquinolone antibiotics at day 7 is advised, as this strategy can reduce antibiotic consumption, length of hospital stay and treatment-related adverse events, and generally yield the same outcome as seen in the patients who continued the antibiotic treatment until day 14[12]. Studies have shown that shorter durations of antibiotic therapy are effective for common infections, such as bacteremia and community-acquired pneumonia and can prevent the rise of antimicrobial resistance[22].

Both meropenem-vaborbactam and piperacillin-tazobactam are effective in treating complicated UTI and acute pyelonephritis, with the overall success rates of 98.4% and 95.6% respectively[10]. Piperacillin-tazobactam has been shown to be effective in patients from whom *Enterobacteriaceae* was isolated, including ESBL-producers[10]. Plazomicin is a aminoglycoside that is effective in treating adult patients with complicated UTI including acute pyelonephritis, with microbiological eradication over 85%[11]. Plazomicin is derived from sisomicin with structural modifications that can

prevent degradation from aminoglycoside-modifying enzymes, which is a common mechanism of aminoglycosides resistance[23]. Therefore, plazomicin has the potential to treat complicated UTI and acute pyelonephritis caused by multidrug-resistant *Enterobacteriaceae*; however, further studies involving larger sample size should be conducted[11].

Tazobactam-ceftolozane is a novel antibiotic therapy that is effective in the treatment of complicated UTI and pyelonephritis, with microbiological response rate and clinical repose rate of 80.7% and 96.6% respectively[13]. Tazobactam-ceftolozane has a favourable safety profile, with a low rate of adverse events (17.5%), and has excellent antibacterial activity against Gram-negative bacteria, which encompass the *Enterobacteriaceae* spp., including ESBL-producing strains and multidrug-resistant *Pseudomonas aeruginosa*[13]. Finally, an initial dose of IV gentamicin has been associated with positive patient outcomes, due to its effectiveness in severe cases of suspected Gram-negative sepsis, especially against *P. aeruginosa*[14]. However, only 54% of *E. coli* strains found in urine have been reported as sensitive to gentamicin[24]. Duration and dose of gentamicin need to be monitored closely, due to increased risk of adverse effects, such as nephrotoxicity[14].

This systematic review has limitations. It is possible that evidence and clinical studies were missed by the search strategy employed. A comparison of efficacy between different antibiotic therapies is difficult, due to the significant variation in study designs, interventions, and outcome measures. Besides that, some novel antibiotic therapies have limited and incomplete clinical data for comparison.

In conclusion, several novel antibiotics and combination therapies have proven to be effective in treating complicated UTI and pyelonephritis. The clinical data have shown that shorter duration of treatment with lower consumption of antibiotics are effective for treatment and can reduce the development of multiple drug resistance bacteria. Ceftazidime-avibactam, piperacillin-tazobactam and tazobactam-ceftolozane can be used as an alternative to carbapenem to treat ESBL-producing *E. coli*. Finally, meropenem-vaborbactam, piperacillin-tazobactam and tazobactam-ceftolozane have high cure rates in treating complicated UTI and pyelonephritis. Therefore, the use of novel antibiotics and combination antibiotic therapy can be considered for treating complicated UTI and acute pyelonephritis when resistance to recommended antibiotics occurs. In future trials, standardized diagnostic criteria and outcome measures should be adopted for direct comparison. Moreover, further research is needed to identify the spectrum of patients in whom different antibiotics offer better clinical outcomes and prognosis.

ARTICLE HIGHLIGHTS

Research background

Antibiotics that are recommended by the current clinical guidelines may not be effective for treatment of complicated urinary tract infection (UTI) and acute pyelonephritis, due to the increasing resistance rates to the antibiotics.

Research motivation

This systematic review is intended to provide comprehensive information to help clinicians in determining suitable antibiotics for the management of complicated UTI and acute pyelonephritis.

Research objectives

The aim of this study was to determine the clinical efficacy and safety of antibiotics for the treatment of complicated UTI and pyelonephritis.

Research methods

A search of three medical literature databases (PubMed, EMBASE and Google Scholar) was conducted for eligible articles describing the use of antibiotics in managing complicated UTI and acute pyelonephritis. The following keywords were used to perform the literature search: "urinary tract infection", "complicated UTI", "pyelonephritis", "treatment", and "antibiotics". Eligibility criteria included diagnosis of either complicated UTI or acute pyelonephritis and use of antibiotics in management. Clinical trials and observational studies were included in this review, while case reports and reviews were excluded.

Research results

Eight studies matched all the eligibility criteria and were included in this review. The antibiotics included in those studies were ceftazidime-avibactam, doripenem, levofloxacin, meropenem-vaborbactam, piperacillin-tazobactam, plazomicin, tazobactam-ceftolozane, and gentamicin. The clinical data have shown that shorter duration of treatment with lower consumption of antibiotics is effective for treatment and can reduce the development of multiple drug resistance bacteria. Ceftazidime-avibactam, piperacillin-tazobactam and tazobactam-ceftolozane can be used as alternatives to carbapenem to treat ESBL-producing *Escherichia coli*. Besides that, meropenem-vaborbactam, piperacillin-tazobactam and tazobactam-ceftolozane have high cure rates in treating complicated UTI and pyelonephritis

Research conclusions

Novel antibiotics and combination antibiotic therapy regimens are effective in managing complicated UTI and acute pyelonephritis when resistance to recommended antibiotics occurs.

Research perspectives

Further research is needed to compare the efficacy of different antibiotic therapies and identify the spectrum of patients in whom different antibiotics offer better clinical outcomes and prognosis.

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CASE REPORT

Abdominal aortic thrombosis as initial presentation of COVID-19 infection: A case report

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Abstract

BACKGROUND

The hypercoagulable state associated with coronavirus disease 2019 (COVID-19) has been shown to complicate the course of this viral illness with both venous and arterial clots. Often presenting after hospitalization and known COVID-19 diagnosis, the etiology of thrombosis has been attributed to the hyperinflammatory state and endothelial dysfunction associated with COVID-19. This report portrays a patient who experienced an aortic thrombosis resulting in back and leg pain with subsequent loss of motor function of his legs as his initial presentation of COVID-19.

CASE SUMMARY

Patient is a 60-year-old Caucasian male with no medical history who presented with sudden onset pain in his lower back and lower extremities. He went on to experience complete motor loss of the lower extremities two hours after admission. Chest pain and shortness of breath developed one day later but were not present at time of presentation. Computed tomography angiography of the chest, abdomen, and pelvis revealed occlusion by thrombosis of the abdominal aorta in addition to multifocal pulmonary ground-glass opacities prompting COVID-19 PCR, which was positive. He was taken to surgery for attempted thrombectomy and the thrombus was retrieved starting from the right common femoral artery, but a second thrombus had immediately reformed in place of the prior thrombectomy site resulting in conclusion of the procedure. He was continued on unfractionated heparin and received a dose of tocilizumab 400 mg, but rapidly developed hemodynamic compromise and expired from cardiac arrest.

CONCLUSION

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This presentation emphasizes the importance of evaluating patients for COVID-19 who experience unusual thromboses without superior explanation.

Key Words: COVID-19; Aortic thrombosis; Arterial thrombosis; Atypical COVID-19 presentation; COVID-19 complication; Case report

Core Tip: Aortic thrombosis preceding respiratory symptoms should raise suspicion for testing for coronavirus disease 2019 in patients with unusual thrombosis presentation.

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INTRODUCTION

The known hypercoagulable state associated with coronavirus disease 2019 (COVID-19) infection has been implicated as a common cause of morbidity and mortality. The presentation can range from microthrombi to large thromboses in both intra- and extrapulmonary vessels. In addition, the diagnosis of thrombosis often occurs days to weeks after the initial onset of respiratory symptoms, resulting in worsening of overall clinical status and prognosis. One study in France showed that pulmonary embolism (PE) was diagnosed with a mean of 12 days since initial onset of symptoms in COVID positive patients[1]. The converse, of having an obvious thrombotic event preceding onset of respiratory symptoms, may lead providers away from testing a patient for COVID-19. There have been two reports of aortic thromboses in patients with COVID-19 pneumonia, but thrombosis occurred after the patient was already known to be positive for COVID-19. The hypercoagulable state associated with this infection should be considered in patients with no obvious risk factors for thrombosis or evidence of thrombosis in an unusual location, as endothelial dysfunction coupled with hyperinflammation are thought to be mediators of this hypercoagulable state. In this case report, we describe a patient who presented with back and leg pain, and further work up revealed extensive thrombosis in the aorta, iliac, and superior mesenteric arteries (SMA). His abnormal chest imaging prompted PCR testing for COVID-19, which was positive. Our case displays the importance of appreciating the hypercoagulability associated with COVID-19 and raises awareness to a variety of possible presentations.

CASE PRESENTATION

Chief complaints

Patient is a 60-year-old incarcerated Caucasian male with no past medical history who presented to the hospital with complaints of sudden onset pain in his lower back and lower extremities.

History of present illness

He went on to experience complete motor loss of the lower extremities two hours after admission. Chest pain and shortness of breath developed one day later but were not present at time of presentation. He did not have any other symptoms indicative of infection including fever, chills, or cough. He was not taking any medications.

History of past illness

Patient has no past medical history.

Physical examination

Vitals at presentation were blood pressure 99/47, pulse 126 beats per minute, temperature 36.8 °C, respirations 15 per minute, and oxygenation 99% on room air. Neurologic exam of the lower extremities initially revealed 3/5 motor strength, but sensation was intact. Repeat exam in 2 hours revealed complete motor loss of the lower extremities. Dorsalis pedis and posterior tibial pulses were not palpable and femoral pulses were weak at 1+. Pulmonary exam revealed diffuse rhonchi in all lung fields. Cardiac exam revealed tachycardia, but no murmurs were noted, and the rhythm was regular. He was alert and oriented to person, place, and time.

Laboratory examinations

Patient had a positive COVID-19 PCR blood test. His laboratory values were remarkable for leukocytosis of 22.3 cells/L (4.5-11.0) with an absolute lymphocyte count of 0.58 K/uL (1.32-3.57), PT 16.4 seconds (12-14.5), INR 1.3 U (< 1.0), PTT 28.9 seconds (23.9-36.6), and d-dimer > 20 μg/mL (< 0.5). Ferritin was significantly elevated at > 40000 μg/L (22-275), C reactive protein was 210 mg/L (0-5), and creatine phosphokinase was 46800 U/L (0-200).

Imaging examinations

Patient underwent computed tomography (CT) angiography of the chest, abdomen, and pelvis which revealed occlusion by thrombosis of the abdominal aorta, depicted in Figures 1 and 2, in the infrarenal segment with extension to his iliac arteries with reconstitution of flow in the bilateral common femoral arteries. Additional nonocclusive thrombosis in the SMA was noted. In addition to these thromboses, multifocal ground-glass opacities were visualized in the bilateral lung fields which prompted COVID-19 PCR testing.

FINAL DIAGNOSIS

This patient suffered from an occlusive abdominal aortic thrombosis secondary to COVID-19 infection.

TREATMENT

Patient was emergently taken to surgery for attempted thrombectomy and a heavy burden of thrombus was retrieved starting initially from the right common femoral artery. After several minutes of closing of vasculature, it was noted that the femoral artery pulsation had weakened and disappeared and it was noted that a second thrombus had formed again in place of the prior thrombectomy site after reevaluation despite running of heparin. At this point, the procedure was concluded as it was clear that the patient was hypercoagulable due to his COVID-19 infection.

Patient remained intubated following the operation due to respiratory compromise in the setting of his known COVID-19 pneumonia. He was continued solely on unfractionated heparin infusion at 18 U/kg/h. He also received a dose of Tocilizumab 400 mg, but continued to worsen from a hemodynamic standpoint, requiring the initiation of vasopressors. No additional anti-viral agents or COVID-19 targeted therapies were employed.

OUTCOME AND FOLLOW-UP

Despite ventilatory support and triple vasopressors with norepinephrine, phenylephrine, and epinephrine, patient continued to deteriorate and soon expired from cardiac arrest in the setting of his occlusive abdominal thrombosis.

DISCUSSION

As a respiratory virus, COVID-19 typically presents with signs of lung infection including shortness of breath, cough, and fever which can progress to acute respiratory distress syndrome. Patients requiring admission to an intensive care unit

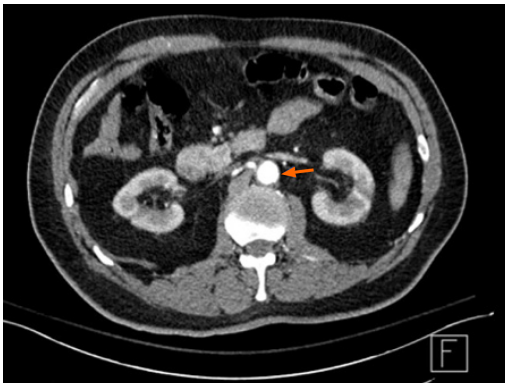

Figure 1 Computed tomography angiogram of the abdomen- portrays a patent aorta with contrast visualized just prior to the complete thrombotic occlusion.

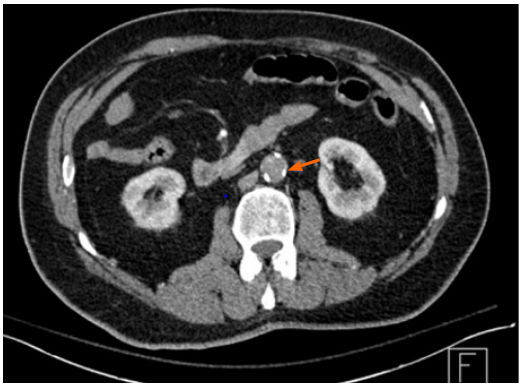

Figure 2 Computed tomography angiogram of the abdomen- shows the abrupt loss of contrast in the aortic lumen indicating the occlusive thrombus that resulted in the patient's symptoms of back pain and loss of lower extremity motor function due to vascular insufficiency. This imaging modality was chosen to visualize the vasculature to evaluate for aortic dissection, aneurysm, or thrombosis given patient's symptoms of crushing back pain with lower extremity motor loss. Aortic calcification incidentally visualized.

(ICU) have been found to have acute thromboses, most commonly being PE in the setting of the severe inflammatory response, endothelial dysfunction, and multi-organ system failure elicited by the virus. Overt thrombosis has been reported to be as high as 25%-50% in this population[2]. Unlike traditional thrombotic events in ICU patients, COVID-19-associated thrombosis has a higher incidence of arterial clot and a greater mortality[3,4]. In a study of three Dutch hospitals, there was a 31% incidence of thrombosis in ICU patients, with 3.7% being arterial[5]. Markers such as D-dimer, lactate dehydrogenase, ferritin, and CRP have been used to stratify patients for risk of thrombosis and potential benefit with prophylactic anticoagulation, but degree of elevation associated with arterial clot has yet to be appreciated.

A report by Berre *et al*[6] presented a patient who was found to have acute aortic thrombosis and concomitant pulmonary embolism after being diagnosed with COVID-19 pneumonia. This patient was found to have a D-dimer of 17.28 μg/mL with normal platelets and prothrombin time. An additional report by Katchanov *et al*[7] described a patient with extensive aortic thrombosis and a D-dimer level of 15.28 μg/mL. Consistent with this trend of severely elevated inflammatory markers, particularly D-dimer, our patient's D-dimer was severely elevated at > 20 μg/mL and ferritin > 40000 μg/L. These findings suggest that extensive thrombosis involving the arterial circulation may be more likely at the far end of the spectrum of extreme inflammation and endothelial dysfunction. Interestingly, both our patient and the above case presented by Katchanov *et al*[7] showed involvement of the abdominal aorta and iliac arteries in addition to occlusion of the SMA. Given these two reports of SMA occlusion and the possibility of intestinal ischemia, providers should consider this in patients to receive the anti-IL-6 agent tocilizumab for severe inflammatory dysregulation, as intestinal perforation is a known side effect despite its single-dose indication[8]. Alternative COVID-19 directed therapies include the anti-viral remdesivir and convalescent plasma, as these agents may have been additional options for this

patient in absence of his rapid clinical decline. Due to the fact that his decline was thought to be more related to his aortic thrombosis and not to COVID-19 induced lung dysfunction, the mainstay of therapy was unfractionated heparin, which was chosen due to rapid reversibility compared to newer direct oral anticoagulants such as apixaban or rivaroxaban.

As arterial thrombi may not always be visualized with routine CT angiography PE protocols, it is important to consider additional scanning for patients with severely elevated inflammatory markers in which suspicion is high for arterial clot. A contrast CT of the abdomen or aortic CT angiography may be necessary to diagnose these aortic thromboses, and our patient was diagnosed with CT angiography of the chest, abdomen, and pelvis given his overt lower back and lower extremity pain as his presenting symptoms. The most impressive finding in our patient was the evidence of a rapidly forming thrombi after successfully removing the initial thrombi and reperfusion of the lower extremities. This echoes the profound hypercoagulable state as a result of COVID-19.

CONCLUSION

Acute thrombosis in the setting of COVID-19 can be a devastating complication with a drastic increase in morbidity and mortality. Our case highlights the profound hypercoagulable state of severe inflammatory response due to COVID-19, with the rapid formation of a thrombi immediately following thrombectomy, compromising perfusion and hastening refractory shock and death. We hope to raise awareness in the importance of recognizing arterial thrombi as a result of COVID-19 in patients with no other obvious explanation, as a prompt diagnosis may influence potential treatment options and lead to better outcomes.

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LETTER TO THE EDITOR

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ABOUT COVER

Peer reviewer for *World Journal of Clinical Infectious Diseases*, Dr. Chun-Feng Xiao is a psychiatrist and medical editor with expertise in Psychiatry and Mental Health, with over 120 publications. He serves as the Bentham Science Ambassador of Bentham Science Publishers and as the Junior Ambassador of the Universal Scientific Education and Research Network (USERN) in China. His research involving the psychological impact of global public health emergencies (particularly of infectious disease pandemic) led to his role as Organizing Committee Member for the upcoming Coronavirus and Research conference (March 29-30, 2021, Rome, Italy). He is the sole inventor of structured letter therapy (SLT), a novel approach to consultation on COVID-19-related psychological and mental problems that is expected to become a bond of transition from traditional psychiatry to digital psychiatry. He is also founder of the SLT Multidisciplinary Collaboration Group, an official organization dedicated to long-term planning and operation for SLT. (L-Editor: Filipodia)

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OPINION REVIEW

COVID-19 risk comorbidities: Time to reappraise our physical inactivity habits (again!)

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Abstract

Infection and mortality rates of coronavirus disease 2019 (COVID-19) are astonishing. As of September 7, 2020, more than 27 million people around the world have already been infected, with more than 890 thousand deaths. Hypertension, diabetes, and obesity are among the most reported comorbidities associated with mortality by this disease. All these comorbidities are also strongly associated with physical inactivity and sedentary behavior. On the other hand, it is known that aerobic and resistive exercises are excellent tools to prevent and manage these comorbidities. Hence, physically active people may have a better prognosis if infected by COVID-19. Also, science tried to warn about mortality and morbidity associated to physical inactivity more than 80 years ago. However, physical inactivity habits are getting more prevalent around the world. Reasons for that include social, technology, and economic development that led to large industrialization and urbanization. Along with these changes, both professional and domestic activities became less active. Consequently, health care costs related to hypokinesis are estimated to increase exponentially in various regions of the planet. Now, while facing COVID-19 pandemic, it is time to reinforce the physiological, social, and economic relevance of regular physical exercise. Therefore, urgent reappraisal of our physical inactivity habits should be done, again!

Key Words: COVID-19; Physical inactivity; Sedentarism; Exercise; Health; Economy

Core Tip: Millions of people have been infected by coronavirus disease 2019 (COVID-19) after its outbreak in December 2019 in China, and thousands of them have died

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around the world. These astonishing happenings forced the World Health Organization to declare a pandemic. Of note, older people and those with comorbidities such as hypertension and diabetes are at higher risk. Regular exercise is an excellent tool to manage all those comorbidities as well as to boost human immune system, preparing people to fight infections. However, people are getting more sedentary in the last decades! During COVID-19 pandemic, we must reappraise our inactivity habits to improve health and to minimize costs to public health systems.

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INTRODUCTION

Infection with severe acute respiratory syndrome coronavirus 2 results in coronavirus disease 2019 (COVID-19)[1]. The outbreak of the disease occurred in China in December 2019. Infection and mortality rates of this pandemic around the world are astonishing. As of September 7, 2020, more than 27 million people around the world have already been infected, with more than 890 thousand deaths. United States, India, Brazil, and Russia are the most infected countries at this point with more than 1 million confirmed cases[2].

Older individuals are at higher risk of poor clinical outcomes related to the disease[1]. However, several other risk factors have been associated to COVID-19 severity. Of note, hypertension, diabetes, and obesity are among the most reported comorbidities associated to high mortality rates[3,4]. For instance, Zhou *et al*[1] found that hypertension (30% of patients) and diabetes (19% of patients) were the most common comorbidities in a sample of 191 patients from Wuhan. A nationwide study from China with more than 1500 patients confirmed those data showing that hypertension was the most prevalent comorbidity (17%), followed by diabetes (8%)[5]. Finally, data from 5700 patients from the New York City area showed that hypertension (57%), obesity (42%), and diabetes (34%) were the most common comorbidities[6].

Interestingly, all these comorbidities, including age, are also strongly associated with physical inactivity and sedentary behavior[7,8]. On the other hand, it is widely known that physical exercise, both aerobic and resistive, is an excellent tool to prevent and manage these comorbidities[9]. In addition, immune system can be boosted with regular exercise, with an additional anti-inflammatory effect[10,11]. This could also contribute to fighting the inflammatory/cytokine storm of COVID-19[3].

Hence, even though there are so many aspects to account for, it is not hard to infer that physically active people may have a better prognosis if infected by COVID-19. However, despite the worldwide awareness about the health benefits of exercise, physical inactivity habits are getting more prevalent around the world[12]. Why is that? And what are we going to do, in terms of physical activity, after this pandemic is under control?

FIRST THINGS FIRST: WE WERE DESIGNED TO MOVE

Some generations ago, physical activity was part of humans' daily life and survival. We "*walked, ran, lifted and carried, we pushed and pulled; we dug, harvested and gathered; we danced, jumped and climbed. But things have changed-We have changed*"[13]. Well, what, and when did we change?

Briefly, we can say that social, technology, and economic development through the last two centuries brought industrialization and urbanization in a large scale to most countries. Along with these changes, both professional and domestic activities became less active[13]: Too much sitting at work, too much time driving inside a car, and too much television at home[8]. More recently, too much smartphones and notebooks are everywhere. Hence, main opportunities to maintain physical activity are in moments of leisure[13]. Unfortunately, it seems like things are worsening.

PHYSICAL ACTIVITY LEVELS PROJECTION

There is growing evidence that sedentarism has increased in the last decade[12]. Thus, projections of physical activity levels (PALs) are not promising. In developed countries, such as United States and United Kingdom, PALs are expected to reduce by 46% and 35%, respectively, until 2030. In countries with emergent economies, such as Brazil, China, India, and Russia, reduction in PALs until 2030 is expected to reach 34%, 51%, 14%, and 32%, respectively[13]. In other words, even though we were *designed to move*, we are getting more sedentary, and counting! The big problem is that this lack of physical activity is not without consequences and costs.

Besides the increased risk of COVID-19 comorbidities associated with physical inactivity[7,8], strong and recent evidence shows that the risk of all-cause mortality is also closely related to it. For instance, individuals watching television ≥ 4 h per day present 80% increased risk of all-cause and cardiovascular disease mortality[14]. If we look closely, we will see that this information is not new. We have been warned of the risks of physical inactivity to the cardiovascular system and mortality since the 1950s with the work of Morris and Crawford[15] and then in the 1980s with the work of Paffenbarger *et al*[16]. Briefly, these publications showed that physical activity may prevent coronary disease and increase longevity compared to sedentary or people engaged in less active work.

Moreover, economic consequences of physical inactivity are high to the public health systems. For instance, in Canada, physical inactivity represents 3.7% of the overall health care costs. In China, more than 15% of both medical and non-medical costs per year are attributable to physical inactivity[8]. In contrast, small changes in physical inactivity levels can be strongly beneficial. In Australia, for example, it was estimated that a 10% reduction in inactivity levels would result in a 96 million (Australian dollars) reduction in health sector costs per year, allied with an increase in work force production[17]. Yet, it is estimated that, until 2030, health care costs related to inactivity will increase around 113% and 61% in the European Union Association and United Kingdom, respectively; whereas in Brazil and China, these values are expected to reach 182% and 453%, respectively. To be more specific, by 2030, the direct costs related to inactivity consequences in United States, Russia, and Brazil are expected to reach 191, 3.4, and 6.2 billion dollars, respectively[13].

TIME TO REAPPRAISE PHYSICAL INACTIVITY HABITS

So, evidence shows that COVID-19 mortality rates are higher among people with comorbidities. Also, it is known that physical inactivity and sedentary behavior lead to the appearance of these COVID-19 deadly comorbidities, especially hypertension, diabetes, and obesity. Yet, even though scientific-based information was already available about physical activity health and economic benefits, humankind is becoming more sedentary and less prepared, from a physiological perspective, to fight a hazardous infection like COVID-19. Thus, while facing this pandemic, it is time to think about the physiological, health, social, and economic relevance of regular and well oriented physical exercise. Of note, literature shows that people who exercise with direct professional supervision and periodized exercise schemes present greater adaptations compared with low supervision and non-periodized training[18,19]. This highlights the relevance of investments in this area. It is also relevant to point that some exercises are safe to be performed indoor while social distance is still recommended. Yoga, low intensity body weight exercises, and active video games are amongst the options.

CONCLUSION

Urgent reappraisal of our physical inactivity habits should be done, again!

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LETTER TO THE EDITOR

COVID-19 and dengue coinfection in Brazil

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Abstract

The case we present here is a man who lives in a dengue-endemic area. Initially, the patient was diagnosed with dengue fever by clinical evaluation and laboratorial confirmation. Subsequently, he presented respiratory symptoms, and a concomitant severe acute respiratory syndrome coronavirus 2 infection was confirmed. He was hospitalized for 17 d and had a satisfactory recovery.

Key Words: COVID-19; Dengue fever; SARS-CoV-2; Dengue virus; Coinfection; Diagnosis

Core Tip: Corona virus disease 2019 represents a big concern for public health. Simultaneously, many countries are also being affected by arbovirus epidemics, which overwhelms the health assistance services from those localities. That scenario calls attention to how these epidemics will affect the health of people living in those geographic areas. In this Letter to the Editor, we report a coinfection by severe acute respiratory syndrome coronavirus 2 and dengue virus that occurred in northeastern Brazil.

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TO THE EDITOR

From the first cases reported in December 2019 in Wuhan, China, to May 13, 2020, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has already infected 4179479 people worldwide[1,2]. Concomitant to the corona virus disease 2019 (COVID-19) pandemic, half of the world population is at increased risk of developing arbovirus infections, and 390 million individuals are infected by the dengue virus (DENV) every year, which makes the world health scenario even more worrying[3]. Here we report a coinfection by SARS-CoV-2 and DENV that occurred in a patient from northeastern Brazil.

This study was approved by the National Commission of Research Ethics, from the National Health Council, Ministry of Health of Brazil (Number 30700320.0.0000.0008), and a signed informed consent was obtained from the patient.

The case is a 59-year-old physician male with well-controlled comorbidities (hypertension and type 2 diabetes, in use of an angiotensin converting enzyme inhibitor and metformin) living in a dengue-endemic area with no history of recent travel. He requested medical home care on March 30, 2020 due to the onset of symptoms such as fever, chills, anorexia, and headache. Three days later, the patient was tested by immunochromatography assay for specific dengue immunoglobulins (94% sensitivity and 96% specificity). Positive IgM and negative IgG results along with the clinical data led to the diagnosis of dengue fever.

On the fourth day, the patient presented dyspnea and cough, and a bilateral ground-glass pattern was observed in his lungs by a thoracic computerized tomography. He was immediately hospitalized and found to be positive for SARS-CoV-2 infection by serology (positive IgM and IgG immunoglobulins) and by RT-PCR of the material obtained by a nasopharyngeal swab. The liver and kidney tests were within the reference values as well as the blood counts.

Two days later, the patient presented a hypoxemic respiratory insufficiency and was transferred to an intensive care unit. He underwent noninvasive oxygen therapy and developed a deep vein thrombosis in the right femoral vein, which was accompanied by increased D-dimer values. He received full-dose heparin therapy followed by full-dose enoxaparin prophylaxis. The patient was kept in the intensive care unit for 12 d and was subsequently transferred to conventional hospital care.

Five days later, a COVID-19 serological test was negative for IgM and positive for IgG, and he was discharged from the hospital with a rivaroxaban (30 mg/d) prescription. Three weeks after hospital discharge, he underwent a new immunochromatography assay for dengue diagnosis (94% sensitivity and 96% specificity), and IgG dengue specific immunoglobulin was positive, representing a seroconversion and confirming the concomitant diagnosis of dengue fever and COVID-19.

It has to be emphasized that there was an increase of about 70% in the number of dengue cases in Brazil in the period from December 30, 2019 to March 12, 2020 (390684 cases reported) compared to the same period in 2018-2019 (229064 cases reported)[4,5]. In addition, according to the Pan American Health Organization, Brazil registered 2226865 dengue cases in 2019, 70% of the total in the Americas[6]. Not only Brazil, but also all of the dengue-endemic world regions are at risk of suffering the consequences of the threatening cocirculation of those viruses[7,8]. As an example, a prior publication called attention to Colombia, which registered 52679 dengue notifications and 14943 COVID-19 cases during the first five months of 2020[9]. Interestingly, a study demonstrated a considerable drop in the number of dengue cases notified during the COVID-19 epidemic in the State of São Paulo, Brazil[10]. This study hypothesized that there might be an under notification of dengue cases due to the impairments in health system functioning because of the COVID-19 epidemic. The authors also theorized that the restriction of the social interactions aiming to limit the SARS-CoV-2 dissemination resulted in a lower circulation of people and could have reduced the propagation of arboviruses, decreasing the risk of dengue outbreaks in various geographic areas.

The case reported here joins some previously published descriptions of dengue and

COVID-19 coinfections. The first reported DENV and SARS-CoV-2 coinfection dates from March 11, 2020 and affected a 44-year-old male living at Mayotte in the Indian Ocean, who traveled to Switzerland and France, where his symptoms started[11]. Verduyn *et al*[12] reported another coinfection in an 18-year-old male who traveled from France to Reunion Island, also located in the Indian ocean. We have just also published a similar coinfection in a Brazilian man aged 39 years, who lived in a small county with no prior register of COVID-19 circulation and had the onset of symptoms three days after a day trip to another city[13]. Interestingly, all of the above mentioned case reports refer to travelers who potentially acquired each of the infections in different geographic areas.

In contrast, the present report describes a coinfection in a patient who had not traveled before falling ill. Such data alerts to the occurrence of a local circulation of both viruses, which can lead to serious impacts in the regional public health. Moreover, all of the previously reported cases are young adults who did not experience severe respiratory symptoms unlike the patient reported here, who also had type 2 diabetes and hypertension, two well-known risk factors for unfavorable COVID-19 outcomes.

Although the patient underwent intensive medical care and supplementary oxygen, he had a satisfactory recovery with no necessity for intubation. Some authors believe that metformin may play a protective role in diabetic COVID-19 patients because this medication has promising results when used in other lung diseases such as asthma and pneumonia[14]. Moreover, a retrospective cohort study analyzing 223 diabetic individuals who had dengue fever found a lower risk of developing severe dengue among metformin users[15]. In addition, we hypothesize that the interplay between those infections may influence the immune response in an idiosyncratic way. However, deeper analysis on that issue could not be performed in the present case, and further studies should be conducted in order to better understand this relationship.

The existence of clinical similarities between COVID-19 and dengue fever can lead to misdiagnoses, which may delay important clinical measures for the management of patients. Waterman *et al*[16] drew attention to the need for physicians in dengue-endemic areas to be alert for recognizing clinical characteristics associated with severe dengue fever in individuals with a suspected SARS-CoV-2 infection. On the other hand, the report of a 35-year-old nurse who likely got COVID-19 while sampling blood of a man who was presumed to have dengue fever highlights the risks of covert SARS-CoV-2 infections in dual viral circulation settings[17]. Complementarily, the occurrence of false-positive serology for dengue in SARS-CoV-2-infected individuals in Singapore reinforce the necessity of careful management of patients with nonspecific clinical presentations in coepidemic scenarios[18]. The discussion above gains even more importance when considering that laboratory parameters such as thrombocytopenia can be observed in both infections[19]. Taken together, health systems of dengue-endemic regions should consider social isolation procedures for patients without a clear etiologic diagnosis aiming to avoid the SARS-CoV-2 dissemination.

In view of the potential risks of a coinfection by SARS-CoV-2 and DENV, we highlight the importance of this Letter to the Editor as a way to alert health professionals to consider both diagnoses in countries simultaneously affected by these epidemics.

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LETTER TO THE EDITOR

Top ten tips for perfect corona-2 prophylaxis

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Abstract

The current corona-2 pandemic has stimulated wide research for hydroxychloroquine (Quine) therapy and lately, prophylaxis. To optimize prophylaxis proper methods of use are explained. The focus is on tools of assessment and robust comparison; defining infection objectively; loading and maintenance dose designing based on pharmaco-viro-kinetics; confirming Quine threshold-levels and its sufficiency; and Quine side-effects vigilance/amelioration. Attention to statistics to study valid endpoints of goals in appropriately-sized population is essential. Mass interactive quine dose auto designer software is built to simplify, optimize and help collaboration of complex Quine dosing system. A similar chloroquine software can be built.

Key Words: Corona-2; COVID-19; Hydroxychloroquine; Prophylaxis; Dose; Mass interactive quine dose auto designer

Core Tip: Quine's role in corona-2 pandemic prophylaxis can be assured *via* designing correct loading doses (LD)/ maintenance doses (MD), therapy duration, and volumetric absorptive microsampling (VAMS) concentrations, assuring human IC_{50} and Liver and Heart safety thresholds of TC_L10 and TC_H10. Surely, good care will translate VeroE6 Viro-kinetics into human Viro-kinetics and help human-tailored dosing; not misguided by improper models, malaria, or rheumatology doses. Mass interactive quine dose auto designer (MIQDAD), viral count, and VAMS test help initial Quine LD/MD designing and human-tailored LD/MD dosing.

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TO THE EDITOR

A good effort to study post-exposure hydroxychloroquine (Quine) prophylaxis was recently published[1]. Despite that it has decent statistical design, it has salient issues that need to be addressed to perfect the outcome of further Quine prophylaxis: (1) The primary outcome should involve viro-conversion from positive at entry to negative on exit (little testing in this study); (2) Primary outcome involves clinical symptoms reported by patients (was its percent reliability factored in sample-size calculations?) and was rated by 4 Infectious Disease doctors (without mention of inter-rater training or kappa of agreement reliability)[2]; (3) No data on further exposure to corona-2; other flu virus or having nasal allergy during the 2-wk study; (4) Using primary outcome as clinical symptoms is subjective, neither sensitive (as 80% are asymptomatic) nor specific to COVID19, (is it another flu virus or hay-fever?); (5) The Quine antimalarial dose is smaller than its antiviral loading doses (LD) calculated from the pharmaco-kinetics data held by FDA[3] (Table 1). Low LD will produce sub-inhibitory levels, so that patients are not protected for 1-4 d pre-enrollment and 4-5 d post-enrollment (treatment start 1 d after enrollment and might take 4 d to reach the level required for protection-threshold); (6) although measuring drug levels by using finger-prick to self-collect 10 µm blood samples (VAMS) is well-known, *in-vivo* Quine IC_{50} (= 50% Inhibitory Concentration) is never assured; pharmacokinetics from one study proposed VeroE6 cell IC_{50} of 4.5 µmol/L in 48 h of post-infection (mcM/hpi)[4,5], and another 6.3-5.9 in 24-48 mcM/hpi[6] requiring higher LD (15 and 20 tablets x 200 mg each, respectively); plus 2-3-wk maintenance doses (MD) or until patients develop their own immuno-protection; (7) Finding of safety-thresholds (10% Toxic Concentration = TC10) for liver enzymes elevation (= $TC_{Liver}10$), for heart QT-prolongation (= $TC_{Heart}10$), clinical hepatitis and dysrhythmia issues; (8) Since Quine is virostatic, its prophylactic-level must be maintained for at least 2-3 wk to build immunity that can clear virion particles (not possible in VeroE6 cell-kinetic cultures). So, dosing for 5 of 14 d is inadequate; (9) the folate-placebo helps one-carbon atom transfer to thymine to produce uracil, the rate-limiting substrate for RNA synthesis –undesired confounder; and (10) Although using sophisticated statistics to end the study early at a priori statistical power outcome is good, extending Quine prophylaxis (following correct LD) to achieve and define human IC_{50}, is a missed historical landmark in the human/corona-2 contest. Sadly, statistical passion forced ending at only 2.4% incidence reduction rather than a 7% reduction –glorifying statistical-significance sacrificed nearby finding/measuring the more clinically important IC_{50} –*cf. McNamara fallacy*.

CONCLUSION

Quine's role in corona-2 pandemic prophylaxis can be assured *via* designing correct LD/MD, therapy duration, and VAMS concentrations, assuring human IC_{50} and Liver and Heart safety thresholds of $TC_{Liver}10$ and $TC_{Heart}10$. Surly, good care will translate VeroE6 Viro-kinetics into human Viro-kinetics and help human-tailored dosing; not misguided by improper models, malaria, or rheumatology doses.

Mass interactive quine dose auto designer, viral count and VAMS test help initial Quine LD/MD designing and human-tailored LD/MD dosing.

Table 1 The mass interactive quine dose auto designer (MIQDAD,Download)

Body weight (kg)	60		Loading dose	Loading days if	Maintenance	Post-protect	Durations	Load/Maint	Protective nadir (mcM)	5
C rise/tab (mcM)	0.386	Target levels	Computed	6	7	5.0	Give to stay on peak doses for	6		
Half-life: T ½	22.4	Level in mcM	Tablets to load	Tablets used/d	Tablets/wk	Post-last-dose		Doses Ratio	Maintenance interval (d)	1
Well indications		1 Tab = 200 mg = 155 × 0.74		2 tablets /6 h						
Protection		5.0	14	2.3	2.5	0	Until becomes immuno-protected or the pandemic ends	5.5	Protective peak (mcM)	5.2
Community helper		6.0	17	2.8	3.0	6		5.5		
Exposed but well		7.0	20	3.3	3.5	11	2 wk	5.6	First dose (200 mg tablets)	13.4
Unwell indications										
Low Infection		8.0	23	4	4	15	2 wk	5.7	Maintain dose (tablets)	0.4
Medium infection		10.4	30	5	5	24	2 wk	5.8		
High infection		13.3	40	7	7	32	2 wk	6.0	PostCourse protected days	1.0

Assuming these doses for weights 40-60 kg: Each 600 mg or 3 tablets is replaced by: Child < 40 kg dose = 12 mg/kg or Adult > 60 kg idealised 3 tablets equivalent = kg/20 Tablets; Micro finger-prick testing (Volumetric Absorptive Micro-Sampling, VAMS) can be used to confirm or guide dosing; All red numbers are editable, so that user's can tailor to the needs and evolving data on effective inhibitory concentrations; Durations: For symptomatic infections, Quine (virostatic) should cover until immune system is able to inactivate the virus; C rise/tab (mcM): Drug concentration rise per tablet in micro-moles/L (mcM).

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