

# World Journal of *Clinical Infectious Diseases*

*World J Clin Infect Dis* 2015 February 25; 5(1): 1-13



## Editorial Board

2011-2015

The World Journal of Clinical Infectious Diseases Editorial Board consists of 291 members, representing a team of worldwide experts in infectious diseases. They are from 56 countries, including Argentina (5), Australia (8), Austria (3), Bangladesh (1), Belgium (2), Bosnia and Herzegovina (1), Brazil (6), Brunei Darussalam (1), Bulgaria (1), Cameroon (1), Canada (7), China (18), Colombia (1), Costa Rica (1), Cuba (1), Denmark (2), Egypt (1), Finland (1), France (11), Germany (4), Greece (8), Hungary (6), India (14), Indonesia (1), Iran (5), Israel (10), Italy (19), Japan (4), Jordan (1), Kosovo (1), Kuwait (1), Lebanon (3), Lithuania (1), Malawi (1), Mexico (5), Morocco (2), Netherlands (4), Nigeria (1), Pakistan (2), Peru (1), Philippines (1), Portugal (5), Russia (1), Saudi Arabia (2), Singapore (3), South Africa (2), South Korea (6), Spain (24), Switzerland (2), Tanzania (1), Thailand (4), Tunisia (1), Turkey (4), United Kingdom (9), United States (59), and Venezuela (1).

### EDITORS-IN-CHIEF

Shyam Sundar, *Varanasi*  
Lihua Xiao, *Atlanta*

### GUEST EDITORIAL BOARD MEMBERS

Huan-Tsung Chang, *Taipei*  
Jia-Ming Chang, *Taipei*  
Kuo-Chin Huang, *Chiayi*  
Wei-Chen Lee, *Taoyuan*  
Hsiu-Jung Lo, *Miaoli*  
Jin-Town Wang, *Taipei*  
Deng-Chyang Wu, *Kaohsiung*  
Jiunn-Jong Wu, *Tainan*

### MEMBERS OF THE EDITORIAL BOARD



#### Argentina

Sergio Angel, *Chascomus*  
Luis Adrian Diaz, *Cordoba*  
Gustavo Daniel Lopardo, *Buenos Aires*  
Emilio L Malchiodi, *Buenos Aires*  
Victor D Rosenthal, *Buenos Aires*



#### Australia

Thea van de Mortel, *Lismore*  
David Llewellyn Gordon, *Bedford Park*  
Asad Khan, *Brisbane*  
Ruiting Lan, *Sydney*  
John McBride, *Cairns*  
David Leslie Paterson, *Brisbane*  
Nitin K Saksena, *Sydney*  
Andrew Slack, *Brisbane*



#### Austria

Ojan Assadian, *Vienna*  
Christian Joukhadar, *Vienna*  
Bernhard Resch, *Graz*



#### Bangladesh

Harunor Rashid, *Cox's Bazar*



#### Belgium

Mickael Aoun, *Bruxelles*  
Paul M Tulkens, *Brussels*



#### Bosnia and Herzegovina

Selma Uzunovic, *Zenica*



#### Brazil

Jane Costa, *Rio de Janeiro*  
Pedro Alves d'Azevedo, *Sao Paulo*  
Gerly Anne de Castro Brito, *Fortaleza*  
RL Dantas Machado, *Sao Paulo*  
Leandro R Rodrigues Perez, *Porto Alegre*  
M de Nazare Correia Soeiro, *Rio de Janeiro*



#### Brunei Darussalam

Vui Heng Chong, *Bandar Seri Begawan*



#### Bulgaria

Iva Christova, *Sofia*



#### Cameroon

Richard Njouom, *Yaounde*



#### Canada

Aranka Anema, *Vancouver*  
Peter C Coyte, *Toronto*  
Pavel Gershkovich, *Vancouver*  
Marcelo Gottschalk, *Quebec*  
Marina Ulanova, *Thunder Bay*  
Jude Uzonna, *Winnipeg*  
Jun Wang, *Halifax*



#### China

Tian-Hua Huang, *Shantou*  
Xi-Tai Huang, *Tianjin*  
Dong-Ming Li, *Beijing*  
Xin-Yong Liu, *Jinan*  
Wu-Bin Pan, *Taichang*  
Kai Wang, *Jinan*  
Patrick Chiu Yat Woo, *Hong Kong*  
Yong-Feng Yang, *Nanjing*  
Chi-Yu Zhang, *Zhenjiang*  
Li-Juan Zhang, *Beijing*



#### Colombia

Jorge Enrique Gomez-Marin, *Armenia*

**Costa Rica**

Adriano Arguedas, *San José*

**Cuba**

Maria G Guzman, *Havana*

**Denmark**

Janne Kudsk Klitgaard, *Odense*  
Henrik Torkil Westh, *Hvidovre*

**Egypt**

Olfat Shaker, *Cairo*

**Finland**

Jari Timo Juhani Nuutila, *Turku*

**France**

Hassane Adakal, *Burkina Faso*  
Pascal Bigey, *Paris*  
Philippe Brouqui, *Marseille*  
Christophe Chevillard, *Marseille*  
Raphaelé Girard, *Pierre Bénite*  
Vincent Pascal Jarlier, *Paris*  
Sandrine Marquet, *Marseille*  
Vayssier-Taussat Muriel, *Maisons-Alfort*  
Thierry Naas, *Le Kremlin-Bicetre*  
Saad Nseir, *Lille*  
Philippe Seguin, *Rennes*

**Germany**

Stefan Borgmann, *Ingolstadt*  
Georg Harter, *Ulm*  
Matthias Imohl, *Aachen*  
Kurt G Naber, *Straubing*

**Greece**

Apostolos Beloukas, *Athens*  
Alex P Betrosian, *Athens*  
George L Daikos, *Athens*  
Helena Maltezou, *Athens*  
Argyris S Michalopoulos, *Athens*  
Maria Moschovi, *Athens*  
George Petrikkos, *Athens*  
Athanasios Tragiannidis, *Thessaloniki*

**Hungary**

Laszlo Galgoczy, *Szeged*  
Viktor Muller, *Budapest*  
Ferenc Orosz, *Budapest*  
Ferenc Rozgonyi, *Budapest*  
Jozsef Soki, *Szeged*

Dezso Peter Virok, *Szeged*

**India**

Ritesh Agarwal, *Chandigarh*  
Syed Imteyaz Alam, *Gwalior*  
Atmaram Hari Bandivdekar, *Mumbai*  
Runu Chakravarty, *Kolkata*  
Dipshikha Chakravorty, *Bangalore*  
Sanjay Chhibber, *Chandigarh*  
BN Harish, *Pondicherry*  
Triveni Krishnan, *Kolkata*  
Rashmi Kumar, *Lucknow*  
Mohammad Owais, *Aligarh*  
Banwarilal Sarkar, *Kolkata*  
Mamta Chawla Sarkar, *Kolkata*  
Akashdeep Singh, *Ludhiana*

**Indonesia**

Jeanne Adiwinata Pawitan, *Jakarta*

**Iran**

Parissa Farnia, *Tehran*  
Seyed Mohammad Jazayeri, *Tehran*  
Morteza Pourahmad, *Jahrom*  
Mohammad Reza Pourshafie, *Tehran*  
Mohammad Hossein Salari, *Tehran*

**Israel**

Jacob Amir, *Petach Tikvah*  
Shai Ashkenazi, *Petach Tikva*  
Gadi Borkow, *Gibton*  
Raul Colodner, *Afula*  
Jacob Moran Gilad, *Jerusalem*  
Noah Isakov, *Beer Sheva*  
Michal Mandelboim, *Hashomer*  
Shifra Shvarts, *Omer*  
Oshri Wasserzug, *Tel-Aviv*  
Pablo Victor Yagupsky, *Beer-Sheva*

**Italy**

Giuseppe Barbaro, *Rome*  
Paolo Bonilauri, *Reggio Emilia*  
Guido Calleri, *Torino*  
Mario Cruciani, *Verona*  
Marco Falcone, *Rome*  
Antonio Fasanella, *Foggia*  
Daniele Focosi, *Pisa*  
Delia Goletti, *Rome*  
Guido Grandi, *Siena*  
Fabio Grizzi, *Rozzano*  
Giuseppe Ippolito, *Rome*  
Roberto Manfredi, *Bologna*  
Claudio M Mastroianni, *Rome*  
Ivano Mezzaroma, *Rome*  
Giuseppe Micali, *Catania*  
Antonella d'Arminio Monforte, *Milano*  
Annamaria Passantino, *Messina*  
Mariagrazia Perilli, *L'Aquila*  
Patrizia Pontisso, *Padova*

**Japan**

Masashi Emoto, *Maebashi*  
Toshi Nagata, *Hamamatsu*  
Ryohei Yamasaki, *Tottori*  
Shin-Ichi Yokota, *Sapporo*

**Jordan**

Asem A Shehabi, *Amman*

**Kosovo**

Lul Raka, *Prishtina*

**Kuwait**

Willias Masocha, *Safat*

**Lebanon**

Ziad Daoud, *Beirut*  
Ghassan M Matar, *Beirut*  
Sami Ramia, *Beirut*

**Lithuania**

Gazim Bizanov, *Vilnius*

**Malawi**

Adamson Sinjani Muula, *Blantyre*

**Mexico**

Agnes Fleury, *Mexico*  
Guadalupe Garcia-Elorriaga, *Mexico*  
Alejandro E Macias, *Mexico*  
Mussaret Zaidi, *Merida*  
Roberto Zenteno-Cuevas, *Veracruz*

**Morocco**

Redouane Abouqal, *Rabat*  
Ezzikouri Sayeh, *Casablanca*

**Netherlands**

Aldert Bart, *Amsterdam*  
John Hays, *Rotterdam*  
Nisar Ahmed Khan, *Rotterdam*  
Rogier Louwen, *Rotterdam*

**Nigeria**

Samuel Sunday Taiwo, *Osogbo*



### **Pakistan**

Muhammad Idrees, *Lahore*  
Muhammad Mukhtar, *Bahawalpur*



### **Peru**

Salim Mohanna, *Lima*



### **Philippines**

Vicente Y Belizario, *Ermita Manila*



### **Portugal**

Ricardo Araujo, *Porto*  
Manuela Canica, *Lisbon*  
Francisco Esteves, *Lisbon*  
Fernando Rodrigues, *Braga*  
Nuno Taveira, *Lisbon*



### **Russia**

Alexander M Shestopalov, *Koltsovo*



### **Saudi Arabia**

Jaffar A Al-Tawfiq, *Dhahran*  
Atef M Shibl, *Riyadh*



### **Singapore**

Yee Sin Leo, *Singapore*  
Laurent Claude Stephane Renia, *Singapore*  
Richard J Sugrue, *Singapore*



### **South Africa**

Carolina H Pohl-Albertyn, *Bloemfontein*  
Natasha Potgieter, *Louis Trichardt*



### **South Korea**

Chong Cho, *Seoul*  
Sang Ho Choi, *Seoul*  
Ju-Young Chung, *Seoul*  
Jung Mogg Kim, *Seoul*  
Kyongmin Kim, *Suwon*  
Sang Hee Lee, *Yongin*



### **Spain**

Alberto Arnedo-Pena, *Castellon*  
Alfredo Berzal-Herranz, *Granada*  
Vicente Brito, *Alicante*

Enrique Calderon, *Seville*

Rafael Canton, *Madrid*

Jose M Cuevas, *Valencia*

Laila Darwich, *Cerdanyola del Valles*

Adela Gonzalez de la Campa, *Madrid*

Pere Domingo, *Barcelona*

Tahia D Fernandez, *Malaga*

Lucia Gallego, *Leioa*

Luis Ignacio Gonzalez-Granado, *Madrid*

Bruno Gonzalez-Zorn, *Madrid*

Eduardo Lopez-Collazo, *Madrid*

Miguel Marcos, *Salamanca*

Antonio Torres Marti, *Barcelona*

Andres Moya, *Valencia*

Rafael Najera, *Madrid*

Maria Mercedes Nogueras-Mas, *Sabadell*

Jose A Oteo, *Logrono*

Pilar Perez-Romero, *Sevilla*

Ruth Gil Raka, *Madrid*

Eduardo Reyes, *Madrid*

Francisco Soriano, *Madrid*



### **Switzerland**

Stephen Hawser, *Epalinges*  
Andrew Hemphill, *Bern*



### **Tanzania**

John Peter Andrea Lusingu, *Tanga*



### **Thailand**

Kosum Chansiri, *Bangkok*  
Subsai Kongsangdao, *Bangkok*  
Niwat Maneeekarn, *Chiang Mai*  
Viroj Wiwanitkit, *Bangkok*



### **Tunisia**

Aouni Mahjoub, *Monastir*



### **Turkey**

Oguz Karabay, *Sakarya*  
Uner Kayabas, *Malatya*  
Gokhan Metan, *Kayseri*  
Oral Oncul, *Istanbul*



### **United Kingdom**

Zainab Al-Doori, *Glasgow*  
David Carmena, *London*  
Ronald Anthony Dixon, *Lincoln*  
Vanya Alasdair Ivan Andre Gant, *London*  
Robin Goodwin, *London*  
Andrew Cunliffe Hayward, *London*  
Laura Anne Hughes, *Neston*  
Michele Esther Murdoch, *Herts*  
Craig William Roberts, *Glasgow*



### **United States**

Majdi N Al-Hasan, *Lexington*  
Ibne KM Ali, *Charlottesville*  
Hossam M Ashour, *Detroit*  
Joseph Urban Becker, *Palo Alto*  
M Eric Benbow, *Dayton*  
Eliahu Bishburg, *Newark*  
Luz P Blanco, *Ann Arbor*  
Robert Bucki, *Philadelphia*  
Steven Dale Burdette, *Dayton*  
Archana Chatterjee, *Omaha*  
Pai-Lien Chen, *Durham*  
Pawel S Ciborowski, *Omaha*  
Michael Cynamon, *Syracuse*  
Siddhartha Das, *El Paso*  
Ralph J DiClemente, *Atlanta*  
Noton Kumar Dutta, *Baltimore*  
Garth D Ehrlich, *Pittsburgh*  
Michael S Firstenberg, *Columbus*  
Walter A Hall, *Syracuse*  
Yongqun He, *Ann Arbor*  
Brenda Lorraine Helms, *Plano*  
Joseph U Igietseme, *Atlanta*  
Mohammad Khalid Ijaz, *Montvale*  
Suresh G Joshi, *Philadelphia*  
Thomas F Kresina, *Rockville*  
Alain B Labrique, *Baltimore*  
Shenghan Lai, *Baltimore*  
Benfang Lei, *Bozeman*  
Jeff G Leid, *Flagstaff*  
Vladimir Leonitiev, *St. Louis*  
Andrea Lisco, *Bethesda*  
James M McMahon, *Rochester*  
Geraldine M McQuillan, *Hyattsville*  
Lawrence F Muscarella, *Ivyland*  
Daniel Musher, *Houston*  
Stella Nowicki, *Nashville*  
M Jacques Nsuami, *New Orleans*  
Phillipe N Nyambi, *New York*  
Raymund Rabe Razonable, *Rochester*  
Anand Reddi, *Denver*  
Michael Switow Saag, *Birmingham*  
Danny J Schust, *Columbia*  
William R Schwan, *La Crosse*  
Richard A Slayden, *Fort Collins*  
Theodore J Standiford, *Ann Arbor*  
William M Switzer, *Atlanta*  
Ashutosh Tamhane, *Birmingham*  
Giorgio E Tarchini, *Weston*  
Carmen Taype, *New York*  
Barbara Van Der Pol, *Bloomington*  
Jose Antonio Vazquez, *Detroit*  
Fernando Villalta, *Nashville*  
Haider J Warraich, *Boston*  
Xianfu Wu, *Atlanta*  
Genyan Yang, *Atlanta*  
Frank X Yang, *Indianapolis*  
Hong Zhang, *Rockville*  
Lyna Zhang, *Atlanta*



### **Venezuela**

Alfonso J Rodriguez-Morales, *Caracas*



**MINIREVIEWS**

- 1**      Pneumococcal disease in adult solid organ transplantation recipients  
*Roca-Oporto C, Pachón-Ibáñez ME, Pachón J, Cordero E*

**CASE REPORT**

- 11**     Non chylous filarial ascites: A rare case report  
*Shah KS, Bhate PA, Solanke D, Pandey V, Ingle MA, Kane SV, Sawant P*



## Contents

*World Journal of Clinical Infectious Diseases*  
Volume 5 Number 1 February 25, 2015

### ABOUT COVER

Editorial Board Member of *World Journal of Clinical Infectious Diseases*, Jiunn-Jong Wu, Professor, Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng-Kung University, Tainan 701, Tawian

### AIM AND SCOPE

*World Journal of Clinical Infectious Diseases* (*World J Clin Infect Dis*, *WJCID*, online ISSN 2220-3176, DOI: 10.5495) is a peer-reviewed open access (OA) academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJCID* will focus on a broad spectrum of topics on infectious diseases that will cover epidemiology, immune-pathogenesis, genetic factors, host susceptibility to infection, vector control, novel approaches of treatment, molecular diagnostic and vaccines. It will provide a common stage to share the visions, new approaches, most advanced techniques, and to discuss research problems that will help everyone working in the field of various infections to exchange their views and to improve public health. *WJCID* will also focus on broad range of infections like opportunistic infections, zoonotic infections, tropical and neglected tropical diseases, emerging infections, *etc.* and following topics related to these issues: (1) Causative agents discussing various pathogens; (2) Vectors and Mode of transmission; (3) Host-pathogen interaction and immune-pathogenesis of the disease; (4) Epidemiology of the infection and vector control strategies; (5) Genetic factors covering both host and pathogen; (6) Molecular diagnostic techniques vaccines; and (7) Recent advances in cell tissue culture, lab techniques, *etc.* Various other related fields like medical microbiology, pharmacology of herbs, bioinformatics, *etc.* will be included.

We encourage authors to submit their manuscripts to *WJCID*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

### INDEXING/ABSTRACTING

*World Journal of Clinical Infectious Diseases* is now indexed in Digital Object Identifier.

### FLYLEAF

I-III Editorial Board

### EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Ya-Jing Lu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xin-Xia Song*

NAME OF JOURNAL  
*World Journal of Clinical Infectious Diseases*

ISSN  
ISSN 2220-3176 (online)

LAUNCH DATE  
December 30, 2011

FREQUENCY  
Quarterly

EDITORS-IN-CHIEF  
Shyam Sundar, MD, FRCP (London), FAMS, FNA Sc, FASc, FNA, Professor, Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India

Lihua Xiao, DVM, PhD, Senior Scientist, Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Bldg 23, Rm 9-168, MS D66, 1600 Clifton

Rd, Atlanta, GA 30333, United States

EDITORIAL OFFICE  
Jin-Lei Wang, Director  
Xiu-Xia Song, Vice Director  
*World Journal of Clinical Infectious Diseases*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-85381891  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esp/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esp/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLICATION DATE  
February 25, 2015

COPYRIGHT  
© 2015 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS  
Full instructions are available online at [http://www.wjgnet.com/2220-3176/g\\_info\\_20100722180909.htm](http://www.wjgnet.com/2220-3176/g_info_20100722180909.htm).

ONLINE SUBMISSION  
<http://www.wjgnet.com/esp/>

## Pneumococcal disease in adult solid organ transplantation recipients

Cristina Roca-Oporto, María Eugenia Pachón-Ibañez, Jerónimo Pachón, Elisa Cordero

Cristina Roca-Oporto, María Eugenia Pachón-Ibañez, Jerónimo Pachón, Elisa Cordero, Clinic Unit of Infectious Diseases, Microbiology and Preventive Medicine, Institute of Biomedicine of Seville, University Hospital Virgen del Rocío/CSIC/University of Seville, 41013 Seville, Spain

**Author contributions:** Roca-Oporto C and Pachón J contributed equally to this work, generated the tables and wrote the manuscript; Pachón-Ibañez ME and Cordero E wrote the manuscript; Roca-Oporto C, Pachón-Ibañez ME, Pachón J and Cordero E designed the aim of the minireview.

**Supported by** Ministerio de Economía y Competitividad, Instituto de Salud Carlos III - co-financed by the European Development Regional Fund "A way to achieve Europe" ERDF, and the Spanish Network for the Research in Infectious Diseases, No. REIPI RD12/00015/0001.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Jerónimo Pachón, MD, PhD, Clinic Unit of Infectious Diseases, Microbiology and Preventive Medicine, Institute of Biomedicine of Seville, University Hospital Virgen del Rocío/CSIC/University of Seville, Av. Manuel Siurot s/n, 41013 Seville, Spain. [pachon@us.es](mailto:pachon@us.es)  
**Telephone:** +34-955-012185

**Fax:** +34-955-013292

**Received:** July 24, 2014

**Peer-review started:** July 24, 2014

**First decision:** August 14, 2014

**Revised:** August 26, 2014

**Accepted:** November 7, 2014

**Article in press:** November 10, 2014

**Published online:** February 25, 2015

and mortality ranging from non-invasive to invasive diseases, including pneumonia, bacteremia, and meningitis, with a risk of invasive pneumococcal disease 12 times higher than that observed in non-immunocompromised patients. Moreover, pneumococcal infection has been related to graft dysfunction. Several factors have been involved in the risk of pneumococcal disease in SOT recipients, such as type of transplant, time since transplantation, influenza activity, and nasopharyngeal colonization. Pneumococcal vaccination is recommended for all SOT recipients with 23-valent pneumococcal polysaccharides vaccine. Although immunological rate response is appropriate, it is lower than in the rest of the population, decreases with time, and its clinical efficacy is variable. Booster strategy with 7-valent pneumococcal conjugate vaccine has not shown benefit in this population. Despite its relevance, there are few studies focused on invasive pneumococcal disease in SOT recipients. Further studies addressing clinical, microbiological, and epidemiological data of pneumococcal disease in the transplant setting as well as new strategies for improving the protection of SOT recipients are warranted.

**Key words:** Transplantation; Pneumococcal infections; Pneumococcal serotypes; Nasopharyngeal carriage; Pneumococcal vaccine

© **The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** *Streptococcus pneumoniae* causes substantial morbidity and mortality in solid organ transplant (SOT) recipients, ranging from non-invasive to invasive diseases, with a 12-fold risk of invasive pneumococcal disease higher than in non-immunocompromised patients. Pneumococcal vaccination is recommended for all SOT recipients with 23-valent pneumococcal polysaccharides vaccine. Although immunological rate response is appropriate, it is lower than in the rest of the population, decreases with time, and its clinical

### Abstract

In solid organ transplant (SOT) recipients, *Streptococcus pneumoniae* can cause substantial morbidity

efficacy is variable. Booster strategy with 7-valent pneumococcal conjugate vaccine has not shown benefit in this population. Despite its relevance, robust evidence on pneumococcal disease in organ transplant recipients is lacking. New strategies for improving the protection of SOT recipients are warranted.

Roca-Oporto C, Pachón-Ibañez ME, Pachón J, Cordero E. Pneumococcal disease in adult solid organ transplantation recipients. *World J Clin Infect Dis* 2015; 5(1): 1-10 Available from: URL: <http://www.wjgnet.com/2220-3176/full/v5/i1/1.htm> DOI: <http://dx.doi.org/10.5495/wjcid.v5.i1.1>

## INCIDENCE

*Streptococcus pneumoniae* (*S. pneumoniae*) is the cause of 3300 deaths every year in the United States, mainly among adults<sup>[1]</sup>. According to the United States Active Bacterial Core Surveillance Database of the Emerging Infections Program Network, in 2012 the incidence of invasive pneumococcal disease (IPD) ranged from 2.8 per 100000 persons aged 18-34 years to 29.6 per 100000 among those older than 65 years<sup>[1]</sup>. In the 2013 Annual Epidemiological Report of the European Centers for Disease Prevention and Control (ECDC) the incidence of IPD in Europe was even higher: 3.8 per 100000 persons per year<sup>[2]</sup>. Invasive pneumococcal disease is an important cause of illness in patients with certain underlying medical conditions<sup>[3]</sup> or demographic risk factors, including age < 2 or ≥ 65 years, chronic diseases, alcohol abuse, smoking, and immunosuppressive conditions such as human immunodeficiency virus (HIV) infection<sup>[4]</sup>, multiple myeloma, or solid organ transplantation (SOT)<sup>[5-10]</sup>. The disease rates for adults with high-risk factors can be more than 20 times that those for adults without high-risk medical conditions. Middle-aged patients with hematological cancer have a rate of IPD of 186 per 100000 persons per year during 2010 and HIV infected patients have a rate of IPD of 173 per 100000 persons per year<sup>[11]</sup>.

In solid organ transplant recipients, *S. pneumoniae* can cause substantial morbidity and mortality, ranging from no invasive diseases such as otitis media, sinusitis, and non-bacteremic pneumonia to invasive diseases, including bacteremic pneumonia and meningitis<sup>[9]</sup>. Data from the literature on SOT recipients are referred almost exclusively to the most severe spectrum of disease. Invasive pneumococcal disease is defined as an isolation of *S. pneumoniae* from a sterile body fluid with a compatible clinical syndrome<sup>[12]</sup>. Sterile sites included blood, cerebrospinal fluid, peritoneal fluid, pleural fluid, or needle aspiration of a collection. Sputum or bronchoalveolar lavage isolates are not considered sterile-site isolates in some studies<sup>[13]</sup>. The risk of developing invasive pneumococcal disease in these

patients has been estimated in studies carried out in the 70s<sup>[14]</sup>, 80s<sup>[15]</sup>, and 90s<sup>[16]</sup> to be 2800 per 100000 patient-years in a cohort of kidney transplant recipients<sup>[14]</sup>, 3600 per 100000 patient-years in heart transplant recipients<sup>[15]</sup>, and 2270 per 100000 patient-years in lung transplant recipients<sup>[16]</sup>. The first two studies were conducted in the pre-vaccine era, justifying higher incidence rates, while in the latter all lung transplant recipients had received pneumococcal vaccine before transplantation. Moreover, in one of the studies most of the kidney recipients were splenectomized, due to the belief at that time that such procedure prolonged graft survival, thus increasing expected IPD cases<sup>[14]</sup>. However, it is difficult to compare the findings of these different studies, due to the disparity of vaccination rates, types of organ transplantation, and immunosuppression protocols according to the time when the studies were carried out. A recent study prospectively determined the incidence of IPD in a population of adult SOT recipients over a period of almost 10 years, with an incidence of 146 per 100000 persons per year, 12.8 times greater than that in the general population. Specific incidences for kidney, lung, and liver transplant recipients were 104, 239, and 354 per 100000 transplant recipients per year, respectively, compared to 11.5 cases per 100000 person-years in the general population. Interestingly, there were no cases of IPD in heart and pancreas transplant recipients<sup>[9]</sup>.

Non-bacteremic pneumococcal pneumonia is the most relevant non-IPD among SOT recipients, due to its morbidity and mortality. *S. pneumoniae* is the second or third leading cause of late community-acquired pneumonia in solid organ transplantation<sup>[17-21]</sup>. As in other infections, pneumococcal pneumonia incidence depends on the type of SOT, being more frequent in lung transplant recipients (3262 per 100000 persons-year) followed by kidney (344 per 100000 persons-year) and liver recipients (304 per 100000 persons-year)<sup>[9]</sup>.

## RISK FACTORS OF PNEUMOCOCCAL DISEASE

Several risk factors of pneumococcal disease in SOT recipients have been described. The type of organ transplanted is one of the most important, as previously stated. Liver transplant recipients have an increased risk of IPD, while lung recipients have the highest rates of pneumococcal pneumonia, followed by heart and kidney recipients<sup>[9,16,21]</sup>.

Time since transplantation is another factor to be considered. The median time since transplantation to the onset of pneumococcal infection is 1.3 to 2.7 years<sup>[9,16,21]</sup>. Although Amber *et al.*<sup>[15]</sup>, in a study carried out in 5 heart recipients, found that the median time for IPD was 58 d (range 1-5 mo), in



most of the studies pneumococcal disease occurs after the first 4 to 6 mo from the transplantation<sup>[9,16]</sup>. In a prospective multicenter study carried out in Canada, 57% of the cases were diagnosed during the first three years since transplantation, while one fifth of them occurred after the first 10 years of the transplant<sup>[9]</sup>.

Although the sizes of most of the studies published are underpowered to establish statistical associations, the type of immunosuppressors has not been related to an increased risk of pneumococcal disease<sup>[9,14-16]</sup>. Neither has been found an increased risk of hospital mortality in patients with classic risk factors in the general population, as age, diabetes mellitus, chronic renal disease, or splenectomy<sup>[9]</sup>. Probably, the small number of the studies in the literature in this group of immunosuppressed patients and their heterogeneity do not allow drawing appropriate conclusions.

The role of universal prophylaxis with trimethoprim-sulfamethoxazole (TMP/SMX) in the prevention of pneumococcal disease in SOT recipients has not been established. Use of TMP/SMX prophylaxis at the moment of pneumococcal disease diagnosis is common among SOT recipients, with a frequency that ranges from 14% to 100% of the cases. However, the rate of TMP/SMX resistant isolates in these patients is high (66% to 71%)<sup>[9,16]</sup>. This high rate of resistance to TMP/SMX invalidates this measure to prevent pneumococcal disease.

Nasopharyngeal colonization is a preliminary step in the development of pneumococcal disease, with transmission only coming from other human carriers<sup>[22]</sup>. Pneumococcal colonization has been mainly studied in children, as the highest rates of colonization are found during childhood. As children grow older, the prevalence of pneumococcal carriage decreases, and the distribution of colonizing serotypes changes. With the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) the colonization rate in children has been reduced from 50%-70%<sup>[23-26]</sup> to 21%-33% in the United States and Europe<sup>[27-30]</sup>. Pneumococcal colonization studies carried out in children have increased the knowledge about the rate of colonization, the circulating serotypes, the serotypes (mostly encapsulated) related to invasive disease, the expected pattern of resistance and, specially, the effectiveness of the recently introduced new pneumococcal vaccines, offering a better understanding of the pneumococcal disease in the children population<sup>[2,31-33]</sup>.

There are very few data regarding *S. pneumoniae* nasopharyngeal carriage in healthy adults, showing colonization rates of 4%-13%<sup>[25,34]</sup>, depending on the geographic area of the population studied<sup>[23,25,26,35-37]</sup>, and the presence of underlying diseases. Patients with asthma, mainly treated with inhaled corticosteroids, have a higher risk of *S. pneumoniae* colonization than the healthy population<sup>[6,38-40]</sup>. In immunosuppressed

patients, the information available is scarce. In HIV-infected adults, the frequency of pneumococcal colonization ranges from 3.4% in United States, to 17% in Brazil and 52% in Malawi<sup>[34,41,42]</sup>. No information of pneumococcal colonization in SOT recipients is available.

Influenza activity has been associated with significant increases in the incidence of invasive pneumococcal pneumonia, both in children and adults. The association was more pronounced among younger adults without co-morbidities<sup>[43]</sup>. In non-immunocompromised patients, influenza is associated with the greatest increase in the incidence of bacteremic pneumococcal pneumonia caused by serotypes with lower invasive potential. The importance of influenza for adult bacteremic pneumococcal pneumonia varies by serotype and host co-morbidity<sup>[44]</sup>. Although there are no studies that address the impact of influenza activity in the incidence of IPD in the transplant setting, it is plausible that there might be a relationship between both diseases.

One of the most important virulence factors of *S. pneumoniae* is the polysaccharide capsule. Chemical and antigenic differences in this capsule result in more than 90 different capsular types or serotypes, 20 of which cause the majority of invasive disease<sup>[45,46]</sup>. According to the results of a meta-analysis of adults with pneumococcal bacteremic pneumonia, the risk of death varies with the serotype and is stable among studies across time and in diverse geographic locations. Patients with pneumonia caused by serotypes 3, 6A, 6B, 9N, and 19F died more frequently than those caused by serotype 14. In contrast, patients infected with serotypes 1, 7F, and 8 were significantly less likely to die than those infected with serotype 14<sup>[31]</sup>. In a study carried out in Spain, during 2012, the most frequent serotypes linked to IPD in the general population were 1, 3, 7F, 19A, 12F, 14, 22F, 24F, 8, 9A, 4, 10A, and 11A<sup>[46]</sup>. In Europe, in adults older 15 years old, the ten most common serotypes of IPD in 2011 ordered by frequency were 7F, 19A, 3, 1, 22F, 8, 14, 12F, 6C and 4, accounting for 61.5% of the typed isolates<sup>[2]</sup>.

In SOT recipients, serotypes distribution in invasive pneumococcal disease is slightly different from general population. Kumar *et al*<sup>[9]</sup> reported that the most frequent serotypes in 21 cases of recent cases of IPD were 23F (4/21, 19%), 22F (3/21, 14.2%) and 19F (2/21, 9.5%), followed by at least one case of 4, 6A, 6B, 8, 9V, 11, 13, 14, 18C and 35V. De Bruyn *et al*<sup>[16]</sup> also reported serotype 23F as the most frequent in the IPD occurring in lung transplant recipients (27.2%), followed by 19A (18.1%), 6A/B (18.1%), and at least one case of 3, 4 and 9V. In these more recent studies, serotype of dead patients because of IPD was not specified.

**Table 1 Outcome of invasive pneumococcal disease in solid organ transplantation recipients**

Ref.	n	Inclusion period	Organ	Time since transplant median (range)	Type of IPD	Prior vaccination	Most frequent isolated serotypes	Graft dysfunction/loss	Mortality
Linnemann <i>et al</i> <sup>[14]</sup>	12	1971-1977	Kidney	No data	Pneumonia: 83% Bacteremia: 41% Meningitis: 16%	No	8, 9, 12, 24	75%/22.2%	8.30% (1/12)
Amber <i>et al</i> <sup>[15]</sup>	5	1985-1987	Heart	0.15 yr (1.2-4.4 mo)	Pneumonia: 80% Bacteremia: 20%	No	3	20%/No data	0%
de Bruyn <i>et al</i> <sup>[16]</sup>	14	1992-2003	Lung	1.3 yr (9.6 mo-2.3 yr)	Pneumonia: 78% Bacteremia: 21%	100%	23F, 19A, 6	No data	7.10% (1/14)
Kumar <i>et al</i> <sup>[9]</sup>	21	1995-2004	Kidney: 11 Liver: 9 Lung: 1	2.7 yr (1.3 mo-23.8 yr)	Pneumonia: 57% Bacteremia: 33% Peritonitis: 5% Parotitis: 5%	23.80%	23F, 22F, 19F	No data	28.60% (6/21)

IPD: Invasive pneumococcal disease.

## CLINICAL MANIFESTATIONS

The clinical features of invasive pneumococcal disease in SOT recipients are similar to those of the general population. Bacteremic pneumococcal pneumonia is the most frequent manifestation followed by primary bacteremia and meningitis<sup>[9,14-16]</sup>. Kumar *et al*<sup>[9]</sup> reported an incidence of 90.5% cases of bacteremia (19/21), 57.1% cases of pneumonia (12/21), and 33.3% cases of primary bacteremia (7/21), with no cases of meningitis. These proportions were similar to those described by de Bruyn *et al*<sup>[16]</sup> and Linnemann *et al*<sup>[14]</sup>, reported 83.3% of pneumonia, 41.6% of bacteremia and 16.6% of meningitis in kidney recipients during the 80s. In the general population in United States, the frequency of bacteremic pneumonia is 69.1%, primary bacteremia 16.8% and meningitis 7%<sup>[1]</sup>. By contrast, in the European general population, there is a lower rate of bacteremic pneumonia (48%), with higher rate of primary bacteremia (29%), and meningitis (18%)<sup>[33]</sup>. Other rare manifestations of IPD has been previously described in case reports or case series of SOT recipients, including peritonitis or parotitis<sup>[9]</sup>, endocarditis, spondylitis, and muscle abscess<sup>[47]</sup>, and necrotizing fasciitis<sup>[48]</sup>. Despite its low frequency, reports of sporadic cases highlight an important clinical aspect: *S. pneumoniae* may cause suppurate infection in virtually any anatomic location, with or without prior detected bacteremia.

## IMPACT ON PATIENT AND GRAFT SURVIVAL

Invasive pneumococcal disease results in significant morbidity and mortality. Kumar *et al*<sup>[9]</sup> reported an in-hospital mortality due to IPD in SOT recipients of 28.6% (6/21), 66.7% (4/6) of them directly attributable to pneumococcal infection. Although they did not find statistically differences of mortality among SOT recipients and non-immunosuppressed patients (28.6% vs 17.8%), this mortality is higher

than that reported in most of the studies carried out in general populations, where the mortality due to pneumococcal bacteremia ranges from 12% to 17%<sup>[49,50]</sup>.

Length of hospitalization is also higher in SOT recipients (16.2 d vs 11.6 d), probably related to long antibiotics course and complications<sup>[9,16,50]</sup>. de Bruyn *et al*<sup>[16]</sup> reported more severe complications, requiring mechanical ventilation, in the group of lung transplant recipients who developed bacteremic pneumococcal pneumonia compared with those with non-bacteremic pneumococcal pneumonia. Allograft dysfunction has been associated to pneumococcal infection, as observed with other infections in SOT recipients<sup>[51]</sup>. Linnemann *et al*<sup>[14]</sup> described a rate of allograft dysfunction in renal transplant recipients with IPD as high as 75% (9/12), with a 22% of cases presenting definitive graft loss (2/9). Amber *et al*<sup>[15]</sup> also reported a 20% (1/5) of allograft dysfunction after IPD, in addition to two cases of graft dysfunction treated with increased immunosuppression and complicated with IPD within 20 d. However, graft dysfunction has not been analyzed in the most recent studies of IPD in SOT recipients<sup>[9,16]</sup>. Therefore, data available of graft dysfunction are referred exclusively to older studies carried out in heart and kidney transplant recipients and on a small number of patients (Table 1). New prospective studies are required to confirm these data and to analyze the risk of rejection and graft dysfunction in SOT recipients after an episode of IPD.

## VACCINATION

Knowledge of the most invasive serotypes allowed the development of the first pneumococcal vaccine. In 1983, a 23-valent pneumococcal polysaccharide vaccine (PPV23) was approved, expanding serotype coverage to more than 85% of the organisms causing IPD<sup>[52]</sup>. This vaccine induces a humoral immune response through the production of specific antibodies, but do not prompt immunological

memory. This aspect, added to the fact that PPV23 induced poor T-cell-independent immunogenicity in infants led to development of the pneumococcal conjugate vaccine<sup>[52]</sup>. The pneumococcal conjugate vaccine consists of capsular polysaccharides from the most common serotypes that cause IPD along with a carrier protein, and induce a T-cell-dependent immune response, so that they are able to produce immunological memory and greater immunogenic response than that produced by the PPV23<sup>[53]</sup>. Another advantage of pneumococcal conjugate vaccine is that stimulates mucosal immunity, resulting in decreased naso-pharynx colonization and also exhibits secondary protection of unvaccinated individuals. Pneumococcal conjugate vaccine (PCV) was initially approved in children less than two years while PPV23 was recommended for children over two years, adults and immunocompromised patients, including SOT recipients.

The first PCV7 was licensed in the United States in 2000 and introduced in Europe in 2001. This vaccine included capsular polysaccharides of serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, representing approximately 80%-90% of IPD in children. With serotype shifts resulting from vaccine pressure, the protective coverage of PCV7 was reduced, as this vaccine did not include serotypes 1, 3, and 5, which are common in Europe, Asia, and Africa. Therefore, a 13-valent conjugate vaccine (PCV13) including serotypes 1, 3, 5, 6A, 7F, and 19A, was licensed in 2009 in Europe. In 2010, the theoretical vaccine preventable proportion of cases using PCV7, PCV10 and PCV13 in children under five years was 19.2%, 46.1% and 73.1% respectively<sup>[33]</sup>.

The use of pneumococcal vaccination determined a reduction in the number of cases of invasive and non-invasive pneumococcal disease in children and adults. Several randomized trials and meta-analyses concluded that PPV23 prevents pneumococcal disease in adult population<sup>[54-56]</sup>. Other studies suggest that PPV23 protects against invasive infection but not from non bacteremic pneumococcal pneumonia<sup>[56-58]</sup> and it did not affect survival<sup>[54]</sup>. However, in most of these studies the etiology of community-acquired pneumonia was not confirmed, assuming the expected higher frequency of pneumococcal etiology. In general, studies with more specific endpoints as IPD caused by vaccine serotypes have been more likely to demonstrate efficacy than studies with less specific ones.

In immunosuppressed population, data are scarce and heterogeneous. In SOT recipients, vaccine efficacy is derived from studies conducted mostly in kidney transplant recipients<sup>[59-65]</sup> (Table 2), with less information available in heart<sup>[15,66,67]</sup> and liver transplant recipients<sup>[68]</sup> (Table 3). Moreover, most studies of vaccination in SOT were performed with the old 14-serotypes polysaccharide vaccine (previous to the introduction of the PPV23 in 1980s) and with

different methods for detecting immunity, such as radioimmunoassay. Nevertheless, practically all studies agrees that pneumococcal vaccine in SOT recipients is effective, but the immune response is weaker than in healthy controls and that there is a greater loss of antibodies over time, especially in the first months after the transplant<sup>[59-62,64,65]</sup>. Studies carried out in SOT recipients with the PPV23 vaccine yield similar conclusions<sup>[66,68-70]</sup>: there was an adequate post-vaccine response but the antibody titers decreased from the sixth month after vaccination<sup>[68,70]</sup>. This decrease in the geometric mean titer of antibodies also occurs after the naturally acquired pneumococcal immunity after transplantation<sup>[15]</sup>. Interestingly, Blumberg *et al.*<sup>[67]</sup> evaluated the impact of prior antibody titers and its relationship with the elicited immune response. In this study, patients with previous antibody titers, although low, obtained better post-vaccine titers after receiving a second vaccine dose (Tables 2 and 3). It is important to emphasize that in all the studies regarding pneumococcal vaccination in SOT recipients, the vaccine was safe, regardless of its effectiveness.

Globally, the knowledge gained from the use of the polysaccharide vaccine in adults reveals that new vaccine strategies are needed to increase vaccine efficacy, especially in vulnerable populations, such as the elderly and immunocompromised patients. In this sense, testing the hypothetical usefulness of the conjugate vaccine (typically used in children) in adult population was performed, supported by the previously discussed benefits. A single study carried out in HIV-infected patients, most of them without antiretroviral therapy, reported protection against pneumococcal disease with PCV7, but not with the polysaccharide vaccine.

Based on these observations, in 2012, the Advisory Committee on Immunization Practices (ACIP) recommended routine PCV13 use for adults aged  $\geq 19$  years with immune compromise conditions, including SOT recipients, functional or anatomic asplenia, cerebrospinal fluid leaks, or cochlear implants. This vaccine should be administered to eligible adults but in addition to PPV23, the vaccine currently recommended for these groups of adults<sup>[71,72]</sup> and SOT recipients, specifically<sup>[73,74]</sup>. This strategy appears to be a cost-effective vaccine policy<sup>[75]</sup>.

However, according to the 2013 ECDC surveillance report, the emergence of non-vaccine serotypes remains an important issue, and continued monitoring in Europe is essential for assessing interventions and informing the development of new vaccines<sup>[2]</sup>. This might be especially relevant in the transplant setting. Immunosuppressed patients can have a different serotype distribution than the general population. In a recent study, carried out in Spain, serotypes not included in the PCV13 and

**Table 2** Pneumococcal vaccination studies in kidney transplantation in adult recipients

Ref.	N	Time since transplant	Vaccine	Technique	Response to immunization	Long term immune response
Silberman <i>et al</i> <sup>[59]</sup>	27 KT vs 24 healthy adults	7-108 mo	14-valent	Indirect hemagglutination	4-fold increase in titers, equivalent to controls	Not studied
Marrie <i>et al</i> <sup>[60]</sup>	63 KT vs 8 healthy adults	3-99 mo	14-valent	RIA	2.8-fold increase in titers in KT vs 3.4-fold in controls	GMT in KT: 872 ng N/mL post-vaccine 659 ng N/mL at 12 mo
Cosio <i>et al</i> <sup>[61]</sup>	25 KT (14 asplenia) vs 14 healthy adults	3-118 mo	14-valent	RIA	2-fold increase in titers in 93% of 14 healthy adults vs 78% of 14 splenectomized KT and 55% of 11 non-splenectomized KT patients	Not studied
Linneman <i>et al</i> <sup>[62,63]</sup>	104 KT (79 asplenia) vs 33 patients in hemodialysis	No data	14-valent	RIA	1.4-fold increase in antibody titers: 91% GMT post-vaccine with lower response in splenectomized KT vs non-splenectomized KT patients (394 vs 626 ng N/mL, $P < 0.05$ ) GMT post-vaccine with lower response in KT patients before 6 mo after transplant vs hemodialysis patients (303 vs 592 ng N/mL, $P < 0.05$ )	GMT at 24 mo in 33 KT: 932 ng N/mL post-vaccine 385 ng N/mL at 24 mo 536 ng N/mL at revaccination
Arnold <i>et al</i> <sup>[64]</sup>	75 KT (32 asplenia) vs 60 healthy controls	> 12 mo	14-valent	Serum opsonizing antibody	2-fold increase in titers to serotypes 12F and 14 No differences in splenectomized vs non-splenectomized KT recipients	Not studied
Rytel <i>et al</i> <sup>[65]</sup>	61 KT (57 asplenia) vs 23 patients in dialysis vs 9 healthy controls	> 6 mo	14-valent	RIA	Equivalent protective GMT titers vs controls	Equivalent at 1, 2, and 3.5 yr vs controls
Kazancioglu <i>et al</i> <sup>[69]</sup>	21 KT vs 25 healthy controls	> 2 mo	PPV23	ELISA	Protective response to vaccination (40% increases in the antibody concentration) in 95.2% of KT at 1.5 and 3 mo after vaccination	Not studied
Kumar <i>et al</i> <sup>[78,79]</sup>	30 KT (PPV23) vs 30 KT (PCV7)	3 mo-3 yr	PPV23 vs PCV7	ELISA, OPA	Response to at least 1 serotype: no significant differences in antibody titers between PPV23 and PVC7, both using ELISA (53.3% vs 73.3%) and OPA (83.3% vs 80%)	Vaccine responses decline significantly after 3 yr and conjugate vaccine does not improve the durability of response

KT: Kidney transplantation; PPV23: 23-valent pneumococcal polysaccharide vaccine; PCV7: 7-valent pneumococcal conjugate vaccine; RIA: Radioimmunoassay; ELISA: Enzyme-Linked ImmunoSorbent Assay; OPA: Opsonophagocytic assay; GMT: Geometric Mean Titer.

PPV23 formulations were more frequently isolated in patients with IPD and cardio-respiratory comorbidities or immunosuppression, including SOT recipients. Indeed, three serotypes (10A, 11A, and 33F), not included in the PCV13 formulation, were the most frequently isolated in immunocompromised patients with IPD, although their prevalence in the complete cohort was low<sup>[8]</sup>. According to Kumar *et al*<sup>[9]</sup>, only 23.8% of the SOT recipients with IPD had been previously vaccinated with PPV23. In this study, 65% of transplant recipients had disease due to pneumococcus serotypes included in the PCV13 and 85% of them had disease due to serotypes included in the PPV23<sup>[9]</sup>.

Clinical failure of pneumococcal vaccine is common in the transplant setting. Thus, 24% to 100% of the SOT recipients with pneumococcal disease had being vaccinated<sup>[9,16]</sup>. One possible explanation could be the different pneumococcal serotypes distribution observed in the transplant setting and the proportion of serotypes not included

in the pneumococcal vaccine. Other aspect to consider is the reduction in the effectiveness of pre-transplant pneumococcal vaccine in the context of end-organ disease previous to the transplant, as kidney, liver or heart disease, and in the post-transplant due to the use of immunosuppressors necessary to prevent graft rejection, as observed in a study, where all SOT recipients with IPD had received the PPV23 vaccination with all the serotypes producing pneumococcal disease covered by the vaccine<sup>[16]</sup>. The required post-transplant immunosuppression may cause progressive decrease of the levels of antibody previously achieved, like other immunizations in SOT recipients, as influenza<sup>[76]</sup>.

New strategies have been proposed to enhance the immunogenic response and clinical efficacy obtained by PPV23 in SOT recipients, which include revaccination, use of pneumococcal conjugate vaccine, or "booster" strategy. Revaccination with polysaccharide antigens does not elicit a suitable



**Table 3** Pneumococcal vaccination studies in heart and liver transplantation in adult recipients

Ref.	n	Type and time since transplant	Vaccine	Technique	Response to immunization	Long term immune response
McCash-land <i>et al</i> <sup>[68]</sup>	25 LT vs 13 healthy controls	LT 1 - 6 mo	PPV23	ELISA	Pneumococcal antibody levels were significantly increased over baseline by 1 mo after vaccination in both groups	At 6th mo: antibody levels declined faster in patients than in control subjects
Amber <i>et al</i> <sup>[15]</sup>	6 HT before and after transplantation	HT 0.6 - 5.3 mo	Unvaccinated	RIA	Protective antibody titers to 12 pneumococcal serotypes contained in PPV23 in a mean of $8.7 \pm 1.2$ serotypes before transplantation vs $6.5 \pm 1.4$ serotypes after transplantation ( $P < 0.05$ )	Not studied
Dengler <i>et al</i> <sup>[66]</sup>	16 HT vs 23 healthy controls	HT > 12 mo	PPV23	ELISA	Protective post-vaccine antibody titers ( $> 1000$ U/mL): 94% in HT recipients vs 100% in controls	Not studied
Blumberg <i>et al</i> <sup>[67]</sup>	35 HT vs 35 healthy controls. Group 2 ( $n = 21$ ), vaccinated before this study The HT patients were classified as: Group 1 ( $n = 11$ ), no vaccinated before this study No data about previous vaccine ( $n = 2$ )	HT 55 - 122 mo	PPV23	ELISA	Post-vaccine antibody titers were higher in group 2 than in group 1 for all pneumococcal serotypes ( $P < 0.05$ for all serotypes, except 3)	Detectable antibody titers at 24 mo (only 7 available patients) in 50% to serotypes 19F and 23F and in 80% to serotype 3

LT: Liver transplantation; HT: Heart transplantation; PPV23: 23-valent pneumococcal polysaccharide vaccine; ELISA: Enzyme-Linked ImmunoSorbent Assay; RIA: Radioimmunoassay.

memory response in healthy people<sup>[77]</sup> and kidney recipients respond less vigorously to revaccination than to primary immunization<sup>[63]</sup>. Trials comparing PCV7 with PPV23 in renal transplant recipients have not succeeded in achieving differences in immunogenicity between these vaccines<sup>[78,79]</sup>. Furthermore, the recommendation made by the ACIP of a prime-boost strategy (PCV13 followed by PPV23 8 wk later) in adult > 50 years old and in immunocompromised patients has been evaluated in SOT recipients with unfavorable results. Kumar *et al*<sup>[80]</sup> randomized 130 adult liver transplant recipients to receive either PCV7 followed by a PPV23 booster 8 wk later (the "primed" group) or placebo followed by a standard single dose of PPV23 (the "unprimed" group, as usual practice). There was no difference in immune response between the two groups. More recently, Tobudic *et al*<sup>[81]</sup> randomized 80 kidney transplants recipients to received either PCV7 or PPV23 followed by PPV23 the following year. There was no benefit in either of the sequential regimens when compared with single-dose PPV23 vaccination as recommended by the guidelines.

## CONCLUSION

*S. pneumoniae* can cause substantial morbidity and mortality in SOT recipients, ranging from non-invasive to invasive diseases, including bacteremic pneumonia and meningitis, with a 12-fold risk of IPD higher than in non-immunocompromised patients. Despite its relevance, there are few studies focused on IPD in SOT recipients. Pneumococcal vaccination

is recommended for all SOT recipients with PPV23. Although immunological rate response is appropriate, it is lower than that in the rest of the population, decreases with time, and its clinical efficacy is variable. Booster strategy with PCV7 has not shown benefit in this population. Further studies addressing clinical, microbiological, and epidemiological data of pneumococcal disease in the transplant setting as well as new strategies for improving the protection of SOT recipients are warranted.

## REFERENCES

- Centers for Disease Control and Prevention (CDC). Active Bacterial Core Surveillance Report, Emerging Infections Program Network, *Streptococcus pneumoniae*, 2010. USA: CDC, 2011
- European Centre for Disease Prevention and Control. Annual Epidemiological Report 2013. Reporting on 2011 surveillance data and 2012 epidemic intelligence data. Stockholm: ECDC, 2013
- Lipsky BA, Boyko EJ, Inui TS, Koepsell TD. Risk factors for acquiring pneumococcal infections. *Arch Intern Med* 1986; **146**: 2179-2185 [PMID: 3778047 DOI: 10.1001/archinte.1986.00360230105016]
- McEllistrem MC, Mendelsohn AB, Pass MA, Elliott JA, Whitney CG, Kolano JA, Harrison LH. Recurrent invasive pneumococcal disease in individuals with human immunodeficiency virus infection. *J Infect Dis* 2002; **185**: 1364-1368 [PMID: 12001059 DOI: 10.1086/339882]
- Gentile JH, Sparo MD, Mercapide ME, Luna CM. Adult bacteremic pneumococcal pneumonia acquired in the community. A prospective study on 101 patients. *Medicina (B Aires)* 2003; **63**: 9-14 [PMID: 12673954]
- Talbot TR, Hartert TV, Mitchel E, Halasa NB, Arbogast PG, Poehling KA, Schaffner W, Craig AS, Griffin MR. Asthma as a risk factor for invasive pneumococcal disease. *N Engl J Med* 2005; **352**: 2082-2090 [PMID: 15901861 DOI: 10.1056/NEJMoa044113]
- Chi RC, Jackson LA, Neuzil KM. Characteristics and outcomes of



- older adults with community-acquired pneumococcal bacteremia. *J Am Geriatr Soc* 2006; **54**: 115-120 [PMID: 16420207 DOI: 10.1111/j.1532-5415.2005.00528.x]
- 8 **Luján M**, Burgos J, Gallego M, Falcó V, Bermudo G, Planes A, Fontanals D, Peghin M, Monsó E, Rello J. Effects of immunocompromise and comorbidities on pneumococcal serotypes causing invasive respiratory infection in adults: implications for vaccine strategies. *Clin Infect Dis* 2013; **57**: 1722-1730 [PMID: 24065334 DOI: 10.1093/cid/cit640]
  - 9 **Kumar D**, Humar A, Plevneshi A, Green K, Prasad GV, Siegal D, McGeer A. Invasive pneumococcal disease in solid organ transplant recipients--10-year prospective population surveillance. *Am J Transplant* 2007; **7**: 1209-1214 [PMID: 17286615 DOI: 10.1111/j.1600-6143.2006.01705.x]
  - 10 **Cordero E**, Pachón J, Rivero A, Girón JA, Gómez-Mateos J, Merino MD, Torres-Tortosa M, González-Serrano M, Aliaga L, Collado A, Hernández-Quero J, Barrera A, Nuño E. Community-acquired bacterial pneumonia in human immunodeficiency virus-infected patients: validation of severity criteria. The Grupo Andaluz para el Estudio de las Enfermedades Infecciosas. *Am J Respir Crit Care Med* 2000; **162**: 2063-2068 [PMID: 11112115 DOI: 10.1164/ajrccm.162.6.9910104]
  - 11 **Centers for Disease Control and Prevention (CDC)**. Use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine for adults with immunocompromising conditions: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2012; **61**: 816-819 [PMID: 23051612]
  - 12 Decision 2002/253/EC laying down case definitions for reporting communicable diseases to the Community network under Decision No 2119/98/EC of the European Parliament and of the Council. *OJEU* 2008; **159**: 46-90
  - 13 **Gill VJ**, Fedorko DP, Witebsky FG. The clinician and the microbiology laboratory. In: Principles and Practice of Infectious Diseases. Mandel GL, Bennett JE, Dolin R, editors. Churchill: Livingstone; 2005: 203-241
  - 14 **Linnemann CC**, First MR. Risk of pneumococcal infections in renal transplant patients. *JAMA* 1979; **241**: 2619-2621 [PMID: 374767 DOI: 10.1001/jama.1979.03290500027016]
  - 15 **Amber IJ**, Gilbert EM, Schiffman G, Jacobson JA. Increased risk of pneumococcal infections in cardiac transplant recipients. *Transplantation* 1990; **49**: 122-125 [PMID: 2301002 DOI: 10.1097/00007890-199001000-00027]
  - 16 **de Bruyn G**, Whelan TP, Mulligan MS, Raghu G, Limaye AP. Invasive pneumococcal infections in adult lung transplant recipients. *Am J Transplant* 2004; **4**: 1366-1371 [PMID: 15268742 DOI: 10.1111/j.1600-6143.2004.00512.x]
  - 17 **Eyüboğlu FÖ**, Küpeli E, Bozbaş SS, Ozen ZE, Akkurt ES, Aydoğan C, Ulubay G, Akçay S, Akbaş M. Evaluation of pulmonary infections in solid organ transplant patients: 12 years of experience. *Transplant Proc* 2013; **45**: 3458-3461 [PMID: 24314931 DOI: 10.1016/j.transproceed.2013.09.024]
  - 18 **Cervera C**, Agustí C, Angeles Marcos M, Pumarola T, Cofán F, Navasa M, Pérez-Villa F, Torres A, Moreno A. Microbiologic features and outcome of pneumonia in transplanted patients. *Diagn Microbiol Infect Dis* 2006; **55**: 47-54 [PMID: 16500066 DOI: 10.1016/j.diagmicrobio.2005.10.014]
  - 19 **Cisneros JM**, Muñoz P, Torre-Cisneros J, Gurgui M, Rodriguez-Hernandez MJ, Aguado JM, Echaniz A. Pneumonia after heart transplantation: a multi-institutional study. Spanish Transplantation Infection Study Group. *Clin Infect Dis* 1998; **27**: 324-331 [PMID: 9709883 DOI: 10.1086/514649]
  - 20 **Fishman JA**, Rubin RH. Infection in organ-transplant recipients. *N Engl J Med* 1998; **338**: 1741-1751 [PMID: 9624195 DOI: 10.1056/NEJM199806113382407]
  - 21 **Giannella M**, Muñoz P, Alarcón JM, Mularoni A, Grossi P, Bouza E. Pneumonia in solid organ transplant recipients: a prospective multicenter study. *Transpl Infect Dis* 2014; **16**: 232-241 [PMID: 24593292 DOI: 10.1111/tid.12193]
  - 22 **van der Poll T**, Opal SM. Pathogenesis, treatment, and prevention of pneumococcal pneumonia. *Lancet* 2009; **374**: 1543-1556 [PMID: 19880020 DOI: 10.1016/S0140-6736(09)61114-4]
  - 23 **Bogaert D**, De Groot R, Hermans PW. Streptococcus pneumoniae colonisation: the key to pneumococcal disease. *Lancet Infect Dis* 2004; **4**: 144-154 [PMID: 14998500 DOI: 10.1016/S1473-3099(04)00938-7]
  - 24 **Greenberg D**, Broides A, Blancovich I, Peled N, Givon-Lavi N, Dagan R. Relative importance of nasopharyngeal versus oropharyngeal sampling for isolation of Streptococcus pneumoniae and Haemophilus influenzae from healthy and sick individuals varies with age. *J Clin Microbiol* 2004; **42**: 4604-4609 [PMID: 15472316 DOI: 10.1128/JCM.42.10.4604-4609.2004]
  - 25 **Regev-Yochay G**, Raz M, Dagan R, Porat N, Shainberg B, Pinco E, Keller N, Rubinstein E. Nasopharyngeal carriage of Streptococcus pneumoniae by adults and children in community and family settings. *Clin Infect Dis* 2004; **38**: 632-639 [PMID: 14986245 DOI: 10.1086/381547]
  - 26 **Neves FP**, Pinto TC, Corrêa MA, dos Anjos Barreto R, de Souza Gouveia Moreira L, Rodrigues HG, Cardoso CA, Barros RR, Teixeira LM. Nasopharyngeal carriage, serotype distribution and antimicrobial resistance of Streptococcus pneumoniae among children from Brazil before the introduction of the 10-valent conjugate vaccine. *BMC Infect Dis* 2013; **13**: 318 [PMID: 23849314 DOI: 10.1186/1471-2334-13-318]
  - 27 **Özdemir H**, Çiftçi E, Durmaz R, Güriz H, Aysev AD, Karbuz A, Gökdemir R, Acar B, Nar-Ötgin S, Ertek M, Köse SK, İnce E. Risk factors for nasopharyngeal carriage of Streptococcus pneumoniae in healthy Turkish children after the addition of heptavalent pneumococcal conjugate vaccine (PCV7) to the national vaccine schedule. *Turk J Pediatr* 2013; **55**: 575-583 [PMID: 24577974 DOI: 10.1007/s00431-013-2156-7]
  - 28 **Wroe PC**, Lee GM, Finkelstein JA, Pelton SI, Hanage WP, Lipsitch M, Stevenson AE, Rifas-Shiman SL, Kleinman K, Dutta-Linn MM, Hinrichsen VL, Lakoma M, Huang SS. Pneumococcal carriage and antibiotic resistance in young children before 13-valent conjugate vaccine. *Pediatr Infect Dis J* 2012; **31**: 249-254 [PMID: 22173142 DOI: 10.1097/INF.0b013e31824214ac]
  - 29 **García-Vera C**, Ruiz Andrés MÁ, Arana Navarro T, Moneo Hernández I, Castillo Laita JA, Macepe Costa R, Revillo Pinilla MJ. [Nasopharyngeal carriage of pneumococcal serotypes in healthy pre-school aged children after 7-valent pneumococcal vaccine]. *Med Clin (Barc)* 2011; **137**: 1-7 [PMID: 21514939 DOI: 10.1016/j.medcli.2010.09.051]
  - 30 **Obando I**, Sánchez-Tatay D, Molinos-Quintana A, Delgado-Pecellin I, Porras A, Morillo-Gutiérrez B, Fenoll A, Lirio MJ. [Epidemiology of nasopharyngeal carriage of Streptococcus pneumoniae in children < 6 years old in Seville]. *Enferm Infecc Microbiol Clin* 2011; **29**: 581-586 [PMID: 21821320 DOI: 10.1016/j.eimc.2011.05.010]
  - 31 **Weinberger DM**, Harboe ZB, Sanders EA, Ndiritu M, Klugman KP, Rückinger S, Dagan R, Adegbola R, Cutts F, Johnson HL, O'Brien KL, Scott JA, Lipsitch M. Association of serotype with risk of death due to pneumococcal pneumonia: a meta-analysis. *Clin Infect Dis* 2010; **51**: 692-699 [PMID: 20715907 DOI: 10.1086/655828]
  - 32 **Brueggemann AB**, Peto TE, Crook DW, Butler JC, Kristinsson KG, Spratt BG. Temporal and geographic stability of the serogroup-specific invasive disease potential of Streptococcus pneumoniae in children. *J Infect Dis* 2004; **190**: 1203-1211 [PMID: 15346329 DOI: 10.1086/423820]
  - 33 **European Centre for Disease Prevention and Control**. Surveillance of invasive pneumococcal disease in Europe, 2010. Stockholm: ECDC, 2012
  - 34 **Glennie SJ**, Banda D, Gould K, Hinds J, Kamngona A, Everett DD, Williams NA, Heyderman RS. Defective pneumococcal-specific Th1 responses in HIV-infected adults precedes a loss of control of pneumococcal colonization. *Clin Infect Dis* 2013; **56**: 291-299 [PMID: 23024291 DOI: 10.1093/cid/cis842]
  - 35 **Farida H**, Severin JA, Gasem MH, Keuter M, Wahyono H, van den Broek P, Hermans PW, Verbrugh HA. Nasopharyngeal carriage of Streptococcus pneumoniae in pneumonia-prone age groups in Semarang, Java Island, Indonesia. *PLoS One* 2014; **9**: e87431

- [PMID: 24498104 DOI: 10.1371/journal.pone.0087431]
- 36 **Park SY**, Moore MR, Bruden DL, Hyde TB, Reasonover AL, Harker-Jones M, Rudolph KM, Hurlburt DA, Parks DJ, Parkinson AJ, Schuchat A, Hennessy TW. Impact of conjugate vaccine on transmission of antimicrobial-resistant *Streptococcus pneumoniae* among Alaskan children. *Pediatr Infect Dis J* 2008; **27**: 335-340 [PMID: 18316986 DOI: 10.1097/INF.0b013e318161434d]
  - 37 **Mehr S**, Wood N. *Streptococcus pneumoniae*--a review of carriage, infection, serotype replacement and vaccination. *Paediatr Respir Rev* 2012; **13**: 258-264 [PMID: 23069126 DOI: 10.1016/j.prrv.2011.12.001]
  - 38 **Cardozo DM**, Nascimento-Carvalho CM, Andrade AL, Silvany-Neto AM, Daltro CH, Brandão MA, Brandão AP, Brandileone MC. Prevalence and risk factors for nasopharyngeal carriage of *Streptococcus pneumoniae* among adolescents. *J Med Microbiol* 2008; **57**: 185-189 [PMID: 18201984 DOI: 10.1099/jmm.0.47470-0]
  - 39 **Jounio U**, Juvonen R, Bloigu A, Silvennoinen-Kassinen S, Kaijalainen T, Kauma H, Peitso A, Saukkoripi A, Vainio O, Harju T, Leinonen M. Pneumococcal carriage is more common in asthmatic than in non-asthmatic young men. *Clin Respir J* 2010; **4**: 222-229 [PMID: 20887345 DOI: 10.1111/j.1752-699X.2009.00179.x]
  - 40 **Zhang L**, Prietsch SO, Mendes AP, Von Groll A, Rocha GP, Carrion L, Da Silva PE. Inhaled corticosteroids increase the risk of oropharyngeal colonization by *Streptococcus pneumoniae* in children with asthma. *Respirology* 2013; **18**: 272-277 [PMID: 23039314 DOI: 10.1111/j.1440-1843.2012.02280.x]
  - 41 **Nicoletti C**, Brandileone MC, Guerra ML, Levin AS. Prevalence, serotypes, and risk factors for pneumococcal carriage among HIV-infected adults. *Diagn Microbiol Infect Dis* 2007; **57**: 259-265 [PMID: 17292578 DOI: 10.1016/j.diagmicrobio.2006.08.021]
  - 42 **Onwubiko C**, Swiatlo E, McDaniel LS. Cross-sectional study of nasopharyngeal carriage of *Streptococcus pneumoniae* in human immunodeficiency virus-infected adults in the conjugate vaccine era. *J Clin Microbiol* 2008; **46**: 3621-3625 [PMID: 18845823 DOI: 10.1128/JCM.01245-08]
  - 43 **Weinberger DM**, Harboe ZB, Viboud C, Krause TG, Miller M, Mølbak K, Konradsen HB. Pneumococcal disease seasonality: incidence, severity and the role of influenza activity. *Eur Respir J* 2014; **43**: 833-841 [PMID: 24036243 DOI: 10.1183/09031936.00056813]
  - 44 **Weinberger DM**, Harboe ZB, Viboud C, Krause TG, Miller M, Mølbak K, Konradsen HB. Serotype-specific effect of influenza on adult invasive pneumococcal pneumonia. *J Infect Dis* 2013; **208**: 1274-1280 [PMID: 23901093 DOI: 10.1093/infdis/jit375]
  - 45 **Microbiologic Information System, National Epidemiology Center, Institute of Health Carlos III**. Annual Report (2012) of Microbiologic Information System. Spain: Madrid, 2014
  - 46 **Hausdorff WP**, Bryant J, Paradiso PR, Siber GR. Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. *Clin Infect Dis* 2000; **30**: 100-121 [PMID: 10619740 DOI: 10.1086/313608]
  - 47 **Belvisi V**, Del Borgo C, Morelli F, Marocco R, Tieghi T, Fabietti P, Vetica A, Lichtner M, Mastroianni CM. Late onset invasive pneumococcal disease in a liver transplanted patient: beyond the Austrian syndrome. *Transpl Infect Dis* 2013; **15**: E111-E114 [PMID: 23581282 DOI: 10.1111/tid.12083]
  - 48 **Imhof A**, Maggiorini M, Zbinden R, Walter RB. Fatal necrotizing fasciitis due to *Streptococcus pneumoniae* after renal transplantation. *Nephrol Dial Transplant* 2003; **18**: 195-197 [PMID: 12480983 DOI: 10.1093/ndt/18.1.195]
  - 49 **Yu VL**, Chiou CC, Feldman C, Ortvist A, Rello J, Morris AJ, Baddour LM, Luna CM, Snyderman DR, Ip M, Ko WC, Chedid MB, Andremon A, Klugman KP. An international prospective study of pneumococcal bacteremia: correlation with in vitro resistance, antibiotics administered, and clinical outcome. *Clin Infect Dis* 2003; **37**: 230-237 [PMID: 12856216 DOI: 10.1086/377534]
  - 50 **Kalin M**, Ortvist A, Almela M, Aufwerber E, Dwyer R, Henriques B, Jorup C, Julander I, Marrie TJ, Mufson MA, Riquelme R, Thalme A, Torres A, Woodhead MA. Prospective study of prognostic factors in community-acquired bacteremic pneumococcal disease in 5 countries. *J Infect Dis* 2000; **182**: 840-847 [PMID: 10950779 DOI: 10.1086/315760]
  - 51 **Dupont PJ**, Manuel O, Pascual M. Infection and chronic allograft dysfunction. *Kidney Int Suppl* 2010; **119**: S47-S53 [PMID: 21116318 DOI: 10.1038/ki.2010.423]
  - 52 **Centers for Disease Control and Prevention**. Immunization of Health-Care Workers: Recommendations of the Advisory Committee on Immunization Practices (ACIP) and the Hospital Infection Control Practices Advisory Committee (HICPAC). *MMWR Recommendations and Reports* 1997; **46**: 1-24
  - 53 **Advisory Committee on Immunization Practices**. Preventing pneumococcal disease among infants and young children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recommendations and Reports* 2000; **49**: 1-35
  - 54 **Moberley S**, Holden J, Tatham DP, Andrews RM. Vaccines for preventing pneumococcal infection in adults. *Cochrane Database Syst Rev* 2013; **1**: CD000422 [PMID: 23440780 DOI: 10.1002/14651858.CD000422.pub3]
  - 55 **Shapiro ED**, Berg AT, Austrian R, Schroeder D, Parcells V, Margolis A, Adair RK, Clemens JD. The protective efficacy of polyvalent pneumococcal polysaccharide vaccine. *N Engl J Med* 1991; **325**: 1453-1460 [PMID: 1944423 DOI: 10.1056/NEJM199111213252101]
  - 56 **Jackson LA**, Neuzil KM, Yu O, Benson P, Barlow WE, Adams AL, Hanson CA, Mahoney LD, Shay DK, Thompson WW. Effectiveness of pneumococcal polysaccharide vaccine in older adults. *N Engl J Med* 2003; **348**: 1747-1755 [PMID: 12724480 DOI: 10.1056/NEJMoa022678]
  - 57 **Musher DM**, Rueda-Jaimes AM, Graviss EA, Rodriguez-Barradas MC. Effect of pneumococcal vaccination: a comparison of vaccination rates in patients with bacteremic and nonbacteremic pneumococcal pneumonia. *Clin Infect Dis* 2006; **43**: 1004-1008 [PMID: 16983612 DOI: 10.1086/507699]
  - 58 **Huss A**, Scott P, Stuck AE, Trotter C, Egger M. Efficacy of pneumococcal vaccination in adults: a meta-analysis. *CMAJ* 2009; **180**: 48-58 [PMID: 19124790 DOI: 10.1503/cmaj.080734]
  - 59 **Silberman H**, Overturf GD, Field RJ, Butler J, Berne TV, Witt R. Pneumococcal vaccination in recipients of renal allografts. *Surg Forum* 1979; **30**: 156-157 [PMID: 44020]
  - 60 **Marrie TJ**, Schiffman G, Bortolussi R, Field C, Whalen A. Humoral immune response of kidney transplant recipients to pneumococcal vaccine. *Proc Soc Exp Biol Med* 1981; **167**: 62-69 [PMID: 7015353]
  - 61 **Cosio FG**, Giebink GS, Le CT, Schiffman G. Pneumococcal vaccination in patients with chronic renal disease and renal allograft recipients. *Kidney Int* 1981; **20**: 254-258 [PMID: 6169871 DOI: 10.1038/ki.1981.128]
  - 62 **Linnemann CC**, First MR, Schiffman G. Response to pneumococcal vaccine in renal transplant and hemodialysis patients. *Arch Intern Med* 1981; **141**: 1637-1640 [PMID: 7030249 DOI: 10.1001/archinte.1981.00340130081018]
  - 63 **Linnemann CC**, First MR, Schiffman G. Revaccination of renal transplant and hemodialysis recipients with pneumococcal vaccine. *Arch Intern Med* 1986; **146**: 1554-1556 [PMID: 3524494 DOI: 10.1001/archinte.1986.00360200116019]
  - 64 **Arnold WC**, Steele RW, Rastogi SP, Flanagan WJ. Response to pneumococcal vaccine in renal allograft recipients. *Am J Nephrol* 1985; **5**: 30-34 [PMID: 3881958 DOI: 10.1159/000166899]
  - 65 **Rytel MW**, Dailey MP, Schiffman G, Hoffmann RG, Piering WF. Pneumococcal vaccine immunization of patients with renal impairment. *Proc Soc Exp Biol Med* 1986; **182**: 468-473 [PMID: 3526355]
  - 66 **Dengler TJ**, Strnad N, Bühring I, Zimmermann R, Girsdies O, Kubler WE, Zielen S. Differential immune response to influenza and pneumococcal vaccination in immunosuppressed patients after heart transplantation. *Transplantation* 1998; **66**: 1340-1347 [PMID: 9846520 DOI: 10.1097/00007890-199811270-00014]
  - 67 **Blumberg EA**, Brozena SC, Stutman P, Wood D, Phan HM,

- Musher DM. Immunogenicity of pneumococcal vaccine in heart transplant recipients. *Clin Infect Dis* 2001; **32**: 307-310 [PMID: 11170924 DOI: 10.1086/318482]
- 68 **McCashland TM**, Preheim LC, Gentry MJ. Pneumococcal vaccine response in cirrhosis and liver transplantation. *J Infect Dis* 2000; **181**: 757-760 [PMID: 10669371 DOI: 10.1086/315245]
- 69 **Kazancioğlu R**, Sever MS, Yüksel-Onel D, Eraksoy H, Yildiz A, Celik AV, Kayacan SM, Badur S. Immunization of renal transplant recipients with pneumococcal polysaccharide vaccine. *Clin Transplant* 2000; **14**: 61-65 [PMID: 10693637 DOI: 10.1034/j.1399-0012.2000.140111.x]
- 70 **Pourfarziani V**, Ramezani MB, Taheri S, Izadi M, Einollahi B. Immunogenicity of pneumococcal vaccination in renal transplant recipients and hemodialysis patients: a comparative controlled trial. *Ann Transplant* 2008; **13**: 43-47 [PMID: 18806734]
- 71 **Centers for Disease Control and Prevention**. Licensure of 13-Valent Pneumococcal Conjugate Vaccine for Adults Aged 50 Years and Older. Available from: URL: <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6121a3.htm>
- 72 **Food and Drug Administration**. FDA expands use of Prevnar 13 vaccine for people ages 50 and older. Silver Spring (MD): US Department of Health and Human Services. USA: Food and Drug Administration, 2011: 26-40
- 73 **Danziger-Isakov L**, Kumar D. Vaccination in solid organ transplantation. *Am J Transplant* 2013; **13** Suppl 4: 311-317 [PMID: 23465023 DOI: 10.1111/ajt.12122]
- 74 **Food and Drug Administration**. Vaccines and Related Biological Products Advisory Committee (VRBPAC) adult indication briefing document: Prevnar 13. Silver Spring, MD: US Department of Health and Human Services. USA: Food and Drug Administration, 2011
- 75 **Chen J**, O'Brien MA, Yang HK, Grabenstein JD, Dasbach EJ. Cost-effectiveness of pneumococcal vaccines for adults in the United States. *Adv Ther* 2014; **31**: 392-409 [PMID: 24718851 DOI: 10.1007/s12325-014-0115-y]
- 76 **Cordero E**, Aydillo TA, Perez-Ordóñez A, Torre-Cisneros J, Lara R, Segura C, Gentil MA, Gomez-Bravo MA, Lage E, Pachon J, Perez-Romero P. Deficient long-term response to pandemic vaccine results in an insufficient antibody response to seasonal influenza vaccination in solid organ transplant recipients. *Transplantation* 2012; **93**: 847-854 [PMID: 22377789 DOI: 10.1097/TP.0b013e318247a6ef]
- 77 **Mufson MA**, Krause HE, Schiffman G. Reactivity and antibody responses of volunteers given two or three doses of pneumococcal vaccine. *Proc Soc Exp Biol Med* 1984; **177**: 220-225 [PMID: 6483857 DOI: 10.3181/00379727-177-41934]
- 78 **Kumar D**, Rotstein C, Miyata G, Arlen D, Humar A. Randomized, double-blind, controlled trial of pneumococcal vaccination in renal transplant recipients. *J Infect Dis* 2003; **187**: 1639-1645 [PMID: 12721944 DOI: 10.1086/374784]
- 79 **Kumar D**, Welsh B, Siegal D, Chen MH, Humar A. Immunogenicity of pneumococcal vaccine in renal transplant recipients--three year follow-up of a randomized trial. *Am J Transplant* 2007; **7**: 633-638 [PMID: 17217436 DOI: 10.1111/j.1600-6143.2007.01668.x]
- 80 **Kumar D**, Chen MH, Wong G, Cobos I, Welsh B, Siegal D, Humar A. A randomized, double-blind, placebo-controlled trial to evaluate the prime-boost strategy for pneumococcal vaccination in adult liver transplant recipients. *Clin Infect Dis* 2008; **47**: 885-892 [PMID: 18715160 DOI: 10.1086/591537]
- 81 **Tobudic S**, Plunger V, Sunder-Plassmann G, Riegersperger M, Burgmann H. Randomized, single blind, controlled trial to evaluate the prime-boost strategy for pneumococcal vaccination in renal transplant recipients. *PLoS One* 2012; **7**: e46133 [PMID: 23029408 DOI: 10.1371/journal.pone.0046133]

**P- Reviewer:** Belliato M, Nosotti M **S- Editor:** Tian YL

**L- Editor:** A **E- Editor:** Lu YJ



## Non chylous filarial ascites: A rare case report

Kaivan S Shah, Prasad A Bhate, Dattatray Solanke, Vikas Pandey, Meghraj A Ingle, Shubhada V Kane, Prabha Sawant

Kaivan S Shah, Prasad A Bhate, Dattatray Solanke, Vikas Pandey, Meghraj A Ingle, Shubhada V Kane, Prabha Sawant, Department Of Gastroenterology, LTMMC and LTMGH, Sion Hospital, Sion West, Mumbai 400022, India

Author contributions: Shah KS and Bhate PA designed the case and wrote the paper; Solanke D and Pandey V collected clinical data; case report was guided by Ingle MA and Sawant P; cytological examination was performed by Kane SV.

Ethics approval: The ethics committee functions as per ICH-GCO, schedule Y guidelines.

Informed consent: The patient is not revealed.

Conflict-of-interest: All the authors of this case report state that there is no conflict of interest involved.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Dr. Kaivan S Shah, MD, Department of Gastroenterology, LTMMC and LTMG, Sion Hospital, College Building, First Floor Endoscopy Room, Room No. 13, Sion West, Mumbai 400022, India. [drkaivanshah26@gmail.com](mailto:drkaivanshah26@gmail.com)

Telephone: +91-98-33622433

Fax: +91-22-24076100

Received: August 22, 2014

Peer-review started: August 23, 2014

First decision: October 5, 2014

Revised: November 18, 2014

Accepted: December 16, 2014

Article in press: December 17, 2014

Published online: February 25, 2015

in ascitic fluid is an extremely uncommon finding. We present a case of non chylous ascites where microfilaria were detected in the ascitic fluid.

**Key words:** Microfilaria; Postpartum ascites; Non chylous ascites

© The Author(s) 2015. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** We report a rare case of postpartum ascites caused by filarial infection. There are only a few case reports where microfilaria were detected in ascitic fluid; among these, non chylous ascitis is even rarer.

Shah KS, Bhate PA, Solanke D, Pandey V, Ingle MA, Kane SV, Sawant P. Non chylous filarial ascites: A rare case report. *World J Clin Infect Dis* 2015; 5(1): 11-13 Available from: URL: <http://www.wjgnet.com/2220-3176/full/v5/i1/11.htm> DOI: <http://dx.doi.org/10.5495/wjcid.v5.i1.11>

## INTRODUCTION

Filarial infection is common in various Asian and African countries. It presents with various manifestations like ascites, pleural effusion and pedal edema; it may even be asymptomatic. We present an interesting and rare case of filariasis in ascitic fluid in postpartum female.

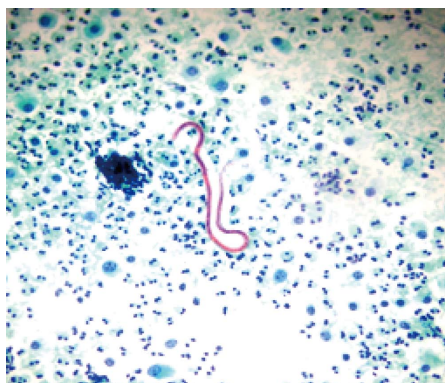
## CASE REPORT

A 26 years old female residing in Uttar Pradesh, India, who was 2 mo postpartum, presented with chief complaints of abdominal pain and vomiting since 1 mo and abdominal distention since 15 d. The pain was in periumbilical, non radiating, dull, mild and continuous. It was associated with non-

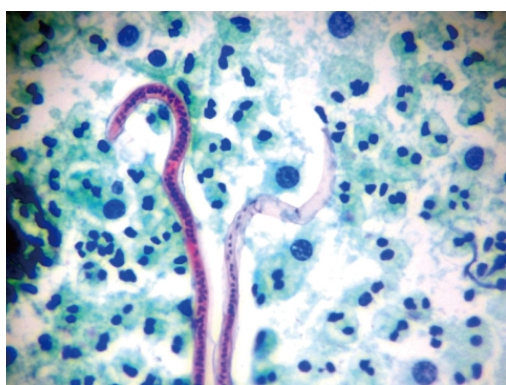
## Abstract

Filariasis is a common health problem in tropical and subtropical regions including India. It commonly presents with lymphatic involvement in form of nonpitting pedal edema, chylous ascites, chyluria, hydrocele and lymphocele. Detection of microfilaria





**Figure 1** Ascitic fluid-Smear shows **Microfilaria**. Background shows inflammatory cells (Pap stain 100 ×).



**Figure 2** **Microfilaria** with nuclei. Note that the tail portion is devoid of the nuclei (pap 400 ×).

bilious vomiting, 2-3 episodes per day. She also complained of abdominal distention from the last 15 d, which was generalized and gradually increasing with increasing abdominal pain. She had undergone a Caesarean section 2 mo ago. The pregnancy had been uneventful. Her general physical, cardiovascular and respiratory systems examination did not reveal any abnormalities. Shifting dullness was present on abdominal examination without tenderness or hepatosplenomegaly.

Laboratory examination revealed haemoglobin of 12.2 gm%, normal mean corpuscular volume (MCV) of 82fl, total leukocyte count of 16500/cmm and platelet count of 253000/cmm. Creatinine was 1.2 mg%, AST (aspartate aminotransferase) 21 IU/L, ALT (alanine aminotransferase) 25 IU/L and Bilirubin 1.1 mg/dL. Her total serum protein was 6.4 g/dL, albumin 3.7 g/dL and INR (international normalised ratio) 1.1, serum cholesterol and TG (triglycerides) were normal. Serum amylase was 45 IU/L and serum LDH (lactate dehydrogenase) was 1241 IU/L. Stool examination was normal. Ascitic fluid analysis revealed straw coloured fluid, total leukocyte count of 876 cells/mm<sup>3</sup> with 65% neutrophils, ascitic protein 3.7 g/dL, ascitic albumin of 2.7 g/dL and SAAG was 1. Ascitic fluid ADA (adenosine deaminase) was 14 U/L,



**Figure 3** **Microfilaria** in peripheral smear. Sample collected at 2 am.

amylase 46 IU/L, LDH 106 IU/L, glucose 40 mg/dL and TG was 55 mg/dL. Ultrasonography of abdomen was unremarkable except for moderate ascites. Portosplenic Doppler was also normal. CT abdomen was suggestive of moderate ascites, omental and mesenteric fat stranding with multiple non necrotic mesenteric lymph nodes and diffuse long segment concentric wall thickening involving small bowel loops, especially the jejunum. Upper GI endoscopy was normal. She was given IV antibiotics and IV fluids. She improved symptomatically but abdominal distention persisted. Ascitic fluid cytology showed numerous eosinophils, few neutrophils, mesothelial cells and few histiocytes. Cytology was negative for malignant cells. But cytological examination revealed presence of sheathed microfilariae consistent with *Wuchereria Bancrofti* (Figures 1 and 2). Subsequently patient's peripheral smear examination showed presence of motile microfilaria which confirmed our diagnosis (Figure 3). She was given diethylcarbamazine 300 mg/d for 21 d along with albendazole 400 mg/d for 7 d. She responded well to treatment; abdominal pain and ascites disappeared in a few days. Peripheral blood smear repeated after two weeks was negative for microfilaria.

## DISCUSSION

We report a case of non chylous filarial ascites positive for microfilaria in ascitic fluid which is a rare finding. To the best of our knowledge, there are only two published cases where microfilaria were detected in ascitic fluid<sup>[1,2]</sup>.

Filariasis is still endemic in many parts of world including India and a predominant cause of health morbidity. It is quite prevalent in many states of India like Jharkhand, Andhra Pradesh, Uttar Pradesh, Gujarat, Orissa, Tamil Nadu, Kerala and Bihar. Many infected patients remain asymptomatic.

The definitive host for filarial infection is man. The parasites have a predilection for lymphatics. The *Culex* mosquito is an intermediate vector. They ingest microfilariae from affected individuals and



this larvae develop into active motile forms in 10-12 d for further transmission into a new host. In the definitive host, the larvae develop into adult worms in lymphatics and give rise up to 50000 worms/d.

The adult worm usually resides in lymphatics while microfilariae traverse in peripheral blood. Microfilaria are visible in specimens of tissue or fluids due to obstruction of lymphatics and vascular channels. Inflammatory conditions, major trauma, even stasis or tumours can precipitate obstruction. Due to this obstruction, there is lymphatic damage and extravasation of microfilariae. Based on the detection of microfilariae in blood samples and body fluids, we establish our diagnosis. On autopsy, adult filarial parasites can be demonstrated.

Moreover, pregnancy is associated with pelvic congestion due to effect of progesterone and other placental hormones. Hypothetically, caesarean section could be a cause of traumatic rupture of lymphatic vessel with subsequent spread of microfilariae into the peritoneal cavity<sup>[3]</sup>.

Ascites and pleural effusion are uncommon findings. Commonly they are chylous in nature due to blockage of lymph from the occluded lymphatic channels. Such microfilarial ascites being non-chylous microfilarial ascites is extremely rare. Lymphangitis because of partial obstruction is a proposed mechanism for such exudative collection<sup>[4]</sup>. Ascitic fluid TG could also be low due to inadequate diet, but ascitic fluid TG content is low in our patient inspite of adequate food intake.

Many authors have reported microfilariae in breast lump as well as in lymph node aspirates<sup>[5,6]</sup>. Microfilariae have been detected in thyroid swelling and rarely in subcutaneous swellings<sup>[7,8]</sup>. Detection of microfilaria in body fluids like pleural effusion and ascites is rare and such ascites being non chylous is extremely rare.

Therefore clinical suspicion and careful cytological examination is extremely important to avoid misdiagnosis. Demonstration of parasite in cytology will be helpful not only in the right diagnosis but also in instituting specific treatment.

## COMMENTS

### Case characteristics

Vomiting since 1 mo and abdominal distention since 15 d.

### Clinical diagnosis

Ascites on percussion of abdomen without organomegaly.

### Differential diagnosis

Twenty six years old female 2 mo postpartum presented with complaints of abdominal pain and Vomiting since 1 mo and abdominal distention since 15 d. Budd chiari syndrome, decompensated chronic liver disease, tuberculosis.

### Laboratory diagnosis

Normal CBC, liver function and metabolic panel except high leukocyte count with ascitic fluid showing high leukocytes with low protein and normal ADA level.

### Imaging diagnosis

CT abdomen was done which was suggestive of moderate ascites, omental and mesenteric fat stranding with multiple non necrotic mesenteric lymph nodes and diffuse long segment concentric wall thickening involving small bowel loops especially jejunum with normal ultrasound and colour Doppler study.

### Pathological diagnosis

Ascitic cytology revealed presence of numerous neutrophils with presence of microfilaria of W Bancrofti.

### Treatment

Patient was treated with diethylcarbamazine for 21 d and albendazole for 7 d.

### Related reports

Presence of microfilaria has been documented in atypical location by FNA has been documented by Yenkeswar PN and others but detection of microfilaria in ascitic fluid is very uncommon.

### Experiences and lessons

Clinical suspicion and careful cytological examination by expert pathologist is extremely important in clinical practice.

### Peer review

Shah and colleagues present an interesting and very rare case of ascites due to filariasis in a young woman a few weeks after giving birth.

## REFERENCES

- 1 **Yenkeswar PN**, Kumbhalkar DT, Bobhate SK. Microfilariae in fine needle aspirates: a report of 22 cases. *Indian J Pathol Microbiol* 2006; **49**: 365-369 [PMID: 17001886]
- 2 **Mitra SK**, Mishra RK, Verma P. Cytological diagnosis of microfilariae in filariasis endemic areas of eastern Uttar Pradesh. *J Cytol* 2009; **26**: 11-14 [PMID: 21938142 DOI: 10.4103/0970-9371.51333]
- 3 **Babic I**, Tulbah M, Ghourab S. Spontaneous resolution of chylous ascites following delivery: a case report. *J Med Case Rep* 2012; **6**: 187 [PMID: 22762446 DOI: 10.1186/1752-1947-6-187]
- 4 **Walter A**, Krishnaswami H, Cariappa A. Microfilariae of Wuchereria bancrofti in cytologic smears. *Acta Cytol* 1983; **27**: 432-436 [PMID: 6349200]
- 5 **Varghese R**, Raghuveer CV, Pai MR, Bansal R. Microfilariae in cytologic smears: a report of six cases. *Acta Cytol* 1996; **40**: 299-301 [PMID: 8629415]
- 6 **Pandit AA**, Shah RK, Shenoy SG. Adult filarial worm in a fine needle aspirate of a soft tissue swelling. *Acta Cytol* 1997; **41**: 944-946 [PMID: 9167733]
- 7 **Joshi AM**, Pangarkar MA, Ballal MM. Adult female Wuchereria bancrofti nematode in a fine needle aspirate of the lymph node. *Acta Cytol* 1995; **39**: 138 [PMID: 7847003]
- 8 **Dey P**, Walker R. Microfilariae in a fine needle aspirate from a skin nodule. *Acta Cytol* 1994; **38**: 114 [PMID: 8291349]

P- Reviewer: Lutz P S- Editor: Ji FF L- Editor: A  
E- Editor: Lu YJ





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

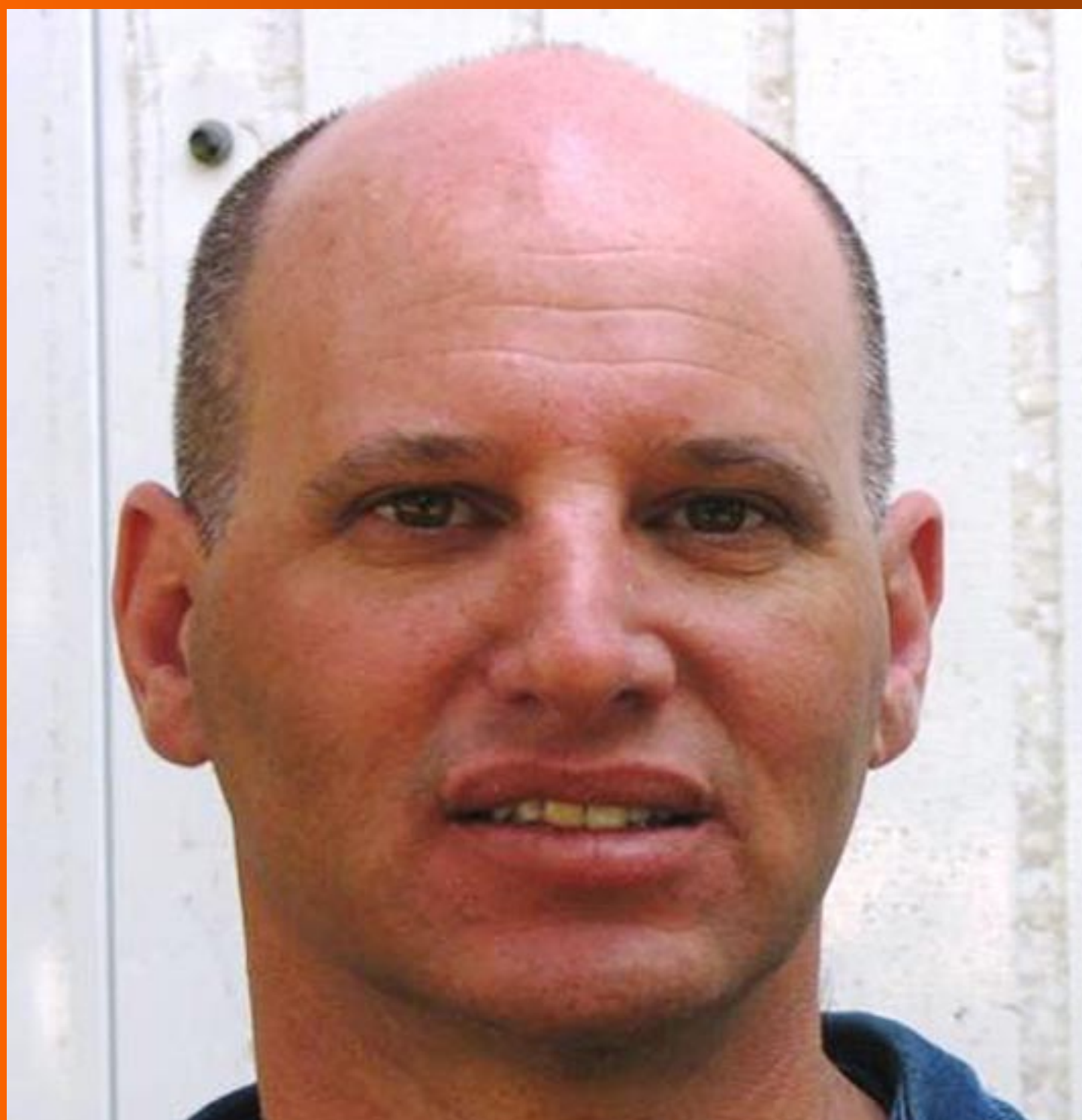
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Clinical Infectious Diseases*

*World J Clin Infect Dis* 2015 May 25; 5(2): 14-50



## Editorial Board

2011-2015

The World Journal of Clinical Infectious Diseases Editorial Board consists of 291 members, representing a team of worldwide experts in infectious diseases. They are from 56 countries, including Argentina (5), Australia (8), Austria (3), Bangladesh (1), Belgium (2), Bosnia and Herzegovina (1), Brazil (6), Brunei Darussalam (1), Bulgaria (1), Cameroon (1), Canada (7), China (18), Colombia (1), Costa Rica (1), Cuba (1), Denmark (2), Egypt (1), Finland (1), France (11), Germany (4), Greece (8), Hungary (6), India (14), Indonesia (1), Iran (5), Israel (10), Italy (19), Japan (4), Jordan (1), Kosovo (1), Kuwait (1), Lebanon (3), Lithuania (1), Malawi (1), Mexico (5), Morocco (2), Netherlands (4), Nigeria (1), Pakistan (2), Peru (1), Philippines (1), Portugal (5), Russia (1), Saudi Arabia (2), Singapore (3), South Africa (2), South Korea (6), Spain (24), Switzerland (2), Tanzania (1), Thailand (4), Tunisia (1), Turkey (4), United Kingdom (9), United States (59), and Venezuela (1).

### EDITORS-IN-CHIEF

Shyam Sundar, *Varanasi*  
Lihua Xiao, *Atlanta*

### GUEST EDITORIAL BOARD MEMBERS

Huan-Tsung Chang, *Taipei*  
Jia-Ming Chang, *Taipei*  
Kuo-Chin Huang, *Chiayi*  
Wei-Chen Lee, *Taoyuan*  
Hsiu-Jung Lo, *Miaoli*  
Jin-Town Wang, *Taipei*  
Deng-Chyang Wu, *Kaohsiung*  
Jiunn-Jong Wu, *Tainan*

### MEMBERS OF THE EDITORIAL BOARD



#### Argentina

Sergio Angel, *Chascomus*  
Luis Adrian Diaz, *Cordoba*  
Gustavo Daniel Lopardo, *Buenos Aires*  
Emilio L Malchioldi, *Buenos Aires*  
Victor D Rosenthal, *Buenos Aires*



#### Australia

Thea van de Mortel, *Lismore*  
David Llewellyn Gordon, *Bedford Park*  
Asad Khan, *Brisbane*  
Ruiting Lan, *Sydney*  
John McBride, *Cairns*  
David Leslie Paterson, *Brisbane*  
Nitin K Saksena, *Sydney*  
Andrew Slack, *Brisbane*



#### Austria

Ojan Assadian, *Vienna*  
Christian Joukhadar, *Vienna*  
Bernhard Resch, *Graz*



#### Bangladesh

Harunor Rashid, *Cox's Bazar*



#### Belgium

Mickael Aoun, *Bruxelles*  
Paul M Tulkens, *Brussels*



#### Bosnia and Herzegovina

Selma Uzunovic, *Zenica*



#### Brazil

Jane Costa, *Rio de Janeiro*  
Pedro Alves d'Azevedo, *Sao Paulo*  
Gerly Anne de Castro Brito, *Fortaleza*  
RL Dantas Machado, *Sao Paulo*  
Leandro R Rodrigues Perez, *Porto Alegre*  
M de Nazare Correia Soeiro, *Rio de Janeiro*



#### Brunei Darussalam

Vui Heng Chong, *Bandar Seri Begawan*



#### Bulgaria

Iva Christova, *Sofia*



#### Cameroon

Richard Njouom, *Yaounde*



#### Canada

Aranka Anema, *Vancouver*  
Peter C Coyte, *Toronto*  
Pavel Gershkovich, *Vancouver*  
Marcelo Gottschalk, *Quebec*  
Marina Ulanova, *Thunder Bay*  
Jude Uzonna, *Winnipeg*  
Jun Wang, *Halifax*



#### China

Tian-Hua Huang, *Shantou*  
Xi-Tai Huang, *Tianjin*  
Dong-Ming Li, *Beijing*  
Xin-Yong Liu, *Jinan*  
Wu-Bin Pan, *Taichang*  
Kai Wang, *Jinan*  
Patrick Chiu Yat Woo, *Hong Kong*  
Yong-Feng Yang, *Nanjing*  
Chi-Yu Zhang, *Zhenjiang*  
Li-Juan Zhang, *Beijing*



#### Colombia

Jorge Enrique Gomez-Marin, *Armenia*

**Costa Rica**

Adriano Arguedas, *San José*

**Cuba**

Maria G Guzman, *Havana*

**Denmark**

Janne Kudsk Klitgaard, *Odense*  
Henrik Torkil Westh, *Hvidovre*

**Egypt**

Olfat Shaker, *Cairo*

**Finland**

Jari Timo Juhani Nuutila, *Turku*

**France**

Hassane Adakal, *Burkina Faso*  
Pascal Bigey, *Paris*  
Philippe Brouqui, *Marseille*  
Christophe Chevillard, *Marseille*  
Raphaelé Girard, *Pierre Bénite*  
Vincent Pascal Jarlier, *Paris*  
Sandrine Marquet, *Marseille*  
Vayssier-Taussat Muriel, *Maisons-Alfort*  
Thierry Naas, *Le Kremlin-Bicetre*  
Saad Nseir, *Lille*  
Philippe Seguin, *Rennes*

**Germany**

Stefan Borgmann, *Ingolstadt*  
Georg Harter, *Ulm*  
Matthias Imohl, *Aachen*  
Kurt G Naber, *Straubing*

**Greece**

Apostolos Beloukas, *Athens*  
Alex P Betrosian, *Athens*  
George L Daikos, *Athens*  
Helena Maltezou, *Athens*  
Argyris S Michalopoulos, *Athens*  
Maria Moschovi, *Athens*  
George Petrikkos, *Athens*  
Athanasios Tragiannidis, *Thessaloniki*

**Hungary**

Laszlo Galgoczy, *Szeged*  
Viktor Muller, *Budapest*  
Ferenc Orosz, *Budapest*  
Ferenc Rozgonyi, *Budapest*  
Jozsef Soki, *Szeged*

Dezso Peter Virok, *Szeged*

**India**

Ritesh Agarwal, *Chandigarh*  
Syed Imteyaz Alam, *Gwalior*  
Atmaram Hari Bandivdekar, *Mumbai*  
Runu Chakravarty, *Kolkata*  
Dipshikha Chakravorty, *Bangalore*  
Sanjay Chhibber, *Chandigarh*  
BN Harish, *Pondicherry*  
Triveni Krishnan, *Kolkata*  
Rashmi Kumar, *Lucknow*  
Mohammad Owais, *Aligarh*  
Banwarilal Sarkar, *Kolkata*  
Mamta Chawla Sarkar, *Kolkata*  
Akashdeep Singh, *Ludhiana*

**Indonesia**

Jeanne Adiwinata Pawitan, *Jakarta*

**Iran**

Parissa Farnia, *Tehran*  
Seyed Mohammad Jazayeri, *Tehran*  
Morteza Pourahmad, *Jahrom*  
Mohammad Reza Pourshafie, *Tehran*  
Mohammad Hossein Salari, *Tehran*

**Israel**

Jacob Amir, *Petach Tikvah*  
Shai Ashkenazi, *Petach Tikva*  
Gadi Borkow, *Gibton*  
Raul Colodner, *Afula*  
Jacob Moran Gilad, *Jerusalem*  
Noah Isakov, *Beer Sheva*  
Michal Mandelboim, *Hashomer*  
Shifra Shvarts, *Omer*  
Oshri Wasserzug, *Tel-Aviv*  
Pablo Victor Yagupsky, *Beer-Sheva*

**Italy**

Giuseppe Barbaro, *Rome*  
Paolo Bonilauri, *Reggio Emilia*  
Guido Calleri, *Torino*  
Mario Cruciani, *Verona*  
Marco Falcone, *Rome*  
Antonio Fasanella, *Foggia*  
Daniele Focosi, *Pisa*  
Delia Goletti, *Rome*  
Guido Grandi, *Siena*  
Fabio Grizzi, *Rozzano*  
Giuseppe Ippolito, *Rome*  
Roberto Manfredi, *Bologna*  
Claudio M Mastroianni, *Rome*  
Ivano Mezzaroma, *Rome*  
Giuseppe Micali, *Catania*  
Antonella d'Arminio Monforte, *Milano*  
Annamaria Passantino, *Messina*  
Mariagrazia Perilli, *L'Aquila*  
Patrizia Pontisso, *Padova*

**Japan**

Masashi Emoto, *Maebashi*  
Toshi Nagata, *Hamamatsu*  
Ryohei Yamasaki, *Tottori*  
Shin-Ichi Yokota, *Sapporo*

**Jordan**

Asem A Shehabi, *Amman*

**Kosovo**

Lul Raka, *Prishtina*

**Kuwait**

Willias Masocha, *Safat*

**Lebanon**

Ziad Daoud, *Beirut*  
Ghassan M Matar, *Beirut*  
Sami Ramia, *Beirut*

**Lithuania**

Gazim Bizanov, *Vilnius*

**Malawi**

Adamson Sinjani Muula, *Blantyre*

**Mexico**

Agnes Fleury, *Mexico*  
Guadalupe Garcia-Elorriaga, *Mexico*  
Alejandro E Macias, *Mexico*  
Mussaret Zaidi, *Merida*  
Roberto Zenteno-Cuevas, *Veracruz*

**Morocco**

Redouane Abouqal, *Rabat*  
Ezzikouri Sayeh, *Casablanca*

**Netherlands**

Aldert Bart, *Amsterdam*  
John Hays, *Rotterdam*  
Nisar Ahmed Khan, *Rotterdam*  
Rogier Louwen, *Rotterdam*

**Nigeria**

Samuel Sunday Taiwo, *Osogbo*





### **Pakistan**

Muhammad Idrees, *Lahore*  
Muhammad Mukhtar, *Bahawalpur*



### **Peru**

Salim Mohanna, *Lima*



### **Philippines**

Vicente Y Belizario, *Ermita Manila*



### **Portugal**

Ricardo Araujo, *Porto*  
Manuela Canica, *Lisbon*  
Francisco Esteves, *Lisbon*  
Fernando Rodrigues, *Braga*  
Nuno Taveira, *Lisbon*



### **Russia**

Alexander M Shestopalov, *Koltsovo*



### **Saudi Arabia**

Jaffar A Al-Tawfiq, *Dhahran*  
Atef M Shibl, *Riyadh*



### **Singapore**

Yee Sin Leo, *Singapore*  
Laurent Claude Stephane Renia, *Singapore*  
Richard J Sugrue, *Singapore*



### **South Africa**

Carolina H Pohl-Albertyn, *Bloemfontein*  
Natasha Potgieter, *Louis Trichardt*



### **South Korea**

Chong Cho, *Seoul*  
Sang Ho Choi, *Seoul*  
Ju-Young Chung, *Seoul*  
Jung Mogg Kim, *Seoul*  
Kyongmin Kim, *Suwon*  
Sang Hee Lee, *Yongin*



### **Spain**

Alberto Arnedo-Pena, *Castellon*  
Alfredo Berzal-Herranz, *Granada*  
Vicente Brito, *Alicante*

Enrique Calderon, *Seville*

Rafael Canton, *Madrid*

Jose M Cuevas, *Valencia*

Laila Darwich, *Cerdanyola del Valles*

Adela Gonzalez de la Campa, *Madrid*

Pere Domingo, *Barcelona*

Tahia D Fernandez, *Malaga*

Lucia Gallego, *Leioa*

Luis Ignacio Gonzalez-Granado, *Madrid*

Bruno Gonzalez-Zorn, *Madrid*

Eduardo Lopez-Collazo, *Madrid*

Miguel Marcos, *Salamanca*

Antonio Torres Marti, *Barcelona*

Andres Moya, *Valencia*

Rafael Najera, *Madrid*

Maria Mercedes Nogueras-Mas, *Sabadell*

Jose A Oteo, *Logrono*

Pilar Perez-Romero, *Sevilla*

Ruth Gil Raka, *Madrid*

Eduardo Reyes, *Madrid*

Francisco Soriano, *Madrid*



### **Switzerland**

Stephen Hawser, *Epalinges*  
Andrew Hemphill, *Bern*



### **Tanzania**

John Peter Andrea Lusingu, *Tanga*



### **Thailand**

Kosum Chansiri, *Bangkok*  
Subsai Kongsangdao, *Bangkok*  
Niwat Maneeakarn, *Chiang Mai*  
Viroj Wiwanitkit, *Bangkok*



### **Tunisia**

Aouni Mahjoub, *Monastir*



### **Turkey**

Oguz Karabay, *Sakarya*  
Uner Kayabas, *Malatya*  
Gokhan Metan, *Kayseri*  
Oral Oncul, *Istanbul*



### **United Kingdom**

Zainab Al-Doori, *Glasgow*  
David Carmena, *London*  
Ronald Anthony Dixon, *Lincoln*  
Vanya Alasdair Ivan Andre Gant, *London*  
Robin Goodwin, *London*  
Andrew Cunliffe Hayward, *London*  
Laura Anne Hughes, *Neston*  
Michele Esther Murdoch, *Herts*  
Craig William Roberts, *Glasgow*



### **United States**

Majdi N Al-Hasan, *Lexington*  
Ibne KM Ali, *Charlottesville*  
Hossam M Ashour, *Detroit*  
Joseph Urban Becker, *Palo Alto*  
M Eric Benbow, *Dayton*  
Eliahu Bishburg, *Newark*  
Luz P Blanco, *Ann Arbor*  
Robert Bucki, *Philadelphia*  
Steven Dale Burdette, *Dayton*  
Archana Chatterjee, *Omaha*  
Pai-Lien Chen, *Durham*  
Pawel S Ciborowski, *Omaha*  
Michael Cynamon, *Syracuse*  
Siddhartha Das, *El Paso*  
Ralph J DiClemente, *Atlanta*  
Noton Kumar Dutta, *Baltimore*  
Garth D Ehrlich, *Pittsburgh*  
Michael S Firstenberg, *Columbus*  
Walter A Hall, *Syracuse*  
Yongqun He, *Ann Arbor*  
Brenda Lorraine Helms, *Plano*  
Joseph U Igietseme, *Atlanta*  
Mohammad Khalid Ijaz, *Montvale*  
Suresh G Joshi, *Philadelphia*  
Thomas F Kresina, *Rockville*  
Alain B Labrique, *Baltimore*  
Shenghan Lai, *Baltimore*  
Benfang Lei, *Bozeman*  
Jeff G Leid, *Flagstaff*  
Vladimir Leonitiev, *St. Louis*  
Andrea Lisco, *Bethesda*  
James M McMahon, *Rochester*  
Geraldine M McQuillan, *Hyattsville*  
Lawrence F Muscarella, *Ivyland*  
Daniel Musher, *Houston*  
Stella Nowicki, *Nashville*  
M Jacques Nsuami, *New Orleans*  
Phillipe N Nyambi, *New York*  
Raymund Rabe Razonable, *Rochester*  
Anand Reddi, *Denver*  
Michael Switow Saag, *Birmingham*  
Danny J Schust, *Columbia*  
William R Schwan, *La Crosse*  
Richard A Slayden, *Fort Collins*  
Theodore J Standiford, *Ann Arbor*  
William M Switzer, *Atlanta*  
Ashutosh Tamhane, *Birmingham*  
Giorgio E Tarchini, *Weston*  
Carmen Taype, *New York*  
Barbara Van Der Pol, *Bloomington*  
Jose Antonio Vazquez, *Detroit*  
Fernando Villalta, *Nashville*  
Haider J Warraich, *Boston*  
Xianfu Wu, *Atlanta*  
Genyan Yang, *Atlanta*  
Frank X Yang, *Indianapolis*  
Hong Zhang, *Rockville*  
Lyna Zhang, *Atlanta*



### **Venezuela**

Alfonso J Rodriguez-Morales, *Caracas*



**REVIEW**

- 14 Treatment of methicillin-resistant *Staphylococcus aureus* infections: Importance of high vancomycin minimum inhibitory concentrations

Morales-Cartagena A, Lalueza A, López-Medrano F, Juan RS, Aguado JM

**MINIREVIEWS**

- 30 Origin of *de novo* daptomycin non susceptible enterococci

Kelesidis T

- 37 Surface adhesion and host response as pathogenicity factors of *Neisseria meningitidis*

Uberos J, Molina-Oya M, Martinez-Serrano S, Fernández-López L

**ORIGINAL ARTICLE**

**Observational Study**

- 44 Improvement in human immunodeficiency virus-1/acquired immune deficiency syndrome patients' well-being following administration of "Phyto V7"

Wernik R, Priore JL, Goldman WF, Elias AC, Borkow G

## Contents

*World Journal of Clinical Infectious Diseases*  
Volume 5 Number 2 May 25, 2015

### ABOUT COVER

Editorial Board Member of *World Journal of Clinical Infectious Diseases*, Gadi Borkow, PhD, Chief Medical Scientist, Cupron Scientific, Hameyasdim 44, Gibton 76910, Israel

### AIM AND SCOPE

*World Journal of Clinical Infectious Diseases* (*World J Clin Infect Dis*, *WJCID*, online ISSN 2220-3176, DOI: 10.5495) is a peer-reviewed open access (OA) academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJCID* will focus on a broad spectrum of topics on infectious diseases that will cover epidemiology, immune-pathogenesis, genetic factors, host susceptibility to infection, vector control, novel approaches of treatment, molecular diagnostic and vaccines. It will provide a common stage to share the visions, new approaches, most advanced techniques, and to discuss research problems that will help everyone working in the field of various infections to exchange their views and to improve public health. *WJCID* will also focus on broad range of infections like opportunistic infections, zoonotic infections, tropical and neglected tropical diseases, emerging infections, *etc.* and following topics related to these issues: (1) Causative agents discussing various pathogens; (2) Vectors and Mode of transmission; (3) Host-pathogen interaction and immune-pathogenesis of the disease; (4) Epidemiology of the infection and vector control strategies; (5) Genetic factors covering both host and pathogen; (6) Molecular diagnostic techniques vaccines; and (7) Recent advances in cell tissue culture, lab techniques, *etc.* Various other related fields like medical microbiology, pharmacology of herbs, bioinformatics, *etc.* will be included.

We encourage authors to submit their manuscripts to *WJCID*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

### INDEXING/ABSTRACTING

*World Journal of Clinical Infectious Diseases* is now indexed in Digital Object Identifier.

### FLYLEAF

I-III Editorial Board

### EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*

Responsible Electronic Editor: *Huan-Liang Wu*

Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*

Proofing Editorial Office Director: *Xiu-Xia Song*

#### NAME OF JOURNAL

*World Journal of Clinical Infectious Diseases*

#### ISSN

ISSN 2220-3176 (online)

#### LAUNCH DATE

December 30, 2011

#### FREQUENCY

Quarterly

#### EDITORS-IN-CHIEF

Shyam Sundar, MD, FRCP (London), FAMS, FNA Sc, FASc, FNA, Professor, Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India

Lihua Xiao, DVM, PhD, Senior Scientist, Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Bldg 23, Rm 9-168, MS D66, 1600 Clifton

Rd, Atlanta, GA 30333, United States

#### EDITORIAL OFFICE

Jin-Lei Wang, Director  
Xiu-Xia Song, Vice Director  
*World Journal of Clinical Infectious Diseases*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-85381891  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esp/helpdesk.aspx>  
<http://www.wjgnet.com>

#### PUBLISHER

Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esp/helpdesk.aspx>  
<http://www.wjgnet.com>

#### PUBLICATION DATE

May 25, 2015

#### COPYRIGHT

© 2015 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

#### SPECIAL STATEMENT

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

#### INSTRUCTIONS TO AUTHORS

Full instructions are available online at [http://www.wjgnet.com/2220-3176/g\\_info\\_20100722180909.htm](http://www.wjgnet.com/2220-3176/g_info_20100722180909.htm).

#### ONLINE SUBMISSION

<http://www.wjgnet.com/esp/>

## Treatment of methicillin-resistant *Staphylococcus aureus* infections: Importance of high vancomycin minimum inhibitory concentrations

Alejandra Morales-Cartagena, Antonio Lalueza, Francisco López-Medrano, Rafael San Juan, José María Aguado

Alejandra Morales-Cartagena, Antonio Lalueza, Francisco López-Medrano, Rafael San Juan, José María Aguado, Infectious Diseases Unit, Department of Medicine, University Hospital 12 de Octubre, 28041 Madrid, Spain

**Author contributions:** Morales-Cartagena A and Lalueza A were the main authors in writing the draft version; López-Medrano F and Aguado JM principal physicians involved in the critical revision of the manuscript; all the authors approved the final version of the manuscript.

**Conflict-of-interest:** The authors declare no conflicts of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Dr. Alejandra Morales-Cartagena, Infectious Diseases Unit, Department of Medicine, University Hospital 12 de Octubre, Av. Córdoba km 5.400, 28041 Madrid, Spain. [a.morales.cartagena@gmail.com](mailto:a.morales.cartagena@gmail.com)

**Telephone:** +34-91-3908247

**Fax:** +34-91-3908112

**Received:** July 3, 2014

**Peer-review started:** July 4, 2014

**First decision:** July 29, 2014

**Revised:** February 26, 2015

**Accepted:** March 5, 2015

**Article in press:** March 9, 2015

**Published online:** May 25, 2015

systemic infections. The increasing incidence of methicillin-resistant strains has granted an increasing use of vancomycin causing a covert progressive increase of its minimum inhibitory concentration (MIC) (dubbed the MIC "creep"). In this way, the emergence of vancomycin-intermediate SA (VISA) strains and heteroresistant-VISA has raised concern for the scarcity of alternative treatment options. Equally alarming, though fortunately less frequent, is the emergence of vancomycin-resistant SA. These strains show different mechanisms of resistance but have similar problems in terms of therapeutic approach. Ultimately, various debate issues have arisen regarding the emergence of SA strains with a minimum inhibitory concentration sitting on the superior limit of the sensitivity range (*i.e.*, MIC = 2 µg/mL). These strains have shown certain resilience to vancomycin and a different clinical behaviour regardless of vancomycin use, both in methicillin-resistant SA and in methicillin-sensitive SA. The aim of this text is to revise the clinical impact and consequences of the emergence of reduced vancomycin susceptibility SA strains, and the different optimal treatment options known.

**Key words:** *Staphylococcus aureus*; Minimum inhibitory concentration; Methicillin-resistant *Staphylococcus aureus*; Vancomycin-intermediate *Staphylococcus aureus*; Heteroresistant-vancomycin-intermediate *Staphylococcus aureus*; Vancomycin resistant *Staphylococcus aureus*

© The Author(s) 2015. Published by Baishideng Publishing Group Inc. All rights reserved.

### Abstract

*Staphylococcus aureus* (SA) infections remain a major cause of morbidity and mortality despite the availability of numerous effective anti-staphylococcal antibiotics. This organism is responsible for both nosocomial and community-acquired infections ranging from relatively minor skin and soft tissue infections to life-threatening

**Core tip:** The emergence of increasing vancomycin-resistance in *Staphylococcus aureus* (SA) isolates, has stirred up the basis of therapeutic approach in staphylococcal infections. Complete vancomycin-resistance is acquired through plasmid transmission of enterococcal gene *vanA*. However, the development of strains with gradual loss of vancomycin-susceptibility

seems to be related to conformational bacterial changes and affects its pathogenicity and even its susceptibility to other antimicrobials (other than vancomycin). It has been observed that the impact of diminished vancomycin susceptibility could not only affect methicillin-resistant SA but has also been related to worse prognosis in methicillin-sensitive SA infections. There is yet much to explore to better define the impact of higher vancomycin minimum inhibitory concentration in staphylococcal infections.

Morales-Cartagena A, Lalueza A, López-Medrano F, Juan RS, Aguado JM. Treatment of methicillin-resistant *Staphylococcus aureus* infections: Importance of high vancomycin minimum inhibitory concentrations. *World J Clin Infect Dis* 2015; 5(2): 14-29 Available from: URL: <http://www.wjgnet.com/2220-3176/full/v5/i2/14.htm> DOI: <http://dx.doi.org/10.5495/wjcid.v5.i2.14>

## STAPHYLOCOCCUS AUREUS, AN EVOLVING AGENT

Little after the beginning of the antibiotic era came the arrival of antibiotic resistance. The first *Staphylococcus aureus* (SA) strains resistant to penicillin appeared in 1942 due to an inducible beta-lactamase, and since then it has been evolving, developing resistance to most other antibiotics used for staphylococcal infections<sup>[1]</sup>. In 1959 methicillin became the best option to surpass penicillin resistance, however, resistance appeared only 2 years later [methicillin-resistant *Staphylococcus aureus* (MRSA)]<sup>[2]</sup>. It took some years to spread, and not until the mid 1980's did MRSA reach alarming figures<sup>[3-5]</sup>.

The mechanisms leading to methicillin resistance involve the expression a chromosomal gene *mecA*, which is found in the staphylococcal cassette chromosome (SCC), a mobile genomic element. This gene encodes penicillin binding protein 2a (PBP2a) that has a low affinity for certain betalactams, including penicillin and methicillin. The origin of methicillin resistance is uncertain, however, studies up to now suggest that it first appeared in coagulase-negative staphylococci, and then was transferred to methicillin-susceptible *Staphylococcus aureus* (MSSA) through horizontal gene transfer. Genes encoded in SCC<sub>mec</sub> have proved to be decisive in antibiotic resistance, however, it is not clear whether they play any relevant role in *S. aureus* virulence<sup>[6]</sup>.

Whereas methicillin-resistance developed in the years following its discovery, *S. aureus* strains showing reduced susceptibility to vancomycin were not described until 1997. However, many reports of similar findings started to appear shortly after<sup>[7]</sup>. Even if vancomycin resistance expansion has taken a different form than other patterns of antibiotic resistance and perhaps less aggressive, it is a growing problematic in staphylococcal infections and deciding the optimal

treatment approach is an on-going challenge.

## EPIDEMIOLOGY OF AS INFECTION

### Incidence of methicillin resistant SA

MRSA has spread like an epidemic, becoming 41.2% of the strains isolated in Europe at the present time<sup>[8]</sup>. More than 25% of *S. aureus* strains isolated in Spanish hospitals are methicillin resistant<sup>[9]</sup>. Prevalence of MRSA in Asian hospitals are globally very high, reaching 60% of the SA isolates in countries like Southern Korea, Vietnam or Taiwan<sup>[10,11]</sup>. In the United States, studies in the last decade declared that more than 94000 MRSA-associated infections occur every year, with an estimation of 18650 MRSA-infections attributable deaths<sup>[12]</sup>. One of the most important risk factors in developing MRSA infection has been observed to be MRSA colonization, detected through positive nasal-carriage. In a recent meta-analysis evaluating the prevalence of MRSA colonization and infection in patients admitted to the intensive care unit (ICU) (studies included from Europe, North and South America, Asia and Australia) they observed a prevalence of MRSA colonization ranging from 5.8% to 8.3%, which was higher in North American studies, with an upward trend. MRSA colonization was found to be associated with an important increased risk for MRSA infections [relative risk (RR) of 8.33]<sup>[13]</sup>.

Lately however, decreasing trends in hospital-onset MRSA infections have been observed in several surveillance studies. In an observational study of all Department of Defence TRICARE beneficiaries from January 2005 to December 2010, they found that annual rates of both community-onset and hospital-onset MRSA bacteraemia decreased (from 0.7 per 100000 person-years in 2005 to 0.4 per 100000 person-years in 2010)<sup>[14]</sup>. In addition, MRSA central line-associated bloodstream infections have been decreasing in United States intensive care units<sup>[15]</sup>. Decline in healthcare-associated invasive MRSA infections have also been recently reported<sup>[16]</sup>. The emergence of community-associated MRSA (CA-MRSA) and its introduction into healthcare settings has changed the epidemiology of *S. aureus* infection in the American continent and worldwide. These isolates are chiefly associated with a wide range of soft tissue infections and are sometimes implicated in severe pneumonias. They are rarely encountered in patients with bacteraemia. In an observational study to analyse the impact of CA-MRSA emergence on *S. aureus* bacteraemia (SAB), they describe a steadily decreasing rate of SAB both for community-associated (especially MSSA bacteraemia) and hospital-onset cases, whereas the rate of community-onset healthcare-associated cases did not change<sup>[17]</sup>. These results emerge in the context of multiple strategies adopted with the objective of reducing device-related and surgical-site infections in hospital settings. Most of these studies and revisions are based on retrospective data and observational evidence, and must therefore be weighed in this



context.

The vast majority of published epidemiological studies about the prevalence and clinical impact of SA infections refer to the American and European continents. There is scarce information about *S. aureus* epidemiology in non-Western parts of the world (Africa, Middle East, Asia and Oceania) as highlighted in Rasigade's review<sup>[18]</sup>.

### **Morbidity and mortality associated to MRSA infections**

MRSA bacteraemia is associated with a considerable mortality. In a recent study that took place in nine different areas of the United States where they analysed almost 9000 MRSA invasive infections, bacteraemia (75%) was the clinical syndrome most frequently associated with invasive MRSA infection. Standardized mortality rate in this study was 6.3 per 100000 (interval estimate 3.3-7.5)<sup>[12]</sup>.

Given that MRSA infections have been historically mainly healthcare-associated, bacteraemia by these pathogens have been found more frequently in patients who are severely ill or with a great number of comorbidities. Thereby there has been a continuing perception that this organism is particularly virulent. However, its virulence compared to that of MSSA remains controversial<sup>[19]</sup>. Earlier studies and meta-analysis described an almost two-fold increase in mortality in patients with MRSA bloodstream infections than those due to MSSA<sup>[19]</sup>. However, other studies analysing healthcare-associated MRSA bacteraemia in that same period and in the following years found no differences<sup>[20-22]</sup>. In a meta-analysis that evaluated the results of 9 international studies comparing MRSA vs MSSA risk factors and mortality, they observed that the risk of death was higher in patients with MRSA bacteraemia than those with MSSA bacteraemia in all but one of the studies, with a RR of death of 2, ranging from 0.89 to 4.94. They described potential risk factors associated to MRSA bacteraemia, such as: prior antibiotic therapy, longer previous hospital stay, older age, male sex, past history of MRSA infection and admittance or treatment in an ICU<sup>[23]</sup>. However once again, this almost two-fold higher mortality risk, seemed to be interfered by the base-line comorbid and nosocomial situation of those patients<sup>[19,23]</sup>.

## **ROLE OF VANCOMYCIN IN METHICILLIN-RESISTANT *S. AUREUS* INFECTIONS AND CONSEQUENCES OF ITS USE**

The use of vancomycin, a glycopeptide discovered in 1952 and approved shortly after, didn't spread until years later with the emergence of pseudomembranous enterocolitis and the spread of MRSA infections<sup>[24]</sup>. Its mechanism of action consists on the inhibition of the bacterial wall synthesis, with a slow bactericidal effect compared to beta-lactams<sup>[24]</sup>. Nephrotoxicity is its main toxicity concern, needing caution for patients with

renal impairment. In these cases, if treatment with vancomycin is unavoidable, the best possible approach would be to confirm serologic levels stay within optimal concentration for bactericidal activity (see section "Risk of nephrotoxicity with elevated vancomycin doses")<sup>[25]</sup>.

Clinical guidelines still recommend intravenous vancomycin as one of the first choice antibiotic therapies for the treatment of MRSA infections including bacteraemia, infective endocarditis, meningitis, central-line associated infection, septic thrombosis, osteomyelitis, and septic arthritis (in the latter, the addition of rifampin is sometimes considered). In specific severe complications such as severe septic shock, toxic syndrome, or necrotizing pneumonia, some experts consider adding adjunctive therapy with clindamycin or linezolid, which are protein synthesis inhibitors. Intravenous immunoglobulin have also shown good results in these situations<sup>[26]</sup>.

Unlike with other antibiotics, *S. aureus* did not start to show resistance to vancomycin until 40 years after its discovery. In 1996 in Japan, the first vancomycin-intermediate *Staphylococcus aureus* (VISA) isolate was reported<sup>[27,28]</sup>, and subsequently heteroresistant VISA (hVISA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) isolates were described (see section "Minimum inhibitory concentration value for vancomycin and mechanisms of resistance"). Thereafter, vancomycin failure and apparently worse clinical outcome in these staphylococcal infections started to be described<sup>[29]</sup>. In the Asian continent VISA has never disseminated widely and has only been sporadically reported. VRSA, on the other hand, with greater MIC than 16 µg/mL (harbouring gene *vanA*), have been increasingly reported from northern India and West Bengal, both in clinical and colonization isolates<sup>[11]</sup>.

### **Minimum inhibitory concentration value for vancomycin and mechanisms of resistance**

More than 20 years ago, the Clinical and Laboratory Standards Institute (CLSI) first set broad minimum inhibitory concentration (MIC) cut-off point and disk diffusion testing of vancomycin in *S. aureus* isolates (resistance set at  $\geq 34$  µg/mL). In 1998, after the appearance of the first *S. aureus* strains with reduced vancomycin susceptibility, they lowered the disk diffusion breakpoints, in order to detect these strains to  $\leq 4$  µg/mL. However, clinical failures with vancomycin in patients with MRSA infections resulted in a re-evaluation of its MIC breakpoints in 2004. Finally in 2006 the CLSI established vancomycin MIC susceptibility cut-off point in  $\leq 2$  µg/mL, 4-8 µg/mL for VISA and finally  $\geq 16$  µg/mL for VRSA. MIC for VRSA was lowered to 16 µg/mL because MICs above that limit had shown high probability of adverse clinical outcome<sup>[30]</sup>. Even with these changes, concerns about the declining susceptibility to glycopeptides in MRSA infections persisted<sup>[31]</sup>.

*S. aureus* cell wall is composed of layers of murein monomers (peptidoglycan) with D-alanine-D-alanine

(D-ala-D-ala) residues. From the cytoplasm, where these monomers are synthesized, a lipidic transporter (lipid II) transfers them through the membrane. It is then built into the peptidoglycan chain by enzymes situated within the membrane. Vancomycin binds to these D-ala-D-ala residues and blocks the assembly of peptidoglycan monomers, stopping bacterial growth<sup>[32]</sup>. VISA and VRSA have shown to have different mechanisms of resistance. In the case of VISA, it has been observed that they form a thickened cell wall, with added peptidoglycan layers, and therefore vancomycin isn't able to saturate its target nor reach to the surface of the cell wall, and becomes entrapped within it, never attaining its disruption<sup>[33,34]</sup>. The term glycopeptide-intermediate SA is sometime used in these strains, given that they frequently show similar patterns of resistance for teicoplanin. Though most VISA strains are also methicillin-resistant, a minority do show susceptibility to methicillin<sup>[6]</sup>. Intermediate vancomycin resistance has been associated to previous exposure to vancomycin and it seems these isolates can regain vancomycin susceptibility when the antibiotic pressure is withdrawn<sup>[35]</sup>.

VRSA, with MIC breakpoint  $\geq 16$   $\mu\text{g/mL}$  considering current standards (CLSI) was first detected in 2002. Fortunately it is yet extremely uncommon<sup>[36]</sup>. VRSA acquire their mechanism of resistance from a gene transferred from vancomycin-resistant enterococci, gene *vanA* (usually transferred by transposon plasmids-Tn1546). The resistance mechanism relays on the change of a peptidoglycan residue (D-ala-D-ala by D-ala-D-lactate), so that vancomycin is not able to bind to exert its blockage of the wall synthesis. VISA strains do not carry *vanA*, *vanB*, or *vanC* genes<sup>[7,37]</sup>.

Little after the description of VISA, arose the observation of subpopulations of MRSA apparently vancomycin-susceptible, showing atypical glycopeptide-resistance patterns, referred to as hVISA. These isolates would fall one step before VISA, in vancomycin resistance. Patients with hVISA were found to have been usually exposed to vancomycin in lower levels than desired therapeutic objectives (*i.e.*,  $< 10$   $\mu\text{g/mL}$ ). The population analysis profile-area under the curve (AUC) calculation is the reference method to identify hVISA strains, which is an arduous process and is not always available in all laboratories. Measuring vancomycin-MIC values of these subpopulations, most of them will show a MIC  $\geq 2$   $\mu\text{g/mL}$ , but some yet show MICs  $< 2$   $\mu\text{g/mL}$ . This increases the difficulties involved in their correct identification. There is evidence of prevalence increase of hVISA strains in selected locations. It has been observed that patients with complicated MRSA infections might be at a greater risk of hVISA. In this context, in a recent international study they found that 29% of the patients with MRSA infective endocarditis had isolates with hVISA subpopulations<sup>[24,36]</sup>. Vancomycin heteroresistant *S. aureus* have been classified with a MIC ranging from 2 to 8  $\mu\text{g/mL}$ .

**Table 1** *Staphylococcus aureus* glycopeptide minimum inhibitory concentration cut-off values ( $\mu\text{g/mL}$ ) as defined by Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing (determined by broth microdilution)

Antibiotic	CLSI (2011)			EUCAST (2011)	
	S	VISA	R	S	R
Vancomycin	$\leq 2$	4-8	$\geq 16$	$\leq 2$	$> 2$
Teicoplanin	$\leq 8$	-	$\geq 32$	$\leq 2$	$> 2$

CLSI: Clinical and Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; S: Sensitive; VISA: Vancomycin-intermediate *Staphylococcus aureus*; R: Resistant.

It has also been recently observed that there are emerging *S. aureus* strains with a MIC sitting on the superior limit of the sensitivity range (*i.e.*, MIC  $> 1.5$   $\mu\text{g/mL}$ ), that could show certain resilience to vancomycin and a different clinical behaviour<sup>[31,38,39]</sup>.

This resistance or loss of sensitivity to glycopeptides has given rise to plenty of debates regarding its clinical and epidemiologic relevance. As previously mentioned, partly due to the concerns of reduced vancomycin efficacy, in 2006 the CLSI lowered the *S. aureus* vancomycin-susceptible MIC cut-off point from 4  $\mu\text{g/mL}$  to 2  $\mu\text{g/mL}$ <sup>[30]</sup>. This cut-off point value is shared by the European Committee on Antimicrobial Susceptibility Testing, yet they do not hold the same MIC classification for VISA strains (which CLSI classify with MIC from 2-4 to 8  $\mu\text{g/mL}$ ) and VRSA (MIC  $\geq 16$   $\mu\text{g/mL}$ ), and consider all *S. aureus* strains with MIC higher than 2  $\mu\text{g/mL}$ , as "clinically" resistant to vancomycin (Table 1).

### Different microbiologic methods to calculate vancomycin MIC

Detection of VISA strains can be difficult, and they may take more than two days of incubation to grow on culture plate<sup>[7]</sup>. Quantitative antimicrobial susceptibility methods are the optimum techniques to correctly identify *S. aureus* isolates with VISA subpopulations. Valid quantitative antibiotic sensitivity test are: broth dilution, agar dilution, and agar gradient diffusion (Etest; AB-Biodisk). To correctly measure vancomycin susceptibility, CLSI recommend broth microdilution test done in cation-fixed Mueller-Hinton broth using a bacterial inoculum of 0.5 in McFarland scale, and incubating the dilution at 35 °C for 24 h<sup>[40]</sup>. In laboratories that use automated systems or disk diffusion testing, they recommend using a commercial set prepared with brain-heart infusion agar plate with a vancomycin concentration of 6  $\mu\text{g/mL}$ . By these standards, when a *S. aureus* isolate shows a MIC of  $\geq 2$   $\mu\text{g/mL}$  (according to the latest MIC classification) it should be confirmed with retesting. If confirmed, this information should be reported as possible VISA (or VRSA if it were  $\geq 16$   $\mu\text{g/mL}$ ), and depending on the country and institution policies, should be communicated to the hospital's infectious diseases

department, and established health authorities<sup>[7]</sup>.

Sometimes microbiology laboratories will only inform of the MIC cut-off point, and some automated antimicrobial sensitivity tests will not detect *S. aureus* isolates with a MIC of 2 µg/mL or less, therefore complicating the detection of VISA and hVISA strains<sup>[40]</sup>. Within VSSA isolates, a higher MIC (2 µg/mL) increases the likelihood of detecting the hVISA phenotype<sup>[41]</sup>. In a recent meta-analysis, prevalence of hVISA was observed to be of 1.67% (14 studies published in different countries between 1997 and 2001). They found a much higher incidence of hVISA within MRSA, and even though some studies were biased because they only included MRSA. They hypothesise MRSA could be more likely to harbour hVISA or VISA, given that MRSA is usually health-care related and hVISA/VISA represent strains that emerge under heavy antibiotic pressure<sup>[42,43]</sup>.

MIC elevation has important consequences for the effectiveness of this antibiotic and therefore has an impact on MRSA bacteraemia mortality. Consequently, it would be reasonable to consider vancomycin as a suboptimal treatment for strains with a vancomycin MIC > 1 µg/mL. The majority of the hospitals and health centres routinely use automated tests to estimate vancomycin MIC. However, these methods aren't always comparable to standardized methods (Etest, broth microdilution), on which the outcome data of most of the studies are based. Automated systems are able to detect only 10% of MRSA isolates with a vancomycin MIC of 2 µg/mL. Given these disparities the reference method (usually broth micro-dilution) must always be used as confirmation<sup>[44]</sup>. Unfortunately, these results may take days to become available, which could delay adequate specific therapy<sup>[45]</sup>.

### MIC "creep"

MIC "creep" is the concept of a surreptitious constant vancomycin MIC elevation in *S. aureus* isolates resulting from various factors such as antibiotic exposure and changes in the *S. aureus* clonal population. The clinical impact of MIC "creep" is yet to be determined, however, a feared consequence could be increased mortality and treatment failure in high vancomycin-MIC *S. aureus* infections, treated with vancomycin<sup>[29]</sup>.

Evidence of this concept is reflected in various studies and epidemiologic analysis that have been carried out in the last ten to fifteen years. For instance, an analysis of 6003 *S. aureus* isolates in Los Angeles-California in 2004, found a trend of increasing vancomycin MIC; the prevalence of *S. aureus* with vancomycin-MIC of 1.0 µg/mL increased from 19.9% in 2000 to 70.4% in 2004, when previously most stood at < 1.0 µg/mL<sup>[39]</sup>. Similarly, in 241605 *S. aureus* isolates tested by the Surveillance Network Database in the United States of America in 2007, they found 16.2% of them had a MIC of 2 µg/mL<sup>[30]</sup>. In an evaluation of more than 35000 strains of *S. aureus* isolated during 1998-2003, 4.7% to 7.8% of *S. aureus* isolates had

a MIC of 2 µg/mL<sup>[46]</sup>. Moreover, despite the tapering in CLSI's vancomycin breakpoint to 2 µg/mL, one study demonstrated that 80% of the organisms with MICs of 2 µg/mL were demonstrated to have hVISA phenotypes<sup>[47]</sup>. Thus, we are witnessing a gradual decrement in vancomycin susceptibility which seems to be greater in settings where the drug is most used<sup>[48]</sup>.

### Risk factors for vancomycin MIC elevation

In Lubin's prospective study they analysed predictive factors for an elevated vancomycin MIC in *S. aureus* bacteraemia. In the univariate analysis, various variables were associated with high vancomycin MIC; age > 50 years, the presence of sepsis or shock at the time of culture, a known history of MRSA bacteraemia, recent exposure to vancomycin or daptomycin, and the presence of a prosthetic heart valve or non-tunnelled central line. However, in the final predictive model, only age > 50 years, history of chronic liver disease, recent vancomycin exposure (> 48 h during the previous 7 d), presence of a non-tunnelled central venous catheter at the time of culture, and a history of MRSA bacteraemia were included<sup>[45]</sup>.

A recent study carried out in the United States identified several risk factors for reduced vancomycin susceptibility *S. aureus* infection. A previous history of vancomycin exposure, in the month prior to *S. aureus* isolation (OR = 13) or in the previous 3-6 mo (OR = 2.8), and having any positive culture for MRSA in the previous 2-3 mo were independently related to reduced vancomycin-susceptibility *S. aureus* infections<sup>[49]</sup>.

In a study analysing bacteraemia due to MRSA isolates comparing those manifesting hVISA to those fully vancomycin susceptible, they found association between hVISA and infections with high bacterial load, (i.e., endocarditis), resulting statistically significant. These strains were also associated to longer duration of fever, longer time to clearance of the bacteraemia, length of hospital stay, and failure of vancomycin treatment. Furthermore, they found these strains had frequently been under initial low serum vancomycin levels<sup>[50]</sup>.

Other risk factors observed in previous studies have been admittance to an intensive care unit, female sex, elevated body mass index, recent surgery, and cardiovascular disease<sup>[51-53]</sup>.

Fortunately, VRSA infection continues to be a rare occurrence. In the analysis of the few cases observed, some predisposing risk factors for VRSA infections have been identified. These are; previous enterococcal or MRSA colonization or infection, comorbidities such as diabetes or chronic skin sores and ulcers, and vancomycin exposure. Infection control and antibiotic stewardship are crucial to avoid the emergence of VRSA. However, more studies are needed to better define the specific microbiological and clinical characteristics of these strains<sup>[54]</sup>.

Recently, the first VRSA was detected in Europe, in a Portuguese hospital. In the epidemiological study, the

patient and 53 contacts were screened for *S. aureus* colonization. All strains recovered were characterized by molecular typing methods, by which they observed that VRSA remained confined to the infected foot of the patient and was not detected in any of the close contacts. Only one of the MRSA isolates detected in the screened population was closely related to the VRSA. The VRSA isolated in Portugal belonged to clonal complex (CC) 5, like most of the characterized VRSA strains from other countries. A recent increase in the incidence of lineages belonging to CC5 has been observed in some European countries. This may result in more frequent opportunities for the emergence of VRSA<sup>[55]</sup>.

## CLINICAL RELEVANCE OF VANCOMYCIN MIC ELEVATION

### Adjusting vancomycin dose to improve AUC/MIC

Vancomycin exhibits concentration-independent and time-dependent killing. Vancomycin efficacy is best measured using the ratio of the 24-h area under the concentration-time curve ( $AUC_{0-24}$ ) to the MIC ratio ( $AUC_{0-24}/MIC$ ), in pharmacodynamic parameters. These findings are based on neutropenic murine thigh-infection models<sup>[56]</sup>. In patients with *S. aureus* pneumonia, treated with vancomycin, it has been observed that attaining an  $AUC_{0-24}/MIC \geq 350$  (MIC determined by broth microdilution) is associated with seven times better odds of clinical success. They found shorter time to bacterial elimination when the  $AUC_{0-24}/MIC$  attained was  $\geq 400$ <sup>[57,58]</sup>. The  $AUC_{0-24}/MIC$  concentration obtained with the usual doses administered (1 g/12 h) and with a trough vancomycin concentration of 10 µg/mL, is approximately 400 mg/h per litre. From these results we could assume that the commonly recommended dose is adequate to treat *S. aureus* infections with a vancomycin MIC  $\leq 1$  µg/mL but suboptimal when it is  $> 1$  µg/mL.

In the last years, there have been several publications regarding diminished efficacy of vancomycin in those cases with *S. aureus* infection with high vancomycin MIC but within the sensitivity range<sup>[38,59,60]</sup>. Based on these findings, some studies suggest the ideal aimed vancomycin-dose for best clinical results should be an  $AUC_{0-24}/MIC$  ratio  $\geq 400$ , and therefore targeted trough concentrations should be increased to 15–20 mg/L<sup>[61]</sup>. However, in a recent study with patients with severe MRSA infections treated with vancomycin in which they adjusted daily dose to reach trough concentrations  $\geq 15$  mg/mL, they observed that the cure rate for the cases with vancomycin MIC = 2 µg/mL was still inferior to those with MIC  $\leq 1$  µg/mL (62% vs 85%;  $P = 0.02$ )<sup>[62]</sup>.

On the other hand, aggressive dosing strategy could possibly enable targeted vancomycin concentrations in most cases of vancomycin-susceptible *S. aureus* infection. However, this could possibly be unachievable

in other clinical settings, such as higher MIC or when limited by vancomycin toxicity. In a recent study, Patel showed that creatinine clearance and vancomycin MIC were inversely related to the probability of achieving adequate  $AUC_{0-24}/MIC$  values. He used Monte Carlo simulations to carry out this study. As an example, when administering 1500 mg of intravenous vancomycin every 12 h, target  $AUC_{0-24}/MIC$  values were attained in 97% of the cases of vancomycin MIC  $\leq 0.5$  mg/L, but they weren't able to reach this target in 38% of the cases with a vancomycin MIC of 2 mg/L<sup>[63]</sup>. When evaluating vancomycin-susceptible *S. aureus*, VISA, and hVISA in this model, the  $AUC_{0-24}/MIC$  ratio required for a static effect was similar for all these organisms. However, the dose required for a 2 log<sup>10</sup> kill was 2.5-fold higher for hVISA, compared with VISA. Therefore, the authors concluded that a  $AUC_{0-24}/MIC$  ratio of at least 500 was needed to optimize vancomycin pharmacodynamics for hVISA. To attain this  $AUC_{0-24}/MIC$  ratio, the needed doses to administer would be extremely high and would involve unacceptable toxicity<sup>[64]</sup>.

In Ghosh's recently published study, they evaluated the utility of previously validated AUC predictions (based on creatinine clearance estimation) and explored the optimal  $AUC_{0-24}/MIC$  targets for vancomycin in patients with MRSA bacteraemia. They also investigated whether observed targets are influenced by the sources of the bacteraemia. Treatment failure (persistent bacteraemia, microbiological failure and 30-d all-cause mortality) in their study occurred more frequently in those cases where the  $AUC_{0-24}/MIC$  (by broth microdilution) was less than 398 (54% vs 23.4%,  $P < 0.01$ ). Other variables associated with treatment failure were chronic lung disease, on-going immunosuppressive treatment, and high-risk sources of bacteraemia (endovascular, pneumonia, complicated intra-abdominal and central nervous system foci). In their study they also observed significant differences between MIC calculated by Etest or microdilution, as previously described. Etest generally yielded MIC results approximately 1–2 dilutions higher than broth microdilution, which could be circumvented by aiming appropriate MIC-method specific  $AUC_{0-24}/MIC$  targets. They also observed that bacteraemic source specific  $AUC_{0-24}/MIC$  thresholds may offer better outcome in high risk bacteraemia, with lower doses required for low risk source of infection. However, no further studies have yet been carried out to include these findings in current guidelines for their implementation. The controversy of this study relies also in the fact that they found no significant differences in clinical outcome in those cases with high vancomycin MIC (both measured by Etest or broth microdilution). Finally, they conclude that vancomycin trough concentrations are unlikely to accurately reflect  $AUC_{0-24}/MIC$  targets and may result in suboptimal outcomes. They suggest AUC estimation based on validated formulas, may allow for individual patient-dose optimisation resulting in increased treat-



ment success when a vancomycin AUC<sub>0-24</sub>/MIC of  $\geq 398$  is achieved<sup>[25]</sup>.

### **Risk of nephrotoxicity with elevated vancomycin doses**

There is limited data suggesting a direct causal relationship between toxicity and specific serum vancomycin concentrations<sup>[61]</sup>. However, targeting vancomycin dosing for trough concentrations of 15-20 mg/L leads to a greater risk of nephrotoxicity<sup>[65]</sup>, especially in those patients who also receive other nephrotoxic drugs<sup>[66]</sup>. In fact, a vancomycin trough concentration  $> 15$  mg/L has shown to be an independent predictor of nephrotoxicity<sup>[67]</sup>. In one of the studies proving this association, they compared nephrotoxicity (defined in their study as a 25% decrease in creatinine-clearance rate) developed in 59% of the patients treated with vancomycin that achieved trough serum concentrations of 15 mg/L, whereas in only 30% of those achieving lower trough concentrations ( $P = 0.0006$ )<sup>[68]</sup>. Nephrotoxicity is frequently a limiting factor for patients to receive the optimal doses in MRSA infections, even when adjusted by AUC<sub>0-24</sub>/MIC, and often forces rotation to other less validated antibiotic schemes.

## **CONSEQUENCES OF AN ELEVATED VANCOMYCIN MIC IN INFECTIONS BY MRSA AND MSSA**

As it has been previously mentioned, glycopeptides, mainly vancomycin has traditionally been the treatment of choice for MRSA infections. However, because of the numerous studies declaring worse outcome in MRSA infections with vancomycin MIC  $> 1.5$  µg/mL, even after adjusting trough concentration to higher thresholds (15-20 mg/L)<sup>[63,69]</sup>, confidence in this treatment option has somewhat declined. This is similarly observed in hetero-resistant strains, observing a loss of bactericidal activity (tolerance) to glycopeptides and more frequent treatment failure in hVISA<sup>[70]</sup>. Infections where hVISA are isolated have been associated with high-inoculum infections, persistent bacteraemia and metastatic complications, however, up to now, there are controversial results regarding the impact on mortality in these patients<sup>[71]</sup>.

Along these lines, previous studies have declared worse clinical outcomes in *S. aureus* infections, especially bacteraemia, with decreased vancomycin susceptibility (within VSSA ranges)<sup>[60,62,69,72]</sup>. These results have been reproduced for VISA and hVISA infections, finding bloodstream infections by these strains were more frequently associated to persistent bacteraemia than those that did not show this phenotype<sup>[50]</sup>. In another study yet, they observed that MRSA bacteraemia was associated with higher mortality when vancomycin was used empirically, on those cases where vancomycin MIC was  $> 2$  µg/mL<sup>[60]</sup>. But not all publications corroborate these results, generating further controversy<sup>[73-75]</sup>.

A recent meta-analysis which included a total of 22 studies<sup>[69]</sup> studied the impact of a vancomycin MIC  $\geq 1.5$  µg/mL on the clinical outcome of *S. aureus* infections. In this meta-analysis they highlighted an association between higher vancomycin MIC in MRSA infections and poorer outcomes (even mortality), regardless of the source of infection or MIC methodology (OR = 1.64; 95%CI: 1.14-2.73,  $P = 0.01$ ). They described an increased all-cause 30-d mortality in MRSA bloodstream infections with a vancomycin MIC of 2 µg/mL (determined by Etest), however, no mortality differences were detected in isolates with a MIC of 1 µg/mL and 1.5 µg/mL. Treatment failure, defined as persistent bacteraemia, was also more frequently observed in cases of high vancomycin MIC. After these results have been published, other authors have approached this association with discordant conclusions.

Therefore, despite some contradictory findings, it seems the observation of a higher vancomycin MIC has been repeatedly shown to confer a worse prognosis for MRSA bacteraemia<sup>[60,62,69]</sup>. This association, however, has been scantily investigated for MSSA strains. The present evidence is scarce, however some studies described a similar association between worse clinical outcome and elevated vancomycin MIC in MSSA bacteraemia, regardless of antibiotic treatment administered (anti-staphylococcal penicillin or vancomycin). In a study of 99 patients with MSSA catheter-related bacteraemia, vancomycin MIC (Etest)  $\geq 1.5$  µg/mL was the only independent risk factor for the development of complicated bacteraemia (OR = 22.9; 95%CI: 6.7-78.1), regardless of the initial antibiotic administered<sup>[76]</sup>. Similarly, another study revealed that mortality increased 2.4-fold in patients with a vancomycin MIC  $> 1.5$  µg/mL, and the choice of antibiotic treatment had no statistical significant effect on 30-d mortality in the multivariable model<sup>[72]</sup>.

*S. aureus* is one of the main causes of infective endocarditis (IE), being MSSA more frequently found to be responsible for native-valve IE (85% vs 15%) compared to MRSA, with a very high morbidity and mortality, that sits around 25%<sup>[77-79]</sup>. In 2009, a study carried out to analyse the effect of vancomycin MIC on the outcome of MRSA endocarditis, revealed persistent bacteraemia, heart failure and mortality were associated to vancomycin MIC  $> 1.5$  µg/mL<sup>[80]</sup>. More recently described, higher vancomycin MIC left-sided MSSA endocarditis were more frequently associated with systemic emboli and a higher in-hospital and one-year mortality. In this study, patients with endocarditis by a MSSA strains with a vancomycin MIC  $\geq 1.5$  µg/mL (determined by E-test) had 3-fold higher mortality (OR = 3.1; 95%CI: 1.2-8.2)<sup>[81]</sup>.

Evidence that the mechanisms underlying worse clinical outcomes in high-MIC VSSA infections go beyond antibiotic failure was given in a recent multi-centre observational cohort study of 532 *S. aureus* bacteraemic patients<sup>[72]</sup>. In this study, increasing vancomycin MIC was associated with increased mortality in vancomycin-

**Table 2 Treatment recommendations in *Staphylococcus aureus* with reduced vancomycin susceptibility infections<sup>1</sup>**

General recommendations	
Removal of indwelling hardware (prosthetic devices, surgical material, intravascular catheter, <i>etc.</i> )	
Surgical debridement of infected wounds and abscess drainage	
Follow specific guidelines and local protocols, based on infection site, for treatment duration decisions	
Antibiotic treatment considerations	
Vancomycin	If used aim: AUC <sub>0-24</sub> /MIC ≥ 400 or trough blood concentrations of 15-20 mg/L Careful monitoring of renal function is imperative
Daptomycin	Bactericidal. Good results with VISA and VRSA endovascular infections Consider administration of higher doses ( <i>i.e.</i> , 10 mg/kg per day) in severe infections and if vancomycin MIC > 2 µg/mL (including VISA) <sup>2</sup> Consider synergic combinations ( <i>i.e.</i> , cloxacillin, aminoglycosides, betalactams, fosfomycin) in infections involving high inoculum (as in IE) and prosthetic devices It is inhibited by pulmonary surfactant, therefore should be avoided in SA respiratory or lung infections Monitor CK and liver function
Linezolid	Bacteriostatic Protein synthesis inhibitor. Inhibits bacterial toxin synthesis High tissue bioavailability Good results in SSTI and pneumonia (including VAP) Oral formulation with similar bioavailability Myelotoxicity: Monitor CBC Severe interactions with SSRIs and MAOIs, must not be given simultaneously
Tigecycline	Low plasma concentrations. Bacteriostatic. Avoid monotherapy

<sup>1</sup>Treatment recommendations for SA with reduced vancomycin susceptibility usually take methicillin resistance for granted. If the strain were methicillin sensitive, the latter would be the treatment of choice; <sup>2</sup>In VISA and SA with MIC > 2 µg/mL, worse results with lower daptomycin doses have been observed, probably related to cell wall thickness changes in these strains. AUC: Area under the curve; MIC: Minimum inhibitory concentration; VISA: Vancomycin-intermediate *Staphylococcus aureus*; VRSA: Vancomycin-resistant *Staphylococcus aureus*; IE: Infective endocarditis; SA: *Staphylococcus aureus*; CK: Creatinine kinase; SSTI: Skin and soft tissue infections; VAP: Ventilator associated pneumonia; CBC: Complete blood count; SSRI: Selective serotonin reuptake inhibitor; MAOI: Monoamine oxidase inhibitor.

treated patients. Moreover, even in patients with MSSA bacteraemia treated with flucloxacillin, mortality was higher if the vancomycin (Etest) MIC of their isolate was > 1.5 µg/mL, compared with those with lower MIC isolates (26.8% vs 12.2%;  $P < 0.001$ ). These results suggest that apart from antibiotic choice, other factors (clinical and microbiological) might be crucial in patient outcome.

Interestingly, despite previous information about poorer prognosis associated to elevated vancomycin MIC, these strains have been found to be associated with a diminished inflammatory response, and therefore less incidence of septic shock. This suggests these strains could have alterations in their pathogenic activity and virulence<sup>[60,73]</sup>. Peleg studied the pathogenesis of *S. aureus* infections using *Galleria mellonella*. Using both clinical and laboratory strains, they demonstrated that with the evolution of reduced susceptibility to vancomycin, the virulence of *S. aureus* becomes attenuated. The degree to which virulence is attenuated appears to be proportional to the vancomycin MIC<sup>[74]</sup>.

Therefore the arguments linking vancomycin resistance with both reduced bacterial fitness and increased virulence, are yet to be proved, and more studies are needed to determine their clinical significance<sup>[6]</sup>.

Other virulence and prognostic factors in *S. aureus* infections that have taken a leading role are the expression and function of certain genes. The dysfunction of accessory gene regulator (*agr*), has been found to possibly play a key role in MRSA virulence, and seems related to vancomycin resistance. The *agr* locus is in charge of regulating the expression of certain

virulence genes and other constitutive genes, required for the maintenance of basic cellular function. The overexpression of *agr* increases the toxin production and reduces the expression of cell surface adhesins<sup>[6]</sup>. The *agr* locus dysfunction has been associated with reduced vancomycin susceptibility<sup>[82]</sup>, persistent MRSA bacteraemia<sup>[58,83]</sup>, and increased mortality<sup>[84]</sup>, and has been considered a subrogated marker of health-care associated situations. However more studies and investigations are needed to establish the impact of these findings and the possible consequences derived in daily clinical practice (treatment modifications it could imply, invasive procedures to assess, *etc.*).

Overall, there seems to be numerous studies and data indicating that elevated vancomycin MIC in both methicillin resistant and sensitive VSSA, could be a subrogated virulence marker, however, these results are yet controversial, and have not been universally proven. In this realm of controversy, evidence based clinical decisions seem like an arduous and complicated task.

## ALTERNATIVE THERAPIES FOR MRSA AND MSSA INFECTIONS WITH REDUCED VANCOMYCIN SUSCEPTIBILITY (TABLE 2)

### Clinical approach to *S. aureus* with > 1.5 vancomycin MIC infections

Up to now, there is no evidence-based unified clinical approach for patients with *S. aureus* infections that have an elevated vancomycin MIC within the sensitivity thresholds (MIC ≥ 1.5 µg/mL). If the

**Table 3** Infection control recommendations for patients colonized or infected by drug-resistant *Staphylococcus aureus* (vancomycin-intermediate *Staphylococcus aureus*, vancomycin-resistant *Staphylococcus aureus*, and methicillin-resistant *Staphylococcus aureus*, Centers for Disease Control and Prevention recommendations<sup>1</sup>)

Spread prevention
Isolate patient in a private room
Facilitate gowns and gloves to enter the room
Facilitate mask protection
If risk of aerosol spread consider mask use
Practice hand hygiene with an antibacterial agent (preferably chlorhexidine-based soaps or solutions)
Avoid sharing equipment among patients
Continue isolation until results of tests of nares and infected sites are negative 3 times over 3 wk (including hospital readmission)
Minimize number of staff caring for patient
Educate staff about appropriate precautions and assess compliance
Infection control in nosocomial spread and evaluation
Perform baseline and weekly cultures of hands and nares of healthcare workers in charge of index patient
Consider baseline and weekly cultures for other healthcare workers and persons with extensive contact
Decolonize index patient and healthcare workers with topical mupirocin
Consider avoiding direct patient-contact of colonized healthcare workers until negative culture

<sup>1</sup>Centers for Disease Control and Prevention Healthcare-associated Infections recommendations and guidelines ([http://www.cdc.gov/HAI/prevent/prevent\\_pubs.html](http://www.cdc.gov/HAI/prevent/prevent_pubs.html)).

decision were to use vancomycin, it seems crucial to beware of the different formulas to attain adequate AUC/MIC targets. More studies are needed to consider the contrasting efficacy of other antibiotic treatments in this situation.

The possibility of it being a surrogated virulence marker possibly implies these cases should be up-scored in the severity scale, and this awareness should be maintained for clinical decision-making. Current recommendations include patient isolation and infection control policies, similar to other cases of multi-drug resistant microorganisms infections or colonization (Table 3)<sup>[7]</sup>. However there are no present studies that define specific indications or clinical algorithms in these cases.

### New drugs for MRSA

**Daptomycin:** Resistance to daptomycin (MIC  $\geq 1$   $\mu\text{g/mL}$ ) is infrequent and the strains that have shown elevated MIC have been found to have mutations associated with cell membrane structure and cell wall thickness (mprF, yycFG). This has been more frequently observed in VISA strains with a lower sensitivity to glycopeptides<sup>[85]</sup>.

The main concern with the use of daptomycin is the emergence of resistance in the course of treatment. This problem seems to appear more frequently in cases where daptomycin is introduced as rescue treatment after vancomycin has failed or in cases of hVISA or VISA infections<sup>[86]</sup>. A study carried out in the United States observed a correlation between *S. aureus* strains with reduced vancomycin-susceptibility and the emergence of intra-treatment daptomycin resistance. This was especially found in cases of MRSA infections with vancomycin MIC of 4  $\mu\text{g/mL}$  or greater<sup>[87]</sup>.

Given that daptomycin's mechanism of action is unique, there doesn't seem to be crossed resistance with other antibiotics. Nevertheless, some studies

have observed that *S. aureus* with higher vancomycin-MIC (4-16  $\mu\text{g/mL}$ ) also show reduced sensitivity to daptomycin<sup>[87]</sup>. In those strains with vancomycin MIC 4-16  $\mu\text{g/mL}$ , daptomycin MIC was  $\geq 2$   $\mu\text{g/mL}$ , and therefore would fall over the sensitive threshold. However, *vanA* resistance to vancomycin does not affect daptomycin sensitivity<sup>[86]</sup>. In short, reduced vancomycin MIC is a call for awareness and precaution before using daptomycin in *S. aureus* infections. In these cases it would be important to know precise daptomycin MIC (broth micro-dilution or Etest) before starting this treatment.

In order to overcome these difficulties and to increase the bacterial-killing activity, it has been recommended to use higher doses of daptomycin in high risk infections (8-10 mg/kg per day) and up to now there has not been more toxicity associated to these doses in healthy volunteers treated for 14 d<sup>[88]</sup>. Another possible option that is lately being considered, especially in infections that involve prosthetic materials is the use of daptomycin in combination with other antibiotics. The combination with aminoglycosides and rifampin, has shown to be synergic<sup>[89]</sup>. Other synergic combinations in experimental models and in preliminary studies with promising results are, cloxacillin<sup>[90,91]</sup> other betalactams<sup>[92-94]</sup> and fosfomycin<sup>[95]</sup>. These options have been mainly studied for MRSA infections and for prosthetic devices associated infections. Future guidelines may contemplate treatment of MRSA infections with borderline vancomycin susceptibility with any of such combinations.

**Linezolid:** The main disadvantages of using linezolid for high-risk infections that need antibiotic therapy for an extended period of time (*i.e.*, endocarditis) are that it is a bacteriostatic anti-staphylococcal and its myelotoxicity. On the other hand, it offers the advantage of the possibility of oral administration and

has high tissue distribution. In an endocarditis study, linezolid was effective in 4 out of 8 patients (50%) with IE by VISA (MIC 2-4 µg/mL) that had failed with vancomycin<sup>[96]</sup>. In another retrospective study, 70% (of 22 patients) with MRSA IE that received linezolid because of failure of vancomycin or as sequential oral treatment were cured<sup>[97]</sup>. Sequential treatment showed 100% cure rate in 8 patients with MRSA IE with early valve surgical replacement (mean 5 d). Linezolid was administered from the fifth day onwards during approximately 3 wk<sup>[98]</sup>. Therefore, linezolid is an option for selected IE cases, when other treatments fail, or in patients that have intolerance to other treatments.

MRSA resistance to linezolid was first described associated to ribosomal mutations, and recently it has been described related to the appearance of *cfr* (for chloramphenicol-florfenicol resistance gene) gene. This is a plasmid-borne methyltransferase-mediated resistance mechanism that leads to resistance to various antibiotics, as well as linezolid<sup>[99,100]</sup>. This gene is responsible for the synthesis of a methylase that interferes with 23S rRNA. Hospital outbreaks of linezolid-resistant infections have been associated to these mutations.

**Other:** There is little clinical experience in the treatment of MRSA severe infections (such as endocarditis or pneumonia) with the “new” glycopeptides such as dalbavancin, oritavancin or telavancin, with tigecycline, or with the new cephalosporins (ceftobiprole or ceftaroline). However the majority of these drugs have shown potential efficacy in experimental models<sup>[101-106]</sup>.

In two recently published trials oritavancin and dalbavancin, showed to be non-inferior to vancomycin and linezolid for the treatment of skin and soft tissue infections (SSTI). These new glycopeptides offer unusual pharmacodynamic and pharmacokinetic properties that allow treating once or twice a week, as has been proved in skin and soft tissue infections, however more studies are needed for other sources of infection<sup>[107,108]</sup>. Telavancin is approved for the treatment of adult patients with complicated SSTIs and nosocomial pneumonia caused by gram-positive bacteria, including MRSA, when no other options are available. Up to now, the use of telavancin is restricted to MRSA infections with a vancomycin MIC ≥ 1 µg/mL, hVISA infections, lack of response to vancomycin treatment or patients who do not tolerate other antistaphylococcal antibiotics<sup>[109]</sup>.

Tigecycline is a semisynthetic drug derived of minocycline. Being a broad spectrum antibiotic, it has anti gram-positive and gram-negative activity<sup>[110]</sup>. Drawbacks to the use of tigecycline in bloodstream infections come from both, intrinsic drug characteristics and clinical experience. It is a bacteriostatic antibiotic and it reaches high tissue concentration but low concentration in plasma. Moreover, in previous studies it has been associated to worse prognosis and higher

mortality rates in patients with severe infections. Consequently, tigecycline is not normally recommended as a first line antibiotic for bloodstream or severe MRSA infections<sup>[111,112]</sup>.

Ceftaroline-fosamil is a cephalosporin with anti-MRSA activity<sup>[113]</sup>. In two clinical trials comparing ceftaroline with vancomycin plus aztreonam for SSTIs, they found treatments were comparable<sup>[114]</sup>. There is still little experience in the use of ceftaroline in other sources of MRSA infections.

Ceftobiprole is a new cephalosporin, that shows a broad-spectrum and strong bactericidal activity even for MRSA<sup>[115]</sup>. Ceftobiprole has high affinity for PBP2a (main PBP responsible for methicillin resistance), and is also stable to class A penicillinases, thence its good anti-MRSA activity<sup>[115]</sup>. In an animal MRSA endocarditis model, ceftobiprole was found superior to vancomycin, daptomycin, and linezolid<sup>[116]</sup>.

### Combination therapies

Rifampin and gentamycin are the two antibiotics that have most frequently been associated to vancomycin. The use of rifampin is based on its activity against *S. aureus* in stationary phase. However this synergy has not been proved *in vitro*<sup>[117,118]</sup> and the clinical benefits of adding rifampin to vancomycin in the treatment of MRSA IE hasn't been proved either<sup>[119]</sup>. The association of vancomycin and an aminoglycoside has been found synergic<sup>[120]</sup> and is therefore contemplated in patients with persistent bacteraemia. However this association hasn't proved a lower mortality rate in IE, and it has shown increased nephrotoxicity<sup>[121]</sup>, so it probably shouldn't be held as a first option treatment.

The combination of vancomycin and linezolid, both *in vitro* and *in vivo*, is indifferent or possibly antagonistic<sup>[122]</sup>. There are experimental studies<sup>[123]</sup> and a scarce clinical experience<sup>[124]</sup> that showed that the combination of vancomycin and quinupristin-dalfopristin was synergic, safe and useful for the treatment of 5 patients with severe MRSA infections.

There is scarce evidence about possible antibiotic combinations with linezolid, and results are contradictory. *In vitro* studies have shown decreased antibiotic activity of both gentamycin and vancomycin when associated to linezolid<sup>[125]</sup>. On the other hand, in animal models of IE they observed advantages in the combination of linezolid and gentamycin vs only linezolid<sup>[126]</sup>. Synergic combinations of linezolid with ertapenem and imipenem have also been communicated, both *in vitro*, and in experimental endocarditis models. However this association only is observed if the carbapenem is given in sub-inhibitory doses, whereas therapeutic doses decreases linezolid's antibiotic activity<sup>[127]</sup>.

Daptomycin at a dose of 10 mg/kg per day (and perhaps higher) may be more effective than the currently approved 6 mg/kg per day dose for severe *S. aureus* infections caused by non-susceptible strains (*i.e.*, those with MICs of > 1 µg/mL)<sup>[128]</sup>. In an experimental



animal aortic valve MRSA endocarditis model, combinations of daptomycin with an aminoglycoside or rifampin didn't show synergy<sup>[129]</sup>.

While fosfomycin is Food and Drug Administration approved only for the treatment of uncomplicated urinary tract infections, it has demonstrated good antimicrobial activity against a broad spectrum of pathogens, including MSSA and MRSA<sup>[130]</sup>. Fosfomycin, which acts by inhibition of an early step in cell wall synthesis, has been used successfully in combination with beta-lactams to treat severe staphylococcal infections<sup>[131]</sup>. It also shows *in vitro* synergy when combined with daptomycin<sup>[132]</sup>. Three cases have been recently published where they observe that the *in vitro* combination of high doses of daptomycin plus fosfomycin can be effective in the treatment of both native- and prosthetic-valve endocarditis caused by MSSA or MRSA<sup>[133]</sup>.

## CONCLUSION

MRSA proves to be a persistently lurking microorganism underlying both community and healthcare associated infection. The emergence of increasing vancomycin resistance patterns and the different consequences derived have created a new area of uncertainty in the clinical and therapeutic approach to these infections. More studies and trials are needed in order to better define these issues.

## REFERENCES

- 1 **Lachowicz TM**. The mechanism of development in vitro of penicillin-resistant variants of *Staphylococcus aureus*. II. Further investigation on the fluctuation test in the study of the origin of penicillin-resistance. *Acta Microbiol Pol* 1960; **9**: 143-150 [PMID: 13758083]
- 2 **McHenry MC**, Gavan TL, Farmer RG, Evarts CM. Infection due to methicillin-resistant *Staphylococcus aureus*. Report of an unusual case. *Cleve Clin Q* 1969; **36**: 9-16 [PMID: 5190707 DOI: 10.3949/ccjm.36.1.9]
- 3 **Kayser FH**. Methicillin-resistant staphylococci 1965-75. *Lancet* 1975; **2**: 650-653 [PMID: 52016 DOI: 10.1016/S0140-6736(75)90129-4]
- 4 **Crossley K**, Loesch D, Landesman B, Mead K, Chern M, Strate R. An outbreak of infections caused by strains of *Staphylococcus aureus* resistant to methicillin and aminoglycosides. I. Clinical studies. *J Infect Dis* 1979; **139**: 273-279 [PMID: 255552 DOI: 10.1093/infdis/139.3.273]
- 5 **Saroglou G**, Cromer M, Bisno AL. Methicillin-resistant *Staphylococcus aureus*: interstate spread of nosocomial infections with emergence of gentamicin-methicillin resistant strains. *Infect Control* 1980; **1**: 81-89 [PMID: 6915016]
- 6 **Stryjewski ME**, Corey GR. Methicillin-resistant *Staphylococcus aureus*: an evolving pathogen. *Clin Infect Dis* 2014; **58** Suppl 1: S10-S19 [PMID: 24343827 DOI: 10.1093/cid/cit613]
- 7 **Cosgrove SE**, Carroll KC, Perl TM. *Staphylococcus aureus* with reduced susceptibility to vancomycin. *Clin Infect Dis* 2004; **39**: 539-545 [PMID: 15356818 DOI: 10.1086/422458]
- 8 **Hawser SP**, Bouchillon SK, Hoban DJ, Dowzicky M, Babinchak T. Rising incidence of *Staphylococcus aureus* with reduced susceptibility to vancomycin and susceptibility to antibiotics: a global analysis 2004-2009. *Int J Antimicrob Agents* 2011; **37**: 219-224 [PMID: 21239146 DOI: 10.1016/j.ijantimicag.2010.10.029]
- 9 **Cuevas O**, Cercenado E, Vindel A, Guinea J, Sánchez-Conde M, Sánchez-Somolinos M, Bouza E. Evolution of the antimicrobial resistance of *Staphylococcus* spp. in Spain: five nationwide prevalence studies, 1986 to 2002. *Antimicrob Agents Chemother* 2004; **48**: 4240-4245 [PMID: 15504847 DOI: 10.1128/AAC.48.11.4240-4245.2004]
- 10 **Song JH**, Hsueh PR, Chung DR, Ko KS, Kang CI, Peck KR, Yeom JS, Kim SW, Chang HH, Kim YS, Jung SI, Son JS, So TM, Lalitha MK, Yang Y, Huang SG, Wang H, Lu Q, Carlos CC, Perera JA, Chiu CH, Liu JW, Chongthaleong A, Thamlikitkul V, Van PH. Spread of methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: an ANSORP study. *J Antimicrob Chemother* 2011; **66**: 1061-1069 [PMID: 21393157 DOI: 10.1093/jac/dkr024]
- 11 **Chen CJ**, Huang YC. New epidemiology of *Staphylococcus aureus* infection in Asia. *Clin Microbiol Infect* 2014; **20**: 605-623 [PMID: 24888414 DOI: 10.1111/1469-0691.12705]
- 12 **Klevens RM**, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyati G, Townes JM, Craig AS, Zell ER, Fosheim GE, McDougal LK, Carey RB, Fridkin SK. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA* 2007; **298**: 1763-1771 [PMID: 17940231 DOI: 10.1001/jama.298.15.1763]
- 13 **Ziakas PD**, Anagnostou T, Mylonakis E. The prevalence and significance of methicillin-resistant *Staphylococcus aureus* colonization at admission in the general ICU Setting: a meta-analysis of published studies. *Crit Care Med* 2014; **42**: 433-444 [PMID: 24145849 DOI: 10.1097/CCM.0b013e3182a66bb8]
- 14 **Landrum ML**, Neumann C, Cook C, Chukwuma U, Ellis MW, Hospenthal DR, Murray CK. Epidemiology of *Staphylococcus aureus* blood and skin and soft tissue infections in the US military health system, 2005-2010. *JAMA* 2012; **308**: 50-59 [PMID: 22760291 DOI: 10.1001/jama.2012.7139]
- 15 **Burton DC**, Edwards JR, Horan TC, Jernigan JA, Fridkin SK. Methicillin-resistant *Staphylococcus aureus* central line-associated bloodstream infections in US intensive care units, 1997-2007. *JAMA* 2009; **301**: 727-736 [PMID: 19224749 DOI: 10.1001/jama.2009.153]
- 16 **MRSA surveillance: active bacterial core (ABCs)** [updated 2015 Feb 11]. Available from: URL: <http://www.cdc.gov/hai/progress-report/index.html>
- 17 **Khatib R**, Sharma M, Iyer S, Fakih MG, Obeid KM, Venugopal A, Fishbain J, Johnson LB, Segireddy M, Jose J, Riederer K. Decreasing incidence of *Staphylococcus aureus* bacteremia over 9 years: greatest decline in community-associated methicillin-susceptible and hospital-acquired methicillin-resistant isolates. *Am J Infect Control* 2013; **41**: 210-213 [PMID: 23040608 DOI: 10.1016/j.ajic.2012.03.038]
- 18 **Rasigade JP**, Dumitrescu O, Lina G. New epidemiology of *Staphylococcus aureus* infections. *Clin Microbiol Infect* 2014; **20**: 587-588 [PMID: 24930666 DOI: 10.1111/1469-0691.12718]
- 19 **Cosgrove SE**, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. *Clin Infect Dis* 2003; **36**: 53-59 [PMID: 12491202 DOI: 10.1086/345476]
- 20 **Crossley K**, Landesman B, Zaske D. An outbreak of infections caused by strains of *Staphylococcus aureus* resistant to methicillin and aminoglycosides. II. Epidemiologic studies. *J Infect Dis* 1979; **139**: 280-287 [PMID: 255553 DOI: 10.1093/infdis/139.3.280]
- 21 **Sorrell TC**, Packham DR, Shanker S, Foldes M, Munro R. Vancomycin therapy for methicillin-resistant *Staphylococcus aureus*. *Ann Intern Med* 1982; **97**: 344-350 [PMID: 7114631]
- 22 **French GL**, Cheng AF, Ling JM, Mo P, Donnan S. Hong Kong strains of methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* have similar virulence. *J Hosp Infect* 1990; **15**: 117-125 [PMID: 1969433]
- 23 **Whitby M**, McLaws ML, Berry G. Risk of death from methicillin-resistant *Staphylococcus aureus* bacteraemia: a meta-analysis. *Med J Aust* 2001; **175**: 264-267 [PMID: 11587259]
- 24 **Levine DP**. Vancomycin: a history. *Clin Infect Dis* 2006; **42** Suppl 1:

- S5-12 [PMID: 16323120 DOI: 10.1086/491709]
- 25 **Ghosh N**, Chavada R, Maley M, van Hal SJ. Impact of source of infection and vancomycin AUC0-24/MICBMD targets on treatment failure in patients with methicillin-resistant *Staphylococcus aureus* bacteraemia. *Clin Microbiol Infect* 2014; **20**: O1098-O1105 [PMID: 24890030 DOI: 10.1111/1469-0691.12695]
- 26 **Liu C**, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, Kaplan SL, Karchmer AW, Levine DP, Murray BE, J Rybak M, Talan DA, Chambers HF. Clinical practice guidelines by the infectious diseases society of america for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. *Clin Infect Dis* 2011; **52**: e18-e55 [PMID: 21208910 DOI: 10.1093/cid/ciq146]
- 27 **Hiramatsu K**, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Reduced susceptibility of *Staphylococcus aureus* to vancomycin--Japan, 1996. *MMWR Morb Mortal Wkly Rep* 1997; **46**: 624-626 [PMID: 9218648]
- 28 **Hiramatsu K**, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother* 1997; **40**: 135-136 [PMID: 9249217]
- 29 **van Hal SJ**, Fowler VG. Is it time to replace vancomycin in the treatment of methicillin-resistant *Staphylococcus aureus* infections? *Clin Infect Dis* 2013; **56**: 1779-1788 [PMID: 23511300 DOI: 10.1093/cid/cit178]
- 30 **Tenover FC**, Moellering RC. The rationale for revising the Clinical and Laboratory Standards Institute vancomycin minimal inhibitory concentration interpretive criteria for *Staphylococcus aureus*. *Clin Infect Dis* 2007; **44**: 1208-1215 [PMID: 17407040 DOI: 10.1086/513203]
- 31 **Steinkraus G**, White R, Friedrich L. Vancomycin MIC creep in non-vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-susceptible clinical methicillin-resistant *S. aureus* (MRSA) blood isolates from 2001-05. *J Antimicrob Chemother* 2007; **60**: 788-794 [PMID: 17623693 DOI: 10.1093/jac/dkm258]
- 32 **Hiramatsu K**. Vancomycin-resistant *Staphylococcus aureus*: a new model of antibiotic resistance. *Lancet Infect Dis* 2001; **1**: 147-155 [PMID: 11871491 DOI: 10.1016/S1473-3099(01)00091-3]
- 33 **Cui L**, Ma X, Sato K, Okuma K, Tenover FC, Mamizuka EM, Gemmell CG, Kim MN, Ploy MC, El-Solh N, Ferraz V, Hiramatsu K. Cell wall thickening is a common feature of vancomycin resistance in *Staphylococcus aureus*. *J Clin Microbiol* 2003; **41**: 5-14 [PMID: 12517819 DOI: 10.1128/JCM.41.1.5-14.2003]
- 34 **Sieradzki K**, Tomasz A. Alterations of cell wall structure and metabolism accompany reduced susceptibility to vancomycin in an isogenic series of clinical isolates of *Staphylococcus aureus*. *J Bacteriol* 2003; **185**: 7103-7110 [PMID: 14645269 DOI: 10.1128/JB.185.24.7103-7110.2003]
- 35 **Boyle-Vavra S**, Berke SK, Lee JC, Daum RS. Reversion of the glycopeptide resistance phenotype in *Staphylococcus aureus* clinical isolates. *Antimicrob Agents Chemother* 2000; **44**: 272-277 [PMID: 10639349]
- 36 **Centers for Disease Control and Prevention (CDC)**. *Staphylococcus aureus* resistant to vancomycin--United States, 2002. *MMWR Morb Mortal Wkly Rep* 2002; **51**: 565-567 [PMID: 12139181]
- 37 **Périchon B**, Courvalin P. VanA-type vancomycin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2009; **53**: 4580-4587 [PMID: 19506057 DOI: 10.1128/AAC.00346-09]
- 38 **Sakoulas G**, Moise-Broder PA, Schentag J, Forrest A, Moellering RC, Eliopoulos GM. Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. *J Clin Microbiol* 2004; **42**: 2398-2402 [PMID: 15184410 DOI: 10.1128/JCM.42.6.2398-2402.2004]
- 39 **Wang G**, Hindler JF, Ward KW, Bruckner DA. Increased vancomycin MICs for *Staphylococcus aureus* clinical isolates from a university hospital during a 5-year period. *J Clin Microbiol* 2006; **44**: 3883-3886 [PMID: 16957043 DOI: 10.1128/JCM.01388-06]
- 40 **Howden BP**, Davies JK, Johnson PD, Stinear TP, Grayson ML. Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. *Clin Microbiol Rev* 2010; **23**: 99-139 [PMID: 20065327]
- 41 **Musta AC**, Riederer K, Shemes S, Chase P, Jose J, Johnson LB, Khatib R. Vancomycin MIC plus heteroresistance and outcome of methicillin-resistant *Staphylococcus aureus* bacteremia: trends over 11 years. *J Clin Microbiol* 2009; **47**: 1640-1644 [PMID: 19369444 DOI: 10.1128/JCM.02135-08]
- 42 **Hussain FM**, Boyle-Vavra S, Shete PB, Daum RS. Evidence for a continuum of decreased vancomycin susceptibility in unselected *Staphylococcus aureus* clinical isolates. *J Infect Dis* 2002; **186**: 661-667 [PMID: 12195353 DOI: 10.1086/342708]
- 43 **Liu C**, Chambers HF. *Staphylococcus aureus* with heterogeneous resistance to vancomycin: epidemiology, clinical significance, and critical assessment of diagnostic methods. *Antimicrob Agents Chemother* 2003; **47**: 3040-3045 [PMID: 14506006 DOI: 10.1128/AAC.47.10.3040-3045.2003]
- 44 **Jenkins SG**, Schuetz AN. Current concepts in laboratory testing to guide antimicrobial therapy. *Mayo Clin Proc* 2012; **87**: 290-308 [PMID: 22386185 DOI: 10.1016/j.mayocp.2012.01.007]
- 45 **Lubin AS**, Snyderman DR, Ruthazer R, Bide P, Golan Y. Predicting high vancomycin minimum inhibitory concentration in methicillin-resistant *Staphylococcus aureus* bloodstream infections. *Clin Infect Dis* 2011; **52**: 997-1002 [PMID: 21460313 DOI: 10.1093/cid/cir118]
- 46 **Jones RN**. Microbiological features of vancomycin in the 21st century: minimum inhibitory concentration creep, bactericidal/static activity, and applied breakpoints to predict clinical outcomes or detect resistant strains. *Clin Infect Dis* 2006; **42** Suppl 1: S13-S24 [PMID: 16323115 DOI: 10.1086/491710]
- 47 **Wootton M**, Walsh TR, MacGowan AP. Evidence for reduction in breakpoints used to determine vancomycin susceptibility in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2005; **49**: 3982-3983 [PMID: 16127089 DOI: 10.1128/AAC.49.9.3982-3983.2005]
- 48 **Deresinski S**. Counterpoint: Vancomycin and *Staphylococcus aureus*--an antibiotic enters obsolescence. *Clin Infect Dis* 2007; **44**: 1543-1548 [PMID: 17516396 DOI: 10.1086/518452]
- 49 **Fridkin SK**, Hageman J, McDougal LK, Mohammed J, Jarvis WR, Perl TM, Tenover FC. Epidemiological and microbiological characterization of infections caused by *Staphylococcus aureus* with reduced susceptibility to vancomycin, United States, 1997-2001. *Clin Infect Dis* 2003; **36**: 429-439 [PMID: 12567300 DOI: 10.1086/346207]
- 50 **Charles PG**, Ward PB, Johnson PD, Howden BP, Grayson ML. Clinical features associated with bacteremia due to heterogeneous vancomycin-intermediate *Staphylococcus aureus*. *Clin Infect Dis* 2004; **38**: 448-451 [PMID: 14727222 DOI: 10.1086/381093]
- 51 **Lodise TP**, Miller CD, Graves J, Evans A, Graffunder E, Helmecke M, Stellrecht K. Predictors of high vancomycin MIC values among patients with methicillin-resistant *Staphylococcus aureus* bacteraemia. *J Antimicrob Chemother* 2008; **62**: 1138-1141 [PMID: 18694905 DOI: 10.1093/jac/dkn329]
- 52 **Moise PA**, Smyth DS, El-Fawal N, Robinson DA, Holden PN, Forrest A, Sakoulas G. Microbiological effects of prior vancomycin use in patients with methicillin-resistant *Staphylococcus aureus* bacteraemia. *J Antimicrob Chemother* 2008; **61**: 85-90 [PMID: 18042628 DOI: 10.1093/jac/dkn445]
- 53 **Maclayton DO**, Suda KJ, Coval KA, York CB, Garey KW. Case-control study of the relationship between MRSA bacteremia with a vancomycin MIC of 2 microg/mL and risk factors, costs, and outcomes in inpatients undergoing hemodialysis. *Clin Ther* 2006; **28**: 1208-1216 [PMID: 16982298 DOI: 10.1016/j.clinthera.2006.08.003]
- 54 **Sievert DM**, Rudrik JT, Patel JB, McDonald LC, Wilkins MJ, Hageman JC. Vancomycin-resistant *Staphylococcus aureus* in the

- United States, 2002-2006. *Clin Infect Dis* 2008; **46**: 668-674 [PMID: 18257700 DOI: 10.1086/527392]
- 55 **Friães A**, Resina C, Manuel V, Lito L, Ramirez M, Melo-Cristino J. Epidemiological survey of the first case of vancomycin-resistant *Staphylococcus aureus* infection in Europe. *Epidemiol Infect* 2015; **143**: 745-748 [PMID: 24901752 DOI: 10.1017/S0950268814001423]
- 56 **Labrou M**, Michail G, Ntokou E, Pittaras TE, Pourmaras S, Tsakris A. Activity of oxacillin versus that of vancomycin against oxacillin-susceptible mecA-positive *Staphylococcus aureus* clinical isolates evaluated by population analyses, time-kill assays, and a murine thigh infection model. *Antimicrob Agents Chemother* 2012; **56**: 3388-3391 [PMID: 22430957 DOI: 10.1128/AAC.00103-12]
- 57 **Moise-Broder PA**, Forrest A, Birmingham MC, Schentag JJ. Pharmacodynamics of vancomycin and other antimicrobials in patients with *Staphylococcus aureus* lower respiratory tract infections. *Clin Pharmacokinet* 2004; **43**: 925-942 [PMID: 15509186]
- 58 **Moise PA**, Sakoulas G, Forrest A, Schentag JJ. Vancomycin in vitro bactericidal activity and its relationship to efficacy in clearance of methicillin-resistant *Staphylococcus aureus* bacteremia. *Antimicrob Agents Chemother* 2007; **51**: 2582-2586 [PMID: 17452488 DOI: 10.1128/AAC.00939-06]
- 59 **Moise PA**, Schentag JJ. Vancomycin treatment failures in *Staphylococcus aureus* lower respiratory tract infections. *Int J Antimicrob Agents* 2000; **16** Suppl 1: S31-S34 [PMID: 11137406]
- 60 **Soriano A**, Marco F, Martínez JA, Pisos E, Almela M, Dimova VP, Alamo D, Ortega M, Lopez J, Mensa J. Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. *Clin Infect Dis* 2008; **46**: 193-200 [PMID: 18171250 DOI: 10.1086/524667]
- 61 **Rybak MJ**, Lomaestro BM, Rotschafer JC, Moellering RC, Craig WA, Billeter M, Dalovisio JR, Levine DP. Vancomycin therapeutic guidelines: a summary of consensus recommendations from the infectious diseases Society of America, the American Society of Health-System Pharmacists, and the Society of Infectious Diseases Pharmacists. *Clin Infect Dis* 2009; **49**: 325-327 [PMID: 19569969 DOI: 10.1086/600877]
- 62 **Hidayat LK**, Hsu DI, Quist R, Shriner KA, Wong-Beringer A. High-dose vancomycin therapy for methicillin-resistant *Staphylococcus aureus* infections: efficacy and toxicity. *Arch Intern Med* 2006; **166**: 2138-2144 [PMID: 17060545 DOI: 10.1001/archinte.166.19.2138]
- 63 **Patel N**, Pai MP, Rodvold KA, Lomaestro B, Drusano GL, Lodise TP. Vancomycin: we can't get there from here. *Clin Infect Dis* 2011; **52**: 969-974 [PMID: 21460308 DOI: 10.1093/cid/cir078]
- 64 **Mohr JF**, Murray BE. Point: Vancomycin is not obsolete for the treatment of infection caused by methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis* 2007; **44**: 1536-1542 [PMID: 17516395 DOI: 10.1086/518451]
- 65 **van Hal SJ**, Paterson DL, Lodise TP. Systematic review and meta-analysis of vancomycin-induced nephrotoxicity associated with dosing schedules that maintain troughs between 15 and 20 milligrams per liter. *Antimicrob Agents Chemother* 2013; **57**: 734-744 [PMID: 23165462 DOI: 10.1128/AAC.01568-12]
- 66 **Lodise TP**, Lomaestro B, Graves J, Drusano GL. Larger vancomycin doses (at least four grams per day) are associated with an increased incidence of nephrotoxicity. *Antimicrob Agents Chemother* 2008; **52**: 1330-1336 [PMID: 18227177 DOI: 10.1128/AAC.01602-07]
- 67 **Lodise TP**, Patel N, Lomaestro BM, Rodvold KA, Drusano GL. Relationship between initial vancomycin concentration-time profile and nephrotoxicity among hospitalized patients. *Clin Infect Dis* 2009; **49**: 507-514 [PMID: 19586413 DOI: 10.1086/600884]
- 68 **Jeffres MN**, Isakow W, Doherty JA, Micek ST, Kollef MH. A retrospective analysis of possible renal toxicity associated with vancomycin in patients with health care-associated methicillin-resistant *Staphylococcus aureus* pneumonia. *Clin Ther* 2007; **29**: 1107-1115 [PMID: 17692725 DOI: 10.1016/j.clinthera.2007.06.014]
- 69 **van Hal SJ**, Lodise TP, Paterson DL. The clinical significance of vancomycin minimum inhibitory concentration in *Staphylococcus aureus* infections: a systematic review and meta-analysis. *Clin Infect Dis* 2012; **54**: 755-771 [PMID: 22302374 DOI: 10.1093/cid/cir935]
- 70 **Holmes NE**, Johnson PD, Howden BP. Relationship between vancomycin-resistant *Staphylococcus aureus*, vancomycin-intermediate *S. aureus*, high vancomycin MIC, and outcome in serious *S. aureus* infections. *J Clin Microbiol* 2012; **50**: 2548-2552 [PMID: 22593595 DOI: 10.1128/JCM.00775-12]
- 71 **van Hal SJ**, Paterson DL. Systematic review and meta-analysis of the significance of heterogeneous vancomycin-intermediate *Staphylococcus aureus* isolates. *Antimicrob Agents Chemother* 2011; **55**: 405-410 [PMID: 21078939 DOI: 10.1128/AAC.01133-10]
- 72 **Holmes NE**, Turnidge JD, Munckhof WJ, Robinson JO, Korman TM, O'Sullivan MV, Anderson TL, Roberts SA, Gao W, Christiansen KJ, Coombs GW, Johnson PD, Howden BP. Antibiotic choice may not explain poorer outcomes in patients with *Staphylococcus aureus* bacteremia and high vancomycin minimum inhibitory concentrations. *J Infect Dis* 2011; **204**: 340-347 [PMID: 21742831 DOI: 10.1093/infdis/jir270]
- 73 **Lalueza A**, Chaves F, San Juan R, Daskalaki M, Otero JR, Aguado JM. Is high vancomycin minimum inhibitory concentration a good marker to predict the outcome of methicillin-resistant *Staphylococcus aureus* bacteremia? *J Infect Dis* 2010; **201**: 311-312; author reply 311-312 [PMID: 20034343 DOI: 10.1086/649572]
- 74 **Peleg AY**, Monga D, Pillai S, Mylonakis E, Moellering RC, Eliopoulos GM. Reduced susceptibility to vancomycin influences pathogenicity in *Staphylococcus aureus* infection. *J Infect Dis* 2009; **199**: 532-536 [PMID: 19125671 DOI: 10.1086/596511]
- 75 **Price J**, Atkinson S, Llewelyn M, Paul J. Paradoxical relationship between the clinical outcome of *Staphylococcus aureus* bacteremia and the minimum inhibitory concentration of vancomycin. *Clin Infect Dis* 2009; **48**: 997-998 [PMID: 19260820 DOI: 10.1086/597359]
- 76 **Aguado JM**, San-Juan R, Lalueza A, Sanz F, Rodríguez-Otero J, Gómez-Gonzalez C, Chaves F. High vancomycin MIC and complicated methicillin-susceptible *Staphylococcus aureus* bacteremia. *Emerg Infect Dis* 2011; **17**: 1099-1102 [PMID: 21749780 DOI: 10.3201/eid1706.101037]
- 77 **Murdoch DR**, Corey GR, Hoen B, Miró JM, Fowler VG, Bayer AS, Karchmer AW, Olaison L, Pappas PA, Moreillon P, Chambers ST, Chu VH, Falcó V, Holland DJ, Jones P, Klein JL, Raymond NJ, Read KM, Tripodi MF, Utili R, Wang A, Woods CW, Cabell CH. Clinical presentation, etiology, and outcome of infective endocarditis in the 21st century: the International Collaboration on Endocarditis-Prospective Cohort Study. *Arch Intern Med* 2009; **169**: 463-473 [PMID: 19273776 DOI: 10.1001/archinternmed.2008.603]
- 78 **Miro JM**, Anguera I, Cabell CH, Chen AY, Stafford JA, Corey GR, Olaison L, Eykyn S, Hoen B, Abrutyn E, Raoult D, Bayer A, Fowler VG. *Staphylococcus aureus* native valve infective endocarditis: report of 566 episodes from the International Collaboration on Endocarditis Merged Database. *Clin Infect Dis* 2005; **41**: 507-514 [PMID: 16028160]
- 79 **Fowler VG**, Miro JM, Hoen B, Cabell CH, Abrutyn E, Rubinstein E, Corey GR, Spelman D, Bradley SF, Barsic B, Pappas PA, Anstrom KJ, Wray D, Fortes CQ, Anguera I, Athan E, Jones P, van der Meer JT, Elliott TS, Levine DP, Bayer AS. *Staphylococcus aureus* endocarditis: a consequence of medical progress. *JAMA* 2005; **293**: 3012-3021 [PMID: 15972563 DOI: 10.1001/jama.293.24.3012]
- 80 **Bae IG**, Federspiel JJ, Miró JM, Woods CW, Park L, Rybak MJ, Rude TH, Bradley S, Bukovski S, de la Maria CG, Kanj SS, Korman TM, Marco F, Murdoch DR, Plesiat P, Rodríguez-Creixems M, Reinbott P, Steed L, Tattévin P, Tripodi MF, Newton KL, Corey GR, Fowler VG. Heterogeneous vancomycin-intermediate susceptibility phenotype in bloodstream methicillin-resistant *Staphylococcus aureus* isolates from an international cohort of patients with infective endocarditis: prevalence, genotype, and clinical significance. *J Infect Dis* 2009; **200**: 1355-1366 [PMID: 19811099 DOI: 10.1086/606027]



- 81 **Cervera C**, Castañeda X, de la Maria CG, del Rio A, Moreno A, Soy D, Pericas JM, Falces C, Armero Y, Almela M, Ninot S, Pare JC, Mestres CA, Gatell JM, Marco F, Miro JM. Effect of vancomycin minimal inhibitory concentration on the outcome of methicillin-susceptible *Staphylococcus aureus* endocarditis. *Clin Infect Dis* 2014; **58**: 1668-1675 [PMID: 24647021 DOI: 10.1093/cid/ciu183]
- 82 **Moise PA**, Forrest A, Bayer AS, Xiong YQ, Yeaman MR, Sakoulas G. Factors influencing time to vancomycin-induced clearance of nonendocarditis methicillin-resistant *Staphylococcus aureus* bacteremia: role of platelet microbicidal protein killing and agr genotypes. *J Infect Dis* 2010; **201**: 233-240 [PMID: 20001853 DOI: 10.1086/649429]
- 83 **Fowler VG**, Sakoulas G, McIntyre LM, Meka VG, Arbeit RD, Cabell CH, Stryjewski ME, Eliopoulos GM, Reller LB, Corey GR, Jones T, Lucindo N, Yeaman MR, Bayer AS. Persistent bacteremia due to methicillin-resistant *Staphylococcus aureus* infection is associated with agr dysfunction and low-level in vitro resistance to thrombin-induced platelet microbicidal protein. *J Infect Dis* 2004; **190**: 1140-1149 [PMID: 15319865 DOI: 10.1086/423145]
- 84 **Schweizer ML**, Furuno JP, Sakoulas G, Johnson JK, Harris AD, Shardell MD, McGregor JC, Thom KA, Perencevich EN. Increased mortality with accessory gene regulator (agr) dysfunction in *Staphylococcus aureus* among bacteremic patients. *Antimicrob Agents Chemother* 2011; **55**: 1082-1087 [PMID: 21173172 DOI: 10.1128/AAC.00918-10]
- 85 **Bayer AS**, Schneider T, Sahl HG. Mechanisms of daptomycin resistance in *Staphylococcus aureus*: role of the cell membrane and cell wall. *Ann N Y Acad Sci* 2013; **1277**: 139-158 [PMID: 23215859 DOI: 10.1111/j.1749-6632.2012.06819.x]
- 86 **Moise PA**, North D, Steenbergen JN, Sakoulas G. Susceptibility relationship between vancomycin and daptomycin in *Staphylococcus aureus*: facts and assumptions. *Lancet Infect Dis* 2009; **9**: 617-624 [PMID: 19778764 DOI: 10.1016/S1473-3099(09)70200-2]
- 87 **Patel JB**, Jevitt LA, Hageman J, McDonald LC, Tenover FC. An association between reduced susceptibility to daptomycin and reduced susceptibility to vancomycin in *Staphylococcus aureus*. *Clin Infect Dis* 2006; **42**: 1652-1653 [PMID: 16652325 DOI: 10.1086/504084]
- 88 **Benvenuto M**, Benziger DP, Yankelov S, Vigliani G. Pharmacokinetics and tolerability of daptomycin at doses up to 12 milligrams per kilogram of body weight once daily in healthy volunteers. *Antimicrob Agents Chemother* 2006; **50**: 3245-3249 [PMID: 17005801 DOI: 10.1128/AAC.00247-06]
- 89 **Credito K**, Lin G, Appelbaum PC. Activity of daptomycin alone and in combination with rifampin and gentamicin against *Staphylococcus aureus* assessed by time-kill methodology. *Antimicrob Agents Chemother* 2007; **51**: 1504-1507 [PMID: 17220402 DOI: 10.1128/AAC.01455-06]
- 90 **Garrigós C**, Murillo O, Lora-Tamayo J, Verdaguer R, Tubau F, Cabellos C, Cabo J, Ariza J. Efficacy of daptomycin-cloxacillin combination in experimental foreign-body infection due to methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2012; **56**: 3806-3811 [PMID: 22585211 DOI: 10.1128/AAC.00127-12]
- 91 **Yang SJ**, Xiong YQ, Boyle-Vavra S, Daum R, Jones T, Bayer AS. Daptomycin-oxacillin combinations in treatment of experimental endocarditis caused by daptomycin-nonsusceptible strains of methicillin-resistant *Staphylococcus aureus* with evolving oxacillin susceptibility (the "seesaw effect"). *Antimicrob Agents Chemother* 2010; **54**: 3161-3169 [PMID: 20547804 DOI: 10.1128/AAC.00487-10]
- 92 **Rand KH**, Houck HJ. Synergy of daptomycin with oxacillin and other beta-lactams against methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2004; **48**: 2871-2875 [PMID: 15273094 DOI: 10.1128/AAC.48.8.2871-2875.2004]
- 93 **Mehta S**, Singh C, Plata KB, Chanda PK, Paul A, Riosa S, Rosato RR, Rosato AE.  $\beta$ -Lactams increase the antibacterial activity of daptomycin against clinical methicillin-resistant *Staphylococcus aureus* strains and prevent selection of daptomycin-resistant derivatives. *Antimicrob Agents Chemother* 2012; **56**: 6192-6200 [PMID: 22985884 DOI: 10.1128/AAC.01525-12]
- 94 **Berti AD**, Sakoulas G, Nizet V, Tewhey R, Rose WE.  $\beta$ -Lactam antibiotics targeting PBP1 selectively enhance daptomycin activity against methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2013; **57**: 5005-5012 [PMID: 23896478 DOI: 10.1128/AAC.00594-13]
- 95 **Garrigós C**, Murillo O, Lora-Tamayo J, Verdaguer R, Tubau F, Cabellos C, Cabo J, Ariza J. Fosfomycin-daptomycin and other fosfomycin combinations as alternative therapies in experimental foreign-body infection by methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2013; **57**: 606-610 [PMID: 23089756 DOI: 10.1128/AAC.01570-12]
- 96 **Howden BP**, Ward PB, Charles PG, Korman TM, Fuller A, du Cros P, Grabsch EA, Roberts SA, Robson J, Read K, Bak N, Hurley J, Johnson PD, Morris AJ, Mayall BC, Grayson ML. Treatment outcomes for serious infections caused by methicillin-resistant *Staphylococcus aureus* with reduced vancomycin susceptibility. *Clin Infect Dis* 2004; **38**: 521-528 [PMID: 14765345 DOI: 10.1086/381202]
- 97 **Muñoz P**, Rodríguez-Creixéns M, Moreno M, Marín M, Ramallo V, Bouza E. Linezolid therapy for infective endocarditis. *Clin Microbiol Infect* 2007; **13**: 211-215 [PMID: 17328738 DOI: 10.1111/j.1469-0691.2006.01585.x]
- 98 **Colli A**, Campodonico R, Gherli T. Early switch from vancomycin to oral linezolid for treatment of gram-positive heart valve endocarditis. *Ann Thorac Surg* 2007; **84**: 87-91 [PMID: 17588391 DOI: 10.1016/j.athoracsur.2007.02.096]
- 99 **Gu B**, Kelesidis T, Tsiodras S, Hindler J, Humphries RM. The emerging problem of linezolid-resistant *Staphylococcus*. *J Antimicrob Chemother* 2013; **68**: 4-11 [PMID: 22949625 DOI: 10.1093/jac/dks354]
- 100 **Long KS**, Vester B. Resistance to linezolid caused by modifications at its binding site on the ribosome. *Antimicrob Agents Chemother* 2012; **56**: 603-612 [PMID: 22143525 DOI: 10.1128/AAC.05702-11]
- 101 **Entenza JM**, Hohl P, Heinze-Krauss I, Glauser MP, Moreillon P. BAL9141, a novel extended-spectrum cephalosporin active against methicillin-resistant *Staphylococcus aureus* in treatment of experimental endocarditis. *Antimicrob Agents Chemother* 2002; **46**: 171-177 [PMID: 11751129 DOI: 10.1128/AAC.46.1.171-177.2002]
- 102 **Jacqueline C**, Caillon J, Le Mabecque V, Miègeville AF, Hamel A, Bugnon D, Ge JY, Potel G. In vivo efficacy of ceftaroline (PPI-0903), a new broad-spectrum cephalosporin, compared with linezolid and vancomycin against methicillin-resistant and vancomycin-intermediate *Staphylococcus aureus* in a rabbit endocarditis model. *Antimicrob Agents Chemother* 2007; **51**: 3397-3400 [PMID: 17591849 DOI: 10.1128/AAC.01242-06]
- 103 **Kaatz GW**, Seo SM, Aeschlimann JR, Houlihan HH, Mercier RC, Rybak MJ. Efficacy of LY333328 against experimental methicillin-resistant *Staphylococcus aureus* endocarditis. *Antimicrob Agents Chemother* 1998; **42**: 981-983 [PMID: 9559828]
- 104 **Lefort A**, Pavie J, Garry L, Chau F, Fantin B. Activities of dalbavancin in vitro and in a rabbit model of experimental endocarditis due to *Staphylococcus aureus* with or without reduced susceptibility to vancomycin and teicoplanin. *Antimicrob Agents Chemother* 2004; **48**: 1061-1064 [PMID: 14982811 DOI: 10.1128/AAC.48.3.1061-1064.2004]
- 105 **Miró JM**, García-de-la-Maria C, Armero Y, de-Lazzari E, Soy D, Moreno A, del Rio A, Almela M, Mestres CA, Gatell JM, Jiménez-de-Anta MT, Marco F. Efficacy of telavancin in the treatment of experimental endocarditis due to glycopeptide-intermediate *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2007; **51**: 2373-2377 [PMID: 17485502 DOI: 10.1128/AAC.01266-06]
- 106 **Murphy TM**, Deitz JM, Petersen PJ, Mikels SM, Weiss WJ. Therapeutic efficacy of GAR-936, a novel glycylcycline, in a rat model of experimental endocarditis. *Antimicrob Agents Chemother* 2000; **44**: 3022-3027 [PMID: 11036017 DOI: 10.1128/AAC.44.11.



- 3022-3027.2000]
- 107 **Boucher HW**, Wilcox M, Talbot GH, Puttagunta S, Das AF, Dunne MW. Once-weekly dalbavancin versus daily conventional therapy for skin infection. *N Engl J Med* 2014; **370**: 2169-2179 [PMID: 24897082 DOI: 10.1056/NEJMoa1310480]
- 108 **Corey GR**, Kabler H, Mehra P, Gupta S, Overcash JS, Porwal A, Giordano P, Lucasti C, Perez A, Good S, Jiang H, Moeck G, O'Riordan W. Single-dose oritavancin in the treatment of acute bacterial skin infections. *N Engl J Med* 2014; **370**: 2180-2190 [PMID: 24897083]
- 109 **Gould IM**, David MZ, Esposito S, Garau J, Lina G, Mazzei T, Peters G. New insights into methicillin-resistant *Staphylococcus aureus* (MRSA) pathogenesis, treatment and resistance. *Int J Antimicrob Agents* 2012; **39**: 96-104 [PMID: 22196394 DOI: 10.1016/j.ijantimicag.2011.09.028]
- 110 **Slover CM**, Rodvold KA, Danziger LH. Tigecycline: a novel broad-spectrum antimicrobial. *Ann Pharmacother* 2007; **41**: 965-972 [PMID: 17519296 DOI: 10.1345/aph.1H543]
- 111 **Rodvold KA**, Gotfried MH, Cwik M, Korth-Bradley JM, Dukart G, Ellis-Grosse EJ. Serum, tissue and body fluid concentrations of tigecycline after a single 100 mg dose. *J Antimicrob Chemother* 2006; **58**: 1221-1229 [PMID: 17012300 DOI: 10.1093/jac/dkl403]
- 112 **Liu C**, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, Kaplan SL, Karchmer AW, Levine DP, Murray BE, J Rybak M, Talan DA, Chambers HF. Clinical practice guidelines by the infectious diseases society of america for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children: executive summary. *Clin Infect Dis* 2011; **52**: 285-292 [PMID: 21217178 DOI: 10.1093/cid/cir034]
- 113 **Lodise TP**, Low DE. Ceftaroline fosamil in the treatment of community-acquired bacterial pneumonia and acute bacterial skin and skin structure infections. *Drugs* 2012; **72**: 1473-1493 [PMID: 22779432 DOI: 10.2165/11635660-000000000-00000]
- 114 **File TM**, Wilcox MH, Stein GE. Summary of ceftaroline fosamil clinical trial studies and clinical safety. *Clin Infect Dis* 2012; **55** Suppl 3: S173-S180 [PMID: 22903949 DOI: 10.1093/cid/cis559]
- 115 **Bush K**, Heep M, Macielag MJ, Noel GJ. Anti-MRSA beta-lactams in development, with a focus on ceftobiprole: the first anti-MRSA beta-lactam to demonstrate clinical efficacy. *Expert Opin Investig Drugs* 2007; **16**: 419-429 [PMID: 17371191 DOI: 10.1517/13543784.16.4.419]
- 116 **Tattevin P**, Basuino L, Bauer D, Diep BA, Chambers HF. Ceftobiprole is superior to vancomycin, daptomycin, and linezolid for treatment of experimental endocarditis in rabbits caused by methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2010; **54**: 610-613 [PMID: 19917746 DOI: 10.1128/aac.00886-09]
- 117 **Krut O**, Sommer H, Krönke M. Antibiotic-induced persistence of cytotoxic *Staphylococcus aureus* in non-phagocytic cells. *J Antimicrob Chemother* 2004; **53**: 167-173 [PMID: 14729736 DOI: 10.1093/jac/dkh076]
- 118 **Shelburne SA**, Musher DM, Hulten K, Ceasar H, Lu MY, Bhaila I, Hamill RJ. In vitro killing of community-associated methicillin-resistant *Staphylococcus aureus* with drug combinations. *Antimicrob Agents Chemother* 2004; **48**: 4016-4019 [PMID: 15388469 DOI: 10.1128/AAC.48.10.4016-4019.2004]
- 119 **Levine DP**, Fromm BS, Reddy BR. Slow response to vancomycin or vancomycin plus rifampin in methicillin-resistant *Staphylococcus aureus* endocarditis. *Ann Intern Med* 1991; **115**: 674-680 [PMID: 1929035]
- 120 **Houlihan HH**, Mercier RC, Rybak MJ. Pharmacodynamics of vancomycin alone and in combination with gentamicin at various dosing intervals against methicillin-resistant *Staphylococcus aureus*-infected fibrin-platelet clots in an in vitro infection model. *Antimicrob Agents Chemother* 1997; **41**: 2497-2501 [PMID: 9371356]
- 121 **Lodise TP**, Drusano GL, Zasowski E, Dihmess A, Lazariu V, Cosler L, McNutt LA. Vancomycin exposure in patients with methicillin-resistant *Staphylococcus aureus* bloodstream infections: how much is enough? *Clin Infect Dis* 2014; **59**: 666-675 [PMID: 24867791 DOI: 10.1093/cid/ciu398]
- 122 **Chiang FY**, Climo M. Efficacy of linezolid alone or in combination with vancomycin for treatment of experimental endocarditis due to methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2003; **47**: 3002-3004 [PMID: 12937013]
- 123 **Pavie J**, Lefort A, Zarrouk V, Chau F, Garry L, Leclercq R, Fantin B. Efficacies of quinupristin-dalfopristin combined with vancomycin in vitro and in experimental endocarditis due to methicillin-resistant *Staphylococcus aureus* in relation to cross-resistance to macrolides, lincosamides, and streptogramin B- type antibiotics. *Antimicrob Agents Chemother* 2002; **46**: 3061-3064 [PMID: 12183272 DOI: 10.1128/AAC.46.9.3061-3064.2002]
- 124 **Sgarabotto D**, Cusinato R, Narne E, Scano F, Zignol M, Gambino A, Cattelan A, Meneghetti F, Cadrobbi P. Synergic plus vancomycin for the treatment of severe methicillin-resistant *Staphylococcus aureus* and coagulase-negative staphylococci infections: evaluation of 5 cases. *Scand J Infect Dis* 2002; **34**: 122-126 [PMID: 11928842 DOI: 10.1080/00365540110077245]
- 125 **Jacqueline C**, Caillon J, Le Mabecque V, Miegerville AF, Donnio PY, Bugnon D, Potel G. In vitro activity of linezolid alone and in combination with gentamicin, vancomycin or rifampicin against methicillin-resistant *Staphylococcus aureus* by time-kill curve methods. *J Antimicrob Chemother* 2003; **51**: 857-864 [PMID: 12654769 DOI: 10.1093/jac/dkg160]
- 126 **Jacqueline C**, Asseray N, Batard E, Le Mabecque V, Kergueris MF, Dube L, Bugnon D, Potel G, Caillon J. In vivo efficacy of linezolid in combination with gentamicin for the treatment of experimental endocarditis due to methicillin-resistant *Staphylococcus aureus*. *Int J Antimicrob Agents* 2004; **24**: 393-396 [PMID: 15380267 DOI: 10.1016/j.ijantimicag.2004.03.013]
- 127 **Jacqueline C**, Caillon J, Grossi O, Le Mabecque V, Miegerville AF, Bugnon D, Batard E, Potel G. In vitro and in vivo assessment of linezolid combined with ertapenem: a highly synergistic combination against methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2006; **50**: 2547-2549 [PMID: 16801442 DOI: 10.1128/AAC.01501-05]
- 128 **Chambers HF**, Basuino L, Diep BA, Steenbergen J, Zhang S, Tattevin P, Alder J. Relationship between susceptibility to daptomycin in vitro and activity in vivo in a rabbit model of aortic valve endocarditis. *Antimicrob Agents Chemother* 2009; **53**: 1463-1467 [PMID: 19171803 DOI: 10.1128/AAC.01307-08]
- 129 **Miró JM**, García-de-la-Maria C, Armero Y, Soy D, Moreno A, del Río A, Almela M, Sarasa M, Mestres CA, Gatell JM, Jiménez de Anta MT, Marco F. Addition of gentamicin or rifampin does not enhance the effectiveness of daptomycin in treatment of experimental endocarditis due to methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2009; **53**: 4172-4177 [PMID: 19620326 DOI: 10.1128/aac.00051-09]
- 130 **Falagas ME**, Roussos N, Gkegkes ID, Rafailidis PI, Karageorgopoulos DE. Fosfomycin for the treatment of infections caused by Gram-positive cocci with advanced antimicrobial drug resistance: a review of microbiological, animal and clinical studies. *Expert Opin Investig Drugs* 2009; **18**: 921-944 [PMID: 19548851 DOI: 10.1517/13543780902967624]
- 131 **Portier H**, Kazmierczak A, Lucht F, Tremieux JC, Chavanet P, Duez JM. Cefotaxime in combination with other antibiotics for the treatment of severe methicillin-resistant staphylococcal infections. *Infection* 1985; **13** Suppl 1: S123-S128 [PMID: 3850854]
- 132 **Descourrouez JL**, Jorgenson MR, Wergin JE, Rose WE. Fosfomycin synergy in vitro with amoxicillin, daptomycin, and linezolid against vancomycin-resistant *Enterococcus faecium* from renal transplant patients with infected urinary stents. *Antimicrob Agents Chemother* 2013; **57**: 1518-1520 [PMID: 23263002 DOI: 10.1128/AAC.02099-12]
- 133 **Miró JM**, Entenza JM, Del Río A, Velasco M, Castañeda X, García de la Maria C, Giddey M, Armero Y, Pericàs JM, Cervera

C, Mestres CA, Almela M, Falces C, Marco F, Moreillon P, Moreno A. High-dose daptomycin plus fosfomycin is safe and effective in treating methicillin-susceptible and methicillin-

resistant *Staphylococcus aureus* endocarditis. *Antimicrob Agents Chemother* 2012; **56**: 4511-4515 [PMID: 22644033 DOI: 10.1128/aac.06449-11]

**P- Reviewer:** Schwan WR **S- Editor:** Gong XM **L- Editor:** A  
**E- Editor:** Wu HL



## Origin of *de novo* daptomycin non susceptible enterococci

Theodoros Kelesidis

Theodoros Kelesidis, Department of Medicine, Division of Infectious Diseases, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, United States

Author contributions: Kelesidis T wrote the paper.

Conflict-of-interest: None.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Theodoros Kelesidis, MD, PhD, Department of Medicine, Division of Infectious Diseases, David Geffen School of Medicine at UCLA, 10833 Le Conte Ave, CHS 37-121, Los Angeles, CA 90095, United States. [tkelesidis@mednet.ucla.edu](mailto:tkelesidis@mednet.ucla.edu)

Telephone: +1-310-8257225

Fax: +1-310-2080140

Received: January 27, 2015

Peer-review started: January 28, 2015

First decision: March 20, 2015

Revised: April 1, 2015

Accepted: April 16, 2015

Article in press: April 20, 2015

Published online: May 25, 2015

suggest that the environmental reservoir for *de novo* DNSE may be larger than previously thought. Herein, the limited available scientific evidence regarding the possible origin of *de novo* DNSE is discussed. The current existing evidence is not sufficient to draw firm conclusions on the origin of DNSE. Further studies to determine the mechanisms of *de novo* daptomycin nonsusceptibility among enterococci are needed.

**Key words:** Daptomycin non-susceptible enterococci; Antimicrobial resistance; Environmental reservoir

© **The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Daptomycin non-susceptible enterococci (DNSE) is an emerging clinical problem and may be isolated from patients with or without (*de novo* DNSE) prior exposure to daptomycin. Recent epidemiological data suggest the presence of a community reservoir for DNSE which may be associated with environmental, foodborne and agricultural exposures and may be larger than previously thought. Herein, the limited available scientific evidence regarding the possible origin of *de novo* DNSE is discussed. Further studies to determine the mechanisms of *de novo* daptomycin nonsusceptibility among enterococci are needed.

### Abstract

The emergence of daptomycin non-susceptible enterococci (DNSE) poses both treatment and infection control challenges. Clinicians should be vigilant that DNSE may be isolated from patients with or without (*de novo* DNSE) prior use of daptomycin. Recent epidemiological data suggest the presence of a community reservoir for DNSE which may be associated with environmental, foodborne and agricultural exposures. The mechanisms of nonsusceptibility to daptomycin have not been well characterized and may not parallel those for *Staphylococcus aureus*. The identification of daptomycin resistance genes in anaerobes, in farm animals and in an ecosystem that has been isolated for million years,

Kelesidis T. Origin of *de novo* daptomycin non susceptible enterococci. *World J Clin Infect Dis* 2015; 5(2): 30-36 Available from: URL: <http://www.wjgnet.com/2220-3176/full/v5/i2/30.htm> DOI: <http://dx.doi.org/10.5495/wjcid.v5.i2.30>

### INTRODUCTION

Antibiotic resistance is a major threat to human health<sup>[1]</sup>. Multidrug-resistant organisms such as vancomycin-resistant enterococci (VRE) may increase morbidity and mortality<sup>[1]</sup>. Daptomycin has bactericidal activity against VRE. However, daptomycin non-susceptible enterococci

(DNSE) are difficult to treat and clinicians often have limited treatment options<sup>[2]</sup>. Enterococci with daptomycin MIC > 4 µg/mL are non-susceptible, according to the Clinical Laboratory Standards Institute<sup>[3]</sup> and the Food and Drug Administration<sup>[4]</sup>. Although most DNSE isolates develop after daptomycin therapy they have also emerged in subjects with no prior use of daptomycin<sup>[5]</sup>. Elucidating the origin of *de novo* DNSE infections may help us understand mechanisms of daptomycin non-susceptibility. Herein, the available scientific evidence regarding the possible origin of *de novo* DNSE is reviewed.

## OVERALL PREVALENCE OF DNSE IS LOW

Despite initial *in vitro* studies that emergence of DNSE is rare<sup>[3,6-10]</sup>, recent studies suggest that DNSE is an emerging infection<sup>[2]</sup>. In large surveys of clinical isolates less than 0.6% of *Enterococcus faecalis* (*E. faecalis*) or *E. faecium* isolates were DNSE<sup>[11-15]</sup>. However, there is lack of data on daptomycin non-susceptible enterococcus isolates from international and national programs<sup>[2]</sup>. In a recent literature review, DNSE were present in 0.6% of all enterococci isolates (range 0%-19.1%)<sup>[2]</sup> and out of 150 DNSE isolates, 93.3% were vancomycin resistant enterococci (VRE), 6.0% were vancomycin susceptible enterococci (VSE), 88% were *E. faecium* and 8.7% were *E. faecalis*<sup>[2]</sup>. Most DNSE isolates were reported in Asia (40.3%) and in Europe (34%) while 26% of isolates were reported in North America<sup>[2]</sup>. Reporting bias, use of different susceptibility testing method among clinical microbiology laboratories such as MicroScan and presence of clones may overestimate the detection of DNSE<sup>[16-21]</sup>. Thus, the overall prevalence of DNSE was low.

## MECHANISMS OF EMERGENCE OF DAPTOMYCIN RESISTANCE IN ENTEROCOCCI ARE COMPLEX

The mechanisms for daptomycin nonsusceptibility in enterococci are different than in staphylococci and are poorly-understood<sup>[22-30]</sup>. Whole-genome sequencing of DNSE<sup>[30-34]</sup> suggest that few genetic mutations may be adequate to induce daptomycin non-susceptibility. Compared to their susceptible counterparts DNSE isolates have mutations in stress response regulators (such as the LiaFSR, yycG and YybT regulatory systems)<sup>[29-39]</sup>, phospholipid composition regulators [such as cardiolipin synthase (CIs), glycerophosphoryl diester phosphodiesterase (GdpD), cyclopropane fatty acid synthase (Cfa)]<sup>[27-33,40]</sup>, and phenotypic changes such as reduced cell membrane fluidity<sup>[28,30,31,41]</sup> and increased septation (*via* Ezr A)<sup>[30,31]</sup>.

## DNSE may develop without prior use of daptomycin

Spontaneous emergence of daptomycin non susceptibility *in vitro* is rare<sup>[24]</sup>. Although DNSE usually emerge in the setting of daptomycin therapy<sup>[2]</sup> DNSE have also been identified in subjects without prior use of daptomycin<sup>[5]</sup> and daptomycin use may not be a risk factor for DNSE in a case control study<sup>[42]</sup>. The risk factors related to emergence of *de novo* DNSE remain unclear.

## FACTORS THAT ARE ASSOCIATED WITH DEVELOPMENT OF DNSE

### Host factors related to isolation of DNSE

In a review of DNSE isolates, the source patients were 54.6 years on an average and 62.5% of them were female<sup>[2]</sup>. Factors that may contribute to emergence of DNSE include a source of DNSE infection such as abscess<sup>[2]</sup>, an intra-abdominal pathological process, recent surgery, a lengthy exposure to daptomycin<sup>[43,44]</sup>, immunosuppression and pharmacokinetics<sup>[43]</sup> and suboptimal drug levels among patients with end stage renal disease<sup>[45-47]</sup>. Observations from a case report suggested that chronic severe hypocalcemia in one patient may have contributed to the even lower calcium levels at the nidus of DNSE infection (abscesses)<sup>[32]</sup>, which may precipitate a loss of daptomycin activity<sup>[48]</sup>. Thus, DNSE may occur in the context of the above disorders and only few mutations may occur in DNSE<sup>[32]</sup>.

### Antimicrobial exposure may also be a risk factor for emergence of DNSE

Recent case controls studies with DNSE isolates have identified that many risk factors for emergence of VRE, including recent antimicrobial exposure, and increased hospitalization, were also present in the majority of DNSE cases<sup>[49]</sup>. Recent use of vancomycin, cephalosporins, or antibiotics active against anaerobes is associated with isolation of both VRE and DNSE<sup>[49]</sup>. VRE often causes colonize the colon<sup>[50,51]</sup> and vancomycin resistant<sup>[52]</sup> and daptomycin resistant gut anaerobes have been identified<sup>[53]</sup>. Resistance to vancomycin in gram-positive bacteria did not affect daptomycin activity<sup>[54]</sup>. Finally, multiple comorbidities, immunosuppression, and prior exposures to antimicrobials such as metronidazole and cephalosporins were independently associated with the isolation of DNSE (VRE) in a recent study<sup>[42]</sup>.

### Exposure to daptomycin has may contribute to emergence of DNSE especially in the setting of end stage renal disease

Although previous studies suggest that daptomycin resistance develops during treatment, MICs for daptomycin were often not reported<sup>[2]</sup>. In a review of DNSE isolates, the dose and duration of daptomycin that was administered prior to isolation of DNSE<sup>[2]</sup>. In one study, daptomycin-exposed DNSE patients received an



average of 44.9 d of daptomycin therapy<sup>[49]</sup>. Patients with end stage renal disease have lower  $C_{max}$  for daptomycin compared to healthy subjects<sup>[55]</sup> and the concentrations of daptomycin used in these patients may be relatively low<sup>[55-58]</sup>. Thus, more research should determine the optimal dosage and frequency of daptomycin administration in patients with end stage renal disease<sup>[43,44]</sup> since enterococci may become DNSE rapidly<sup>[32]</sup>.

## FACTORS RELATED TO ISOLATION OF DE NOVO DNSE

### **Limited data suggest that host factors are not known to be related to isolation of de novo DNSE**

We found no significant differences in terms of age, sex and underlying immunosuppressive illnesses between patients with *de novo* DNSE infections and DNSE infections following exposure to daptomycin<sup>[49]</sup>.

### **Environmental factors related to emergence of de novo DNSE**

In our series, 45% of patients with DNSE had no prior use of daptomycin and clonally-related DNSE were isolated in patients with no prior hospitalization<sup>[49]</sup> suggesting an environmental reservoir of DNSE<sup>[5]</sup>. Shorter duration of hospitalization, less frequent exposure to antimicrobials associated with isolation of VRE, were associated with *de novo* DNSE infection<sup>[49]</sup> but since DNSE may persist for years<sup>[59]</sup>, nosocomial acquisition of DNSE is possible. Factors that may contribute to formation of an environmental reservoir of DNSE include exchange of genetic material between enterococci, soil bacteria and bacteria of animal origin, foodborne origin of DNSE and agricultural exposures of humans to DNSE.

### **Transfer of genes that determine antimicrobial resistance between soil bacteria and DNSE may contribute to emergence of de novo DNSE**

Daptomycin resistance genes were found in bacteria from an ancient ecosystem<sup>[60]</sup>. Soil actinomycetes may inactivate daptomycin<sup>[6,61]</sup> and we have also identified found mutations in DNSE isolates in genes that are also present in soil bacteria<sup>[31]</sup>. Soil bacteria and enterococci may exchange genetic material<sup>[62]</sup>. However in another study, mechanisms of inactivation of daptomycin found in soil bacteria were not identified in DNSE *E. faecium*<sup>[22]</sup>. Thus, it remained to be elucidated whether the interplay between soil bacteria and enterococci may contribute to emergence of DNSE.

### **Bacteria in animals may mediate acquired daptomycin resistance in enterococci**

Humans and animals may exchange daptomycin resistance genes and this may lead to emergence of *de novo* DNSE<sup>[63]</sup>. The gut of humans and most animals

harbors enterococci and VRE can spread from farm animals<sup>[64,65]</sup>. Enterococci of animal origin may transfer antimicrobial resistance genes to other enterococci<sup>[66]</sup>. Recombination between repetitive nucleotide sequences<sup>[30]</sup> that may encode resistance cassettes in enterococci<sup>[62,64,65]</sup> may contribute to emergence of DNSE. Finally, we also found similar nucleotide mutations in genes that are common between DNSE and bacteria found in poultry<sup>[31,67-69]</sup>.

### **Limited data suggest that DNSE infections in humans may be foodborne**

DNSE may have passed to humans *via* ingestion of meat<sup>[5]</sup>. Up to 25% of enterococci isolated from beef were DNSE<sup>[65]</sup>. Daptomycin resistant Enterococci were recently identified in cows<sup>[70]</sup>. *E. faecalis* may harbor resistance genes and can be passed to humans through meat consumption<sup>[71]</sup>. Poultry might be a source for *E. faecalis* infections<sup>[72]</sup> and may harbor *E. gallinarum*<sup>[73]</sup> which may also be daptomycin non-susceptible<sup>[49]</sup>. Similarly, all three *de novo* urine DNSE isolates, were *E. faecalis*, may cause zoonosis<sup>[74]</sup>. In our study 4/9 (44.4%) subjects with *de novo* DNSE infections reported consumption of beef<sup>[5]</sup>. Thus, it remains to be shown whether DNSE may be foodborne pathogens<sup>[5,65]</sup>.

### **Limited data from epidemiological studies and case series suggest that DNSE may have a zoonotic potential**

Humans who are exposed to farm animals may be at risk increase to be colonized with multidrug resistant bacteria<sup>[75]</sup>. We found that in contrast to patients with daptomycin-exposed DNSE, the majority (78%) of *de novo* DNSE infections lived in areas with increased prevalence of agricultural exposures<sup>[76]</sup>. In our study of *de novo* DNSE infections 33.3% of patients had prior exposure to farm animals<sup>[5]</sup>. Thus, further epidemiological studies need to confirm if it is possible that exposure of humans to farm animals may increase the risk for isolation of DNSE<sup>[63]</sup>.

### **Limited data from observational studies suggest that transfer of genes that determine antimicrobial resistance between anaerobes and DNSE may contribute to emergence of de novo DNSE**

Enterococci and anaerobes are gastrointestinal tract flora in humans and may exchange antibiotic resistance genes<sup>[77,78]</sup>. Mutations in phospholipid biosynthesis and lac operon expression exist in facultative anaerobic<sup>[79]</sup> and anaerobic bacteria<sup>[80]</sup> may also lead to emergence of DNSE<sup>[30,34]</sup>. In addition, the use of antibiotics with activity against anaerobes may increase the spread of VRE and DNSE<sup>[81]</sup> while recent use of metronidazole may be a risk factor for emergence of DNSE<sup>[42]</sup>. Use of prior antibiotics with activity against anaerobes was found less in patients with *de novo* DNSE compared to daptomycin-exposed patients with DNSE infection<sup>[49]</sup>. Finally, daptomycin nonsusceptibility has been

described in anaerobes<sup>[53]</sup>. Thus, further studies need to confirm that the cross talk among anaerobic bacteria and enterococci may contribute to dissemination of DNSE<sup>[82]</sup>.

## CONCLUSION

Treatment of DNSE infections is a challenge for clinicians. Daptomycin non-susceptible enterococcal strains may develop after exposure to daptomycin. Since DNSE are usually isolated from patients with many comorbidities such as immunocompromised and end stage renal disease patients, strict infection control and prudent use of daptomycin are needed for these patients to limit the emergence and spread of DNSE.

However, DNSE may emerge without prior use of daptomycin. Recent epidemiological data suggest the presence of a community reservoir for DNSE which may be associated with environmental, foodborne and agricultural exposures. The mechanisms of development of daptomycin resistance remain unclear. The identification of daptomycin resistance genes in an ancient ecosystem<sup>[60]</sup>, in anaerobes<sup>[53]</sup> and in farm animals<sup>[70]</sup> suggest that the environmental reservoir for *de novo* DNSE may be larger than previously thought. In most of the studies with reported DNSE isolates complete medical records were not reviewed and interview of patients was not performed and thus potentially relevant occupational or dietary exposures among patients with DNSE were not identified. Epidemiological investigations focused on environmental exposures in the community may help elucidate the origin of *de novo* DNSE. Further studies to identify the mechanisms of *de novo* daptomycin nonsusceptibility in enterococci are needed.

## REFERENCES

1. Eliopoulos GM. Microbiology of drugs for treating multiply drug-resistant Gram-positive bacteria. *J Infect* 2009; **59** Suppl 1: S17-S24 [PMID: 19766885 DOI: 10.1016/S0163-4453]
2. Kelesidis T, Humphries R, Uslan DZ, Pegues DA. Daptomycin nonsusceptible enterococci: an emerging challenge for clinicians. *Clin Infect Dis* 2011; **52**: 228-234 [PMID: 21288849 DOI: 10.1093/cid/ciq113]
3. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; Twentieth Informational Supplement [assessed 2012 Jan]. Available from: URL: <http://antimicrobianos.com.ar/ATB/wp-content/uploads/2012/11/M100S22E.pdf>
4. Humphries RM, Pollett S, Sakoulas G. A current perspective on daptomycin for the clinical microbiologist. *Clin Microbiol Rev* 2013; **26**: 759-780 [PMID: 24092854 DOI: 10.1128/CMR.00030-13]
5. Kelesidis T, Humphries R, Uslan DZ, Pegues D. De novo daptomycin-nonsusceptible enterococcal infections. *Emerg Infect Dis* 2012; **18**: 674-676 [PMID: 22469288 DOI: 10.3201/eid1804.110932]
6. Debono M, Abbott BJ, Molloy RM, Fukuda DS, Hunt AH, Daupert VM, Counter FT, Ott JL, Carrell CB, Howard LC. Enzymatic and chemical modifications of lipopeptide antibiotic A21978C: the synthesis and evaluation of daptomycin (LY146032). *J Antibiot* (Tokyo) 1988; **41**: 1093-1105 [PMID: 2844711 DOI: 10.7164/antibiotics.41.1093]
7. Carpenter CF, Chambers HF. Daptomycin: another novel agent for treating infections due to drug-resistant gram-positive pathogens. *Clin Infect Dis* 2004; **38**: 994-1000 [PMID: 15034832 DOI: 10.1086/383472]
8. Debbia E, Pesce A, Schito GC. In vitro activity of LY146032 alone and in combination with other antibiotics against gram-positive bacteria. *Antimicrob Agents Chemother* 1988; **32**: 279-281 [PMID: 2834999 DOI: 10.1128/AAC.32.2.279]
9. Leclercq R, Bingen E, Su QH, Lambert-Zechovski N, Courvalin P, Duval J. Effects of combinations of beta-lactams, daptomycin, gentamicin, and glycopeptides against glycopeptide-resistant enterococci. *Antimicrob Agents Chemother* 1991; **35**: 92-98 [PMID: 1849711 DOI: 10.1128/AAC.35.1.92]
10. Louie A, Baltch AL, Ritz WJ, Smith RP, Asperilla M. Comparison of in vitro inhibitory and bactericidal activities of daptomycin (LY 146032) and four reference antibiotics, singly and in combination, against gentamicin-susceptible and high-level-gentamicin-resistant enterococci. *Chemotherapy* 1993; **39**: 302-309 [PMID: 8396526 DOI: 10.1159/000239141]
11. Sader HS, Moet GJ, Farrell DJ, Jones RN. Antimicrobial susceptibility of daptomycin and comparator agents tested against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci: trend analysis of a 6-year period in US medical centers (2005-2010). *Diagn Microbiol Infect Dis* 2011; **70**: 412-416 [PMID: 21546202 DOI: 10.1016/j.diagmicrobio.2011.02.008]
12. Pfaller MA, Sader HS, Jones RN. Evaluation of the in vitro activity of daptomycin against 19615 clinical isolates of Gram-positive cocci collected in North American hospitals (2002-2005). *Diagn Microbiol Infect Dis* 2007; **57**: 459-465 [PMID: 17240105 DOI: 10.1016/j.diagmicrobio.2006.10.007]
13. Sader HS, Jones RN, Stilwell MG, Dowzicky MJ, Fritsche TR. Tigecycline activity tested against 26,474 bloodstream infection isolates: a collection from 6 continents. *Diagn Microbiol Infect Dis* 2005; **52**: 181-186 [PMID: 16105562 DOI: 10.1016/j.diagmicrobio.2005.05.005]
14. Sader HS, Fritsche TR, Streit JM, Jones RN. Daptomycin in vitro activity tested against Gram-positive strains collected from European and Latin American medical centers in 2003. *J Chemother* 2005; **17**: 477-483 [PMID: 16323435 DOI: 10.1179/joc.2005.17.5.477]
15. Sader HS, Flamm RK, Jones RN. Antimicrobial activity of daptomycin tested against Gram-positive pathogens collected in Europe, Latin America, and selected countries in the Asia-Pacific Region (2011). *Diagn Microbiol Infect Dis* 2013; **75**: 417-422 [PMID: 23514757 DOI: 10.1016/j.diagmicrobio.2013.01.001]
16. Wang JT, Chen YC, Chang SC, Chen ML, Pan HJ, Chang YY, Sun CC, Wang LH, Wang SH, Lin HC, Chien SF, Tseng MS. Control of vancomycin-resistant enterococci in a hospital: a five-year experience in a Taiwanese teaching hospital. *J Hosp Infect* 2004; **58**: 97-103 [PMID: 15474179 DOI: 10.1016/j.jhin.2004.06.005]
17. Edelsberg J, Weycker D, Barron R, Li X, Wu H, Oster G, Badre S, Langeberg WJ, Weber DJ. Prevalence of antibiotic resistance in US hospitals. *Diagn Microbiol Infect Dis* 2014; **78**: 255-262 [PMID: 24360267 DOI: 10.1016/j.diagmicrobio.2013.11.011]
18. Biedenbach DJ, Bell JM, Sader HS, Fritsche TR, Jones RN, Turnidge JD. Antimicrobial susceptibility of Gram-positive bacterial isolates from the Asia-Pacific region and an in vitro evaluation of the bactericidal activity of daptomycin, vancomycin, and teicoplanin: a SENTRY Program Report (2003-2004). *Int J Antimicrob Agents* 2007; **30**: 143-149 [PMID: 17531446 DOI: 10.1016/j.ijantimicag.2007.03.015]
19. Snyderman DR, McDermott LA, Jacobus NV. Evaluation of in vitro interaction of daptomycin with gentamicin or beta-lactam antibiotics against *Staphylococcus aureus* and Enterococci by FIC index and timed-kill curves. *J Chemother* 2005; **17**: 614-621 [PMID: 16433191 DOI: 10.1179/joc.2005.17.6.614]
20. Fluit AC, Schmitz FJ, Verhoef J, Milatovic D. Daptomycin in vitro susceptibility in European Gram-positive clinical isolates. *Int J Antimicrob Agents* 2004; **24**: 59-66 [PMID: 15225863 DOI: 10.1016/j.ijantimicag.2004.03.005]

- 10.1016/j.ijantimicag.2003.12.014]
- 21 **Bryant KA**, Roberts AL, Rupp ME, Anderson JR, Lyden ER, Fey PD, Van Schooneveld TC. Susceptibility of enterococci to daptomycin is dependent upon testing methodology. *Diagn Microbiol Infect Dis* 2013; **76**: 497-501 [PMID: 23719086 DOI: 10.1016/j.diagmicrobio.2013.04.019]
- 22 **Montero CI**, Stock F, Murray PR. Mechanisms of resistance to daptomycin in *Enterococcus faecium*. *Antimicrob Agents Chemother* 2008; **52**: 1167-1170 [PMID: 18180351 DOI: 10.1128/AAC.00774-07]
- 23 **Critchley IA**, Blosser-Middleton RS, Jones ME, Thornsberry C, Sahm DF, Karlowsky JA. Baseline study to determine in vitro activities of daptomycin against gram-positive pathogens isolated in the United States in 2000-2001. *Antimicrob Agents Chemother* 2003; **47**: 1689-1693 [PMID: 12709341 DOI: 10.1128/AAC.47.5.1689-1693.2003]
- 24 **Silverman JA**, Oliver N, Andrew T, Li T. Resistance studies with daptomycin. *Antimicrob Agents Chemother* 2001; **45**: 1799-1802 [PMID: 11353628 DOI: 10.1128/AAC.45.6.1799-1802.2001]
- 25 **Friedman L**, Alder JD, Silverman JA. Genetic changes that correlate with reduced susceptibility to daptomycin in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2006; **50**: 2137-2145 [PMID: 16723576 DOI: 10.1128/AAC.00039-06]
- 26 **Sakoulas G**, Alder J, Thauvin-Eliopoulos C, Moellering RC, Eliopoulos GM. Induction of daptomycin heterogeneous susceptibility in *Staphylococcus aureus* by exposure to vancomycin. *Antimicrob Agents Chemother* 2006; **50**: 1581-1585 [PMID: 16569891 DOI: 10.1128/AAC.50.4.1581-1585.2006]
- 27 **Sakoulas G**, Bayer AS, Pogliano J, Tsuji BT, Yang SJ, Mishra NN, Nizet V, Yeaman MR, Moise PA. Ampicillin enhances daptomycin- and cationic host defense peptide-mediated killing of ampicillin- and vancomycin-resistant *Enterococcus faecium*. *Antimicrob Agents Chemother* 2012; **56**: 838-844 [PMID: 22123698 DOI: 10.1128/AAC.05551-11]
- 28 **Mishra NN**, Bayer AS, Tran TT, Shamoo Y, Mileykovskaya E, Dowhan W, Guan Z, Arias CA. Daptomycin resistance in enterococci is associated with distinct alterations of cell membrane phospholipid content. *PLoS One* 2012; **7**: e43958 [PMID: 22952824 DOI: 10.1371/journal.pone.0043958]
- 29 **Munita JM**, Panesso D, Diaz L, Tran TT, Reyes J, Wanger A, Murray BE, Arias CA. Correlation between mutations in *liaFSR* of *Enterococcus faecium* and MIC of daptomycin: revisiting daptomycin breakpoints. *Antimicrob Agents Chemother* 2012; **56**: 4354-4359 [PMID: 22664970 DOI: 10.1128/AAC.00509-12]
- 30 **Arias CA**, Panesso D, McGrath DM, Qin X, Mojica MF, Miller C, Diaz L, Tran TT, Rincon S, Barbu EM, Reyes J, Roh JH, Lobos E, Sodergren E, Pasqualini R, Arap W, Quinn JP, Shamoo Y, Murray BE, Weinstock GM. Genetic basis for in vivo daptomycin resistance in enterococci. *N Engl J Med* 2011; **365**: 892-900 [PMID: 21899450 DOI: 10.1056/NEJMoa1011138]
- 31 **Humphries RM**, Kelesidis T, Tewhey R, Rose WE, Schork N, Nizet V, Sakoulas G. Genotypic and phenotypic evaluation of the evolution of high-level daptomycin nonsusceptibility in vancomycin-resistant *Enterococcus faecium*. *Antimicrob Agents Chemother* 2012; **56**: 6051-6053 [PMID: 22948885 DOI: 10.1128/AAC.01318-12]
- 32 **Kelesidis T**, Tewhey R, Humphries RM. Evolution of high-level daptomycin resistance in *Enterococcus faecium* during daptomycin therapy is associated with limited mutations in the bacterial genome. *J Antimicrob Chemother* 2013; **68**: 1926-1928 [PMID: 23580562 DOI: 10.1093/jac/dkt117]
- 33 **Tran TT**, Panesso D, Gao H, Roh JH, Munita JM, Reyes J, Diaz L, Lobos EA, Shamoo Y, Mishra NN, Bayer AS, Murray BE, Weinstock GM, Arias CA. Whole-genome analysis of a daptomycin-susceptible *enterococcus faecium* strain and its daptomycin-resistant variant arising during therapy. *Antimicrob Agents Chemother* 2013; **57**: 261-268 [PMID: 23114757 DOI: 10.1128/AAC.01454-12]
- 34 **Palmer KL**, Daniel A, Hardy C, Silverman J, Gilmore MS. Genetic basis for daptomycin resistance in enterococci. *Antimicrob Agents Chemother* 2011; **55**: 3345-3356 [PMID: 21502617 DOI: 10.1128/AAC.00207-11]
- 35 **Rice LB**, Carias LL, Rudin S, Hutton R, Marshall S, Hassan M, Josseume N, Dubost L, Marie A, Arthur M. Role of class A penicillin-binding proteins in the expression of beta-lactam resistance in *Enterococcus faecium*. *J Bacteriol* 2009; **191**: 3649-3656 [PMID: 19304851 DOI: 10.1128/JB.01834-08]
- 36 **Zhang X**, Paganelli FL, Bierschenk D, Kuipers A, Bonten MJ, Willems RJ, van Schaik W. Genome-wide identification of ampicillin resistance determinants in *Enterococcus faecium*. *PLoS Genet* 2012; **8**: e1002804 [PMID: 22761597 DOI: 10.1371/journal.pgen.1002804]
- 37 **Sakoulas G**, Okumura CY, Thienphrapa W, Olson J, Nonejuie P, Dam Q, Dhand A, Pogliano J, Yeaman MR, Hensler ME, Bayer AS, Nizet V. Nafcillin enhances innate immune-mediated killing of methicillin-resistant *Staphylococcus aureus*. *J Mol Med (Berl)* 2014; **92**: 139-149 [PMID: 24297496 DOI: 10.1007/s00109-013-1100-7]
- 38 **Munita JM**, Tran TT, Diaz L, Panesso D, Reyes J, Murray BE, Arias CA. A *liaF* codon deletion abolishes daptomycin bactericidal activity against vancomycin-resistant *Enterococcus faecalis*. *Antimicrob Agents Chemother* 2013; **57**: 2831-2833 [PMID: 23507277 DOI: 10.1128/AAC.00021-13]
- 39 **Miller C**, Kong J, Tran TT, Arias CA, Saxer G, Shamoo Y. Adaptation of *Enterococcus faecalis* to daptomycin reveals an ordered progression to resistance. *Antimicrob Agents Chemother* 2013; **57**: 5373-5383 [PMID: 23959318 DOI: 10.1128/AAC.01473-13]
- 40 **Tran TT**, Panesso D, Mishra NN, Mileykovskaya E, Guan Z, Munita JM, Reyes J, Diaz L, Weinstock GM, Murray BE, Shamoo Y, Dowhan W, Bayer AS, Arias CA. Daptomycin-resistant *Enterococcus faecalis* diverts the antibiotic molecule from the division septum and remodels cell membrane phospholipids. *MBio* 2013; **4**: pii: e00281-13 [PMID: 23882013]
- 41 **Steed ME**, Vidaillac C, Rose WE, Winterfield P, Kaatz GW, Rybak MJ. Characterizing vancomycin-resistant *Enterococcus* strains with various mechanisms of daptomycin resistance developed in an in vitro pharmacokinetic/pharmacodynamic model. *Antimicrob Agents Chemother* 2011; **55**: 4748-4754 [PMID: 21788457 DOI: 10.1128/AAC.00084-11]
- 42 **Judge T**, Pogue JM, Marchaim D, Ho K, Kamatam S, Parveen S, Tiwari N, Nanjireddy P, Bheemreddy S, Biedron C, Reddy SM, Khammam V, Chalana IK, Tumma RS, Collins V, Yousuf A, Lephart PR, Martin ET, Rybak MJ, Kaye KS, Hayakawa K. Epidemiology of vancomycin-resistant enterococci with reduced susceptibility to daptomycin. *Infect Control Hosp Epidemiol* 2012; **33**: 1250-1254 [PMID: 23143365 DOI: 10.1086/668438]
- 43 **Bubalo JS**, Munar MY, Cherala G, Hayes-Lattin B, Maziarz R. Daptomycin pharmacokinetics in adult oncology patients with neutropenic fever. *Antimicrob Agents Chemother* 2009; **53**: 428-434 [PMID: 19015332 DOI: 10.1128/AAC.00943-08]
- 44 **Enoch DA**, Bygott JM, Daly ML, Karas JA. Daptomycin. *J Infect* 2007; **55**: 205-213 [PMID: 17629567 DOI: 10.1016/j.jinf.2007.05.180]
- 45 **Mushatt DM**, Mihm LB, Dreisbach AW, Simon EE. Antibiotic dosing in slow extended daily dialysis. *Clin Infect Dis* 2009; **49**: 433-437 [PMID: 19580416 DOI: 10.1086/600390]
- 46 **Kielstein JT**, Eugbers C, Bode-Boeger SM, Martens-Lobenhoffer J, Haller H, Joukhadar C, Traunmüller F, Knitsch W, Hafer C, Burkhardt O. Dosing of daptomycin in intensive care unit patients with acute kidney injury undergoing extended dialysis-a pharmacokinetic study. *Nephrol Dial Transplant* 2010; **25**: 1537-1541 [PMID: 20031929 DOI: 10.1093/ndt/gfp704]
- 47 **Burkhardt O**, Joukhadar C, Traunmüller F, Hadem J, Welte T, Kielstein JT. Elimination of daptomycin in a patient with acute renal failure undergoing extended daily dialysis. *J Antimicrob Chemother* 2008; **61**: 224-225 [PMID: 17965030 DOI: 10.1093/jac/dkm405]
- 48 **Hanberger H**, Nilsson LE, Maller R, Isaksson B. Pharmacodynamics of daptomycin and vancomycin on *Enterococcus faecalis* and *Staphylococcus aureus* demonstrated by studies of initial killing and postantibiotic effect and influence of Ca<sup>2+</sup> and albumin on these drugs. *Antimicrob Agents Chemother* 1991; **35**: 1710-1716 [PMID: 1659305 DOI: 10.1128/AAC.35.9.1710]
- 49 **Kelesidis T**, Chow AL, Humphries R, Uslan DZ, Pegues D. Case-control study comparing de novo and daptomycin-exposed



- daptomycin-nonsusceptible *Enterococcus* infections. *Antimicrob Agents Chemother* 2012; **56**: 2150-2152 [PMID: 22252808 DOI: 10.1128/AAC.05918-11]
- 50 **Hume ME**, Poole TL, Pultz NJ, Hanrahan JA, Donskey CJ. Inhibition of vancomycin-resistant enterococcus by continuous-flow cultures of human stool microflora with and without anaerobic gas supplementation. *Curr Microbiol* 2004; **48**: 364-367 [PMID: 15060733 DOI: 10.1007/s00284-003-4112-7]
- 51 **Sun Y**, Smith E, Wolcott R, Dowd SE. Propagation of anaerobic bacteria within an aerobic multi-species chronic wound biofilm model. *J Wound Care* 2009; **18**: 426-431 [PMID: 19816382 DOI: 10.12968/jowc.2009.18.10.44604]
- 52 **Ballard SA**, Grabsch EA, Johnson PD, Grayson ML. Comparison of three PCR primer sets for identification of vanB gene carriage in feces and correlation with carriage of vancomycin-resistant enterococci: interference by vanB-containing anaerobic bacilli. *Antimicrob Agents Chemother* 2005; **49**: 77-81 [PMID: 15616278 DOI: 10.1128/AAC.49.1.77-81.2005]
- 53 **Goldstein EJ**, Citron DM, Merriam CV, Warren YA, Tyrrell KL, Fernandez HT. In vitro activities of daptomycin, vancomycin, quinupristin-dalfopristin, linezolid, and five other antimicrobials against 307 gram-positive anaerobic and 31 *Corynebacterium* clinical isolates. *Antimicrob Agents Chemother* 2003; **47**: 337-341 [PMID: 12499210 DOI: 10.1128/AAC.47.1.337-341.2003]
- 54 **Sader HS**, Streit JM, Fritsche TR, Jones RN. Antimicrobial activity of daptomycin against multidrug-resistant Gram-positive strains collected worldwide. *Diagn Microbiol Infect Dis* 2004; **50**: 201-204 [PMID: 15541606 DOI: 10.1016/j.diagmicrobio.2004.07.002]
- 55 **Salama NN**, Segal JH, Churchwell MD, Patel JH, Gao L, Heung M, Mueller BA. Intradialytic administration of daptomycin in end stage renal disease patients on hemodialysis. *Clin J Am Soc Nephrol* 2009; **4**: 1190-1194 [PMID: 19541812 DOI: 10.2215/CJN.01650309]
- 56 **Salama NN**, Segal JH, Churchwell MD, Patel JH, Gao L, Heung M, Mueller BA. Single-dose daptomycin pharmacokinetics in chronic haemodialysis patients. *Nephrol Dial Transplant* 2010; **25**: 1279-1284 [PMID: 20007981 DOI: 10.1093/ndt/gfp655]
- 57 **Butterfield JM**, Mueller BA, Patel N, Cardone KE, Grabe DW, Salama NN, Lodise TP. Daptomycin pharmacokinetics and pharmacodynamics in a pooled sample of patients receiving thrice-weekly hemodialysis. *Antimicrob Agents Chemother* 2013; **57**: 864-872 [PMID: 23208714 DOI: 10.1128/AAC.02000-12]
- 58 **Vilay AM**, Griot M, Depestel DD, Sowinski KM, Gao L, Heung M, Salama NN, Mueller BA. Daptomycin pharmacokinetics in critically ill patients receiving continuous venovenous hemodialysis. *Crit Care Med* 2011; **39**: 19-25 [PMID: 20890189 DOI: 10.1097/CCM.0b013e3181fa36fb]
- 59 **Baden LR**, Thienke W, Skolnik A, Chambers R, Strymish J, Gold HS, Moellering RC, Eliopoulos GM. Prolonged colonization with vancomycin-resistant *Enterococcus faecium* in long-term care patients and the significance of "clearance". *Clin Infect Dis* 2001; **33**: 1654-1660 [PMID: 11595985 DOI: 10.1086/323762]
- 60 **Bhullar K**, Waglechner N, Pawlowski A, Koteva K, Banks ED, Johnston MD, Barton HA, Wright GD. Antibiotic resistance is prevalent in an isolated cave microbiome. *PLoS One* 2012; **7**: e34953 [PMID: 22509370 DOI: 10.1371/journal.pone.0034953]
- 61 **D'Costa VM**, McGrann KM, Hughes DW, Wright GD. Sampling the antibiotic resistome. *Science* 2006; **311**: 374-377 [PMID: 16424339 DOI: 10.1126/science.1120800]
- 62 **Johnston LM**, Jaykus LA. Antimicrobial resistance of *Enterococcus* species isolated from produce. *Appl Environ Microbiol* 2004; **70**: 3133-3137 [PMID: 15128577 DOI: 10.1128/AEM.70.5.3133-3137.2004]
- 63 **Kelesidis T**. The zoonotic potential of daptomycin non-susceptible enterococci. *Zoonoses Public Health* 2015; **62**: 1-6 [PMID: 24274811]
- 64 **van den Bogaard AE**, Stobberingh EE. Epidemiology of resistance to antibiotics. Links between animals and humans. *Int J Antimicrob Agents* 2000; **14**: 327-335 [PMID: 10794955 DOI: 10.1016/S0924-8579(00)00145-X]
- 65 **Zhang J**, Wall SK, Xu L, Ebner PD. Contamination rates and antimicrobial resistance in bacteria isolated from "grass-fed" labeled beef products. *Foodborne Pathog Dis* 2010; **7**: 1331-1336 [PMID: 20618073 DOI: 10.1089/fpd.2010.0562]
- 66 **Hammerum AM**. Enterococci of animal origin and their significance for public health. *Clin Microbiol Infect* 2012; **18**: 619-625 [PMID: 22487203 DOI: 10.1111/j.1469-0691.2012.03829.x]
- 67 **Johnson TJ**, Fernandez-Alarcon C, Bojesen AM, Nolan LK, Trampel DW, Seemann T. Complete genome sequence of *Gallibacterium anatis* strain UMN179, isolated from a laying hen with peritonitis. *J Bacteriol* 2011; **193**: 3676-3677 [PMID: 21602325 DOI: 10.1128/JB.05177-11]
- 68 **Voget S**, Klippel B, Daniel R, Antranikian G. Complete genome sequence of *Carnobacterium* sp. 17-4. *J Bacteriol* 2011; **193**: 3403-3404 [PMID: 21551290 DOI: 10.1128/JB.05113-11]
- 69 **Lowder BV**, Guinane CM, Ben Zakour NL, Weinert LA, Conway-Morris A, Cartwright RA, Simpson AJ, Rambaut A, Nübel U, Fitzgerald JR. Recent human-to-poultry host jump, adaptation, and pandemic spread of *Staphylococcus aureus*. *Proc Natl Acad Sci USA* 2009; **106**: 19545-19550 [PMID: 19884497 DOI: 10.1073/pnas.0909285106]
- 70 **Kateete DP**, Kabugo U, Baluku H, Nyakarahuka L, Kyobe S, Okee M, Najjuka CF, Joloba ML. Prevalence and antimicrobial susceptibility patterns of bacteria from milkmen and cows with clinical mastitis in and around Kampala, Uganda. *PLoS One* 2013; **8**: e63413 [PMID: 23667611 DOI: 10.1371/journal.pone.0063413]
- 71 **Aslam M**, Diarra MS, Checkley S, Bohaychuk V, Masson L. Characterization of antimicrobial resistance and virulence genes in *Enterococcus* spp. isolated from retail meats in Alberta, Canada. *Int J Food Microbiol* 2012; **156**: 222-230 [PMID: 22520502 DOI: 10.1016/j.jfoodmicro.2012.03.026]
- 72 **Poulsen LL**, Bisgaard M, Son NT, Trung NV, An HM, Dalsgaard A. *Enterococcus faecalis* clones in poultry and in humans with urinary tract infections, Vietnam. *Emerg Infect Dis* 2012; **18**: 1096-1100 [PMID: 22709904 DOI: 10.3201/eid1807.111754]
- 73 **Klein G**. Taxonomy, ecology and antibiotic resistance of enterococci from food and the gastro-intestinal tract. *Int J Food Microbiol* 2003; **88**: 123-131 [PMID: 14596985 DOI: 10.1016/S0168-1605(03)00175-2]
- 74 **Kelesidis T**, Humphries R, Chow AL, Tsiodras S, Uslan DZ. Emergence of daptomycin-non-susceptible enterococci urinary tract isolates. *J Med Microbiol* 2013; **62**: 1103-1105 [PMID: 23598376 DOI: 10.1099/jmm.0.056630-0]
- 75 **Geenen PL**, Graat EA, Haenen A, Hengeveld PD, Van Hoek AH, Huijsdens XW, Kappert CC, Lammers GA, Van Duikeren E, Van De Giessen AW. Prevalence of livestock-associated MRSA on Dutch broiler farms and in people living and/or working on these farms. *Epidemiol Infect* 2013; **141**: 1099-1108 [PMID: 22831886 DOI: 10.1017/S0950268812001616]
- 76 **Kelesidis T**, Chow AL. Proximity to animal or crop operations may be associated with de novo daptomycin-non-susceptible *Enterococcus* infection. *Epidemiol Infect* 2014; **142**: 221-224 [PMID: 23587411 DOI: 10.1017/S0950268813000885]
- 77 **Scott KP**. The role of conjugative transposons in spreading antibiotic resistance between bacteria that inhabit the gastrointestinal tract. *Cell Mol Life Sci* 2002; **59**: 2071-2082 [PMID: 12568333]
- 78 **Garnier F**, Taourit S, Glaser P, Courvalin P, Galimand M. Characterization of transposon Tn1549, conferring VanB-type resistance in *Enterococcus* spp. *Microbiology* 2000; **146** (Pt 6): 1481-1489 [PMID: 10846226]
- 79 **Lapierre L**, Mollet B, Germond JE. Regulation and adaptive evolution of lactose operon expression in *Lactobacillus delbrueckii*. *J Bacteriol* 2002; **184**: 928-935 [PMID: 11807052 DOI: 10.1128/jb.184.4.928-935.2002]
- 80 **Silber P**, Borie RP, Mikowski EJ, Goldfine H. Phospholipid biosynthesis in some anaerobic bacteria. *J Bacteriol* 1981; **147**: 57-61 [PMID: 6263870]
- 81 **Bhalla A**, Pultz NJ, Ray AJ, Huyen CK, Eckstein EC, Donskey CJ. Antianaerobic antibiotic therapy promotes overgrowth of antibiotic-resistant, gram-negative bacilli and vancomycin-resistant enterococci in the stool of colonized patients. *Infect Control*



Kelesidis T. *De novo* daptomycin non susceptible enterococci

*Hosp Epidemiol* 2003; **24**: 644-649 [PMID: 14510245 DOI: 10.1086/502267]

82 **Kelesidis T.** Comment on: Successful therapy of treatment-

emergent, non-clonal daptomycin-non-susceptible *Enterococcus faecium* infections. *J Antimicrob Chemother* 2012; **67**: 515-516 [PMID: 22052687 DOI: 10.1093/jac/dkr465]

**P- Reviewer:** Blanco LP, Krishnan T **S- Editor:** Tian YL  
**L- Editor:** A **E- Editor:** Wu HL



## Surface adhesion and host response as pathogenicity factors of *Neisseria meningitidis*

Jose Uberos, M Molina-Oya, S Martinez-Serrano, L Fernández-López

Jose Uberos, M Molina-Oya, S Martinez-Serrano, L Fernández-López, Department of Paediatrics, School of Medicine, University of Granada, 18012 Granada, Spain

**Author contributions:** All authors contributed to this manuscript.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Dr. Jose Uberos, Department of Paediatrics, School of Medicine, University of Granada, Avda. de Madrid s/n, 18012 Granada, Spain. [joseuberos@telefonica.net](mailto:joseuberos@telefonica.net)  
**Telephone:** +34-95-8243066

**Received:** July 3, 2014

**Peer-review started:** July 3, 2014

**First decision:** July 21, 2014

**Revised:** January 26, 2015

**Accepted:** March 5, 2015

**Article in press:** March 9, 2015

**Published online:** May 25, 2015

### Abstract

*Neisseria meningitidis* (*N. meningitidis*) is an exclusively human pathogen that has been identified in 10%-35% of the adult population and in 5.9% of the child population. Despite the high prevalence of carriers of *N. meningitidis*, it only occasionally causes meningococcal disease in the context of endemic disease, in certain geographic areas or in isolated epidemic outbreaks. After the *N. meningitidis* genome is described, progress has been made toward understanding the pathogenic mechanisms of the bacteria, although some aspects concerning its interaction with the environment and the host remain unclear. Some studies have reported that oxidative stress in the environment can modify the surface characteristics of *N. meningitidis*, increasing its adhesive properties and favouring an asymptomatic

carrier state. The antigenic structure of *N. meningitidis* can be modified by its importing genetic material from other bacteria in its ecological niche. Some structures of lipopolysaccharides help it to evade the immune response, and these are observed more frequently in *N. meningitidis* isolated from blood than in healthy nasopharyngeal carriers. There is evidence that pili and capsule are downregulated upon contact with target cells. This paper reviews current knowledge on host-environment-bacteria mechanisms and interactions, with the aim of contributing to our understanding of the pathogenic mechanisms of *N. meningitidis*.

**Key words:** Bacterial adhesion; *Neisseria meningitidis*; Virulence

© The Author(s) 2015. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** After the *Neisseria meningitidis* (*N. meningitidis*) genome is described, progress has been made toward understanding the pathogenic mechanisms of the bacteria, although some aspects concerning its interaction with the environment and the host remain unclear. This paper reviews current knowledge on host-environment-bacteria mechanisms and interactions, with the aim of contributing to our understanding of the pathogenic mechanisms of *N. meningitidis*.

Uberos J, Molina-Oya M, Martinez-Serrano S, Fernández-López L. Surface adhesion and host response as pathogenicity factors of *Neisseria meningitidis*. *World J Clin Infect Dis* 2015; 5(2): 37-43  
Available from: URL: <http://www.wjgnet.com/2220-3176/full/v5/i2/37.htm> DOI: <http://dx.doi.org/10.5495/wjid.v5.i2.37>

### BACTERIAL ADHESION AND PATHOGENICITY

The ability of bacteria to attach and grow on almost

any surface has been known for decades. The importance of adhesion in the colonisation of specific substrates, and its role in the pathogenesis of bacterial infections and in the maintenance of the carrier state has been studied widely in recent years<sup>[1]</sup>. *Neisseria meningitidis* (*N. meningitidis*) is only found in humans, suggesting that its ability to cause disease is likely an casual side effect of its life cycle. Globally, the carrier rate of *N. meningitidis* ranges from 10%-35% among healthy adults<sup>[2]</sup>; the mean carrier rate in children is 5.9%, peaking at 10.3% in children aged under 3 years<sup>[3]</sup>. This situation has been associated with the genetic characteristics of the circulating strains of *N. meningitidis*, the immune pressure exerted by vaccination programmes and the hygienic and social conditions within a community. Compared with the rates of colonisation, meningococcal disease is less common, its development being affected by interacting factors such as the virulence of the bacterium, host defence mechanisms, the age of the host and the history of previous viral infections<sup>[2]</sup>. The best-defined virulence factor of *N. meningitidis* is its polysaccharide capsule that indicates its serogroup. Although 13 serogroups of *N. meningitidis* have been described (A, B, C, D, 29E, H, I, K, L, Y, W-135, X and Z), invasive meningococcal disease is most frequently caused by serogroups A, B, C, Y and W-135, to which 10% mortality has been attributed.

The adhesion of bacteria to epithelial surfaces is an initial step in the colonisation of microbial habitats, and it ensures the survival of *N. meningitidis* in its ecological niche<sup>[4]</sup>. Adherence can be defined as a phenomenon resulting from the interaction between two surfaces, with the participation of physical, chemical and biological factors, with contact between the bacterium and the cell being necessary for adherence to take place. The adhesion occurs in several steps: (1) *N. meningitidis* attaches to the surface of target cells to form small colonies. This step is essentially a pilus-mediated process; and (2) after *N. meningitidis* has been attached, it comes into close contact with surface of the target cells (intimate adhesion). The adhesive interaction is present both in commensal bacteria and in pathogens and so for *N. meningitidis* to adhere, it has developed proprietary adhesive mechanisms that allow it to compete with flora of the same ecological niche<sup>[5]</sup>. Meningococcal pili are of type IV and are composed of pilin subunits that are encoded by *pilE* gene. Other homologous proteins, PilC1 and PilC2, are also involved in pilus assembly and adhesion. PilC1-containing may involve interaction with CD46, a human trans-membrane glycoprotein involved in complement regulation. The expression of *pilC1* is induced following the contact of *N. meningitidis* with viable target cells. Both pili and capsule are downregulated upon contact with target cells. This downregulation seems to be associated with intimate adhesion of *N. meningitidis* to target cells<sup>[5,6]</sup>.

The "adhesion process" can be defined in terms of

the adhesion affinity of bacteria to epithelial surfaces, as has been described in the Michaelis-Menten equations. The maximum point of adhesion (and affinity) can be determined graphically by the Lineaweaver-Burk equations, using simple experimental models<sup>[7]</sup>. This first phase would be a reversible process in which Van der Waals and electrostatic forces are responsible for a wide range of interactions, including chemical bonding, dipolar interaction and hydrophobicity. Surface molecules as *N*-acetylneuraminic acid may alter the initial adhesion strength, reducing electrostatic repulsive forces or increasing attractive ones<sup>[8]</sup>. The cell surfaces of both prokaryotic and eukaryotic cells are negatively charged. Electrostatic repulsive forces between the cell and the bacteria can be overcome by long and short-range attractive forces, and so the specific binding of fimbriae with cell surface receptors must overcome the repulsive forces between the two surfaces. According to Smyth *et al*<sup>[9]</sup>, the reduction of the surface bacteria potential by the intervention of hydrophobic adhesins probably facilitates adhesion, with the hydrophobic forces being a first step in the interaction of the organism with mucosal surfaces. Surface hydrophobicity is a non-specific adhesion factor which is important to the adhesion and growth of microorganisms on epithelial surfaces. The hydrophobic-hydrophilic environment of the bacterial surface is modulated by hydrophobic or hydrophilic agents (increasing or reducing hydrophobicity, respectively), which may co-exist on the surface of the outer membrane<sup>[5]</sup>. Generally, the strains that formed biofilms show high-level cell surface hydrophobicity. Many studies have examined the contribution of surface hydrophobicity to bacterial adhesion, with particular attention to *Salmonella*<sup>[10,11]</sup>, *Escherichia coli* (*E. coli*), *N. gonorrhoeae*<sup>[12,13]</sup> and *N. meningitidis*<sup>[14,15]</sup>.

The type IV pili of *N. meningitidis* are crucial determinants of the adhesion of these pathogens to epithelial and endothelial cells<sup>[16]</sup>. Under natural conditions, pili are the only means by which encapsulated *N. meningitidis* can adhere to human mucosal surfaces<sup>[17,18]</sup>.

## SURFACE MODULATION AND INTERACTION WITH THE HOST

When *N. meningitidis* adheres to epithelial cells, it becomes resistant to the bactericidal effect of the antimicrobial peptide LL-37, which is the first line of defence of innate immunity. The decreased binding of LL-37 to the adhered bacteria can result from its degradation by proteases released at the site of infection. Furthermore, *N. meningitidis* induces the formation in the nasopharynx of cholesterol-rich membrane microdomains, which are essential to the antimicrobial resistance induced by bacterial adhesion<sup>[2]</sup>.

To avoid immune detection, the surface components of *N. meningitidis* may be modified. The structural and antigenic modification of surface molecules can

involve changes in gene alleles. Studies have reported the import of genetic material from other bacteria or *via* intragenomic recombination<sup>[2]</sup>. *N. meningitidis* may be encapsulated or unencapsulated. *N. meningitidis* isolated from blood or cerebrospinal fluid is invariably encapsulated. The existence of the capsule enables it to withstand the effects of antibodies and complements and to resist serum opsonic activity. Some lipopolysaccharide structures help *N. meningitidis* to evade the immune response, and are more frequently observed in *N. meningitidis* isolated from blood than in healthy nasopharyngeal carriers. Both the capsule and some lipopolysaccharide immunotypes (L<sub>1</sub>, L<sub>8</sub> and L<sub>10</sub>) of *N. meningitidis* may influence bacterial adhesion and invasive capacity. It has been found the inhibitory role of capsule in biofilm formation<sup>[14]</sup>. The capsule genes are located in a single chromosomal locus (*cps*) divided into three regions. The capsular polysaccharides B, C, W-135 and Y contain sialic acid, which contributes to make the lipopolysaccharides of the capsule less visible to the immune system, since sialic acid is a common component of the host cell surfaces. Moreover, the serogroup B capsule contains a homopolymer that is structurally identical to the neural adhesion molecule, which is responsible for the poor immune response generated by serogroup B in humans. However, the genetic similarities of the loci of serogroups B, C, W and Y (not A) favour the horizontal exchange of fragments of the capsule between different serogroups<sup>[2]</sup>.

*N. meningitidis* expresses and secretes various surface molecules that bind to epithelial molecules, and some of these proteins include lactoferrin and the proteins bound to the transferrin that enable the meningococcus to acquire iron from the environment. Iron is a crucial element for bacterial growth in the surface colonisation stage and during the production of disease<sup>[19,20]</sup>, although some adherent properties, such as hydrophobicity and adherence to inert surfaces like nitrocellulose remain unchanged after incubation in culture media supplemented with Fe<sup>[21]</sup>. Other authors have described nine porin complexes formed by different combinations of the meningococcal porin protein (Por) A, PorB and RmpM proteins<sup>[22]</sup>. *N. meningitidis* expresses two types of outer membrane proteins (Opa and Opc) which give an opaque appearance to colonies in agar. Opa and Opc are of a similar size (27-31 kDa). Most Opa molecules recognise one or more members of the family of carcinoembryonic antigen-related cell adhesion molecules (CEACAM). The CEACAM1 receptor is found in epithelial and endothelial cells, while other family members such as CEACAM3 and CEACAM6 are expressed in neutrophils. CEACAM receptor density in the epithelial cells is modulated by the secretion of inflammatory cytokines, such that a high expression of CEACAM receptors takes place in response to inflammation, which could influence the development of meningococcal disease. Furthermore, some Opa proteins can interact with the heparan sulphate proteoglycan that is present in most epithelial

cells<sup>[2,23]</sup>.

Over the past 50 years, our understanding of the importance of serogroup B (MenB) disease *per se*, the social impact of fear caused by the devastating effects of the disease. The difficulty of inducing an effective immune response against the MenB capsular polysaccharide has lead to the search in vaccines for this serogroup based on outer membrane proteins (OMP). Public health interventions in Cuba, Norway and New Zealand have demonstrated that these protein-based vaccines can prevent MenB disease.

By combining a pangenome analysis with an extensive experimental validation to identify new potential vaccine candidates, genes coding for antigens likely to be exposed on the surface of the MenB were selected after a multistep comparative analysis of entire *Neisseria* genomes. Again, in the quest for vaccine candidates are successfully identified a significant number of new genes. Recent studies with meningococcal membrane proteins have centered on conserved antigens in order to obtain a universal vaccine that confers protection against a broad range of strains. There are several recent reports about the use of conserved minor OMP from *N. meningitidis* as immunogens<sup>[24]</sup>.

The classical bioinformatic approaches, in combination with proteomic data, conventional protein purification and immunological evaluation are powerful tools for the identification of novel meningococcal antigens and open reading frames and potential vaccine components<sup>[25]</sup>.

We now know that OMP based vaccines are most effective when are used against epidemics due to a homologous or clonal strain carrying the same PorA as that present in the vaccine. When used against endemic disease or outbreaks due to a number of different strains (heterologous epidemiologic situations), the level of effectiveness will generally be too low to rely on the effects of a conventional OMP vaccine alone for protection.

The general strategy in the Pajon *et al*<sup>[25]</sup> study, was to maximize the chance of identifying bacterial surface components by selecting not only proteins predicted by protein localization algorithms in outer membrane components of gram-negative bacteria, but also those predicted as periplasmic or inner membrane proteins. However, we must stress that while the most attention in the development of meningococcal vaccines has been devoted to major OMPs. The impact of conserved protein components in the induction of a significant immune response, and their potential as adjuvants, it must not be overlooked. The success in expressing all cloned genes came from the use of a highly optimized expression/purification platform designed precisely for this scenario, but also from the stringent selection procedure of potential vaccine candidates. Finally, five proteins are capable of inducing a functional antibody response *vs N. meningitidis* strain CU385: NMB0606 a potential YajC orthologue, NMB0928 the



neisserial NlpB (BamC), NMB0873 a LolB orthologue, NMB1163 a protein belonging to a curli-like assembly machinery, and NMB0938 (a neisserial specific antigen) with evidence of positive selection appreciated for NMB0928<sup>[25]</sup>.

The new set of vaccine candidates and the novel proposed functions will open a new wave of research in the search for the elusive neisserial vaccine. The key limitation of conventional wild-type outer membrane vesicle (wtOMV) vaccines is their lack of broad protective activity against the large diversity of MenB strains circulating globally. The experience with wtOMV vaccines also provide important information for the next generation of MenB vaccines designed to give more comprehensive protection against multiple strains.

## BACTERIAL ADHESION AND OXIDATIVE STRESS

It has been shown that the *N. meningitidis* loci involved in defence against oxidative stress are also involved in biofilm formation and contribute to the colonisation of epithelial surfaces<sup>[26]</sup>. Incubation of *N. meningitidis* *in vitro* with antioxidant molecules increases their adherence to inert surfaces and therefore the ability to generate biofilm, and at the same time increases their surface hydrophobicity<sup>[14,21]</sup>. Similar observations regarding adherence to nitrocellulose have been demonstrated in *E. coli*<sup>[27]</sup>. These observations are an example of how environmental conditions can modulate in *N. meningitidis* the expression of molecules to make it more virulent or more adherent. *In vivo*, plasma antioxidant levels are lower in children who are asymptomatic carriers of *N. meningitidis*<sup>[3]</sup>. We have analysed the association between total antioxidant capacity in plasma and the carrier state of *N. meningitidis*. In the carrier state, the odds ratio for this association (total antioxidant capacity in plasma < 0.25) was 8.44 (95%CI: 1.5-48.9). These observations are in the line with Jamet *et al.*<sup>[26]</sup>, who reported that the activation in *N. meningitidis* of genes involved in defence against oxidative stress (lower levels of plasma antioxidants) favours the adhesion of the bacteria and nasopharyngeal carrier status. Other studies have shown that oxidative stress can be induced experimentally with cysteine depletion, can trigger growth arrest and release of outer membrane vesicles (sOMV). Outer membrane vesicles contain immunogenic proteins and contribute to *in vivo* survival and virulence of bacterial pathogens<sup>[28]</sup>.

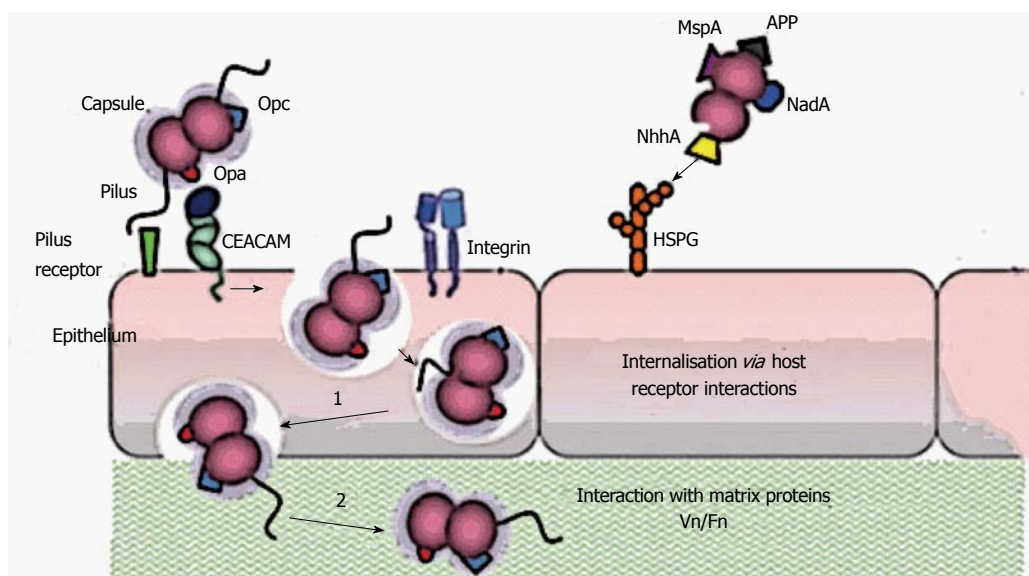
## BACTERIAL ADHESION AND VIRULENCE

Virulence, defined as the degree of aggressiveness of a pathogen, is a highly variable condition. The degree of virulence fluctuates according to the conditions in which microorganisms and their genetic makeup

are located. In general, a pathogen becomes less virulent on passing from a natural environment to an artificial culture medium; in these circumstances, it is said to be attenuated, and the same effect can be observed in unfavourable environmental conditions. The virulence of a microorganism can be reduced, either by the use of certain culture media, or by exploiting its successive passage through animals. Numerous studies support this; thus, Horská *et al.*<sup>[29]</sup> have reported that three different bacterial strains of *Pseudomonas* are capable of changing the surface charge and their hydrophobicity. By contrast, an attenuated microorganism can acquire greater virulence by its prior passage through certain animal species; specifically, pneumococcal virulence is enhanced by its passage through mice<sup>[30]</sup>. *N. meningitidis* requires iron, and in the absence of iron alters its gene expression to increase iron acquisition<sup>[19,31]</sup>. When *N. meningitidis* has grown in an iron-restricted environment, it synthesises new outer membrane proteins, which are necessary for its survival. Some of these proteins Tbp A and Tbp B are examples of meningococcal surface antigens regulated by iron, which are not expressed after culture in common laboratory media<sup>[32]</sup>. TbpA was found to possess a similar architecture to the siderophore and is highly immunogenic, allowing for prediction of potentially important ligand-binding epitopes<sup>[33]</sup>.

The passage of microorganisms through the epithelial layer is not a passive phenomenon (Figure 1). Microorganisms, after overcoming the first hurdle, consisting of the surface epithelium, are exposed to various host defence mechanisms, of which the most important is the inflammatory response. In the course of this response, the blood vessels dilate, thereby increasing their permeability and allowing various serum factors (immunoglobulins and other proteins) to come into contact with the infectious agent. Moreover, the activation of fibrinogen to fibrin delays the diffusion of the microorganisms.

In general, type-1 somatic fimbriae are encoded by chromosomal genes and are found both in commensal bacteria and in pathogenic strains of *E. coli*. The adhesion factors that are most frequently associated with pathogenicity are usually coded by plasmids<sup>[34]</sup>, although this may take place chromosomally. The bacterial surface appendages related to association functions are generally termed "fimbriae", with the term "pili" being reserved for cases in which their presence is related to the exchange of genetic material among microorganisms. The pili of *N. meningitidis* are 6 nm in diameter and extend several micrometres into the surface of the bacterium. They are, therefore, sexual or conjunctive fimbriae. The genes for the bacterial adhesion factors that have been most thoroughly studied, such as K-88, K-99 and CFA/I, are located in plasmids. Of these, the genes for factor K-88 are known to have a total length of 75-135 Kb, and are frequently associated with genes for the fermentation of raffinose. The gene for the CFA/I factor has a size



**Figure 1** Schematic representation of the interaction mechanisms of *Neisseria meningitidis* with cellular receptors. The first adherence phase would be a reversible process in which Van der Waals and electrostatic forces are responsible for a wide range of interactions, including chemical bonding. Finally we added a summary at the ending, dipolar interaction and hydrophobicity. Pili extending beyond the capsule are considered to mediate the primary interaction with epithelial cells. Opa proteins may bind to carcinoembryonic antigen-related cell-adhesion molecule (CEACAMs) and heparan sulphate proteoglycan (HSPGs), and Opc proteins can interact with HSPGs and, via vitronectin and fibronectin, to their integrin receptors. Engagement of CEACAMs, integrins and HSPGs can result in meningococcal internalization by epithelial cells. MSP: Meningococcal serine protease A; App: Adhesion and penetration protein; NadA: Neisserial adhesin; NhhA: Neisseria hia/hsf homologue A.

of approximately 90 Kb, and it is bound to a gene for a stable enterotoxin<sup>[35]</sup>. For adhesion factor K-88, three plasmids have been shown to be responsible for the three known antigenic variants: K-88ab, K-88ac and K-88ad. Mooi *et al.*<sup>[36]</sup>, designed experiments to determine which genes of the plasmid chain were responsible for the formation of the K-88 factor in each of the variants. For this purpose, each of the three K-88 plasmids was digested with restriction enzymes, and the fragments obtained from each one were then cloned by inclusion in the PBR-322 vector. The bacterial clones carrying each of the K-88 antigens were then identified. This procedure revealed that the expression of the K-88 factor depends of the orientation of the DNA chain responsible and on the variant in question. In the case of K-88ab, its insertion into the vector PBR-322 in a direction or another modifies the quantity of antigen expressed. The lipopolysaccharide of *N. meningitidis* is known as the major determinant of its virulence, and the use of monoclonal antibodies, together with structural studies, have highlighted the heterogeneity and complexity of meningococcal lipopolysaccharides, which can be divided into 12 immunotypes<sup>[37]</sup>.

Studies by McGee *et al.*<sup>[38]</sup>, have underlined the importance of gonococcal fimbriae in cell colonisation and destruction in cultures of cells from the human fallopian tube. These assays show that both fimbriate and non-fimbriate gonococci bind epithelial cells, although in the former case cell destruction is produced more quickly, this process being mediated by one or more toxic factors, such as surface lipopolysaccharides. Type IV pili, which are protein structures associated

with the surface, have also been associated with the adhesion of *N. meningitidis* to endothelial cells and the development of fulminant meningococcal disease<sup>[39,40]</sup>. The pili of *E. coli*, which have been studied in detail, consist of protein subunits that are thought to play an important role in the interaction with specific surface carbohydrates in eukaryotic cells, and some of them are K antigens. D-(+)-Mannose inhibits the *in vitro* adhesion of bacteria with type-1 fimbriae on the surface of eukaryotic cells containing mannose residue<sup>[41]</sup>. This is an indiscriminate mechanism of adhesion, as oligosaccharide chains containing mannose are very commonly present in cell surface oligoproteins, including phagocytic cells. Preincubation of bacteria with inhibitor sugars does not affect the adhesiveness, while the pretreatment of cells with carbohydrates effectively prevents adhesion. This indicates that the cell surface structures recognise the radicals of fucose and glucose in the bacterial lipopolysaccharides.

Some authors<sup>[42,43]</sup>, have analysed phenotypic changes in bacteria associated with epigenetic changes. Aspects such as virulence, response to oxidative stress and the formation of biofilm have been observed among epigenetic modifications. Unfortunately, these processes and their relationship with pathogenic changes in *N. meningitidis* are as yet incompletely understood.

Despite the high prevalence of carriers of *N. meningitidis*, it only occasionally causes meningococcal disease in the context of endemic disease, in certain geographic areas or in isolated epidemic outbreaks. Some studies have reported that oxidative stress in the environment can modify the surface characteristics

of *N. meningitidis*. Also the antigenic structure can be modified by its importing genetic material from other bacteria in its ecological niche, and some structures of lipopolysaccharides, pili and capsule change the immune response. This paper reviews current knowledge on host-environment-bacteria mechanisms and interactions, with the aim of contributing to our understanding of the pathogenic mechanisms of *N. meningitidis*.

## REFERENCES

- Hernandez DM, Matos PP, Hernandez JC, Muñoz JL, Villasana Lde C. Persistence of an infected urachus presenting as acute abdominal pain. Case report. *Arch Esp Urol* 2009; **62**: 589-592 [PMID: 19815963]
- Geörg M, Maudsdotter L, Tavares R, Jonsson AB. Meningococcal resistance to antimicrobial peptides is mediated by bacterial adhesion and host cell RhoA and Cdc42 signalling. *Cell Microbiol* 2013; **15**: 1938-1954 [PMID: 23834289]
- Uberos J, Molina-Carballo A, Galdo-Muñoz G, Muñoz-Hoyos A. Total antioxidant capacity of plasma in asymptomatic carrier state of *Neisseria meningitidis*. *Epidemiol Infect* 2007; **135**: 857-860 [PMID: 17109775 DOI: 10.1017/S0950268806007539]
- Van Wamel WJ, Vandenbroucke-Grauls CM, Verhoef J, Fluit AC. The effect of culture conditions on the in-vitro adherence of methicillin-resistant *Staphylococcus aureus*. *J Med Microbiol* 1998; **47**: 705-709 [PMID: 9877191 DOI: 10.1099/00222615-47-8-705]
- Bartley SN, Tzeng YL, Heel K, Lee CW, Mowlaboccus S, Seemann T, Lu W, Lin YH, Ryan CS, Peacock C, Stephens DS, Davies JK, Kahler CM. Attachment and invasion of *Neisseria meningitidis* to host cells is related to surface hydrophobicity, bacterial cell size and capsule. *PLoS One* 2013; **8**: e55798 [PMID: 23405216 DOI: 10.1371/journal.pone.0055798]
- Marrie TJ, Lam J, Costerton JW. Bacterial adhesion to uroepithelial cells: a morphologic study. *J Infect Dis* 1980; **142**: 239-246 [PMID: 6774033 DOI: 10.1093/infdis/142.2.239]
- Cohen C, Phillips GN. Spikes and fimbriae: alpha-helical proteins form surface projections on microorganisms. *Proc Natl Acad Sci USA* 1981; **78**: 5303-5304 [PMID: 6117855 DOI: 10.1073/pnas.78.9.5303]
- Liu F, Lee HJ, Strynadka NC, Tanner ME. Inhibition of *Neisseria meningitidis* sialic acid synthase by a tetrahedral intermediate analogue. *Biochemistry* 2009; **48**: 9194-9201 [PMID: 19719325 DOI: 10.1021/bi9012758]
- Smyth CJ, Siegel J, Salton MR, Owen P. Immunochemical analysis of inner and outer membranes of *Escherichia coli* by crossed immunoelectrophoresis. *J Bacteriol* 1978; **133**: 306-319 [PMID: 338583]
- Edebo L, Hed J, Kihlström E, Magnusson KE, Stendahl O. The adhesion of enterobacteria and the effect of antibodies of different immunoglobulin classes. *Scand J Infect Dis Suppl* 1980; **Suppl 24**: 93-99 [PMID: 7010568]
- Grundström T, Jaurin B, Edlund T, Normark S. Physical mapping and expression of hybrid plasmids carrying chromosomal beta-lactamase genes of *Escherichia coli* K-12. *J Bacteriol* 1980; **143**: 1127-1134 [PMID: 6251026]
- Lambden PR, Heckels JE, James LT, Watt PJ. Variations in surface protein composition associated with virulence properties in opacity types of *Neisseria gonorrhoeae*. *J Gen Microbiol* 1979; **114**: 305-312 [PMID: 120407 DOI: 10.1099/00221287-114-2-305]
- Ellen RP, Gibbons RJ. M protein-associated adherence of *Streptococcus pyogenes* to epithelial surfaces: prerequisite for virulence. *Infect Immun* 1972; **5**: 826-830 [PMID: 4564883]
- Yi K, Rasmussen AW, Gudlavalleti SK, Stephens DS, Stojiljkovic I. Biofilm formation by *Neisseria meningitidis*. *Infect Immun* 2004; **72**: 6132-6138 [PMID: 15385518 DOI: 10.1128/IAI.72.10.6132-6138.2004]
- Beachey EH. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surface. *J Infect Dis* 1981; **143**: 325-345 [PMID: 7014727 DOI: 10.1093/infdis/143.3.325]
- Nassif X, Beretti JL, Lowy J, Stenberg P, O'Gaora P, Pfeifer J, Normark S, So M. Roles of pilin and PilC in adhesion of *Neisseria meningitidis* to human epithelial and endothelial cells. *Proc Natl Acad Sci USA* 1994; **91**: 3769-3773 [PMID: 7909606 DOI: 10.1073/pnas.91.9.3769]
- Virji M, Makepeace K, Peak IR, Ferguson DJ, Jennings MP, Moxon ER. Opc- and pilus-dependent interactions of meningococci with human endothelial cells: molecular mechanisms and modulation by surface polysaccharides. *Mol Microbiol* 1995; **18**: 741-754 [PMID: 8817495 DOI: 10.1111/j.1365-2958.1995.mmi\_18040741.x]
- Koomey M, Gotschlich EC, Robbins K, Bergström S, Swanson J. Effects of recA mutations on pilus antigenic variation and phase transitions in *Neisseria gonorrhoeae*. *Genetics* 1987; **117**: 391-398 [PMID: 2891588]
- Jordan PW, Saunders NJ. Host iron binding proteins acting as niche indicators for *Neisseria meningitidis*. *PLoS One* 2009; **4**: e5198 [PMID: 19352437 DOI: 10.1371/journal.pone.0005198]
- Criado MT, del Río MC, Ferreirós CM, Pintor M, Sáinz V, Carballo J. Iron and outer membrane proteins in the susceptibility of *Neisseria meningitidis* to human serum. *FEMS Microbiol Lett* 1990; **58**: 145-150 [PMID: 2121585 DOI: 10.1111/j.1574-6968.1990.tb13968.x]
- Uberos J, Molina A, Liébana J, Augustin MC, Muñoz A. The influence of different concentrations of melatonin on the cell surface hydrophobic characteristics of *Neisseria meningitidis*. *Lett Appl Microbiol* 2000; **31**: 294-298 [PMID: 11068910 DOI: 10.1046/j.1472-765x.2000.00813.x]
- Marzoa J, Sánchez S, Ferreirós CM, Criado MT. Identification of *Neisseria meningitidis* outer membrane vesicle complexes using 2-D high resolution clear native/SDS-PAGE. *J Proteome Res* 2010; **9**: 611-619 [PMID: 19888731 DOI: 10.1021/pr9006409]
- Johsrich KO, McCaw SE, Islam E, Sintsova A, Gu A, Shively JE, Gray-Owen SD. In vivo adaptation and persistence of *Neisseria meningitidis* within the nasopharyngeal mucosa. *PLoS Pathog* 2013; **9**: e1003509 [PMID: 23935487 DOI: 10.1371/journal.ppat.1003509]
- Holst J, Oster P, Arnold R, Tatley MV, Næss LM, Aaberge IS, Galloway Y, McNicholas A, O'Hallahan J, Rosenqvist E, Black S. Vaccines against meningococcal serogroup B disease containing outer membrane vesicles (OMV): lessons from past programs and implications for the future. *Hum Vaccin Immunother* 2013; **9**: 1241-1253 [PMID: 23857274 DOI: 10.4161/hv.24129]
- Pajon R, Yero D, Niebla O, Climent Y, Sardiñas G, García D, Perera Y, Llanes A, Delgado M, Cobas K, Caballero E, Taylor S, Brookes C, Gorrige A. Identification of new meningococcal serogroup B surface antigens through a systematic analysis of neisserial genomes. *Vaccine* 2009; **28**: 532-541 [PMID: 19837092 DOI: 10.1016/j.vaccine.2009.09.128]
- Jamet A, Euphrasie D, Martin P, Nassif X. Identification of genes involved in *Neisseria meningitidis* colonization. *Infect Immun* 2013; **81**: 3375-3381 [PMID: 23817612 DOI: 10.1128/IAI.00421-13]
- Uberos J, Augustin C, Liébana J, Molina A, Muñoz-Hoyos A. Comparative study of the influence of melatonin and vitamin E on the surface characteristics of *Escherichia coli*. *Lett Appl Microbiol* 2001; **32**: 303-306 [PMID: 11328494 DOI: 10.1046/j.1472-765X.2001.00908.x]
- van de Waterbeemd B, Zomer G, van den Ijssel J, van Keulen L, Eppink MH, van der Ley P, van der Pol LA. Cysteine depletion causes oxidative stress and triggers outer membrane vesicle release by *Neisseria meningitidis*; implications for vaccine development. *PLoS One* 2013; **8**: e54314 [PMID: 23372704 DOI: 10.1371/journal.pone.0054314]
- Horská E, Pokorný J, Labajová M. Effect of cultivation medium on some physicochemical parameters of outer bacterial membrane. *Microbios* 1995; **81**: 203-211 [PMID: 7770007]
- Gibbons RJ, Qureshi JV. Virulence-related physiological changes and antigenic variation in populations of *Streptococcus mutans*

- colonizing gnotobiotic rats. *Infect Immun* 1980; **29**: 1082-1091 [PMID: 7429627]
- 31 **Livorsi DJ**, Stenehjem E, Stephens DS. Virulence factors of gram-negative bacteria in sepsis with a focus on *Neisseria meningitidis*. *Contrib Microbiol* 2011; **17**: 31-47 [PMID: 21659746 DOI: 10.1159/000324008]
- 32 **Pintor M**, Ferrón L, Gómez JA, Powell NB, Ala'Aldeen DA, Borriello SP, Criado MT, Ferreirós CM. Blocking of iron uptake from transferrin by antibodies against the transferrin binding proteins in *Neisseria meningitidis*. *Microb Pathog* 1996; **20**: 127-139 [PMID: 8965674 DOI: 10.1006/mpat.1996.0012]
- 33 **Oakhill JS**, Sutton BJ, Gorringe AR, Evans RW. Homology modelling of transferrin-binding protein A from *Neisseria meningitidis*. *Protein Eng Des Sel* 2005; **18**: 221-228 [PMID: 15820975 DOI: 10.1093/protein/gzi024]
- 34 McNeish AS, Turner P, Fleming J, Evans N. Mucosal adherence of human enteropathogenic *Escherichia coli*. *Lancet* 1975; **2**: 946-948 [DOI: 10.1016/S0140-6736(75)90360-8]
- 35 **Smith HW**, Parsell Z. Transmissible substrate-utilizing ability in enterobacteria. *J Gen Microbiol* 1975; **87**: 129-140 [PMID: 1094091 DOI: 10.1099/00221287-87-1-129]
- 36 **Mooi FR**, de Graaf FK, van Embden JD. Cloning, mapping and expression of the genetic determinant that encodes for the K88ab antigen. *Nucleic Acids Res* 1979; **6**: 849-865 [PMID: 375197 DOI: 10.1093/nar/6.3.849]
- 37 **Verheul AF**, Snippe H, Poolman JT. Meningococcal lipopolysaccharides: virulence factor and potential vaccine component. *Microbiol Rev* 1993; **57**: 34-49 [PMID: 8464406]
- 38 **McGee ZA**, Gross J, Dourmashkin RR, Taylor-Robinson D. Nonpilar surface appendages of colony type 1 and colony type 4 gonococci. *Infect Immun* 1976; **14**: 266-270 [PMID: 820643]
- 39 **Melican K**, Duménil G. A humanized model of microvascular infection. *Future Microbiol* 2013; **8**: 567-569 [PMID: 23642111 DOI: 10.2217/fmb.13.35]
- 40 **Ryll RR**, Rudel T, Scheuerpflug I, Barten R, Meyer TF. PilC of *Neisseria meningitidis* is involved in class II pilus formation and restores pilus assembly, natural transformation competence and adherence to epithelial cells in PilC-deficient gonococci. *Mol Microbiol* 1997; **23**: 879-892 [PMID: 9076726 DOI: 10.1046/j.1365-2958.1997.2631630.x]
- 41 **Firon N**, Ofek I, Sharon N. Interaction of mannose-containing oligosaccharides with the fimbrial lectin of *Escherichia coli*. *Biochem Biophys Res Commun* 1982; **105**: 1426-1432 [PMID: 6125146 DOI: 10.1016/0006-291X(82)90947-0]
- 42 **Chen P**, Jeannotte R, Weimer BC. Exploring bacterial epigenomics in the next-generation sequencing era: a new approach for an emerging frontier. *Trends Microbiol* 2014; **22**: 292-300 [PMID: 24725482 DOI: 10.1016/j.tim.2014.03.005]
- 43 **Davis BM**, Chao MC, Waldor MK. Entering the era of bacterial epigenomics with single molecule real time DNA sequencing. *Curr Opin Microbiol* 2013; **16**: 192-198 [PMID: 23434113 DOI: 10.1016/j.mib.2013.01.011]

**P- Reviewer:** Callegan MC, Weng CF **S- Editor:** Yu J **L- Editor:** A  
**E- Editor:** Wu HL





## Observational Study

# Improvement in human immunodeficiency virus-1/acquired immune deficiency syndrome patients' well-being following administration of "Phyto V7"

Ruben Wernik, Jose L Priore, Walter F Goldman, Adriana del Carmen Elias, Gadi Borkow

Ruben Wernik, Facultad de Medicina, Universidad de la República, Montevideo CP 11800, Uruguay

Jose L Priore, Uruguay Servicio Médico Penitenciario, Dirección Nacional de Cárceles, Penitenciarias y Centros de Recuperación, Montevideo CP 11100, Uruguay

Walter F Goldman, Gadi Borkow, Immune Nutrition Incorporated, Gibton 76910, Israel

Adriana del Carmen Elias, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán 4000, Argentina

**Author contributions:** Wernik R and Goldman WF were involved in the design of the study and interaction with the Uruguay Government and General Direction of Prisons; Priore JL was in charge of the actual implementation of the trial; Elias AC and Borkow G analyzed the data and wrote the manuscript.

**Ethics approval:** The protocol was reviewed and approved by the Ethical Medical Committee of the Ministry of Health of Uruguay.

**Informed consent:** All study participants provided informed written consent prior to study enrollment.

**Conflict-of-interest:** Dr. Walter F Goldman and Dr. Gadi Borkow are members of the Immune Nutrition Incorporated, the company that produces the Phyto V7 complex. All other authors do not have a conflict of interest.

**Data sharing:** Technical appendix, statistical analyses, and dataset are available from the corresponding author at [dr.borkow@gmail.com](mailto:dr.borkow@gmail.com). Consent was not obtained but the presented data are anonymized and risk of identification is nonexistent.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Dr. Gadi Borkow, Chief Scientist, Immune Nutrition Incorporated, Hameyasdim 44, Gibton 76910, Israel. [dr.borkow@gmail.com](mailto:dr.borkow@gmail.com)

Telephone: +972-546-611287

Received: September 17, 2014

Peer-review started: September 18, 2014

First decision: December 17, 2014

Revised: January 29, 2015

Accepted: April 27, 2015

Article in press: April 29, 2015

Published online: May 25, 2015

## Abstract

**AIM:** To corroborate the capacity of Phyto V7, a complex of phytochemicals, to improve the physical well-being of human immunodeficiency virus-1 (HIV-1) infected and acquired immune deficiency syndrome (AIDS) patients not undergoing antiretroviral treatment.

**METHODS:** Two hundred and thirty nine HIV-1 sero-positive male and female voluntary inmates were recruited through the Uruguay National Program of AIDS. The study participants received for 90 consecutive days every eight hours two tablets (760 mg/each) of Phyto V7, containing a mix of the following phytochemicals: flavonols (Kaempferol, Quercetin), flavones (Apigenin, Luteolin), hydroxycinnamic acids (ferrulic acid), carotenoids (Lutein, Lycopene, Beta carotene) and organosulfur compounds, all from vegetal origin. The participants did not receive any antiretroviral treatment during the study. At days 0, 30, 60 and 90 ( $\pm 2$  d) the participants were evaluated for body mass index (BMI), tolerance to Phyto V7 and Index of Quality of Life based on the Karnofsky scale. ANOVA, Tukey Post-test,  $\chi^2$  test and Wilcoxon Signed Rank test were used to analyze the effect of treatment.

**RESULTS:** One hundred and eighty nine study participants finished the study. Already after 30 d of Phyto V7 consumption, the weight, BMI and Karnofsky score statistically significantly improved ( $P < 0.001$ ), and continued to improve until the end of the study. The mean weight gain per participant during the 90 d was

of 1.21 kg (approximately 2% of body weight). The overall increase in the mean Karnofsky score after 90 d was 14.08%. The lower the BMI and Karnofsky score of the participants were at the beginning of the study, the more notorious was the improvement over time. For example, the mean increment of Index of Quality of Life, among the participants with an initial Karnofsky score of 5 or below ( $n = 33$ ) from day 0 to day 90, was of 35.67% ( $0.476 \pm 0.044$  vs  $0.645 \pm 0.09$ ;  $P < 0.001$ ). The tolerability to Phyto V7 was very good and no adverse reactions were recorded or reported.

**CONCLUSION:** Administration of the Phyto V7 can be an important tool to improve the well-being of HIV-1 seropositive individuals and AIDS patients, not undergoing antiretroviral treatment.

**Key words:** Phytochemicals; Karnofsky score; Nutrition; Human immunodeficiency virus-1; Acquired immune deficiency syndrome

© **The Author(s)** 2015. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Phyto V7 is a complex of phytochemicals and micronutrients. Phyto V7 has been found to stimulate the immune system and dramatically improve the physical well-being of terminal acquired immune deficiency syndrome (AIDS) patients. The current study demonstrates the capacity of Phyto V7 to improve the physical well-being of human immunodeficiency virus-1 (HIV-1) infected and AIDS patients not undergoing antiretroviral treatment, as demonstrated in 199 individuals. We conclude that administration of the food supplement Phyto V7 can be an important tool to improve the well-being of HIV-1 seropositive individuals and AIDS patients, not undergoing antiretroviral treatment.

Wernik R, Priore JL, Goldman WF, Elias AC, Borkow G. Improvement in human immunodeficiency virus-1/acquired immune deficiency syndrome patients' well-being following administration of "Phyto V7". *World J Clin Infect Dis* 2015; 5(2): 44-50 Available from: URL: <http://www.wjgnet.com/2220-3176/full/v5/i2/44.htm> DOI: <http://dx.doi.org/10.5495/wjcid.v5.i2.44>

## INTRODUCTION

The energy needed for physical activity and for maintaining the body weight is higher in human immunodeficiency virus-1 (HIV-1) infected individuals than in non-HIV infected individuals<sup>[1,2]</sup>. Acquired immune deficiency syndrome (AIDS) patients spend approximately 20% to 30% more energy than healthy individuals in order to maintain their body weight, including when receiving highly active antiretroviral treatment (HAART)<sup>[3,4]</sup>. The World Health Organization has recommended including micronutrient supplementation as an integral part of all HIV treatment programs<sup>[5]</sup>. Micronutrient supplementation trials

demonstrated a reduced mortality and improved clinical outcomes in HIV-1 infected individuals, regardless of their clinical stage and use of antiretrovirals<sup>[6-9]</sup>.

Phytochemicals, chemical compounds that occur naturally in plants, in addition of serving as micronutrients, enhance nonspecific immunity<sup>[10]</sup>, down regulate inflammatory diseases<sup>[11]</sup>, possess radical scavenging activities<sup>[12]</sup>, and inhibit disease progression<sup>[13-19]</sup>. For example, administration of phytochemicals reduced hepatotoxic, lithic, and hepatitis related adverse symptoms<sup>[19]</sup>. Some phytochemicals inhibit HIV-1 protease and integrase, and inhibit viral entry to target cells<sup>[12,20-24]</sup>. Phyto V7 is a complex of phytochemicals, which also contains micronutrients, registered as a nutritional supplement in several countries. Administration of Phyto V7 to chicks enhances their humoral immune responses against Newcastle Disease Virus following vaccination<sup>[25]</sup>. Furthermore, its administration to human papilloma virus (HPV) affected women undergoing electrosurgical excision of cervical lesions resulted in approximately two-fold higher elimination of HPV than in the control group of women. In the group of woman receiving Phyto V7 there was an increase in the local cellular immune responses, as exemplified by much higher elevated presence of NK cells and cytotoxic T-cells (CD8<sup>+</sup>) in the cervical smears 90 d after the electrosurgical excisional procedure<sup>[26]</sup>. We have also found an increase in CD4<sup>+</sup> T-cells in HIV-1 infected individuals taking Phyto V7, without affecting their viral loads titers (manuscript in press). Taken together, the above findings indicate that Phyto V7 has immunestimulatory properties. Remarkably, administration of Phyto V7 to 9 terminally ill AIDS patients resulted in a dramatic improvement in their physical status<sup>[27]</sup>.

Antiretroviral treatment, which can effectively control viremia, requires high patient adherence for life. Low patient adherence results in the appearance of drug resistant viral isolates and necessitates different treatment protocols and salvage therapy options. Unfortunately, in many developing countries HIV-1 infected individuals are not treated at all. Many reasons account for that, such as inappropriate or non-existent centralized government treatment programs and elevated costs of antiretroviral treatments. One of the treatment neglected populations, in many developing countries, is prison inmates. The rates of HIV-1 infection are very high in this population<sup>[28,29]</sup>. Prison inmates are at higher risk of HIV-1 infection due to increased intravenous drug use, unprotected sexual activity, exposure to blood during fights, and tattooing.

In the current manuscript we report the very significant improvement in the well-being of 199 HIV-1 infected prison inmates, who did not receive any antiretroviral treatment while in prison, receiving only a daily administration of Phyto V7.

## MATERIALS AND METHODS

The methodological design of the study was analytical

**Table 1** Karnofsky score used

10	No complaints, no signs of disease
9	Capable of normal activity, few symptoms or signs of disease
8	Normal activity with some difficulty, some symptoms or signs
7	Caring for self, not capable of normal activity or work
6	Requiring some help, can take care of most personal requirements
5	Requires help often, requires frequent medical care
4	Disabled, requires special care and help
3	Severely disabled, hospital admission indicated but no risk of death
2	Very ill, urgently requiring hospital admission, requires supportive measures or treatment
1	Moribund, rapidly progressive fatal disease processes

**Table 2** Frequency of increase in the weight of the study participants over time

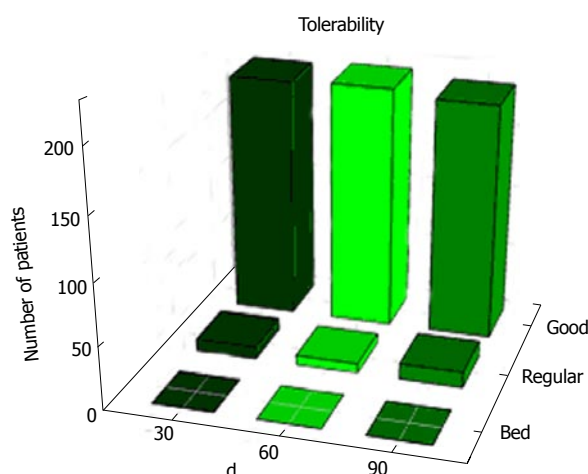
Weight	30 d		60 d		90 d	
	n	%	n	%	n	%
Decrease	27	13.6	19	9.5	12	6
Equal	65	32.7	36	18.1	24	12.1
Increase	107	53.8	144	72.4	163	81.9

and longitudinal, conducted by mid-2010 in Uruguay through the patronage of Dr. Tabaré Vazquez, President of Uruguay, by the General Direction of Prisons and the Uruguay Association of Seropositives. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was reviewed and approved by the Ethical Medical Committee of the Ministry of Health of Uruguay. HIV-1 seropositive male and female inmates were recruited from the Libertad, La Tablada and Cabillo prisons. All study participants gave their informed consent prior to the commencement of the study.

Phyto V7 was donated by the Israel Project Life Foundation and Immune Nutrition Incorporated. Phyto V7 was registered as a food supplement (Registration Number 54221) at the Division of Health Products, Department of Food. Each Phyto V7 tablet contained 760 mg of the following phytochemicals: flavonols (Kaempferol, Quercetin), flavones (Apigenin, Luteolin), hydroxy-cinnamic acids (ferrulic acid), carotenoids (Lutein, Lycopene, Beta carotene) and organosulfur compounds, all from vegetal origin.

During the study, each participant was given every 8 h two Phyto V7 tablets. At days 0, 30, 60 and 90 ( $\pm 2$  d) the participants were evaluated for body mass index (BMI), tolerance to Phyto V7 and well-being. The well-being was estimated according to the modified Karnofsky scale (Table 1). Each time the doctor in charge filled the questionnaire while examining and consulting each study participant, without seeing the previous already filled questionnaires. No data regarding the viral load or immune profile of the participants could be gathered. The participants did not receive any antiretroviral treatment during the study.

The differences between weight and BMI were analyzed with Kruskal-Wallis One Way Analysis of



**Figure 1** Assessment of Phyto V7 tolerability. The assessment of tolerability is based on the medical examination and the participant's feedback and general feeling.

Variance on Ranks (ANOVA) and Tukey Post test. The proportions of levels of quality of life were analyzed with the  $\chi^2$  test. An Index of Quality of Life was defined by dividing the levels of the Karnofsky score by the maximal level (10) and applied the Wilcoxon Signed Rank Test to analyze the differences. The SigmaPlot 12 software was used to conduct the statistical analyses.

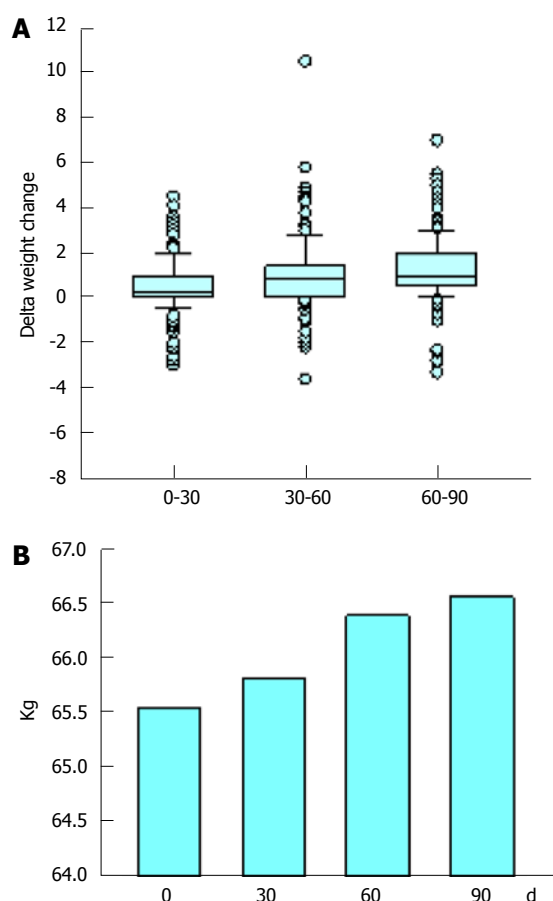
## RESULTS

A total of 239 HIV-1 seropositive inmates were recruited. Forty participants did not finish the study due to various reasons, such as being transferred to other facilities and being released from prison. Thus, the data presented is of 199 participants.

As reported by the study participants, after taking Phyto V7 for 30, 60 and 90 d, the tolerability to Phyto V7 was very good (Figure 1). No adverse reactions were recorded or reported.

As can be seen in Table 2, the proportion of individuals that participated in the study in whom there was an increase in their weight was 53.8%, 72.4% and 81.9% after 30, 60 and 90 d, respectively. The increase in weight was statistically significant ( $P < 0.001$ ). After 90 ds there was a decrease in weight in only 6% of the patients. The increase in the mean weight of the study participants can be appreciated in Figure 2B. The mean weight gain per participant during the 90 d was of 1.21 kg (approximately 2% of body weight).

In accordance with the increase in the weight, also the BMI of the participants increased over time (Figure 3A). The mean of BMI increased from 23.18 on day 0 to 23.64 on day 90, a 1.98% increase. When analyzing the mean increase in the BMI of the group of participants that had a BMI of below 21 at the beginning of the study ( $n = 60$ ), the increase in BMI is even more impressive (Figure 3B) - the mean in BMI among this group increased from 19.69 on day 0 to 20.24 on day 90, a 2.75% increase. Similarly, when

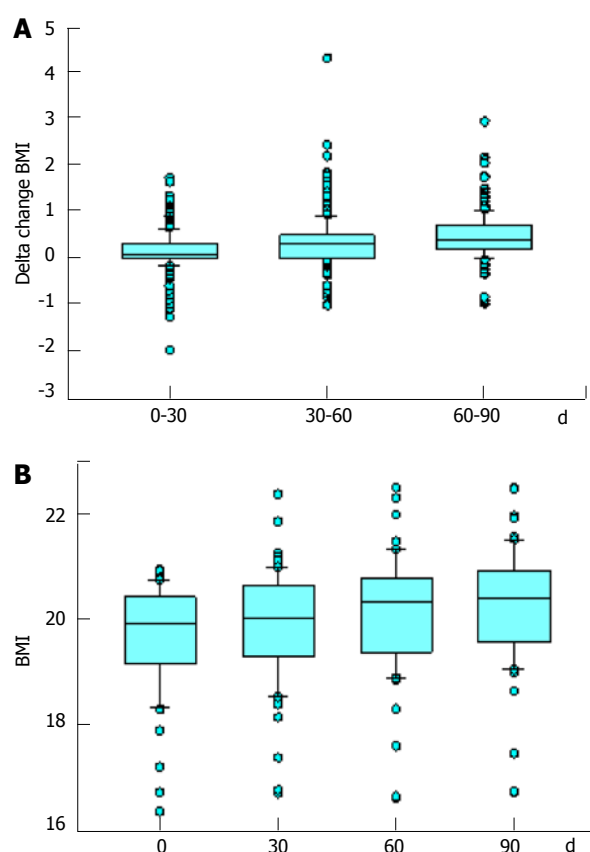


**Figure 2 Participant body weight.** A: Box plots describing the delta change in the weight of the study participants. The boxes represent the middle 50% of the data values. The horizontal line across the box marks the median value. The error bars show the 10<sup>th</sup> and 90<sup>th</sup> percentiles of the population. Individual data-points falling beyond these boundaries are shown as dots; B: The mean weight of the study participants.

looking into the 11 participants that had a BMI of below 19 at the beginning of the study ( $n = 11$ ), the mean BMI increased from 18.02 on day 0 to 18.62 on day 90, a 3.04% increase.

The overall quality of life of the participants increased over time, as determined by the Karnofsky scale determinations, and as exemplified for the Index of Quality of Life in Figure 4A. The Index of Quality of Life was already statistically significantly higher at day 30 compared to day 0 (mean of 0.657 vs 0.632;  $P < 0.001$ ). The Index of Quality of Life continued to increase with Phyto V7 consumption, from a mean of 0.657 to 0.7 and 0.721 at days 30, 60 and 90, respectively ( $P < 0.001$  between each data point). The overall increase in the mean Karnofsky score after 90 d was 14.08%.

When analyzing the changes in the Index of Quality of Life among the participants that at day 0 had a Karnofsky score of 5 or below ( $n = 33$ ), the changes in the score from day 0 to day 90 are even more impressive, *i.e.*, 35.67%, from  $0.476 \pm 0.044$  to  $0.645 \pm 0.09$  ( $P < 0.001$ ; Figure 4B). The clear increase in the proportions of the Karnofsky score over time is depicted in Figure 4C. For example, the level score 8



**Figure 3 Body mass index of study participants.** Box plots describing (A) the delta change in body mass index (BMI) of all the study participants over time and (B) the BMI of the participants who had a BMI of less than 21 at the onset of the study.

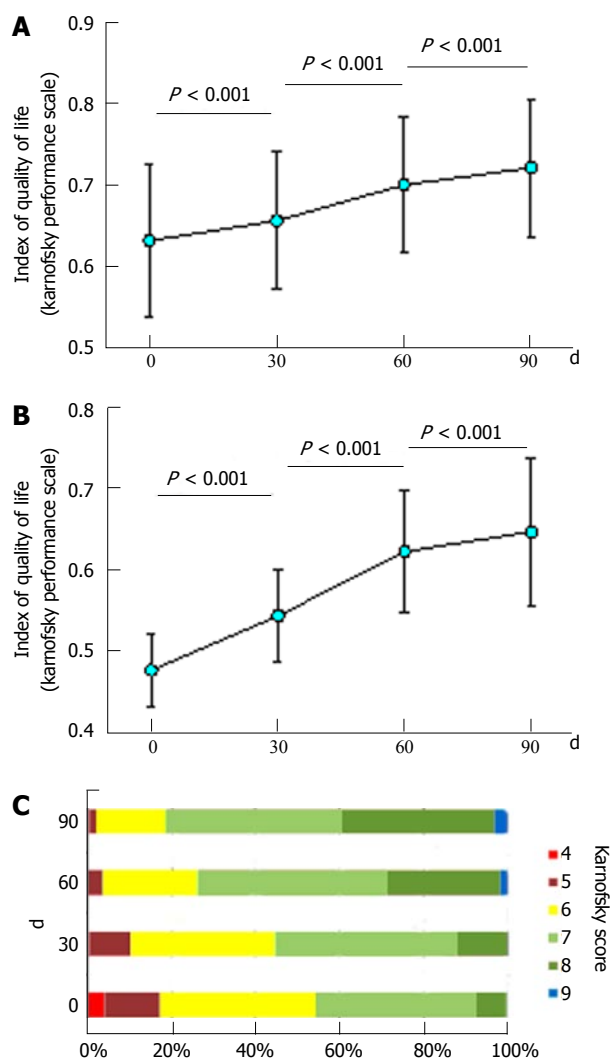
increased from day 0 to day 90 by approximately 5 fold, from 7.5% to 36.7% for all study participants. In contrast, the level score 5 decreased from day 0 to day 90 from 13.1% to 1.5%.

At day 90, approximately 73% of the study participant's felt that consumption of Phyto V7 was beneficial to them, while approximately 25% felt the same. This is in accordance with an increase in weight in 81.9% of the study participants. Two percent of the patients felt that their situation worsened during the 90 d study.

## DISCUSSION

Since the institution of HAART, the number of individuals becoming ill with AIDS has declined significantly and the prognosis of AIDS patients has improved notably. However, low compliance, viral cross-resistance, and significant side effects caused by HAART, serve as reason to postpone HAART. In developing countries, wide implementation of HAART may be even more problematic due to high costs, infrastructure problems and high prevalence of other ailments such as anemia and co-infections<sup>[30,31]</sup>. Thus, new, non-expensive, safe, easy to take alternative or complementary remedies, that can improve the patient's well-being, are very attractive for the treatment of individuals that fail HAART or antiretroviral naïve patients that can not get





**Figure 4** Quality of life of study participants based on the Karnofsky score. A: The mean and standard deviation of the Index of Quality of Life score of all study participants and of (B) participants who had a Karnofsky score of 5 or less at the onset of the study; C: The proportions of Karnofsky score at days 0, 30, 60 and 90. The *P* values of Wilcoxon Signed Rank Tests between each day are shown.

antiretroviral therapy.

Recently we published the results of a study that was conducted with 9 terminally ill AIDS patients living in a hospice<sup>[27]</sup>. All patients had very high HIV-1 viral loads and 8 out of the 9 patients were scored as C3 according to the United States Centers for Disease Control status index. Seven out of the 9 patients were antiretroviral naïve patients. During the study they did not receive antiretroviral treatment but only received the food supplement Phyto V7. While most of the patients at the commencement of the study could not eat, stand, dress or shower by themselves, after 3 mo of Phyto V7 supplementation all patients could eat, sit down, shower, stand up and dress without help. The well-being of the patients improved dramatically, both physically and mentally. The success of this trial was the incentive to conduct the current study.

As with the terminally ill AIDS patients, the administration of Phyto V7 to HIV-1 infected, asymptomatic and

symptomatic individuals in the current study, resulted in a very significant improvement in the individuals' well-being. The weight, BMI and Karnofsky score of the study participants increased notably, especially in those who had a low BMI and low Karnofsky score at the onset of the study. Increase in appetite, weight, and individuals mood, has a positive outcome in the individual well-being. Notably 83% of the participants adhered until the end of the trial and took Phyto V7, indicating the high likelihood that they will continue using Phyto V7 also finalizing the study. Part of the positive effect of Phyto V7 can also be explained as phytochemicals having radical scavenging activities<sup>[12]</sup>, stimulating nonspecific immunity<sup>[10]</sup>, and down regulating inflammatory responses<sup>[11]</sup>. Indeed, Phyto V7 has been shown to enhance humoral and cellular immune responses<sup>[25,26]</sup>. It is not clear from this study if crucial parameters relevant to the progression to AIDS were affected, such as the CD4<sup>+</sup> T-cell counts and viremia. However, in a another study (manuscript in press) the administration of Phyto V7 resulted in the upregulation of CD4<sup>+</sup> T-cell counts without affecting viral loads, indicating that Phyto V7 has an immuno-stimulating effect and no direct antiviral effect.

Administration of a food supplement, such as the Phyto V7, is extremely inexpensive as compared to HAART. Phyto V7 is from a natural source and as opposed to antiretrovirals, does not affect directly HIV-1. Thus its uptake with low adherence would not result in appearance of drug resistant viruses. Obviously, in order to increase its efficacy, high compliance is desired. Administration of Phyto V7 may potentially postpone the need to treat HIV-1 infected individuals with HAART, postponing the potential complications associated with this treatment. It may well be that Phyto V7 can be given in conjunction with HAART resulting in better prognosis. These assumptions need to be examined in placebo controlled studies.

## ACKNOWLEDGMENTS

We would like to thank Dr. Tabaré Vazquez, President of Uruguay during the study, who approved the Phyto V7 donation and encouraged all involved to conduct the study. We also thank Laboratorios Haymann, especially Prof. Nelson Lago, Technical Director of Laboratorios Haymann, who registered Phyto V7 under the name of GT+ in Uruguay. The study was funded by the Uruguay National Program of AIDS. This study is dedicated to Dr. Simon Raul Goldman who recently passed away. Dr. Goldman was the CEO of Immune Nutrition Incorporated and the driving force behind the development, testing and introduction of Phyto V7.

## COMMENTS

### Background

The immune system of human immunodeficiency virus-1 (HIV-1) infected individuals decays with the progression of time until they develop immu-

nodeficiency. HIV-1 infected individuals also have increased energy needs than non-HIV infected individuals and many suffer from significant weight loss and wasting. Micronutrient supplementation is thus recommended as an integral part of all HIV treatment programs.

### Research frontiers

Micronutrient supplementation improves the physical condition of HIV-1 infected individuals and acquired immune deficiency syndrome (AIDS) patients regardless of their clinical status and antiretroviral treatment, as was demonstrated in several studies. The administration of micronutrients that also enhance the immune system may be significantly advantageous to the HIV-1 infected individuals.

### Innovations and breakthroughs

The food supplement Phyto V7 is a complex of phytochemicals and micronutrients. Phyto V7 has been found to stimulate cellular and antibody immune responses against viruses both in humans and in chicks. Importantly, administration of Phyto V7 to 9 terminal AIDS patients resulted in dramatic improvement in their physical well-being. The current study corroborated the significant positive effect on Phyto V7 on the physical well-being of HIV-1 infected individuals. This was demonstrated by the significant increase in the body weight and physical well-being a very large group of HIV-1 infected individuals not undergoing antiviral treatment that only received a daily dose of Phyto V7 for a period of 90 consecutive days.

### Applications

Administration of the Phyto V7 can be an important tool to improve the well-being of HIV-1 seropositive individuals and AIDS patients, not undergoing antiretroviral treatment. It may well be that administration of Phyto V7 together with antiviral treatment is highly advantageous. Further studies should test this hypothesis.

### Terminology

Phytochemicals are chemical compounds that occur naturally in plants. These chemicals, in addition of serving as micronutrients, have been found to enhance nonspecific immunity, down regulate inflammatory diseases, and inhibit disease progression. The Karnofsky score is a well-accepted scale used to assess the quality of life of patients. It was used by the examining physicians to address the well-being of the study participants during the study.

### Peer-review

This is an interesting work with promising results in which Phyto V7, a phytochemical mix, quickly and effectively improves the weight and makes most of the HIV patients treated to feel better. For these kinds of patients it is good to be able to help them and this treatment might prepare them for a future more aggressive antiviral therapy.

## REFERENCES

- Barron MA, Makhija M, Hagen LE, Pencharz P, Grunebaum E, Roifman CM. Increased resting energy expenditure is associated with failure to thrive in infants with severe combined immunodeficiency. *J Pediatr* 2011; **159**: 628-632.e1 [PMID: 21592502 DOI: 10.1016/j.jpeds.2011.03.041]
- Batterham MJ. Investigating heterogeneity in studies of resting energy expenditure in persons with HIV/AIDS: a meta-analysis. *Am J Clin Nutr* 2005; **81**: 702-713 [PMID: 15755842]
- Sutinen J, Yki-Järvinen H. Increased resting energy expenditure, fat oxidation, and food intake in patients with highly active antiretroviral therapy-associated lipodystrophy. *Am J Physiol Endocrinol Metab* 2007; **292**: E687-E692 [PMID: 17062843 DOI: 10.1152/ajpendo.00219.2006]
- Shevitz AH, Knox TA, Spiegelman D, Roubenoff R, Gorbach SL, Skolnik PR. Elevated resting energy expenditure among HIV-seropositive persons receiving highly active antiretroviral therapy. *AIDS* 1999; **13**: 1351-1357 [PMID: 10449288 DOI: 10.1097/00002030-199907300-00012]
- World Health Organization. Nutrient requirements for people living with HIV/AIDS: Report of a technical consultation. Geneva, Switzerland: 2003. Available from: URL: [http://www.who.int/nutrition/publications/Content\\_nutrient\\_requirements.pdf](http://www.who.int/nutrition/publications/Content_nutrient_requirements.pdf)
- Fawzi WW, Msamanga GI, Spiegelman D, Wei R, Kapiga S, Villamor E, Mwagagile D, Mugusi F, Hertzmark E, Essex M, Hunter DJ. A randomized trial of multivitamin supplements and HIV disease progression and mortality. *N Engl J Med* 2004; **351**: 23-32 [PMID: 15229304 DOI: 10.1056/NEJMoa040541]
- Forrester JE, Sztam KA. Micronutrients in HIV/AIDS: is there evidence to change the WHO 2003 recommendations? *Am J Clin Nutr* 2011; **94**: 1683S-1689S [PMID: 22089440 DOI: 10.3945/ajcn.111.011999]
- Kaiser JD, Campa AM, Ondercin JP, Leoung GS, Pless RF, Baum MK. Micronutrient supplementation increases CD4 count in HIV-infected individuals on highly active antiretroviral therapy: a prospective, double-blinded, placebo-controlled trial. *J Acquir Immune Defic Syndr* 2006; **42**: 523-528 [PMID: 16868496 DOI: 10.1097/01.qai.0000230529.25083.42]
- Siegfried N, Irlam JH, Visser ME, Rollins NN. Micronutrient supplementation in pregnant women with HIV infection. *Cochrane Database Syst Rev* 2012; **3**: CD009755 [PMID: 22419344]
- Sun LZ, Currier NL, Miller SC. The American coneflower: a prophylactic role involving nonspecific immunity. *J Altern Complement Med* 1999; **5**: 437-446 [PMID: 10537243 DOI: 10.1089/acm.1999.5.437]
- Aggarwal BB, Shishodia S. Suppression of the nuclear factor-kappaB activation pathway by spice-derived phytochemicals: reasoning for seasoning. *Ann N Y Acad Sci* 2004; **1030**: 434-441 [PMID: 15659827 DOI: 10.1196/annals.1329.054]
- Wang X, Liu Z, Qiao W, Cheng R, Liu B, She G. Phytochemicals and biological studies of plants from the genus *Balanophora*. *Chem Cent J* 2012; **6**: 79 [PMID: 22853440 DOI: 10.1186/1752-153X-6-79]
- de Mejía EG, Ramírez-Mares MV. Ardisia: health-promoting properties and toxicity of phytochemicals and extracts. *Toxicol Mech Methods* 2011; **21**: 667-674 [PMID: 22003924 DOI: 10.3109/15376516.2011.601355]
- Rao BN. Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention. *Asia Pac J Clin Nutr* 2003; **12**: 9-22 [PMID: 12737006]
- Kennedy DO, Wightman EL. Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. *Adv Nutr* 2011; **2**: 32-50 [PMID: 22211188 DOI: 10.3945/an.110.000117]
- Kumar GP, Khanum F. Neuroprotective potential of phytochemicals. *Pharmacogn Rev* 2012; **6**: 81-90 [PMID: 23055633 DOI: 10.4103/0973-7847.99898]
- Traka MH, Mithen RF. Plant science and human nutrition: challenges in assessing health-promoting properties of phytochemicals. *Plant Cell* 2011; **23**: 2483-2497 [PMID: 21803940 DOI: 10.1105/tpc.111.087916]
- Rajaram S. The effect of vegetarian diet, plant foods, and phytochemicals on hemostasis and thrombosis. *Am J Clin Nutr* 2003; **78**: 552S-558S [PMID: 12936949]
- Bagalkotkar G, Sagineedu SR, Saad MS, Stanslas J. Phytochemicals from *Phyllanthus niruri* Linn. and their pharmacological properties: a review. *J Pharm Pharmacol* 2006; **58**: 1559-1570 [PMID: 17331318 DOI: 10.1211/jpp.58.12.0001]
- Berginc K, Trdan T, Trontelj J, Kristl A. HIV protease inhibitors: garlic supplements and first-pass intestinal metabolism impact on the therapeutic efficacy. *Biopharm Drug Dispos* 2010; **31**: 495-505 [PMID: 21104925 DOI: 10.1002/bdd.730]
- Bunluepuech K, Sudsai T, Wattanapiromsakul C, Tewtrakul S. Inhibition on HIV-1 integrase activity and nitric oxide production of compounds from *Ficus glomerata*. *Nat Prod Commun* 2011; **6**: 1095-1098 [PMID: 21922907]
- Mushi NF, Mbwapo ZH, Innocent E, Tewtrakul S. Antibacterial, anti-HIV-1 protease and cytotoxic activities of aqueous ethanolic extracts from *Combretum adenogonium* Steud. Ex A. Rich (Combretaceae). *BMC Complement Altern Med* 2012; **12**: 163 [PMID: 23013240 DOI: 10.1186/1472-6882-12-163]
- Tewtrakul S, Subhadhirasakul S, Cheenpracha S, Karalai C. HIV-1 protease and HIV-1 integrase inhibitory substances from *Eclipta prostrata*. *Phytother Res* 2007; **21**: 1092-1095 [PMID: 17696192 DOI: 10.1002/ptr.2252]
- Xia CL, Mao QC, Li RM, Chen ZP, Jiang SB, Jiang ZH, Liu SW. Study of the mechanism of caffeoyl glucopyranosides in inhibiting HIV-1 entry using pseudotyped virus system. *Nanfang Yike Daxue*

- Xuebao 2010; **30**: 720-723 [PMID: 20423834]
- 25 **Perelman D**, Goldman WF, Wernik JR, Borkow G. Enhancement of Antibody Titers against Newcastle Disease Virus in Vaccinated Chicks by Administration of Phyto V7. *Journal of Vaccines and Vaccination* 2013; **4**: 7-8
  - 26 **Goldman WF**, Wernik R, Carmen-Elias A, Borkow G. Immunomodulating effect of Phyto V7 in preneoplastic cervical lesions. *Med J Obstet Gynecol* 2014; **2**: 1038-1042 [DOI: 10.4172/2157-7560.1000203]
  - 27 **Lavandera DMM**, Jiminian FAC, Wernik R, Goldman WF, Borkow G. Dramatic improvement in physical well-being of terminal AIDS patients following administration of phytochemicals. *World J AIDS* 2013; **3**: 287-293 [DOI: 10.4236/wja.2013.33036]
  - 28 **Prellwitz IM**, Alves BM, Ikeda ML, Kuhleis D, Picon PD, Jarczewski CA, Osório MR, Sánchez A, Seuánez HN, Larouzé B, Soares MA, Soares EA. HIV behind bars: human immunodeficiency virus cluster analysis and drug resistance in a reference correctional unit from southern Brazil. *PLoS One* 2013; **8**: e69033 [PMID: 23874857 DOI: 10.1371/journal.pone.0069033]
  - 29 **Kebede Y**, Pickering J, McDonald JC, Wotton K, Zewde D. HIV infection in an Ethiopian prison. *Am J Public Health* 1991; **81**: 625-627 [PMID: 2014865 DOI: 10.2105/AJPH.81.5.625]
  - 30 **Subbaraman R**, Chaguturu SK, Mayer KH, Flanigan TP, Kumarasamy N. Adverse effects of highly active antiretroviral therapy in developing countries. *Clin Infect Dis* 2007; **45**: 1093-1101 [PMID: 17879931 DOI: 10.1086/521150]
  - 31 **Obiako OR**, Muktar HM. Challenges of HIV treatment in resource-poor countries: a review. *Niger J Med* 2010; **19**: 361-368 [PMID: 21526621 DOI: 10.4314/njm.v19i4.69785]

**P- Reviewer:** Blanco LP, Louwen R **S- Editor:** Tian YL  
**L- Editor:** A **E- Editor:** Wu HL





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>





# World Journal of *Clinical Infectious Diseases*

*World J Clin Infect Dis* 2015 August 25; 5(3): 51-54



## Editorial Board

2011-2015

The World Journal of Clinical Infectious Diseases Editorial Board consists of 291 members, representing a team of worldwide experts in infectious diseases. They are from 56 countries, including Argentina (5), Australia (8), Austria (3), Bangladesh (1), Belgium (2), Bosnia and Herzegovina (1), Brazil (6), Brunei Darussalam (1), Bulgaria (1), Cameroon (1), Canada (7), China (18), Colombia (1), Costa Rica (1), Cuba (1), Denmark (2), Egypt (1), Finland (1), France (11), Germany (4), Greece (8), Hungary (6), India (14), Indonesia (1), Iran (5), Israel (10), Italy (19), Japan (4), Jordan (1), Kosovo (1), Kuwait (1), Lebanon (3), Lithuania (1), Malawi (1), Mexico (5), Morocco (2), Netherlands (4), Nigeria (1), Pakistan (2), Peru (1), Philippines (1), Portugal (5), Russia (1), Saudi Arabia (2), Singapore (3), South Africa (2), South Korea (6), Spain (24), Switzerland (2), Tanzania (1), Thailand (4), Tunisia (1), Turkey (4), United Kingdom (9), United States (59), and Venezuela (1).

### EDITORS-IN-CHIEF

Shyam Sundar, *Varanasi*  
Lihua Xiao, *Atlanta*

### GUEST EDITORIAL BOARD MEMBERS

Huan-Tsung Chang, *Taipei*  
Jia-Ming Chang, *Taipei*  
Kuo-Chin Huang, *Chiayi*  
Wei-Chen Lee, *Taoyuan*  
Hsiu-Jung Lo, *Miaoli*  
Jin-Town Wang, *Taipei*  
Deng-Chyang Wu, *Kaohsiung*  
Jiunn-Jong Wu, *Tainan*

### MEMBERS OF THE EDITORIAL BOARD



#### Argentina

Sergio Angel, *Chascomus*  
Luis Adrian Diaz, *Cordoba*  
Gustavo Daniel Lopardo, *Buenos Aires*  
Emilio L Malchioldi, *Buenos Aires*  
Victor D Rosenthal, *Buenos Aires*



#### Australia

Thea van de Mortel, *Lismore*  
David Llewellyn Gordon, *Bedford Park*  
Asad Khan, *Brisbane*  
Ruiting Lan, *Sydney*  
John McBride, *Cairns*  
David Leslie Paterson, *Brisbane*  
Nitin K Saksena, *Sydney*  
Andrew Slack, *Brisbane*



#### Austria

Ojan Assadian, *Vienna*  
Christian Joukhadar, *Vienna*  
Bernhard Resch, *Graz*



#### Bangladesh

Harunor Rashid, *Cox's Bazar*



#### Belgium

Mickael Aoun, *Bruxelles*  
Paul M Tulkens, *Brussels*



#### Bosnia and Herzegovina

Selma Uzunovic, *Zenica*



#### Brazil

Jane Costa, *Rio de Janeiro*  
Pedro Alves d'Azevedo, *Sao Paulo*  
Gerly Anne de Castro Brito, *Fortaleza*  
RL Dantas Machado, *Sao Paulo*  
Leandro R Rodrigues Perez, *Porto Alegre*  
M de Nazare Correia Soeiro, *Rio de Janeiro*



#### Brunei Darussalam

Vui Heng Chong, *Bandar Seri Begawan*



#### Bulgaria

Iva Christova, *Sofia*



#### Cameroon

Richard Njouom, *Yaounde*



#### Canada

Aranka Anema, *Vancouver*  
Peter C Coyte, *Toronto*  
Pavel Gershkovich, *Vancouver*  
Marcelo Gottschalk, *Quebec*  
Marina Ulanova, *Thunder Bay*  
Jude Uzonna, *Winnipeg*  
Jun Wang, *Halifax*



#### China

Tian-Hua Huang, *Shantou*  
Xi-Tai Huang, *Tianjin*  
Dong-Ming Li, *Beijing*  
Xin-Yong Liu, *Jinan*  
Wu-Bin Pan, *Taichang*  
Kai Wang, *Jinan*  
Patrick Chiu Yat Woo, *Hong Kong*  
Yong-Feng Yang, *Nanjing*  
Chi-Yu Zhang, *Zhenjiang*  
Li-Juan Zhang, *Beijing*



#### Colombia

Jorge Enrique Gomez-Marin, *Armenia*

**Costa Rica**

Adriano Arguedas, *San José*

**Cuba**

Maria G Guzman, *Havana*

**Denmark**

Janne Kudsk Klitgaard, *Odense*  
Henrik Torkil Westh, *Hvidovre*

**Egypt**

Olfat Shaker, *Cairo*

**Finland**

Jari Timo Juhani Nuutila, *Turku*

**France**

Hassane Adakal, *Burkina Faso*  
Pascal Bigey, *Paris*  
Philippe Brouqui, *Marseille*  
Christophe Chevillard, *Marseille*  
Raphaelé Girard, *Pierre Bénite*  
Vincent Pascal Jarlier, *Paris*  
Sandrine Marquet, *Marseille*  
Vayssier-Taussat Muriel, *Maisons-Alfort*  
Thierry Naas, *Le Kremlin-Bicetre*  
Saad Nseir, *Lille*  
Philippe Seguin, *Rennes*

**Germany**

Stefan Borgmann, *Ingolstadt*  
Georg Harter, *Ulm*  
Matthias Imohl, *Aachen*  
Kurt G Naber, *Straubing*

**Greece**

Apostolos Beloukas, *Athens*  
Alex P Betrosian, *Athens*  
George L Daikos, *Athens*  
Helena Maltezou, *Athens*  
Argyris S Michalopoulos, *Athens*  
Maria Moschovi, *Athens*  
George Petrikkos, *Athens*  
Athanasios Tragiannidis, *Thessaloniki*

**Hungary**

Laszlo Galgoczy, *Szeged*  
Viktor Muller, *Budapest*  
Ferenc Orosz, *Budapest*  
Ferenc Rozgonyi, *Budapest*  
Jozsef Soki, *Szeged*

Dezso Peter Virok, *Szeged*

**India**

Ritesh Agarwal, *Chandigarh*  
Syed Imteyaz Alam, *Gwalior*  
Atmaram Hari Bandivdekar, *Mumbai*  
Runu Chakravarty, *Kolkata*  
Dipshikha Chakravorty, *Bangalore*  
Sanjay Chhibber, *Chandigarh*  
BN Harish, *Pondicherry*  
Triveni Krishnan, *Kolkata*  
Rashmi Kumar, *Lucknow*  
Mohammad Owais, *Aligarh*  
Banwarilal Sarkar, *Kolkata*  
Mamta Chawla Sarkar, *Kolkata*  
Akashdeep Singh, *Ludhiana*

**Indonesia**

Jeanne Adiwinata Pawitan, *Jakarta*

**Iran**

Parissa Farnia, *Tehran*  
Seyed Mohammad Jazayeri, *Tehran*  
Morteza Pourahmad, *Jahrom*  
Mohammad Reza Pourshafie, *Tehran*  
Mohammad Hossein Salari, *Tehran*

**Israel**

Jacob Amir, *Petach Tikvah*  
Shai Ashkenazi, *Petach Tikva*  
Gadi Borkow, *Gibton*  
Raul Colodner, *Afula*  
Jacob Moran Gilad, *Jerusalem*  
Noah Isakov, *Beer Sheva*  
Michal Mandelboim, *Hashomer*  
Shifra Shvarts, *Omer*  
Oshri Wasserzug, *Tel-Aviv*  
Pablo Victor Yagupsky, *Beer-Sheva*

**Italy**

Giuseppe Barbaro, *Rome*  
Paolo Bonilauri, *Reggio Emilia*  
Guido Calleri, *Torino*  
Mario Cruciani, *Verona*  
Marco Falcone, *Rome*  
Antonio Fasanella, *Foggia*  
Daniele Focosi, *Pisa*  
Delia Goletti, *Rome*  
Guido Grandi, *Siena*  
Fabio Grizzi, *Rozzano*  
Giuseppe Ippolito, *Rome*  
Roberto Manfredi, *Bologna*  
Claudio M Mastroianni, *Rome*  
Ivano Mezzaroma, *Rome*  
Giuseppe Micali, *Catania*  
Antonella d'Arminio Monforte, *Milano*  
Annamaria Passantino, *Messina*  
Mariagrazia Perilli, *L'Aquila*  
Patrizia Pontisso, *Padova*

**Japan**

Masashi Emoto, *Maebashi*  
Toshi Nagata, *Hamamatsu*  
Ryohei Yamasaki, *Tottori*  
Shin-Ichi Yokota, *Sapporo*

**Jordan**

Asem A Shehabi, *Amman*

**Kosovo**

Lul Raka, *Prishtina*

**Kuwait**

Willias Masocha, *Safat*

**Lebanon**

Ziad Daoud, *Beirut*  
Ghassan M Matar, *Beirut*  
Sami Ramia, *Beirut*

**Lithuania**

Gazim Bizanov, *Vilnius*

**Malawi**

Adamson Sinjani Muula, *Blantyre*

**Mexico**

Agnes Fleury, *Mexico*  
Guadalupe Garcia-Elorriaga, *Mexico*  
Alejandro E Macias, *Mexico*  
Mussaret Zaidi, *Merida*  
Roberto Zenteno-Cuevas, *Veracruz*

**Morocco**

Redouane Abouqal, *Rabat*  
Ezzikouri Sayeh, *Casablanca*

**Netherlands**

Aldert Bart, *Amsterdam*  
John Hays, *Rotterdam*  
Nisar Ahmed Khan, *Rotterdam*  
Rogier Louwen, *Rotterdam*

**Nigeria**

Samuel Sunday Taiwo, *Osogbo*



### **Pakistan**

Muhammad Idrees, *Lahore*  
Muhammad Mukhtar, *Bahawalpur*



### **Peru**

Salim Mohanna, *Lima*



### **Philippines**

Vicente Y Belizario, *Ermita Manila*



### **Portugal**

Ricardo Araujo, *Porto*  
Manuela Canica, *Lisbon*  
Francisco Esteves, *Lisbon*  
Fernando Rodrigues, *Braga*  
Nuno Taveira, *Lisbon*



### **Russia**

Alexander M Shestopalov, *Koltsovo*



### **Saudi Arabia**

Jaffar A Al-Tawfiq, *Dhahran*  
Atef M Shibl, *Riyadh*



### **Singapore**

Yee Sin Leo, *Singapore*  
Laurent Claude Stephane Renia, *Singapore*  
Richard J Sugrue, *Singapore*



### **South Africa**

Carolina H Pohl-Albertyn, *Bloemfontein*  
Natasha Potgieter, *Louis Trichardt*



### **South Korea**

Chong Cho, *Seoul*  
Sang Ho Choi, *Seoul*  
Ju-Young Chung, *Seoul*  
Jung Mogg Kim, *Seoul*  
Kyongmin Kim, *Suwon*  
Sang Hee Lee, *Yongin*



### **Spain**

Alberto Arnedo-Pena, *Castellon*  
Alfredo Berzal-Herranz, *Granada*  
Vicente Brito, *Alicante*

Enrique Calderon, *Seville*

Rafael Canton, *Madrid*

Jose M Cuevas, *Valencia*

Laila Darwich, *Cerdanyola del Valles*

Adela Gonzalez de la Campa, *Madrid*

Pere Domingo, *Barcelona*

Tahia D Fernandez, *Malaga*

Lucia Gallego, *Leioa*

Luis Ignacio Gonzalez-Granado, *Madrid*

Bruno Gonzalez-Zorn, *Madrid*

Eduardo Lopez-Collazo, *Madrid*

Miguel Marcos, *Salamanca*

Antonio Torres Marti, *Barcelona*

Andres Moya, *Valencia*

Rafael Najera, *Madrid*

Maria Mercedes Nogueras-Mas, *Sabadell*

Jose A Oteo, *Logrono*

Pilar Perez-Romero, *Sevilla*

Ruth Gil Raka, *Madrid*

Eduardo Reyes, *Madrid*

Francisco Soriano, *Madrid*



### **Switzerland**

Stephen Hawser, *Epalinges*  
Andrew Hemphill, *Bern*



### **Tanzania**

John Peter Andrea Lusingu, *Tanga*



### **Thailand**

Kosum Chansiri, *Bangkok*  
Subsai Kongsangdao, *Bangkok*  
Niwat Maneeakarn, *Chiang Mai*  
Viroj Wiwanitkit, *Bangkok*



### **Tunisia**

Aouni Mahjoub, *Monastir*



### **Turkey**

Oguz Karabay, *Sakarya*  
Uner Kayabas, *Malatya*  
Gokhan Metan, *Kayseri*  
Oral Oncul, *Istanbul*



### **United Kingdom**

Zainab Al-Doori, *Glasgow*  
David Carmena, *London*  
Ronald Anthony Dixon, *Lincoln*  
Vanya Alasdair Ivan Andre Gant, *London*  
Robin Goodwin, *London*  
Andrew Cunliffe Hayward, *London*  
Laura Anne Hughes, *Neston*  
Michele Esther Murdoch, *Herts*  
Craig William Roberts, *Glasgow*



### **United States**

Majdi N Al-Hasan, *Lexington*  
Ibne KM Ali, *Charlottesville*  
Hossam M Ashour, *Detroit*  
Joseph Urban Becker, *Palo Alto*  
M Eric Benbow, *Dayton*  
Eliahu Bishburg, *Newark*  
Luz P Blanco, *Ann Arbor*  
Robert Bucki, *Philadelphia*  
Steven Dale Burdette, *Dayton*  
Archana Chatterjee, *Omaha*  
Pai-Lien Chen, *Durham*  
Pawel S Ciborowski, *Omaha*  
Michael Cynamon, *Syracuse*  
Siddhartha Das, *El Paso*  
Ralph J DiClemente, *Atlanta*  
Noton Kumar Dutta, *Baltimore*  
Garth D Ehrlich, *Pittsburgh*  
Michael S Firstenberg, *Columbus*  
Walter A Hall, *Syracuse*  
Yongqun He, *Ann Arbor*  
Brenda Lorraine Helms, *Plano*  
Joseph U Igietseme, *Atlanta*  
Mohammad Khalid Ijaz, *Montvale*  
Suresh G Joshi, *Philadelphia*  
Thomas F Kresina, *Rockville*  
Alain B Labrique, *Baltimore*  
Shenghan Lai, *Baltimore*  
Benfang Lei, *Bozeman*  
Jeff G Leid, *Flagstaff*  
Vladimir Leonitiev, *St. Louis*  
Andrea Lisco, *Bethesda*  
James M McMahon, *Rochester*  
Geraldine M McQuillan, *Hyattsville*  
Lawrence F Muscarella, *Ivyland*  
Daniel Musher, *Houston*  
Stella Nowicki, *Nashville*  
M Jacques Nsuami, *New Orleans*  
Phillipe N Nyambi, *New York*  
Raymund Rabe Razonable, *Rochester*  
Anand Reddi, *Denver*  
Michael Switow Saag, *Birmingham*  
Danny J Schust, *Columbia*  
William R Schwan, *La Crosse*  
Richard A Slayden, *Fort Collins*  
Theodore J Standiford, *Ann Arbor*  
William M Switzer, *Atlanta*  
Ashutosh Tamhane, *Birmingham*  
Giorgio E Tarchini, *Weston*  
Carmen Taype, *New York*  
Barbara Van Der Pol, *Bloomington*  
Jose Antonio Vazquez, *Detroit*  
Fernando Villalta, *Nashville*  
Haider J Warraich, *Boston*  
Xianfu Wu, *Atlanta*  
Genyan Yang, *Atlanta*  
Frank X Yang, *Indianapolis*  
Hong Zhang, *Rockville*  
Lyna Zhang, *Atlanta*



### **Venezuela**

Alfonso J Rodriguez-Morales, *Caracas*





**EDITORIAL**

- 51 New tools, new tick-borne diseases?

*Portillo A, Oteo JA*

ABOUT COVER

Editorial Board Member of *World Journal of Clinical Infectious Diseases*, Shai Ashkenazi, Professor, Pediatrics A, Schneider Children Hospital, Petach Tikva 49202, Israel

AIM AND SCOPE

*World Journal of Clinical Infectious Diseases* (*World J Clin Infect Dis*, *WJCID*, online ISSN 2220-3176, DOI: 10.5495) is a peer-reviewed open access (OA) academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJCID* will focus on a broad spectrum of topics on infectious diseases that will cover epidemiology, immune-pathogenesis, genetic factors, host susceptibility to infection, vector control, novel approaches of treatment, molecular diagnostic and vaccines. It will provide a common stage to share the visions, new approaches, most advanced techniques, and to discuss research problems that will help everyone working in the field of various infections to exchange their views and to improve public health. *WJCID* will also focus on broad range of infections like opportunistic infections, zoonotic infections, tropical and neglected tropical diseases, emerging infections, *etc.* and following topics related to these issues: (1) Causative agents discussing various pathogens; (2) Vectors and Mode of transmission; (3) Host-pathogen interaction and immune-pathogenesis of the disease; (4) Epidemiology of the infection and vector control strategies; (5) Genetic factors covering both host and pathogen; (6) Molecular diagnostic techniques vaccines; and (7) Recent advances in cell tissue culture, lab techniques, *etc.* Various other related fields like medical microbiology, pharmacology of herbs, bioinformatics, *etc.* will be included.

We encourage authors to submit their manuscripts to *WJCID*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

INDEXING/ABSTRACTING

*World Journal of Clinical Infectious Diseases* is now indexed in Digital Object Identifier.

FLYLEAF

I-III Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: Xiang Li  
Responsible Electronic Editor: Su-Qing Liu  
Proofing Editor-in-Chief: Lian-Sheng Ma

Responsible Science Editor: Xue-Mei Gong  
Proofing Editorial Office Director: Xiu-Xia Song

NAME OF JOURNAL  
*World Journal of Clinical Infectious Diseases*

ISSN  
ISSN 2220-3176 (online)

LAUNCH DATE  
December 30, 2011

FREQUENCY  
Quarterly

EDITORS-IN-CHIEF  
Shyam Sundar, MD, FRCP (London), FAMS, FNA Sc, FASc, FNA, Professor, Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India

Lihua Xiao, DVM, PhD, Senior Scientist, Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Bldg 23, Rm 9-168, MS D66, 1600 Clifton

Rd, Atlanta, GA 30333, United States

EDITORIAL OFFICE  
Jin-Lei Wang, Director  
Xiu-Xia Song, Vice Director  
*World Journal of Clinical Infectious Diseases*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-85381891  
Fax: +86-10-85381893  
E-mail: editorialoffice@wjgnet.com  
Help Desk: <http://www.wjgnet.com/esp/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esp/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLICATION DATE  
August 25, 2015

COPYRIGHT  
© 2015 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS  
Full instructions are available online at [http://www.wjgnet.com/2220-3176/g\\_info\\_20100722180909.htm](http://www.wjgnet.com/2220-3176/g_info_20100722180909.htm).

ONLINE SUBMISSION  
<http://www.wjgnet.com/esp/>

## New tools, new tick-borne diseases?

Aránzazu Portillo, José A Oteo

Aránzazu Portillo, José A Oteo, Infectious Diseases Department, Hospital San Pedro-Center for Biomedical Research from La Rioja (CIBIR), Center of Rickettsioses and Arthropod-Borne Diseases, 26006 Logroño, La Rioja, Spain

**Author contributions:** Portillo A and Oteo JA contributed to this paper.

**Conflict-of-interest statement:** Authors declare no conflict of interests.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** José A Oteo, MD, PhD, Head of the Infectious Diseases Department, Hospital San Pedro-Center for Biomedical Research from La Rioja (CIBIR), Center of Rickettsioses and Arthropod-Borne Diseases, C/Piqueras, 98, 26006 Logroño, La Rioja, Spain. [jaoteo@riojasalud.es](mailto:jaoteo@riojasalud.es)  
Telephone: +34-941-278871  
Fax: +34-941-298667

Received: February 8, 2015  
Peer-review started: February 9, 2015  
First decision: March 6, 2015  
Revised: March 17, 2015  
Accepted: July 29, 2015  
Article in press: August 3, 2015  
Published online: August 25, 2015

### Abstract

Tick-borne diseases (TBDs) are a major public health concern that has increased in the past three decades. Nevertheless, emerging or reemerging TBDs may be still misdiagnosed. Molecular biology techniques for the screening of ticks, use of "Omics" approaches and

the incorporation of analytical methods such as mass spectrometry or nuclear magnetic resonance, to the study of ticks and their associated pathogens or potential pathogens are promising tools for a more accurate differential diagnosis of TBDs. However, this huge amount of data needs to be carefully interpreted before being incorporated to the routine of clinical practice. In the meantime, a clinical approach and high level of suspicion keep being essential for the diagnosis and proper handling of TBDs.

**Key words:** Ticks; Tick-borne diseases; Tick-borne pathogens; Molecular biology tools; DNA-arrays; "Omics" approaches; Analytical tools; Mass spectrometry; Nuclear magnetic resonance

© The Author(s) 2015. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Tick-borne diseases (TBDs) are a major public health concern that has increased in the past three decades. Molecular biology techniques for the screening of ticks, use of "Omics" approaches and the incorporation of analytical methods to the study of ticks and their associated microorganisms are promising tools for a more accurate differential diagnosis of TBDs. Nevertheless, a clinical approach and high level of suspicion remain essential for the diagnosis and proper handling of TBDs before the incorporation of these innovative technologies to the routine in clinical practice.

Portillo A, Oteo JA. New tools, new tick-borne diseases? *World J Clin Infect Dis* 2015; 5(3): 51-54 Available from: URL: <http://www.wjgnet.com/2220-3176/full/v5/i3/51.htm> DOI: <http://dx.doi.org/10.5495/wjcid.v5.i3.51>

### TEXT

Tick-borne diseases (TBDs) are a major public health concern that has increased in the past three decades.

Thus, for instance, in 1988 only Mediterranean spotted fever caused by *Rickettsia conorii*, and babesiosis caused by *Babesia* spp., were recognized as TBDs in Spain (southern Europe). Nowadays the list of known TBDs has grown thanks to clinical observation together with the use and development of new microbiological techniques. To date, patients diagnosed of Lyme borreliosis, Spotted Fever due to *Rickettsia* spp. (*R. conorii*, *Rickettsia monacensis*, *Rickettsia sibirica mongolitimonae* and *Rickettsia massiliae*), *Dermacentor*-borne necrosis, erythema and lymphadenopathy/Tick-borne lymphadenopathy (DEBONEL/TIBOLA) caused by *Rickettsia slovaca*, *Rickettsia rioja* and *Rickettsia raoultii*, besides human anaplasmosis, human babesiosis and tick paralysis have been reported in our country<sup>[1-3]</sup>. The discovery of new TBDs and the identification of TBDs in new geographical regions have also occurred in other parts of the world. Rickettsioses caused by *Rickettsia parkeri* or by *R. massiliae* or the Bourbon virus disease caused by Bourbon virus, a new virus thought to be transmitted by ticks, serve as examples<sup>[4-6]</sup>. Not only we have involved microorganisms in different syndromes and diseases but also, using new techniques, we have discovered "new microorganisms" that are good candidates to be considered pathogens for humans and animals<sup>[7-13]</sup>. Nevertheless, we assume that other TBDs in our environment may have been misdiagnosed due to the lack of clinical suspicion or diagnostic tools, or because to date they have been absent.

In the last years, the identification of tick-associated pathogens has been frequently based on polymerase chain reaction screening, sequencing and subsequent nucleotide sequence analyses. Supported on the aphorism "If you do not look for it, you do not find it", our team has been able to detect tick-borne bacteria and viruses, such as *Candidatus* Neoehrlichia mikurensis and crimean-congo hemorrhagic fever virus, for the first time in ticks from Spain<sup>[14,15]</sup>. As it has occurred with other human pathogens previously described in arthropods worldwide, these evidences of the presence of microorganisms are useful tools to be aware of the risk of exposure to certain infections in an area. Clinicians must include unexpected TBDs in the differential diagnosis of patients with epidemiological background and unspecific clinical manifestations, especially if they are elderly people with underlying diseases. In these cases, failure of diagnosis may lead to a fatal outcome<sup>[16]</sup>.

Microbial culture is the gold-standard diagnostic method in microbiology. However, bacteria transmitted by ticks are fastidious and difficult to grow in axenic media, and many of them are obligate intracellular bacteria. Cell-culture procedures are time-consuming and isolation of microorganisms is not always successful<sup>[17]</sup>.

Serologic methods, especially immunofluorescence assays, support the diagnosis of TBDs but sera samples of patients in acute and convalescent phases of the disease are needed and cross-reactions are a common problem. Hence and up to date, serologic assays are

accepted as valid to confirm a rickettsial syndrome but not an infection caused by a certain *Rickettsia* spp.<sup>[17]</sup>.

More recent, and still non-commercial for diagnosis, are sandwich immunoassays for the quantification of IgE specific antibodies that have enabled to associate tick bites and food allergy. Patients frequently bitten by ticks have shown an increased level of IgE antibodies to the oligosaccharide galactose- $\alpha$ -1,3-galactose (alpha-gal) that seems related to delayed red meat anaphylaxis<sup>[18]</sup>. In our area (northern Spain), where ticks are endemic, a study performed with risk population by our group revealed nearly 30% sensitization to alpha-gal<sup>[19]</sup>.

In the post-genomic era, DNA microarrays-based technologies have enabled simultaneous identification of several tick-borne pathogens in ticks<sup>[20]</sup>. Nevertheless, the application of DNA arrays to the diagnoses of TBDs using human samples is still in progress<sup>[21]</sup>. Based on complete genome sequences, these methods have been also used for the global analysis of gene expression patterns (transcriptome) in *R. conorii*<sup>[22,23]</sup> and *Borrelia burgdorferi*<sup>[24]</sup>. DNA microarrays can be useful for the identification of markers to provide a guide on the etiology and virulence or to monitor the course or treatment of a TBD.

Recently, the analysis of the tick microbiome (bacterial communities associated with ticks) is possible using next generation sequencing (NGS) methods based on 16S rRNA sequencing<sup>[25,26]</sup>. It has been evidenced that interactions among microbes within the tick vector can at least modulate pathogen transmission, vector competence and tick reproductive fitness<sup>[27-30]</sup>. Also in this context, the study of relationships between tick-transmitted pathogens and their environment (a term coined as pathobiome) is increasing the knowledge on TBDs from a new multidisciplinary point of view.

Other novel "Omics" technologies such as proteomics, metabolomics, immunomics, and vaccinomics provide a huge amount of data with relative low cost and effort<sup>[31,32]</sup>. These innovative approaches will contribute to predict emerging TBDs in a near future that has already started.

Moreover, in the last few years matrix-assisted laser desorption/ionization time-of-flight mass spectrometry has become a powerful tool for the rapid one-shot identification of ticks and cultured tick-borne bacteria such as *Borrelia* spp. and *Rickettsia* spp.<sup>[33-35]</sup>.

Other techniques, such as nuclear magnetic resonance spectroscopic methods applied to the study of the metabolism of tick-borne bacteria such as *Rickettsia* species also provide a challenging approach in this research field<sup>[36]</sup>.

Despite the development of molecular and analytical tools, there are still patients bitten by ticks with unspecific clinical manifestations of unknown etiology. Nowadays, NGS methods combined with bioinformatics are providing an inventory of predicted and/or unexpected pathogenic bacteria harbored by ticks. These findings will enable to include novel potential pathogens, in addition to known species, in the differential diagnosis of TBDs. At the same



time that these tools are incorporated into the routine, a clinical approach and high level of suspicion remain necessary for diagnosis and proper handling of TBDs.

## REFERENCES

- Oteo JA, Portillo A. Tick-borne rickettsioses in Europe. *Ticks Tick Borne Dis* 2012; **3**: 271-278 [PMID: 23177355 DOI: 10.1016/j.ttbdis.2012.10.035]
- Portillo A, Santibáñez S, Oteo JA. Lyme disease. *Enferm Infecc Microbiol Clin* 2014; **32** Suppl 1: 37-42 [PMID: 24630582 DOI: 10.1016/S0213-005X(14)70148-X]
- García JC, Núñez MJ, Portillo A, Oteo JA. Human anaplasmosis: two case-reports. *Enferm Infecc Microbiol Clin* 2015; **33**: 68-69 [PMID: 25073813 DOI: 10.1016/j.eimc.2014.05.009]
- Paddock CD, Sumner JW, Comer JA, Zaki SR, Goldsmith CS, Goddard J, McLellan SL, Tamminga CL, Ohl CA. Rickettsia parkeri: a newly recognized cause of spotted fever rickettsiosis in the United States. *Clin Infect Dis* 2004; **38**: 805-811 [PMID: 14999622]
- García-García JC, Portillo A, Núñez MJ, Santibáñez S, Castro B, Oteo JA. A patient from Argentina infected with Rickettsia massiliae. *Am J Trop Med Hyg* 2010; **82**: 691-692 [PMID: 20348520 DOI: 10.4269/ajtmh.2010.09-0662]
- Kosoy OI, Lambert AJ, Hawkinson DJ, Pastula DM, Goldsmith CS, Hunt DC, Staples JE. Novel Thogotovirus species associated with febrile illness and death, United States, 2014. *Emerg Infect Dis* 2015; **21**: 760-764 [DOI: 10.3201/eid2105.150150]
- Portillo A, Santibáñez P, Santibáñez S, Pérez-Martínez L, Oteo JA. Detection of Rickettsia spp. in Haemaphysalis ticks collected in La Rioja, Spain. *Vector Borne Zoonotic Dis* 2008; **8**: 653-658 [PMID: 18454590 DOI: 10.1089/vbz.2007.0272]
- Portillo A, Ibarra V, Santibáñez S, Pérez-Martínez L, Blanco JR, Oteo JA. Genetic characterisation of ompA, ompB and gltA genes from Candidatus Rickettsia rioja. *Clin Microbiol Infect* 2009; **15** Suppl 2: 307-308 [PMID: 19438649 DOI: 10.1111/j.1469-0691.2008.02250.x]
- Miranda J, Portillo A, Oteo JA, Mattar S. Rickettsia sp. strain colombianensi (Rickettsiales: Rickettsiaceae): a new proposed Rickettsia detected in Amblyomma dissimile (Acari: Ixodidae) from iguanas and free-living larvae ticks from vegetation. *J Med Entomol* 2012; **49**: 960-965 [PMID: 22897060]
- Palomar AM, Portillo A, Santibáñez P, Santibáñez S, García-Álvarez L, Oteo JA. Genetic characterization of Candidatus Rickettsia vini, a new rickettsia amplified in ticks from La Rioja, Spain. *Ticks Tick Borne Dis* 2012; **3**: 319-321 [PMID: 23140892 DOI: 10.1016/j.ttbdis.2012.10.025]
- Lopez-Velez R, Palomar AM, Oteo JA, Norman FF, Pérez-Molina JA, Portillo A. Novel Candidatus rickettsia species detected in nostril tick from human, Gabon, 2014. *Emerg Infect Dis* 2015; **21**: 325-327 [PMID: 25625886 DOI: 10.3201/eid2102.141048]
- Palomar AM, Portillo A, Crespo A, Santibáñez S, Mazuelas D, Oteo JA. Prevalence of 'Candidatus Rickettsia vini' in Ixodes arboricola ticks in the North of Spain, 2011-2013. *Parasit Vectors* 2015; **8**: 110 [PMID: 25889739 DOI: 10.1186/s13071-015-0724-6]
- Pesquera C, Portillo A, Palomar AM, Oteo JA. Investigation of tick-borne bacteria (Rickettsia spp., Anaplasma spp., Ehrlichia spp. and Borrelia spp.) in ticks collected from Andean tapirs, cattle and vegetation from a protected area in Ecuador. *Parasit Vectors* 2015; **8**: 46 [PMID: 25616567 DOI: 10.1186/s13071-015-0662-3]
- Palomar AM, García-Álvarez L, Santibáñez S, Portillo A, Oteo JA. Detection of tick-borne 'Candidatus Neoehrlichia mikurensis' and Anaplasma phagocytophilum in Spain in 2013. *Parasit Vectors* 2014; **7**: 57 [PMID: 24484637 DOI: 10.1186/1756-3305-7-57]
- Estrada-Peña A, Palomar AM, Santibáñez P, Sánchez N, Habela MA, Portillo A, Romero L, Oteo JA. Crimean-Congo hemorrhagic fever virus in ticks, Southwestern Europe, 2010. *Emerg Infect Dis* 2012; **18**: 179-180 [PMID: 22261502 DOI: 10.3201/eid1801.111040]
- Grankvist A, Andersson PO, Mattsson M, Sender M, Vaht K, Höper L, Sakiniene E, Trysberg E, Stenson M, Fehr J, Pekova S, Bogdan C, Bloemberg G, Wennerås C. Infections with the tick-borne bacterium "Candidatus Neoehrlichia mikurensis" mimic noninfectious conditions in patients with B cell malignancies or autoimmune diseases. *Clin Infect Dis* 2014; **58**: 1716-1722 [PMID: 24647019 DOI: 10.1093/cid/ciu189]
- Brouqui P, Bacellar F, Baranton G, Birtles RJ, Bjoersdorff A, Blanco JR, Caruso G, Cinco M, Fournier PE, Francavilla E, Jensenius M, Kazar J, Laferl H, Lakos A, Lotric Furlan S, Maurin M, Oteo JA, Parola P, Perez-Eid C, Peter O, Postic D, Raoult D, Tellez A, Tselentis Y, Wilske B. Guidelines for the diagnosis of tick-borne bacterial diseases in Europe. *Clin Microbiol Infect* 2004; **10**: 1108-1132 [PMID: 15606643]
- Commings SP, James HR, Kelly LA, Pochan SL, Workman LJ, Perzanowski MS, Kocan KM, Fahy JV, Nanga LW, Ronmark E, Cooper PJ, Platts-Mills TA. The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose- $\alpha$ -1,3-galactose. *J Allergy Clin Immunol* 2011; **127**: 1286-93.e6 [PMID: 21453959 DOI: 10.1016/j.jaci.2011.02.019]
- Venturini M, Lobera T, Portillo A, Oteo JA, Blasco A, González I. Sensibilización a alfa-gal en pacientes con múltiples picaduras por garrapata en La Rioja. *J Investig Allergol Clin Immunol* 2013; **23** Suppl 2: 170
- Melníčáková J, Derdáková M, Barák I. A system to simultaneously detect tick-borne pathogens based on the variability of the 16S ribosomal genes. *Parasit Vectors* 2013; **6**: 269 [PMID: 24330462 DOI: 10.1186/1756-3305-6-269]
- Jääskeläinen AJ, Viitala SM, Kurkela S, Hepojoki S, Sillanpää H, Kallio-Kokko H, Bergström T, Suni J, Närvänen A, Vapalahti O, Vaheri A. Performance of a multiplexed serological microarray for the detection of antibodies against central nervous system pathogens. *J Microbiol Methods* 2014; **100**: 27-31 [PMID: 24594410 DOI: 10.1016/j.mimet.2014.02.011]
- La MV, François P, Rovey C, Robineau S, Barbry P, Schrenzel J, Raoult D, Renesto P. Development of a method for recovering rickettsial RNA from infected cells to analyze gene expression profiling of obligate intracellular bacteria. *J Microbiol Methods* 2007; **71**: 292-297 [PMID: 17964675]
- Renesto P, Rovey C, Schrenzel J, Leroy Q, Huyghe A, Li W, Lepidi H, François P, Raoult D. Rickettsia conorii transcriptional response within inoculation eschar. *PLoS One* 2008; **3**: e3681 [PMID: 18997861 DOI: 10.1371/journal.pone.0003681]
- Ellis TC, Jain S, Linowski AK, Rike K, Bestor A, Rosa PA, Halpern M, Kurhanewicz S, Jewett MW. In vivo expression technology identifies a novel virulence factor critical for Borrelia burgdorferi persistence in mice. *PLoS Pathog* 2013; **9**: e1003567 [PMID: 24009501 DOI: 10.1371/journal.ppat.1003567]
- Carpi G, Cagnacci F, Wittekindt NE, Zhao F, Qi J, Tomsho LP, Drautz DI, Rizzoli A, Schuster SC. Metagenomic profile of the bacterial communities associated with Ixodes ricinus ticks. *PLoS One* 2011; **6**: e25604 [PMID: 22022422 DOI: 10.1371/journal.pone.0025604]
- Vayssier-Taussat M, Moutailler S, Michelet L, Devillers E, Bonnet S, Cheval J, Hébert C, Eloit M. Next generation sequencing uncovers unexpected bacterial pathogens in ticks in western Europe. *PLoS One* 2013; **8**: e81439 [PMID: 24312301 DOI: 10.1371/journal.pone.0081439]
- Macaluso KR, Sonenshine DE, Ceraul SM, Azad AF. Rickettsial infection in Dermacentor variabilis (Acari: Ixodidae) inhibits transovarial transmission of a second Rickettsia. *J Med Entomol* 2002; **39**: 809-813 [PMID: 12495176]
- Burgdorfer W, Brinton LP, Hughes LE. Isolation and characterization of symbiotes from the Rocky Mountain wood tick, Dermacentor andersoni. *J Invertebr Pathol* 1973; **22**: 424-434 [PMID: 4202564]
- Clay K, Klyachko O, Grindle N, Civitello D, Oleske D, Fuqua C. Microbial communities and interactions in the lone star tick, Amblyomma americanum. *Mol Ecol* 2008; **17**: 4371-4381 [PMID: 19378409]
- Zhong J, Jasinskas A, Barbour AG. Antibiotic treatment of the tick vector Amblyomma americanum reduced reproductive fitness.

- PLoS One* 2007; **2**: e405 [PMID: 17476327]
- 31 **Marcelino I**, de Almeida AM, Ventosa M, Pruneau L, Meyer DF, Martinez D, Lefrançois T, Vachiéry N, Coelho AV. Tick-borne diseases in cattle: applications of proteomics to develop new generation vaccines. *J Proteomics* 2012; **75**: 4232-4250 [PMID: 22480908 DOI: 10.1016/j.jprot.2012.03.026]
  - 32 **de la Fuente J**, Merino O. Vaccinomics, the new road to tick vaccines. *Vaccine* 2013; **31**: 5923-5929 [PMID: 24396872]
  - 33 **Yssouf A**, Flaudrops C, Drali R, Kernif T, Socolovschi C, Berenger JM, Raoult D, Parola P. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid identification of tick vectors. *J Clin Microbiol* 2013; **51**: 522-528 [PMID: 23224087 DOI: 10.1128/JCM.02665-12]
  - 34 **Calderaro A**, Gorrini C, Piccolo G, Montecchini S, Buttrini M, Rossi S, Piergianni M, Arcangeletti MC, De Conto F, Chezzi C, Medici MC. Identification of *Borrelia* species after creation of an in-house MALDI-TOF MS database. *PLoS One* 2014; **9**: e88895 [PMID: 24533160 DOI: 10.1371/journal.pone.0088895]
  - 35 **Yssouf A**, Almeras L, Terras J, Socolovschi C, Raoult D, Parola P. Detection of *Rickettsia* spp in ticks by MALDI-TOF MS. *PLoS Negl Trop Dis* 2015; **9**: e0003473 [PMID: 25659152 DOI: 10.1371/journal.pntd.0003473]
  - 36 **García-Álvarez L**, Busto JH, Peregrina JM, Fernández Recio MA, Avenoza A, Oteo JA. Nuclear magnetic resonance applied to antimicrobial drug susceptibility. *Future Microbiol* 2013; **8**: 537-547 [PMID: 23534364 DOI: 10.2217/fmb.13.8]

**P- Reviewer:** Bonilauri P, Garcia-Elorriaga G, Moschovi M

**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Liu SQ





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Clinical Infectious Diseases*

*World J Clin Infect Dis* 2015 November 25; 5(4): 55-93





## Editorial Board

2011-2015

The World Journal of Clinical Infectious Diseases Editorial Board consists of 291 members, representing a team of worldwide experts in infectious diseases. They are from 56 countries, including Argentina (5), Australia (8), Austria (3), Bangladesh (1), Belgium (2), Bosnia and Herzegovina (1), Brazil (6), Brunei Darussalam (1), Bulgaria (1), Cameroon (1), Canada (7), China (18), Colombia (1), Costa Rica (1), Cuba (1), Denmark (2), Egypt (1), Finland (1), France (11), Germany (4), Greece (8), Hungary (6), India (14), Indonesia (1), Iran (5), Israel (10), Italy (19), Japan (4), Jordan (1), Kosovo (1), Kuwait (1), Lebanon (3), Lithuania (1), Malawi (1), Mexico (5), Morocco (2), Netherlands (4), Nigeria (1), Pakistan (2), Peru (1), Philippines (1), Portugal (5), Russia (1), Saudi Arabia (2), Singapore (3), South Africa (2), South Korea (6), Spain (24), Switzerland (2), Tanzania (1), Thailand (4), Tunisia (1), Turkey (4), United Kingdom (9), United States (59), and Venezuela (1).

### EDITORS-IN-CHIEF

Shyam Sundar, *Varanasi*  
Lihua Xiao, *Atlanta*

### GUEST EDITORIAL BOARD MEMBERS

Huan-Tsung Chang, *Taipei*  
Jia-Ming Chang, *Taipei*  
Kuo-Chin Huang, *Chiayi*  
Wei-Chen Lee, *Taoyuan*  
Hsiu-Jung Lo, *Miaoli*  
Jin-Town Wang, *Taipei*  
Deng-Chyang Wu, *Kaohsiung*  
Jiunn-Jong Wu, *Tainan*

### MEMBERS OF THE EDITORIAL BOARD



#### Argentina

Sergio Angel, *Chascomus*  
Luis Adrian Diaz, *Cordoba*  
Gustavo Daniel Lopardo, *Buenos Aires*  
Emilio L Malchiodi, *Buenos Aires*  
Victor D Rosenthal, *Buenos Aires*



#### Australia

Thea van de Mortel, *Lismore*  
David Llewellyn Gordon, *Bedford Park*  
Asad Khan, *Brisbane*  
Ruiting Lan, *Sydney*  
John McBride, *Cairns*  
David Leslie Paterson, *Brisbane*  
Nitin K Saksena, *Sydney*  
Andrew Slack, *Brisbane*



#### Austria

Ojan Assadian, *Vienna*  
Christian Joukhadar, *Vienna*  
Bernhard Resch, *Graz*



#### Bangladesh

Harunor Rashid, *Cox's Bazar*



#### Belgium

Mickael Aoun, *Bruxelles*  
Paul M Tulkens, *Brussels*



#### Bosnia and Herzegovina

Selma Uzunovic, *Zenica*



#### Brazil

Jane Costa, *Rio de Janeiro*  
Pedro Alves d'Azevedo, *Sao Paulo*  
Gerly Anne de Castro Brito, *Fortaleza*  
RL Dantas Machado, *Sao Paulo*  
Leandro R Rodrigues Perez, *Porto Alegre*  
M de Nazare Correia Soeiro, *Rio de Janeiro*



#### Brunei Darussalam

Vui Heng Chong, *Bandar Seri Begawan*



#### Bulgaria

Iva Christova, *Sofia*



#### Cameroon

Richard Njouom, *Yaounde*



#### Canada

Aranka Anema, *Vancouver*  
Peter C Coyte, *Toronto*  
Pavel Gershkovich, *Vancouver*  
Marcelo Gottschalk, *Quebec*  
Marina Ulanova, *Thunder Bay*  
Jude Uzonna, *Winnipeg*  
Jun Wang, *Halifax*



#### China

Tian-Hua Huang, *Shantou*  
Xi-Tai Huang, *Tianjin*  
Dong-Ming Li, *Beijing*  
Xin-Yong Liu, *Jinan*  
Wu-Bin Pan, *Taichang*  
Kai Wang, *Jinan*  
Patrick Chiu Yat Woo, *Hong Kong*  
Yong-Feng Yang, *Nanjing*  
Chi-Yu Zhang, *Zhenjiang*  
Li-Juan Zhang, *Beijing*



#### Colombia

Jorge Enrique Gomez-Marin, *Armenia*

**Costa Rica**

Adriano Arguedas, *San José*

**Cuba**

Maria G Guzman, *Havana*

**Denmark**

Janne Kudsk Klitgaard, *Odense*  
Henrik Torkil Westh, *Hvidovre*

**Egypt**

Olfat Shaker, *Cairo*

**Finland**

Jari Timo Juhani Nuutila, *Turku*

**France**

Hassane Adakal, *Burkina Faso*  
Pascal Bigey, *Paris*  
Philippe Brouqui, *Marseille*  
Christophe Chevillard, *Marseille*  
Raphaelé Girard, *Pierre Bénite*  
Vincent Pascal Jarlier, *Paris*  
Sandrine Marquet, *Marseille*  
Vayssier-Taussat Muriel, *Maisons-Alfort*  
Thierry Naas, *Le Kremlin-Bicetre*  
Saad Nseir, *Lille*  
Philippe Seguin, *Rennes*

**Germany**

Stefan Borgmann, *Ingolstadt*  
Georg Harter, *Ulm*  
Matthias Imohl, *Aachen*  
Kurt G Naber, *Straubing*

**Greece**

Apostolos Beloukas, *Athens*  
Alex P Betrosian, *Athens*  
George L Daikos, *Athens*  
Helena Maltezou, *Athens*  
Argyris S Michalopoulos, *Athens*  
Maria Moschovi, *Athens*  
George Petrikkos, *Athens*  
Athanasios Tragiannidis, *Thessaloniki*

**Hungary**

Laszlo Galgoczy, *Szeged*  
Viktor Muller, *Budapest*  
Ferenc Orosz, *Budapest*  
Ferenc Rozgonyi, *Budapest*  
Jozsef Soki, *Szeged*

Dezso Peter Virok, *Szeged*

**India**

Ritesh Agarwal, *Chandigarh*  
Syed Imteyaz Alam, *Gwalior*  
Atmaram Hari Bandivdekar, *Mumbai*  
Runu Chakravarty, *Kolkata*  
Dipshikha Chakravorty, *Bangalore*  
Sanjay Chhibber, *Chandigarh*  
BN Harish, *Pondicherry*  
Triveni Krishnan, *Kolkata*  
Rashmi Kumar, *Lucknow*  
Mohammad Owais, *Aligarh*  
Banwarilal Sarkar, *Kolkata*  
Mamta Chawla Sarkar, *Kolkata*  
Akashdeep Singh, *Ludhiana*

**Indonesia**

Jeanne Adiwinata Pawitan, *Jakarta*

**Iran**

Parissa Farnia, *Tehran*  
Seyed Mohammad Jazayeri, *Tehran*  
Morteza Pourahmad, *Jahrom*  
Mohammad Reza Pourshafie, *Tehran*  
Mohammad Hossein Salari, *Tehran*

**Israel**

Jacob Amir, *Petach Tikvah*  
Shai Ashkenazi, *Petach Tikva*  
Gadi Borkow, *Gibton*  
Raul Colodner, *Afula*  
Jacob Moran Gilad, *Jerusalem*  
Noah Isakov, *Beer Sheva*  
Michal Mandelboim, *Hashomer*  
Shifra Shvarts, *Omer*  
Oshri Wasserzug, *Tel-Aviv*  
Pablo Victor Yagupsky, *Beer-Sheva*

**Italy**

Giuseppe Barbaro, *Rome*  
Paolo Bonilauri, *Reggio Emilia*  
Guido Calleri, *Torino*  
Mario Cruciani, *Verona*  
Marco Falcone, *Rome*  
Antonio Fasanella, *Foggia*  
Daniele Focosi, *Pisa*  
Delia Goletti, *Rome*  
Guido Grandi, *Siena*  
Fabio Grizzi, *Rozzano*  
Giuseppe Ippolito, *Rome*  
Roberto Manfredi, *Bologna*  
Claudio M Mastroianni, *Rome*  
Ivano Mezzaroma, *Rome*  
Giuseppe Micali, *Catania*  
Antonella d'Arminio Monforte, *Milano*  
Annamaria Passantino, *Messina*  
Mariagrazia Perilli, *L'Aquila*  
Patrizia Pontisso, *Padova*

**Japan**

Masashi Emoto, *Maebashi*  
Toshi Nagata, *Hamamatsu*  
Ryohei Yamasaki, *Tottori*  
Shin-Ichi Yokota, *Sapporo*

**Jordan**

Asem A Shehabi, *Amman*

**Kosovo**

Lul Raka, *Prishtina*

**Kuwait**

Willias Masocha, *Safat*

**Lebanon**

Ziad Daoud, *Beirut*  
Ghassan M Matar, *Beirut*  
Sami Ramia, *Beirut*

**Lithuania**

Gazim Bizanov, *Vilnius*

**Malawi**

Adamson Sinjani Muula, *Blantyre*

**Mexico**

Agnes Fleury, *Mexico*  
Guadalupe Garcia-Elorriaga, *Mexico*  
Alejandro E Macias, *Mexico*  
Mussaret Zaidi, *Merida*  
Roberto Zenteno-Cuevas, *Veracruz*

**Morocco**

Redouane Abouqal, *Rabat*  
Ezzikouri Sayeh, *Casablanca*

**Netherlands**

Aldert Bart, *Amsterdam*  
John Hays, *Rotterdam*  
Nisar Ahmed Khan, *Rotterdam*  
Rogier Louwen, *Rotterdam*

**Nigeria**

Samuel Sunday Taiwo, *Osogbo*



### **Pakistan**

Muhammad Idrees, *Lahore*  
Muhammad Mukhtar, *Bahawalpur*



### **Peru**

Salim Mohanna, *Lima*



### **Philippines**

Vicente Y Belizario, *Ermita Manila*



### **Portugal**

Ricardo Araujo, *Porto*  
Manuela Canica, *Lisbon*  
Francisco Esteves, *Lisbon*  
Fernando Rodrigues, *Braga*  
Nuno Taveira, *Lisbon*



### **Russia**

Alexander M Shestopalov, *Koltsovo*



### **Saudi Arabia**

Jaffar A Al-Tawfiq, *Dhahran*  
Atef M Shibl, *Riyadh*



### **Singapore**

Yee Sin Leo, *Singapore*  
Laurent Claude Stephane Renia, *Singapore*  
Richard J Sugrue, *Singapore*



### **South Africa**

Carolina H Pohl-Albertyn, *Bloemfontein*  
Natasha Potgieter, *Louis Trichardt*



### **South Korea**

Chong Cho, *Seoul*  
Sang Ho Choi, *Seoul*  
Ju-Young Chung, *Seoul*  
Jung Mogg Kim, *Seoul*  
Kyongmin Kim, *Suwon*  
Sang Hee Lee, *Yongin*



### **Spain**

Alberto Arnedo-Pena, *Castellon*  
Alfredo Berzal-Herranz, *Granada*  
Vicente Brito, *Alicante*

Enrique Calderon, *Seville*

Rafael Canton, *Madrid*

Jose M Cuevas, *Valencia*

Laila Darwich, *Cerdanyola del Valles*

Adela Gonzalez de la Campa, *Madrid*

Pere Domingo, *Barcelona*

Tahia D Fernandez, *Malaga*

Lucia Gallego, *Leioa*

Luis Ignacio Gonzalez-Granado, *Madrid*

Bruno Gonzalez-Zorn, *Madrid*

Eduardo Lopez-Collazo, *Madrid*

Miguel Marcos, *Salamanca*

Antonio Torres Marti, *Barcelona*

Andres Moya, *Valencia*

Rafael Najera, *Madrid*

Maria Mercedes Nogueras-Mas, *Sabadell*

Jose A Oteo, *Logrono*

Pilar Perez-Romero, *Sevilla*

Ruth Gil Raka, *Madrid*

Eduardo Reyes, *Madrid*

Francisco Soriano, *Madrid*



### **Switzerland**

Stephen Hawser, *Epalinges*  
Andrew Hemphill, *Bern*



### **Tanzania**

John Peter Andrea Lusingu, *Tanga*



### **Thailand**

Kosum Chansiri, *Bangkok*  
Subsai Kongsangdao, *Bangkok*  
Niwat Maneeakarn, *Chiang Mai*  
Viroj Wiwanitkit, *Bangkok*



### **Tunisia**

Aouni Mahjoub, *Monastir*



### **Turkey**

Oguz Karabay, *Sakarya*  
Uner Kayabas, *Malatya*  
Gokhan Metan, *Kayseri*  
Oral Oncul, *Istanbul*



### **United Kingdom**

Zainab Al-Doori, *Glasgow*  
David Carmena, *London*  
Ronald Anthony Dixon, *Lincoln*  
Vanya Alasdair Ivan Andre Gant, *London*  
Robin Goodwin, *London*  
Andrew Cunliffe Hayward, *London*  
Laura Anne Hughes, *Neston*  
Michele Esther Murdoch, *Herts*  
Craig William Roberts, *Glasgow*



### **United States**

Majdi N Al-Hasan, *Lexington*  
Ibne KM Ali, *Charlottesville*  
Hossam M Ashour, *Detroit*  
Joseph Urban Becker, *Palo Alto*  
M Eric Benbow, *Dayton*  
Eliahu Bishburg, *Newark*  
Luz P Blanco, *Ann Arbor*  
Robert Bucki, *Philadelphia*  
Steven Dale Burdette, *Dayton*  
Archana Chatterjee, *Omaha*  
Pai-Lien Chen, *Durham*  
Pawel S Ciborowski, *Omaha*  
Michael Cynamon, *Syracuse*  
Siddhartha Das, *El Paso*  
Ralph J DiClemente, *Atlanta*  
Noton Kumar Dutta, *Baltimore*  
Garth D Ehrlich, *Pittsburgh*  
Michael S Firstenberg, *Columbus*  
Walter A Hall, *Syracuse*  
Yongqun He, *Ann Arbor*  
Brenda Lorraine Helms, *Plano*  
Joseph U Igietseme, *Atlanta*  
Mohammad Khalid Ijaz, *Montvale*  
Suresh G Joshi, *Philadelphia*  
Thomas F Kresina, *Rockville*  
Alain B Labrique, *Baltimore*  
Shenghan Lai, *Baltimore*  
Benfang Lei, *Bozeman*  
Jeff G Leid, *Flagstaff*  
Vladimir Leonitiev, *St. Louis*  
Andrea Lisco, *Bethesda*  
James M McMahon, *Rochester*  
Geraldine M McQuillan, *Hyattsville*  
Lawrence F Muscarella, *Ivyland*  
Daniel Musher, *Houston*  
Stella Nowicki, *Nashville*  
M Jacques Nsuami, *New Orleans*  
Phillipe N Nyambi, *New York*  
Raymund Rabe Razonable, *Rochester*  
Anand Reddi, *Denver*  
Michael Switow Saag, *Birmingham*  
Danny J Schust, *Columbia*  
William R Schwan, *La Crosse*  
Richard A Slayden, *Fort Collins*  
Theodore J Standiford, *Ann Arbor*  
William M Switzer, *Atlanta*  
Ashutosh Tamhane, *Birmingham*  
Giorgio E Tarchini, *Weston*  
Carmen Taype, *New York*  
Barbara Van Der Pol, *Bloomington*  
Jose Antonio Vazquez, *Detroit*  
Fernando Villalta, *Nashville*  
Haider J Warraich, *Boston*  
Xianfu Wu, *Atlanta*  
Genyan Yang, *Atlanta*  
Frank X Yang, *Indianapolis*  
Hong Zhang, *Rockville*  
Lyna Zhang, *Atlanta*



### **Venezuela**

Alfonso J Rodriguez-Morales, *Caracas*



**EDITORIAL**

- 55      How could we reduce antifungal use in the intensive care unit?  
*Rouzé A, Jaffal K, Nseir S*
- 59      New biomarkers for clinical management of hepatitis C virus infected patients  
*Biasiolo A, Martini A, Pontisso P*

**REVIEW**

- 67      Interplay between rabies virus and the mammalian immune system  
*Johnson N, Cunningham AF*
- 77      Epidemiological perspective of drug resistant extrapulmonary tuberculosis  
*Singh PK, Jain A*

**ORIGINAL ARTICLE**

**Basic Study**

- 86      *Tuf* mRNA rather than 16S rRNA is associated with culturable *Staphylococcus aureus*  
*Loonen AJM, Wolffs PFG, de Bresser M, Habraken M, Bruggeman CA, Hermans MHA, van den Brule AJC*



**ABOUT COVER**

Editorial Board Member of *World Journal of Clinical Infectious Diseases*, Geraldine M McQuillan, PhD, Senior Infectious Disease Epidemiologist, National Center for Health Statistics, Centers for Disease Control and Prevention, Hyattsville, MD 20782, United States

**AIM AND SCOPE**

*World Journal of Clinical Infectious Diseases* (*World J Clin Infect Dis*, *WJCID*, online ISSN 2220-3176, DOI: 10.5495) is a peer-reviewed open access (OA) academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJCID* will focus on a broad spectrum of topics on infectious diseases that will cover epidemiology, immune-pathogenesis, genetic factors, host susceptibility to infection, vector control, novel approaches of treatment, molecular diagnostic and vaccines. It will provide a common stage to share the visions, new approaches, most advanced techniques, and to discuss research problems that will help everyone working in the field of various infections to exchange their views and to improve public health. *WJCID* will also focus on broad range of infections like opportunistic infections, zoonotic infections, tropical and neglected tropical diseases, emerging infections, *etc.* and following topics related to these issues: (1) Causative agents discussing various pathogens; (2) Vectors and Mode of transmission; (3) Host-pathogen interaction and immune-pathogenesis of the disease; (4) Epidemiology of the infection and vector control strategies; (5) Genetic factors covering both host and pathogen; (6) Molecular diagnostic techniques vaccines; and (7) Recent advances in cell tissue culture, lab techniques, *etc.* Various other related fields like medical microbiology, pharmacology of herbs, bioinformatics, *etc.* will be included.

We encourage authors to submit their manuscripts to *WJCID*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/ABSTRACTING**

*World Journal of Clinical Infectious Diseases* is now indexed in Digital Object Identifier.

**FLYLEAF**

**I-III Editorial Board**

**EDITORS FOR THIS ISSUE**

**Responsible Assistant Editor:** *Xiang Li*  
**Responsible Electronic Editor:** *Dan Li*  
**Proofing Editor-in-Chief:** *Lian-Sheng Ma*

**Responsible Science Editor:** *Jin-Xin Kong*  
**Proofing Editorial Office Director:** *Xin-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Clinical Infectious Diseases*

**ISSN**  
ISSN 2220-3176 (online)

**LAUNCH DATE**  
December 30, 2011

**FREQUENCY**  
Quarterly

**EDITORS-IN-CHIEF**  
**Shyam Sundar, MD, FRCP (London), FAMS, FNA Sc, FASc, FNA, Professor**, Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India

**Lihua Xiao, DVM, PhD, Senior Scientist**, Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Bldg 23, Rm 9-168, MS D66, 1600 Clifton

Rd, Atlanta, GA 30333, United States

**EDITORIAL OFFICE**  
Jin-Lei Wang, Director  
Xiu-Xia Song, Vice Director  
*World Journal of Clinical Infectious Diseases*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-85381891  
Fax: +86-10-85381893  
E-mail: editorialoffice@wjgnet.com  
Help Desk: <http://www.wjgnet.com/esp/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esp/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
November 25, 2015

**COPYRIGHT**  
© 2015 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**  
Full instructions are available online at [http://www.wjgnet.com/2220-3176/g\\_info\\_20100722180909.htm](http://www.wjgnet.com/2220-3176/g_info_20100722180909.htm).

**ONLINE SUBMISSION**  
<http://www.wjgnet.com/esp/>

## How could we reduce antifungal use in the intensive care unit?

Anahita Rouzé, Karim Jaffal, Saad Nseir

Anahita Rouzé, Karim Jaffal, Saad Nseir, Critical Care Center, R. Salengro Hospital, University Hospital of Lille, 59037 Lille cedex, France

Anahita Rouzé, Saad Nseir, Medical School, Lille University, 59000 Lille, France

**Author contributions:** Rouzé A, Jaffal K and Nseir S drafted the manuscript; all authors read and approved the final version of the manuscript.

**Conflict-of-interest statement:** The authors declare no conflicts of interest regarding this manuscript.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Saad Nseir, Professor, Critical Care Center, R. Salengro Hospital, University Hospital of Lille, boulevard du Pr Leclercq, 59037 Lille cedex, France. [s-nseir@chru-lille.fr](mailto:s-nseir@chru-lille.fr)  
Telephone: +33-3-20444084  
Fax: +33-3-20445094

Received: June 4, 2015  
Peer-review started: June 8, 2015  
First decision: August 10, 2015  
Revised: September 12, 2015  
Accepted: October 12, 2015  
Article in press: October 13, 2015  
Published online: November 25, 2015

### Abstract

Fungal infection is common in critically ill patients. However, this infection is difficult to diagnose, and a

large proportion of patients receive empirical antifungal treatment without further confirmation of invasive fungal disease. Whilst prompt appropriate antifungal treatment is associated with better outcome in patients with confirmed infections, this treatment has several drawbacks. In addition, no clear beneficial effect of empirical antifungal treatment was found in patients without confirmed infection. Reducing antifungal treatment in the intensive care unit (ICU) is feasible, and would allow avoiding drawbacks of this treatment without negative impact on outcome. Antifungal stewardship, preemptive antifungal treatment, based on colonization index and fungal biomarkers; and de-escalation of antifungal treatment based on microbiology results and fungal biomarkers could be suggested to reduce antifungal use in the ICU, and are currently under investigation.

**Key words:** Antifungals; Biomarkers; Colonization; Infection; Preemptive treatment

© **The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Prompt appropriate antifungal treatment is associated with better outcome in patients with confirmed infections, this treatment has several drawbacks. Reducing antifungal treatment in the intensive care unit (ICU) is feasible, and would allow avoiding drawbacks of this treatment without negative impact on outcome. Antifungal stewardship, preemptive antifungal treatment, based on colonization index and fungal biomarkers; and de-escalation of antifungal treatment based on microbiology results and fungal biomarkers could be suggested to reduce antifungal use in the ICU, and are currently under investigation.

Rouzé A, Jaffal K, Nseir S. How could we reduce antifungal use in the intensive care unit? *World J Clin Infect Dis* 2015; 5(4): 55-58  
Available from: URL: <http://www.wjgnet.com/2220-3176/full/v5/>

## INTRODUCTION

Fungal infection is common in critically ill patients<sup>[1]</sup>. Based on the results of the large EPIC II international study, candida spp. represented 17% of all microorganisms isolated in the cohort of 7087 critically ill patients with confirmed infection<sup>[2]</sup>. The subgroup analysis of patients with bloodstream infection (BSI) related to candida reported that intensive care unit (ICU) mortality was extremely high (43%), compared with that of BSI related to Gram-negative (29%), or to Gram-positive (25%) microorganisms<sup>[3]</sup>. Further, a recent epidemiological study, performed in Paris area, showed worrisome trends in the incidence of BSI related to candida, and mortality associated with this infection during the last decade<sup>[4]</sup>. Antifungal treatment could be classified into prophylactic, empirical, pre-emptive, and targeted. Prophylactic treatment is usually given to at high-risk patients without clinical signs of infection. Empirical treatment is prescribed to patients with clinical signs of infection. Antifungal treatment is considered as preemptive, in presence of clinical signs, risk factors for invasive fungal disease, and fungal colonization or high levels of fungal biomarkers. Targeted treatment is defined as a treatment given to patients with documented infection.

### **Pros and cons of empirical antifungal treatment**

In patients with septic shock related to invasive fungal disease, prompt adequate antifungal treatment is the main prognosis factor. In a cohort of 223 patients with septic shock and Candida BSI, Kollef and colleagues identified delayed antifungal treatment, and inadequate source control as the two major independent risk factors for mortality<sup>[5]</sup>. Other risk factors included metastatic cancer, severe heart failure, red-blood cell transfusion, APACHE II score, and serum albumin level.

Diagnosis of invasive fungal disease is still challenging in critically ill patients, because of the low sensitivity of clinical signs and blood cultures, and the difficulties in performing biopsies or other invasive procedure in these patients. Therefore, empirical antifungal treatment is frequently prescribed in the ICU. A recent one-day cross-sectional cohort study was performed in 169 ICUs<sup>[6]</sup>. Antifungal treatment was given to 154 patients of the 2047 included patients, including two thirds of patients who received antifungal without any proven invasive fungal disease. Another retrospective study, performed during a 1-year-period, reported a higher rate of empirical antifungal treatment (28% of the 560 included patients)<sup>[7]</sup>. Chemotherapy, and infection at admission were associated with prolonged empirical antifungal treatment. A recent multicenter study reported that 100 (6.7%) of the 1491 included ICU patients received empirical antifungal treatment. No significant difference

was found in mortality rate between patients who received empirical antifungal treatment, and those who did not receive antifungals<sup>[8]</sup>.

Drawbacks of empirical antifungal treatment include antifungal resistance, side effects, drug interaction, and cost (Figure 1). Whilst fungal resistance is less frequent than bacterial resistance, recent reports suggest an increase in resistance rate among *Candida* spp<sup>[9]</sup>. Further, antifungal resistance was also reported to be associated with worse outcome in patients with BSI related to *Candida glabrata*<sup>[10]</sup>.

### **Strategies to reduce antifungal use**

Several strategies could be suggested to improve antifungal treatment in critically ill patients, and reduce unnecessary treatment.

#### **At the start of antifungal treatment**

Antifungal stewardship could be suggested in patients at higher risk for invasive fungal disease. A recent study performed in hematology department of our hospital during a 10-year-period reported that a decrease of 40% in antifungal consumption was possible using local guidelines, including decision algorithms and preprinted prescriptions allowing only-guideline recommended drugs for a given indication<sup>[11]</sup>. These local guidelines were based on national and international recommendations on antifungal treatment. The incidence of invasive fungal disease, and mortality rate remained stable during the study period.

A preemptive antifungal treatment could also be suggested to reduce antifungal treatment. Such a strategy could be based on clinical rules, colonization index, and/or fungal biomarkers. Whilst the negative predictive value of clinical rules, and colonization index is very good (72-100), the positive predictive value is low (6%-67%), suggesting that a large proportion of patients receiving empirical or preemptive treatment will be free of invasive fungal disease<sup>[12]</sup>. A multicenter randomized controlled double-blind study is currently ongoing in France to determine the impact of mycalfungin in multi-colonized candida patients with nosocomial sepsis. Its results will be very helpful to determine the impact of such a strategy in critically ill patients<sup>[13]</sup>.

Initial empirical antifungal treatment should be based on local epidemiology and sensitivity patterns, in order to avoid using large-spectrum antifungals in settings where the incidence of resistant fungi is low.

#### **De-escalation of antifungal treatment**

$\beta$ -D-glucan is a most studied biomarker for invasive fungal disease. As for clinical rule and colonization index, its negative predictive value is very good (77%-98%), but its positive predictive value is low (59%-72%), suggesting that a treatment strategy based on this biomarker would also result in unnecessary use of antifungals<sup>[12]</sup>. A recent study suggested that combined use of  $\beta$ -D 1, 3-glucan, mannan, mannan-

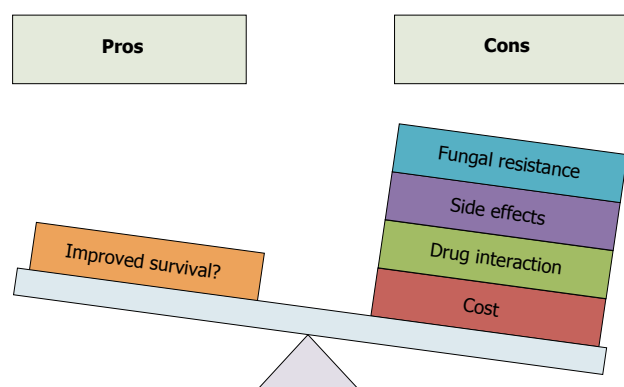


Figure 1 Pros and cons of empirical antifungal treatment.

antibody was helpful in improving the predictive value of candidemia<sup>[14]</sup>. Another recent prospective study performed in 56 patients with candidemia and 200 controls, reported that a combination of  $\beta$ -D-glucan, and mannan had a very good sensitivity (89%), and specificity (85%)<sup>[15]</sup>. Ostrosky-Zeichner *et al.*<sup>[16]</sup> performed a randomized controlled double-blind placebo-controlled study to determine the impact caspofungin prophylaxis followed by preemptive treatment on the incidence of invasive fungal disease. Whilst prophylaxis treatment had no impact on the incidence of invasive candidiasis, preemptive treatment based on  $\beta$ -D-glucan results was associated with significantly decreased incidence of invasive candidiasis. However, the impact of preemptive treatment on invasive candidiasis incidence was a secondary objective.

Another strategy to reduce antifungal treatment in critically ill patients is to deescalate antifungal treatment, as soon as results of blood cultures and biomarkers are available. In fact, based on the excellent negative predictive value of the combination of  $\beta$ -D-glucan, mannan, and antimannan, empirical antifungal treatment could be safely stopped in patients with negative blood cultures and negative fungal biomarker results. Our group is conducting a randomized controlled unblind study to determine the impact of a strategy based on fungal biomarker results on the early stop of antifungal treatment in critically ill patients<sup>[17]</sup>. We plan to include 110 patients in this feasibility study, and to evaluate the safety of such a strategy, including its impact on mortality and recurrence of fungal infection (Figure 2). However, fungal biomarkers are not available in all ICUs, and the cost/effectiveness of such a strategy should be evaluated.

### Reduction of duration of antifungal treatment

To our knowledge, no study has specifically evaluated the best duration of antifungal treatment in critically ill patients with confirmed fungal infections. However, recent guidelines clearly recommend different durations, based on the site of infection. Therefore, one potential strategy to reduce duration of targeted treatment in these patients is to follow the guidelines, and to evaluate

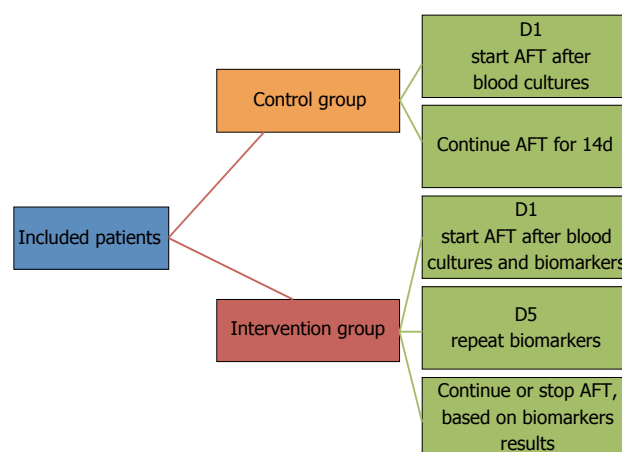


Figure 2 Use of biomarkers to deescalate antifungal treatment. AFT: Antifungal treatment; D: Day.

adherence to local guidelines repeatedly.

## CONCLUSION

Antifungal treatment is frequently used in critically ill patients. Prompt adequate antifungal treatment is required in patients with septic shock related to candida infection. However, the beneficial effects of empirical antifungal treatment in patients with suspected invasive fungal infection have not been demonstrated. In addition, fungal resistance, drug interaction, side effects, and cost should be taken into account when starting such a treatment in ICU patients. Further studies should determine the impact of targeted strategies, and de-escalation of empirical treatment on outcome of critically ill patients.

## REFERENCES

- 1 Cornely OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortholary O, Meersseman W, Akova M, Arendrup MC, Arisan-Akdagli S, Bille J, Castagnola E, Cuenca-Estrella M, Donnelly JP, Groll AH, Herbrecht R, Hope WW, Jensen HE, Lass-Flörl C, Petrikos G, Richardson MD, Roilides E, Verweij PE, Viscoli C, Ullmann AJ. ESCMID guideline for the diagnosis and management of Candida diseases 2012: non-neutropenic adult patients. *Clin Microbiol Infect* 2012; **18** Suppl 7: 19-37 [PMID: 23137135 DOI: 10.1111/1469-0691.12039]
- 2 Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, Moreno R, Lipman J, Gomersall C, Sakr Y, Reinhart K. International study of the prevalence and outcomes of infection in intensive care units. *JAMA* 2009; **302**: 2323-2329 [PMID: 19952319 DOI: 10.1001/jama.2009.1754]
- 3 Kett DH, Azoulay E, Echeverria PM, Vincent JL. Candida bloodstream infections in intensive care units: analysis of the extended prevalence of infection in intensive care unit study. *Crit Care Med* 2011; **39**: 665-670 [PMID: 21169817 DOI: 10.1097/CCM.0b013e318206c1ca]
- 4 Lortholary O, Renaudat C, Sitbon K, Madec Y, Denoeud-Ndam L, Wolff M, Fontanet A, Bretagne S, Dromer F. Worrisome trends in incidence and mortality of candidemia in intensive care units (Paris area, 2002-2010). *Intensive Care Med* 2014; **40**: 1303-1312 [PMID: 25097069 DOI: 10.1007/s00134-014-3408-3]
- 5 Kollef M, Micek S, Hampton N, Doherty JA, Kumar A. Septic



- shock attributed to *Candida* infection: importance of empiric therapy and source control. *Clin Infect Dis* 2012; **54**: 1739-1746 [PMID: 22423135 DOI: 10.1093/cid/cis305]
- 6 **Azoulay E**, Dupont H, Tabah A, Lortholary O, Stahl J-P, Francois A, Martin C, Guidet B, Timsit J-F. Systemic antifungal therapy in critically ill patients without invasive fungal infection. *Crit Care Med* 2012; **40**: 813-822 [PMID: 22297630 DOI: 10.1097/CCM.0b013e318236f297]
- 7 **Zein M**, Parmentier-Decrucq E, Kalaoun A, Bouton O, Wallyn F, Baranzelli A, Elmanser D, Sendid B, Nseir S. Factors predicting prolonged empirical antifungal treatment in critically ill patients. *Ann Clin Microbiol Antimicrob* 2014; **13**: 11 [PMID: 24621182 DOI: 10.1186/1476-0711-13-11]
- 8 **Bailly S**, Bouadma L, Azoulay E, Orgeas MG, Adrie C, Souweine B, Schwebel C, Maubon D, Hamidfar-Roy R, Darmon M, Wolff M, Cornet M, Timsit JF. Failure of empirical systemic antifungal therapy in mechanically ventilated critically ill patients. *Am J Respir Crit Care Med* 2015; **191**: 1139-1146 [PMID: 25780856 DOI: 10.1164/rccm.201409-1701OC]
- 9 **Fournier P**, Schwebel C, Maubon D, Vesin A, Lebeau B, Foroni L, Hamidfar-Roy R, Cornet M, Timsit JF, Pelloux H. Antifungal use influences *Candida* species distribution and susceptibility in the intensive care unit. *J Antimicrob Chemother* 2011; **66**: 2880-2886 [PMID: 21980066 DOI: 10.1093/jac/dkr394]
- 10 **Alexander BD**, Johnson MD, Pfeiffer CD, Jiménez-Ortígoza C, Catania J, Booker R, Castanheira M, Messer SA, Perlin DS, Pfaller MA. Increasing echinocandin resistance in *Candida glabrata*: clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. *Clin Infect Dis* 2013; **56**: 1724-1732 [PMID: 23487382 DOI: 10.1093/cid/cit136]
- 11 **Alfandari S**, Berthon C, Coiteux V. Antifungal stewardship: implementation in a French teaching hospital. *Med Mal Infect* 2014; **44**: 154-158 [PMID: 24612504 DOI: 10.1016/j.medmal.2014.01.012]
- 12 **León C**, Ostrosky-Zeichner L, Schuster M. What's new in the clinical and diagnostic management of invasive candidiasis in critically ill patients. *Intensive Care Med* 2014; **40**: 808-819 [PMID: 24718642 DOI: 10.1007/s00134-014-3281-0]
- 13 **Timsit JF**, Azoulay E, Cornet M, Gangneux JP, Jullien V, Vesin A, Schir E, Wolff M. EMPIRICUS micafungin versus placebo during nosocomial sepsis in *Candida* multi-colonized ICU patients with multiple organ failures: study protocol for a randomized controlled trial. *Trials* 2013; **14**: 399 [PMID: 24261608 DOI: 10.1186/1745-6215-14-399]
- 14 **Poissy J**, Sendid B, Damiens S, Ichi Ishibashi K, François N, Kauv M, Favory R, Mathieu D, Poulain D. Presence of *Candida* cell wall derived polysaccharides in the sera of intensive care unit patients: relation with candidaemia and *Candida* colonisation. *Crit Care* 2014; **18**: R135 [PMID: 24975380 DOI: 10.1186/cc13953]
- 15 **Held J**, Kohlberger I, Rappold E, Busse Grawitz A, Häcker G. Comparison of (1->3)- $\beta$ -D-glucan, mannan/anti-mannan antibodies, and Cand-Tec *Candida* antigen as serum biomarkers for candidemia. *J Clin Microbiol* 2013; **51**: 1158-1164 [PMID: 23363830 DOI: 10.1128/JCM.02473-12]
- 16 **Ostrosky-Zeichner L**, Shoham S, Vazquez J, Reboli A, Betts R, Barron MA, Schuster M, Judson MA, Revankar SG, Caeiro JP, Mangino JE, Mushatt D, Bedimo R, Freifeld A, Nguyen MH, Kauffman CA, Dismukes WE, Westfall AO, Deerman JB, Wood C, Sobel JD, Pappas PG. MSG-01: A randomized, double-blind, placebo-controlled trial of caspofungin prophylaxis followed by preemptive therapy for invasive candidiasis in high-risk adults in the critical care setting. *Clin Infect Dis* 2014; **58**: 1219-1226 [PMID: 24550378 DOI: 10.1093/cid/ciu074]
- 17 Fungal Biomarkers to Reduce Duration of Empirical Antifungal Therapy: a Randomized Comparative Study (STAFE). Available from: URL: <https://www.clinicaltrials.gov/ct2/show/NCT02154178>. Accessed by Nov 21, 2015

**P- Reviewer:** Geraldine MM, Hideo I, James L, Mohan G  
**S- Editor:** Qiu S **L- Editor:** A **E- Editor:** Li D



## New biomarkers for clinical management of hepatitis C virus infected patients

Alessandra Biasiolo, Andrea Martini, Patrizia Pontisso

Alessandra Biasiolo, Andrea Martini, Patrizia Pontisso,  
Department of Medicine, University of Padua, 35128 Padua, Italy

**Author contributions:** Biasiolo A designed the study, acquired data and drafted the article; Martini A acquired data and reviewed the manuscript; Pontisso P conceived the study and made the final critical revision of the manuscript; all authors had seen and approved the final version of the manuscript.

**Supported by** University of Padua (found 2011 - prot. No. STPD11RYPT\_003).

**Conflict-of-interest statement:** All the authors declare no conflicting interests.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Patrizia Pontisso, Professor, Department of Medicine, University of Padua, Via Giustiniani, 2, 35128 Padua, Italy. [patrizia@unipd.it](mailto:patrizia@unipd.it)  
Telephone: +39-49-8217872  
Fax: +39-49-8754179

Received: July 11, 2015  
Peer-review started: July 15, 2015  
First decision: August 25, 2015  
Revised: August 28, 2015  
Accepted: October 16, 2015  
Article in press: October 19, 2015  
Published online: November 25, 2015

### Abstract

Hepatocellular carcinoma (HCC) is the third most frequent oncological cause of death worldwide, prin-

cipally a consequence of hepatitis C virus (HCV) infection and its prognosis is mostly poor. For early identification and surveillance of HCV patients with liver disease progression, the availability of suitable diagnostic and prognostic biomarkers is still an unmet clinical need. Alfa-fetoprotein together with imaging techniques is commonly used, however its specificity and sensitivity are not satisfactory. Several clinical and serological data have been proposed to define the risk of disease progression in HCV infected patients and new biomarkers have been proposed, including post-transcriptionally modified molecules and genetic biomarkers. The present editorial article attempts to summarize the current knowledge on the new promising tools for effective early diagnosis of HCV-related liver disease progression and for the surveillance of HCC.

**Key words:** Hepatitis C virus infection; Biomarkers; Liver disease progression; Hepatocellular carcinoma

© **The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Hepatitis C virus (HCV) infection is a major cause of cirrhosis and hepatocellular carcinoma (HCC), leading to liver failure and/or liver transplantation. The current knowledge on the new promising biomarkers, able to predict the progression of HCV-related liver disease and HCC, has been the focus of this editorial.

Biasiolo A, Martini A, Pontisso P. New biomarkers for clinical management of hepatitis C virus infected patients. *World J Clin Infect Dis* 2015; 5(4): 59-66 Available from: URL: <http://www.wjgnet.com/2220-3176/full/v5/i4/59.htm> DOI: <http://dx.doi.org/10.5495/wjcid.v5.i4.59>

### INTRODUCTION

To date, the natural history of hepatitis C virus (HCV)

infection is still difficult to define because of the insidious onset of the disease and the absence or paucity of symptoms during the generally prolonged (20–40 years) chronic phase of the illness. Moreover, the methodologic differences used to study the clinical course of disease (prospective-retrospective cohort, community-based or cross-sectional studies), in addition to the different selected study populations (patients referring to specialist liver clinics or tertiary care centers, blood donors) or different phases of the disease, determine heterogeneous results in terms of rates of disease progression<sup>[1–6]</sup>. The ideal study to define the natural history of HCV infection would be to closely monitor a representative group of patients from the onset of the acute infection, refrain from treating their liver disease, and then monitor their untreated course to a liver disease end point and/or death, whether from liver disease or from other causes. Since such kind of study cannot be performed, reliance must be placed on surrogate markers of disease progression.

The long-term sequelae of HCV infection, include the transition from not perceivable acute to chronic hepatitis up to cirrhosis, which may progress to end-stage liver disease and/or to hepatocellular carcinoma (HCC), frequently leading to liver transplantation or death<sup>[7–11]</sup>. The recent Global Burden of Disease project estimated that in 2010, among 170 million people with chronic HCV, more than 30 million suffer from cirrhosis and the incidence of HCC is about 1–2 million new cases/year. The actual estimated incidence has been markedly decreased, and this is mainly attributable to the employment of a safe transfusion screening policy, which has markedly decreased the number of new infection. Several reports have identified that among persons with chronic hepatitis C (CHC), cirrhosis has developed in 20% and HCC in 1%–5%, approximately 20 years after disease onset. These data indicate that not all persons with CHC will develop cirrhosis or complications of the disease. The detection of specific markers, able to predict the progression of the disease, has been therefore the focus of this editorial.

## HCV INFECTION MARKERS AND RISK OF LIVER DISEASE PROGRESSION

A long-lasting elevated necroinflammatory activity seems to play a crucial role in the progression of the liver disease, as supported by data from patients with persistently normal transaminase levels<sup>[12–14]</sup>. The biochemistry profile is indeed only partially indicative to predict the disease's progression and the clinical outcome might be modified by different variables and many factors, either virus-related, host-related and environmental associated.

The role of viral-dependent factors as viral load and genotype is still debated and controversial. Several studies have evaluated the relationship between serum concentration of HCV-RNA and liver disease severity

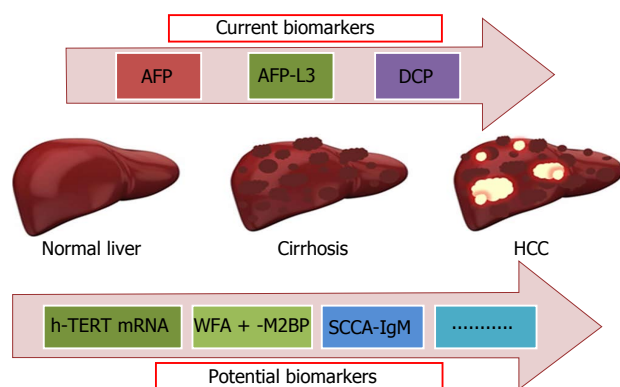
with conflicting results. While some reports have demonstrated a positive correlation between HCV-RNA load and histopathological abnormalities<sup>[15–17]</sup>, others have found no association with hepatic inflammation<sup>[18]</sup> or liver fibrosis<sup>[19,20]</sup>. These studies were conducted as cross-sectional design, resulting in limitations of casual temporality or including a particular type of person [*i.e.*, drug users coinfecting with human immunodeficiency virus (HIV)], thus the generalization of the obtained results to the population in the community was limited<sup>[21]</sup>.

Regardless from the evidence that genotype 1b was reported to be more associated with the development of HCC than genotype 2<sup>[22]</sup> and with a poor outcome of disease<sup>[22–24]</sup>, no data are available, so far, for other HCV genotypes. It should be noted that most of the studies were cross-sectional or included patients enrolled in clinical trials<sup>[25–29]</sup>. In a prospective study, Martinot-Peignoux *et al.*<sup>[25]</sup>, followed 163 patients with liver cirrhosis for seventeen years and reported that HCV genotype 1b was a major risk factor for HCC development. This genotype was confirmed to have three times higher risk of liver tumor development, compared with patients infected with other genotypes<sup>[26]</sup>. Within the community based study REVEAL-HCV study cohort, that recruited 1095 subjects seropositive for antibodies against HCV followed for fifteen years, the multivariate analysis selected serum HCV-RNA, alanine aminotransferase (ALT) levels and HCV genotype as independent risk predictors of HCC. These seromarkers have been proposed as pretreatment markers in clinical decision to classify high-risk patients who need particular clinical care<sup>[30]</sup>.

The presence of other viral coinfections (*i.e.*, HIV, HBV) speeds up the clinical course of the disease with a negative impact on the natural history of HCV<sup>[31,32]</sup>. The progression to cirrhosis is higher in HCV-HIV coinfecting patients<sup>[33,34]</sup> and is associated to other complications, such as hematologic disorders<sup>[35]</sup>, kidney disease<sup>[36]</sup>, cardiovascular disease<sup>[37]</sup> and neurologic abnormalities<sup>[38]</sup>. In addition, the coinfection with HBV determines a more severe liver injury in terms of progression of fibrosis, liver cirrhosis and hepatic decompensation<sup>[39,40]</sup>.

Among the host-related factors, age at infection, male gender and heavy alcohol intake seem to influence the outcome of CHC<sup>[41–43]</sup>. Freeman *et al.*<sup>[44]</sup>, using an ecologic analysis to estimate relative risk of cirrhosis progression across four study methodologies, have found an association between male sex (RR = 1.08) with heavy alcohol consumption (RR = 1.61) and elevated serum ALT levels (RR = 1.23) with higher histological activity index.

In the last few years, another important aspect, represented by the presence of comorbidities, was shown to impact on the evolution of chronic HCV infection. Hepatic steatosis, obesity and insulin resistance must be considered important determinants of liver disease progression, although the relationship between these metabolic factors and clinical outcomes is still complicated. In a long-term peginterferon treatment (HALT-C study) the modifiable risk factors for liver



**Figure 1** Current and newly proposed biomarkers of liver disease progression and hepatocellular carcinoma development. AFP:  $\alpha$ -fetoprotein; AFP-L3: *Lens culinaris* agglutinin A (LCA) - reactive fraction of  $\alpha$ -AFP; DCP: Des- $\gamma$ -carboxyprothrombin; h-TERTmRNA: Human telomerase reverse transcriptase mRNA; WFA + M2BP: *Wisteria floribunda* agglutinin-positive human Mac-2-binding protein; SCCA-IgM: Squamous cell carcinoma antigen-immunoglobulin M; HCC: Hepatocellular carcinoma.

disease progression were studied, and insulin resistance was found strongly associated with clinical outcomes<sup>[45]</sup>. Similar results were found in a large scale community-based study in which an association between diabetes and HCC was observed<sup>[46]</sup>. However, the association between HCV infection and the development of diabetes remains controversial<sup>[47,48]</sup>.

Major advances in genetics during the last decade allow the identification of specific markers associated with viral response and consequently with HCV infection outcomes. Among them, *IL28B* gene variants (*rs12979860* and *rs8099917*) have been strongly associated with favourable response to standard antiviral treatment in patients with CHC<sup>[49-51]</sup>. These treatment response findings were confirmed by many studies in different populations such as HCV-1 patients<sup>[52-57]</sup> and reproduced in several meta-analyses<sup>[58-60]</sup>. Therefore, both host and virus factors are important determinants of liver diseases outcome.

## NEW BIOMARKERS OF LIVER DISEASE PROGRESSION AND HCC DEVELOPMENT

The most relevant consequence of HCV infection is HCC development<sup>[61]</sup>. This primary liver tumor is the third leading cause of cancer deaths worldwide, with an incidence in United States more than doubled in the past 25 years<sup>[62]</sup>. Screening strategies for detection of early HCC have relied primarily on radiologic imaging<sup>[63,64]</sup> and serum biomarkers<sup>[65]</sup>. Over the past two decades, considerable number of studies have been published in order to identify suitable biomarkers, however the results are frequently contradictory. For this reason, tumor marker levels are not included in the screening recommendations of international guidelines<sup>[66,67]</sup>. In this setting,  $\alpha$ -fetoprotein (AFP) is the most commonly used biomarker, but its sensitivity and specificity in detecting HCC is poor. AFP levels are often increased in

patients with cirrhosis without HCC<sup>[68,69]</sup> and the positive rate of AFP in HCC is about 60%, making it a diagnostic limitation.

Other tumor biomarkers have been proposed to complement or replace AFP in HCC detection. In clinical practice, *Lens culinaris* agglutinin A - reactive fraction of  $\alpha$ -AFP ( $\alpha$ -AFP-L3), a glycoform of AFP<sup>[70,71]</sup>, and des- $\gamma$ -carboxyprothrombin (DCP), an abnormal prothrombin molecule generated by the absence of vitamin K responsible of an insufficient post-traslational carboxylation of the prothrombin precursor in malignant cells<sup>[72,73]</sup> have been used. These biomarkers represent independent tumor proteins and, as reported in several studies, they may be complementary in the detection of HCC<sup>[74-76]</sup>. Few prospective studies have been addressed to evaluate the usefulness of these new biomarkers in terms of prognosis. Sterling *et al*<sup>[77]</sup>, in an ancillary study of the prospective HALT-C trial including 855 patients, demonstrated that mild-moderate elevation of total AFP and DCP, but not AFP-L3 occurs in patients with CHC and advanced fibrosis in absence of HCC. However, marked increase of these biomarkers were uncommon in subjects without HCC, although several factors other than HCC, such as gender, age, race and the presence of more advanced liver disease, could be responsible for these increased values. Since sensitivity, specificity and predictive values of these biomarkers were low, the authors concluded that they are poor predictors of HCC development. More recently, the usefulness of these three biomarkers as diagnostic tool for HCC<sup>[78]</sup> and as predictors of outcome in patients with HCC has been reviewed. The combination of these biomarkers resulted to provide a good predictive ability of survival after diagnosis and when considered at time of diagnosis, together with serum albumin and bilirubin levels, they could be used for HCC staging and to predict HCC prognosis<sup>[79]</sup>.

Among the new class of genetic biomarkers, reflecting the presence of circulating HCC cells, serum h-TERT mRNA detection showed higher sensitivity and specificity compared with AFP-mRNA in HCC patients (90% and 85% vs 69% and 50%, respectively) and a close correlation with tumor size and number also in early tumor stage<sup>[80]</sup>. Circulating h-TERT mRNA was indeed detectable in small size tumors, indicating that h-TERT mRNA was up-regulated during rapid proliferation of the tumor, at the early phase of oncogenesis-differentiation.

In the last 10 years a large number of new molecules potentially clinically useful as markers of liver disease progression in HCV infected patients has been identified (Figure 1). One of them is *Wisteria floribunda* agglutinin-positive human Mac-2-binding protein (WFA + -M2BP), a liver fibrosis glyco-biomarker with a unique fibrosis-related glyco-alteration. Using fully automated immunoassay Yamasaki *et al*<sup>[81]</sup> tested serum samples of 707 patients infected with HCV and found increased serum WFA + -M2BP levels in parallel with the progression of liver fibrosis stage. In each distinctive



stage of fibrosis, the risk of HCC development was increased, according to elevation of WFA + -M2BP. The diagnostic performance of this protein, based on the AUROC values, was superior to that of AFP for predicting the development of HCC at 3, 5, and 7 years. The WFA + -M2BP values are proposed as noninvasive predictors of HCC development and could be considered a surrogate marker of liver fibrosis to be added to FibroScan technique.

Several studies have demonstrated in recent years that tumor released antigens can react with natural IgM class of immunoglobulins and form circulating immune complexes in different human tumors. The circulating immune complex composed of squamous cell carcinoma antigen (SCCA) linked with IgM (SCCA-IgM) has been recently discovered as a promising tool to identify patients with progressive liver disease in HCV infected patients. SCCA-IgM complexes were undetectable in the sera of healthy subjects, but the detection rates and the levels consistently increased with liver disease progression<sup>[82]</sup>. In another study, SCCA-IgM complexes were detectable in 33% of the patients with chronic hepatitis<sup>[83]</sup> and in this study a significant increase over time of the immune complex levels was observed in patients with significant increase of liver fibrosis within a time frame of four years, but not in those without histologic progression, suggesting that monitoring the immune complex over time allowed to identify patients at higher risk of cirrhosis progression. In agreement with these findings, significant decrease of the immune complex was observed in sera of patients with HCV infection and persistent virologic response to antiviral therapy<sup>[84,85]</sup>. The positivity of SCCA-IgM has been found correlated with histologic non-alcoholic steatohepatitis (NASH) in patients with CHC<sup>[86]</sup>. It is worth to note that NASH has been recognized as risk factor of liver disease worsening and of HCC development<sup>[87]</sup>. On the basis of these considerations, it is likely that patients with HCV infection and SCCA-IgM positivity present a more fibrogenic and tumorigenic liver condition that should be accurately monitored and therapeutically managed, if possible.

In line with these results, the behaviour profile of SCCA-IgM was different in patient with early HCV-related cirrhosis with or without HCC progression. In a longitudinal, retrospective study a progressive increase of this immune complex was described in the majority of the patients with histological diagnosis of cirrhosis C who developed HCC after at least one year from the end of the study, while the levels of this biomarker remained unchanged or decreased in the majority of the patients without evidence of HCC development during the same time interval. Conversely, the increase of AFP, which was chosen as reference biomarker, was not significantly different between the two groups. The diagnostic accuracy, measured as AUROC values, was higher for SCCA-IgM than for AFP and the former biomarker performed better to identify cirrhotic patients at higher risk of HCC development<sup>[88]</sup>. This behaviour

was observed at least one year before clinical diagnosis of HCC, suggesting that this preclinical phase might become a suitable window to specifically address new potentially effective therapies. A multicenter study, performed in HCV infected patients with overt cirrhosis, started from an opposite approach and demonstrated that the SCCA-IgM value  $\leq 200$  AU/mL accurately identifies patients with low risk of HCC development in the subsequent year (sensitivity 75%, specificity 62%). On the basis of the obtained results the authors concluded that this biomarker might be utilized to tailor surveillance timing<sup>[89]</sup>.

## CONCLUSION

Identifying new factors that could influence clinical outcome of HCV infection is important in order to counsel individuals regarding prognosis and to facilitate decisions related to clinical management. This point is crucial mostly in the current scenarios of new antiviral treatments that include various direct acting antiviral drugs<sup>[90,91]</sup>. Given the high cost of treatment and the increased possibility of adverse events, identification of factors predicting sustained virological response to individualize HCV therapy in clinical decision-making is urgent. While prioritizing treatment to patients who are at risk of future problems seems the optimal solution to deliver most benefits at the lowest costs, the problem still lies in the identification of those patients who should be included in the target population<sup>[92]</sup>. In this context, the above new biomarkers might become useful tools, as part of personalized medicine, for the surveillance of HCV infected patients with chronic hepatitis and/or cirrhosis, in order to better define follow up timing and suitable therapeutic management of the patients at higher risk of liver disease worsening.

## REFERENCES

- 1 **Alberti A**, Chemello L, Benvegñù L. Natural history of hepatitis C. *J Hepatol* 1999; **31** Suppl 1: 17-24 [PMID: 10622555]
- 2 **Mattsson L**, Sönnernborg A, Weiland O. Outcome of acute symptomatic non-A, non-B hepatitis: a 13-year follow-up study of hepatitis C virus markers. *Liver* 1993; **13**: 274-278 [PMID: 7505044]
- 3 **Hopf U**, Möller B, Küther D, Stemerowicz R, Lobeck H, Lütke-Handjery A, Walter E, Blum HE, Roggendorf M, Deinhardt F. Long-term follow-up of posttransfusion and sporadic chronic hepatitis non-A, non-B and frequency of circulating antibodies to hepatitis C virus (HCV). *J Hepatol* 1990; **10**: 69-76 [PMID: 2106548]
- 4 **Tremolada F**, Casarin C, Alberti A, Drago C, Tagger A, Ribero ML, Realdi G. Long-term follow-up of non-A, non-B (type C) post-transfusion hepatitis. *J Hepatol* 1992; **16**: 273-281 [PMID: 1487603]
- 5 **Rai R**, Wilson LE, Astemborski J, Anania F, Torbenson M, Spoler C, Vlahov D, Strathdee SA, Boitnott J, Nelson KE, Thomas DL. Severity and correlates of liver disease in hepatitis C virus-infected injection drug users. *Hepatology* 2002; **35**: 1247-1255 [PMID: 11981775]
- 6 **Niedermaier C**, Lange S, Heintges T, Erhardt A, Buschkamp M, Hürter D, Nawrocki M, Kruska L, Hensel F, Petry W, Häussinger D. Prognosis of chronic hepatitis C: results of a large, prospective

- cohort study. *Hepatology* 1998; **28**: 1687-1695 [PMID: 9828236]
- 7 **Fattovich G**, Pantalena M, Zagni I, Realdi G, Schalm SW, Christensen E. Effect of hepatitis B and C virus infections on the natural history of compensated cirrhosis: a cohort study of 297 patients. *Am J Gastroenterol* 2002; **97**: 2886-2895 [PMID: 12425564 DOI: 10.1111/j.1572-0241.2002.07057]
- 8 **Benvegnù L**, Gios M, Boccato S, Alberti A. Natural history of compensated viral cirrhosis: a prospective study on the incidence and hierarchy of major complications. *Gut* 2004; **53**: 744-749 [PMID: 15082595]
- 9 **Serfaty L**, Aumaître H, Chazouillères O, Bonnand AM, Rosmorduc O, Poupon RE, Poupon R. Determinants of outcome of compensated hepatitis C virus-related cirrhosis. *Hepatology* 1998; **27**: 1435-1440 [PMID: 9581703]
- 10 **Fattovich G**, Giustina G, Degos F, Tremolada F, Diodati G, Almasio P, Nevens F, Solinas A, Mura D, Brouwer JT, Thomas H, Njapoum C, Casarin C, Bonetti P, Fuschi P, Basho J, Tocco A, Bhalla A, Galassini R, Noventa F, Schalm SW, Realdi G. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology* 1997; **112**: 463-472 [PMID: 9024300]
- 11 **Perz JF**, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol* 2006; **45**: 529-538 [PMID: 16879891]
- 12 **Persico M**, Persico E, Suozzo R, Conte S, De Seta M, Coppola L, Palmentieri B, Sasso FC, Torella R. Natural history of hepatitis C virus carriers with persistently normal aminotransferase levels. *Gastroenterology* 2000; **118**: 760-764 [PMID: 10734027]
- 13 **Rossi S**, De Filippi F, Saibeni S, Persico M, Bollani S, Camera A, Rizzolo L, Maria Croce A, Bruno S. A 15-Yr Prospective Histological Follow-Up Study in Patients With Persistently Normal Aminotransferase Levels (PNAL) Carrying HCV Infection. *Am J Gastroenterol* 2007; **102**: 2604-2606 [PMID: 17958768]
- 14 **Rumi MG**, De Filippi F, Donato MF, Del Ninno E, Colombo M. Progressive hepatic fibrosis in healthy carriers of hepatitis C virus with a transaminase breakthrough. *J Viral Hepat* 2002; **9**: 71-74 [PMID: 11851905]
- 15 **Fanning L**, Kenny E, Sheehan M, Cannon B, Whelton M, O'Connell J, Collins JK, Shanahan F. Viral load and clinicopathological features of chronic hepatitis C (1b) in a homogeneous patient population. *Hepatology* 1999; **29**: 904-907 [PMID: 10051496]
- 16 **Lau JY**, Davis GL, Kniffen J, Qian KP, Urdea MS, Chan CS, Mizokami M, Neuwald PD, Wilber JC. Significance of serum hepatitis C virus RNA levels in chronic hepatitis C. *Lancet* 1993; **341**: 1501-1504 [PMID: 8099380]
- 17 **Naito M**, Hayashi N, Hagiwara H, Hiramatsu N, Kasahara A, Fusamoto H, Kamada T. Serum hepatitis C virus RNA quantity and histological features of hepatitis C virus carriers with persistently normal ALT levels. *Hepatology* 1994; **19**: 871-875 [PMID: 8138259]
- 18 **De Moliner L**, Pontisso P, De Salvo GL, Cavalletto L, Chemello L, Alberti A. Serum and liver HCV RNA levels in patients with chronic hepatitis C: correlation with clinical and histological features. *Gut* 1998; **42**: 856-860 [PMID: 9691926]
- 19 **Duvoux C**, Pawlotsky JM, Bastie A, Cherqui D, Soussy CJ, Dhumeaux D. Low HCV replication levels in end-stage hepatitis C virus-related liver disease. *J Hepatol* 1999; **31**: 593-597 [PMID: 10551380]
- 20 **Lagging LM**, Garcia CE, Westin J, Wejstål R, Norkrans G, Dhillon AP, Lindh M. Comparison of serum hepatitis C virus RNA and core antigen concentrations and determination of whether levels are associated with liver histology or affected by specimen storage time. *J Clin Microbiol* 2002; **40**: 4224-4229 [PMID: 12409402]
- 21 **Hisada M**, Chatterjee N, Kalaylioglu Z, Battjes RJ, Goedert JJ. Hepatitis C virus load and survival among injection drug users in the United States. *Hepatology* 2005; **42**: 1446-1452 [PMID: 16317675 DOI: 10.1002/hep.20938]
- 22 **Raimondi S**, Bruno S, Mondelli MU, Maisonneuve P. Hepatitis C virus genotype 1b as a risk factor for hepatocellular carcinoma development: a meta-analysis. *J Hepatol* 2009; **50**: 1142-1154 [PMID: 19395111 DOI: 10.1016/j.jhep.2009.01.019]
- 23 **Bruno S**, Zuin M, Crosignani A, Rossi S, Zadra F, Roffi L, Borzio M, Redaelli A, Chiesa A, Silini EM, Almasio PL, Maisonneuve P. Predicting mortality risk in patients with compensated HCV-induced cirrhosis: a long-term prospective study. *Am J Gastroenterol* 2009; **104**: 1147-1158 [PMID: 19352340 DOI: 10.1038/ajg.2009.31]
- 24 **Bruno S**, Crosignani A, Faccioto C, Rossi S, Roffi L, Redaelli A, de Franchis R, Almasio PL, Maisonneuve P. Sustained virologic response prevents the development of esophageal varices in compensated, Child-Pugh class A hepatitis C virus-induced cirrhosis. A 12-year prospective follow-up study. *Hepatology* 2010; **51**: 2069-2076 [PMID: 20196120 DOI: 10.1002/hep.23528]
- 25 **Martinot-Peignoux M**, Roudot-Thoraval F, Mendel I, Coste J, Izopet J, Duverlie G, Payan C, Pawlotsky JM, Defer C, Bogard M, Gerolami V, Halfon P, Buisson Y, Fouqueray B, Loiseau P, Lamoril J, Lefrere JJ, Marcellin P. Hepatitis C virus genotypes in France: relationship with epidemiology, pathogenicity and response to interferon therapy. The GEMHEP. *J Viral Hepat* 1999; **6**: 435-443 [PMID: 10607261]
- 26 **Bruno S**, Crosignani A, Maisonneuve P, Rossi S, Silini E, Mondelli MU. Hepatitis C virus genotype 1b as a major risk factor associated with hepatocellular carcinoma in patients with cirrhosis: a seventeen-year prospective cohort study. *Hepatology* 2007; **46**: 1350-1356 [PMID: 17680653 DOI: 10.1002/hep.21826]
- 27 **Fattovich G**, Ribero ML, Pantalena M, Diodati G, Almasio P, Nevens F, Tremolada F, Degos F, Rai J, Solinas A, Mura D, Tocco A, Zagni I, Fabris F, Lomonaco L, Noventa F, Realdi G, Schalm SW, Tagger A. Hepatitis C virus genotypes: distribution and clinical significance in patients with cirrhosis type C seen at tertiary referral centres in Europe. *J Viral Hepat* 2001; **8**: 206-216 [PMID: 11380799]
- 28 **Kobayashi M**, Tanaka E, Sodeyama T, Urushihara A, Matsumoto A, Kiyosawa K. The natural course of chronic hepatitis C: a comparison between patients with genotypes 1 and 2 hepatitis C viruses. *Hepatology* 1996; **23**: 695-699 [PMID: 8666319]
- 29 **Silini E**, Bottelli R, Asti M, Bruno S, Candusso ME, Brambilla S, Bono F, Iamoni G, Tinelli C, Mondelli MU, Ideo G. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a case-control study. *Gastroenterology* 1996; **111**: 199-205 [PMID: 8698200]
- 30 **Lee MH**, Yang HI, Yuan Y, L'Italien G, Chen CJ. Epidemiology and natural history of hepatitis C virus infection. *World J Gastroenterol* 2014; **20**: 9270-9280 [PMID: 25071320 DOI: 10.3748/wjg.v20.i28.9270]
- 31 **Soto B**, Sánchez-Quijano A, Rodrigo L, del Olmo JA, García-Bengoechea M, Hernández-Quero J, Rey C, Abad MA, Rodríguez M, Sales Gilabert M, González F, Mirón P, Caruz A, Relimpio F, Torronteras R, Leal M, Lissen E. Human immunodeficiency virus infection modifies the natural history of chronic parenterally-acquired hepatitis C with an unusually rapid progression to cirrhosis. *J Hepatol* 1997; **26**: 1-5 [PMID: 9147999]
- 32 **Pontisso P**, Gerotto M, Benvegnù L, Chemello L, Alberti A. Coinfection by hepatitis B virus and hepatitis C virus. *Antivir Ther* 1998; **3**: 137-142 [PMID: 10726063]
- 33 **Kirk GD**, Lesi OA, Mendy M, Akano AO, Sam O, Goedert JJ, Hainaut P, Hall AJ, Whittle H, Montesano R. The Gambia Liver Cancer Study: Infection with hepatitis B and C and the risk of hepatocellular carcinoma in West Africa. *Hepatology* 2004; **39**: 211-219 [PMID: 14752840 DOI: 10.1002/hep.20027]
- 34 **Thompson MA**, Mugavero MJ, Amico KR, Cargill VA, Chang LW, Gross R, Orrell C, Altice FL, Bangsberg DR, Bartlett JG, Beckwith CG, Dowshen N, Gordon CM, Horn T, Kumar P, Scott JD, Stirratt MJ, Remien RH, Simoni JM, Nachega JB. Guidelines for improving entry into and retention in care and antiretroviral adherence for persons with HIV: evidence-based recommendations from an International Association of Physicians in AIDS Care panel. *Ann Intern Med* 2012; **156**: 817-833, W-284, W-285, W-286, W-287, W-288, W-289, W-290, W-291, W-292, W-293, W-294 [PMID: 22393036 DOI: 10.7326/0003-4819-156-11-201206050-0

- 0419]
- 35 **Lapinski TW**, Parfieniuk A, Rogalska-Plonska M, Czajkowska J, Flisiak R. Prevalence of cryoglobulinaemia in hepatitis C virus- and hepatitis C virus/human immunodeficiency virus-infected individuals: implications for renal function. *Liver Int* 2009; **29**: 1158-1161 [PMID: 19602133 DOI: 10.1111/j.1478-3231.2009.02052.x]
- 36 **Wyatt CM**, Malvestutto C, Coca SG, Klotman PE, Parikh CR. The impact of hepatitis C virus coinfection on HIV-related kidney disease: a systematic review and meta-analysis. *AIDS* 2008; **22**: 1799-1807 [PMID: 18753863 DOI: 10.1097/QAD.0b013e32830e0152]
- 37 **Bedimo R**, Westfall AO, Mugavero M, Drechsler H, Khanna N, Saag M. Hepatitis C virus coinfection and the risk of cardiovascular disease among HIV-infected patients. *HIV Med* 2010; **11**: 462-468 [PMID: 20163481 DOI: 10.1111/j.1468-1293.2009.00815.x]
- 38 **Aronow HA**, Weston AJ, Pezeshki BB, Lazarus TS. Effects of coinfection with HIV and hepatitis C virus on the nervous system. *AIDS Read* 2008; **18**: 43-48 [PMID: 18240452]
- 39 **Zarski JP**, Bohn B, Bastie A, Pawlotsky JM, Baud M, Bost-Bezeaux F, Tran van Nhieu J, Seigneurin JM, Buffet C, Dhumeaux D. Characteristics of patients with dual infection by hepatitis B and C viruses. *J Hepatol* 1998; **28**: 27-33 [PMID: 9537860]
- 40 **Fattovich G**, Tagger A, Brollo L, Giustina G, Pontisso P, Realdi G, Alberti A, Ruol A. Hepatitis C virus infection in chronic hepatitis B virus carriers. *J Infect Dis* 1991; **163**: 400-402 [PMID: 1846394]
- 41 **Minola E**, Prati D, Suter F, Maggiolo F, Caprioli F, Sonzogni A, Fraquelli M, Paggi S, Conte D. Age at infection affects the long-term outcome of transfusion-associated chronic hepatitis C. *Blood* 2002; **99**: 4588-4591 [PMID: 12036892 DOI: 10.1182/blood-2001-12-0192]
- 42 **Wiley TE**, McCarthy M, Breidi L, McCarthy M, Layden TJ. Impact of alcohol on the histological and clinical progression of hepatitis C infection. *Hepatology* 1998; **28**: 805-809 [PMID: 9731576]
- 43 **Powell EE**, Edwards-Smith CJ, Hay JL, Clouston AD, Crawford DH, Shorthouse C, Purdie DM, Jonsson JR. Host genetic factors influence disease progression in chronic hepatitis C. *Hepatology* 2000; **31**: 828-833 [PMID: 10733535]
- 44 **Freeman AJ**, Law MG, Kaldor JM, Dore GJ. Predicting progression to cirrhosis in chronic hepatitis C virus infection. *J Viral Hepat* 2003; **10**: 285-293 [PMID: 12823595]
- 45 **Everhart JE**, Lok AS, Kim HY, Morgan TR, Lindsay KL, Chung RT, Bonkovsky HL, Ghany MG. Weight-related effects on disease progression in the hepatitis C antiviral long-term treatment against cirrhosis trial. *Gastroenterology* 2009; **137**: 549-557 [PMID: 19445938 DOI: 10.1053/j.gastro.2009.05.007]
- 46 **Davila JA**, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. *Gut* 2005; **54**: 533-539 [PMID: 15753540]
- 47 **Ruhl CE**, Menke A, Cowie CC, Everhart JE. Relationship of hepatitis C virus infection with diabetes in the U.S. population. *Hepatology* 2014; **60**: 1139-1149 [PMID: 24500979 DOI: 10.1002/hep.27047]
- 48 **Mason AL**, Lau JY, Hoang N, Qian K, Alexander GJ, Xu L, Guo L, Jacob S, Regenstein FG, Zimmerman R, Everhart JE, Wasserfall C, Maclaren NK, Perrillo RP. Association of diabetes mellitus and chronic hepatitis C virus infection. *Hepatology* 1999; **29**: 328-333 [PMID: 9918906]
- 49 **Suppiah V**, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; **41**: 1100-1104 [PMID: 19749758 DOI: 10.1038/ng.447]
- 50 **Tanaka Y**, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**: 1105-1109 [PMID: 19749757 DOI: 10.1038/ng.449]
- 51 **Ge D**, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399-401 [PMID: 19684573 DOI: 10.1038/nature08309]
- 52 **Thompson AJ**, Muir AJ, Sulkowski MS, Ge D, Fellay J, Shianna KV, Urban T, Afdhal NH, Jacobson IM, Esteban R, Poordad F, Lawitz EJ, McCone J, Shiffman ML, Galler GW, Lee WM, Reindollar R, King JW, Kwo PY, Ghalib RH, Freilich B, Nyberg LM, Zeuzem S, Poynard T, Vock DM, Pieper KS, Patel K, Tillmann HL, Novello S, Koury K, Pedicone LD, Brass CA, Albrecht JK, Goldstein DB, McHutchison JG. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. *Gastroenterology* 2010; **139**: 120-129.e18 [PMID: 20399780 DOI: 10.1053/j.gastro.2010.04.013]
- 53 **McCarthy JJ**, Li JH, Thompson A, Suchindran S, Lao XQ, Patel K, Tillmann HL, Muir AJ, McHutchison JG. Replicated association between an IL28B gene variant and a sustained response to pegylated interferon and ribavirin. *Gastroenterology* 2010; **138**: 2307-2314 [PMID: 20176026 DOI: 10.1053/j.gastro.2010.02.009]
- 54 **Montes-Cano MA**, García-Lozano JR, Abad-Molina C, Romero-Gómez M, Barroso N, Aguilar-Reina J, Núñez-Roldán A, González-Escribano MF. Interleukin-28B genetic variants and hepatitis virus infection by different viral genotypes. *Hepatology* 2010; **52**: 33-37 [PMID: 20578254 DOI: 10.1002/hep.23624]
- 55 **Hayes CN**, Kobayashi M, Akuta N, Suzuki F, Kumada H, Abe H, Miki D, Imamura M, Ochi H, Kamatani N, Nakamura Y, Chayama K. HCV substitutions and IL28B polymorphisms on outcome of peg-interferon plus ribavirin combination therapy. *Gut* 2011; **60**: 261-267 [PMID: 21068134 DOI: 10.1136/gut.2010.223495]
- 56 **Venegas M**, Villanueva RA, González K, Brahm J. IL28B polymorphisms associated with therapy response in Chilean chronic hepatitis C patients. *World J Gastroenterol* 2011; **17**: 3636-3639 [PMID: 21987611 DOI: 10.3748/wjg.v17.i31.3636]
- 57 **Cieřla A**, Bocięga-Jasik M, Sobczyk-Krupiarz I, Głowacki MK, Owczarek D, Cibor D, Sanak M, Mach T. IL28B polymorphism as a predictor of antiviral response in chronic hepatitis C. *World J Gastroenterol* 2012; **18**: 4892-4897 [PMID: 23002361 DOI: 10.3748/wjg.v18.i35.4892]
- 58 **Jia Z**, Ding Y, Tian S, Niu J, Jiang J. Test of IL28B polymorphisms in chronic hepatitis C patients treated with PegIFN and ribavirin depends on HCV genotypes: results from a meta-analysis. *PLoS One* 2012; **7**: e45698 [PMID: 23029188 DOI: 10.1371/journal.pone.0045698]
- 59 **Luo Y**, Jin C, Ling Z, Mou X, Zhang Q, Xiang C. Association study of IL28B: rs12979860 and rs8099917 polymorphisms with SVR in patients infected with chronic HCV genotype 1 to PEG-IFN/RBV therapy using systematic meta-analysis. *Gene* 2013; **513**: 292-296 [PMID: 23142377 DOI: 10.1016/j.gene.2012.10.030]
- 60 **Jiménez-Sousa MA**, Fernández-Rodríguez A, Guzmán-Fulgencio M, García-Álvarez M, Resino S. Meta-analysis: implications of interleukin-28B polymorphisms in spontaneous and treatment-related clearance for patients with hepatitis C. *BMC Med* 2013; **11**: 6 [PMID: 23298311 DOI: 10.1186/1741-7015-11-6]
- 61 **El-Serag HB**. Hepatocellular carcinoma: recent trends in the United States. *Gastroenterology* 2004; **127**: S27-S34 [PMID: 15508094]
- 62 **Everhart JE**, Ruhl CE. Burden of digestive diseases in the United States Part III: Liver, biliary tract, and pancreas. *Gastroenterology* 2009; **136**: 1134-1144 [PMID: 19245868 DOI: 10.1053/j.gastro.2009.02.038]
- 63 **Ikeda K**, Saitoh S, Koida I, Tsubota A, Arase Y, Chayama K, Kumada H. Imaging diagnosis of small hepatocellular carcinoma.



- Hepatology 1994; **20**: 82-87 [PMID: 8020908]
- 64 **Takayasu K**, Furukawa H, Wakao F, Muramatsu Y, Abe H, Terauchi T, Winter TC, Sakamoto M, Hirohashi S. CT diagnosis of early hepatocellular carcinoma: sensitivity, findings, and CT-pathologic correlation. *AJR Am J Roentgenol* 1995; **164**: 885-890 [PMID: 7726041 DOI: 10.2214/ajr.164.4.7726041]
- 65 **Oka H**, Tamori A, Kuroki T, Kobayashi K, Yamamoto S. Prospective study of alpha-fetoprotein in cirrhotic patients monitored for development of hepatocellular carcinoma. *Hepatology* 1994; **19**: 61-66 [PMID: 7506227]
- 66 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
- 67 **European Association For The Study Of The Liver**, European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]
- 68 **Bayati N**, Silverman AL, Gordon SC. Serum alpha-fetoprotein levels and liver histology in patients with chronic hepatitis C. *Am J Gastroenterol* 1998; **93**: 2452-2456 [PMID: 9860408]
- 69 **Di Bisceglie AM**, Sterling RK, Chung RT, Everhart JE, Dienstag JL, Bonkovsky HL, Wright EC, Everson GT, Lindsay KL, Lok AS, Lee WM, Morgan TR, Ghany MG, Gretch DR. Serum alpha-fetoprotein levels in patients with advanced hepatitis C: results from the HALT-C Trial. *J Hepatol* 2005; **43**: 434-441 [PMID: 16136646]
- 70 **Oka H**, Saito A, Ito K, Kumada T, Satomura S, Kasugai H, Osaki Y, Seki T, Kudo M, Tanaka M. Multicenter prospective analysis of newly diagnosed hepatocellular carcinoma with respect to the percentage of Lens culinaris agglutinin-reactive alpha-fetoprotein. *J Gastroenterol Hepatol* 2001; **16**: 1378-1383 [PMID: 11851836]
- 71 **Taketa K**, Endo Y, Sekiya C, Tanikawa K, Koji T, Taga H, Satomura S, Matsuura S, Kawai T, Hirai H. A collaborative study for the evaluation of lectin-reactive alpha-fetoproteins in early detection of hepatocellular carcinoma. *Cancer Res* 1993; **53**: 5419-5423 [PMID: 7693340]
- 72 **Mita Y**, Aoyagi Y, Yanagi M, Suda T, Suzuki Y, Asakura H. The usefulness of determining des-gamma-carboxy prothrombin by sensitive enzyme immunoassay in the early diagnosis of patients with hepatocellular carcinoma. *Cancer* 1998; **82**: 1643-1648 [PMID: 9576283]
- 73 **Ishii M**, Gama H, Chida N, Ueno Y, Shinzawa H, Takagi T, Toyota T, Takahashi T, Kasukawa R. Simultaneous measurements of serum alpha-fetoprotein and protein induced by vitamin K absence for detecting hepatocellular carcinoma. South Tohoku District Study Group. *Am J Gastroenterol* 2000; **95**: 1036-1040 [PMID: 10763956]
- 74 **Marrero JA**, Feng Z, Wang Y, Nguyen MH, Befeler AS, Roberts LR, Reddy KR, Harnois D, Llovet JM, Normolle D, Dalhgren J, Chia D, Lok AS, Wagner PD, Srivastava S, Schwartz M. Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. *Gastroenterology* 2009; **137**: 110-118 [PMID: 19362088 DOI: 10.1053/j.gastro.2009.04.005]
- 75 **Volk ML**, Hernandez JC, Su GL, Lok AS, Marrero JA. Risk factors for hepatocellular carcinoma may impair the performance of biomarkers: a comparison of AFP, DCP, and AFP-L3. *Cancer Biomark* 2007; **3**: 79-87 [PMID: 17522429]
- 76 **Lok AS**, Sterling RK, Everhart JE, Wright EC, Hoefs JC, Di Bisceglie AM, Morgan TR, Kim HY, Lee WM, Bonkovsky HL, Dienstag JL. Des-gamma-carboxy prothrombin and alpha-fetoprotein as biomarkers for the early detection of hepatocellular carcinoma. *Gastroenterology* 2010; **138**: 493-502 [PMID: 19852963 DOI: 10.1053/j.gastro.2009.10.031]
- 77 **Sterling RK**, Wright EC, Morgan TR, Seeff LB, Hoefs JC, Di Bisceglie AM, Dienstag JL, Lok AS. Frequency of elevated hepatocellular carcinoma (HCC) biomarkers in patients with advanced hepatitis C. *Am J Gastroenterol* 2012; **107**: 64-74 [PMID: 21931376 DOI: 10.1038/ajg.2011.312]
- 78 **Johnson PJ**, Pirrie SJ, Cox TF, Berhane S, Teng M, Palmer D, Morse J, Hull D, Patman G, Kagebayashi C, Hussain S, Graham J, Reeves H, Satomura S. The detection of hepatocellular carcinoma using a prospectively developed and validated model based on serological biomarkers. *Cancer Epidemiol Biomarkers Prev* 2014; **23**: 144-153 [PMID: 24220911 DOI: 10.1158/1055-9965.EPI-13-0870]
- 79 **Toyoda H**, Kumada T, Tada T, Sone Y, Kaneoka Y, Maeda A. Tumor Markers for Hepatocellular Carcinoma: Simple and Significant Predictors of Outcome in Patients with HCC. *Liver Cancer* 2015; **4**: 126-136 [PMID: 26020034 DOI: 10.1159/000367735]
- 80 **Miura N**, Osaki Y, Nagashima M, Kohno M, Yoroze K, Shomori K, Kanbe T, Oyama K, Kishimoto Y, Maruyama S, Noma E, Horie Y, Kudo M, Sakaguchi S, Hirooka Y, Ito H, Kawasaki H, Hasegawa J, Shiota G. A novel biomarker TERTmRNA is applicable for early detection of hepatoma. *BMC Gastroenterol* 2010; **10**: 46 [PMID: 20482774 DOI: 10.1186/1471-230X-10-46]
- 81 **Yamasaki K**, Tateyama M, Abiru S, Komori A, Nagaoka S, Saeki A, Hashimoto S, Sasaki R, Bekki S, Kugiyama Y, Miyazoe Y, Kuno A, Korenaga M, Togayachi A, Ocho M, Mizokami M, Narimatsu H, Yatsushashi H. Elevated serum levels of Wisteria floribunda agglutinin-positive human Mac-2 binding protein predict the development of hepatocellular carcinoma in hepatitis C patients. *Hepatology* 2014; **60**: 1563-1570 [PMID: 25042054 DOI: 10.1002/hep.27305]
- 82 **Beneduce L**, Castaldi F, Marino M, Quarta S, Ruvoletto M, Benvegnù L, Calabrese F, Gatta A, Pontisso P, Fassina G. Squamous cell carcinoma antigen-immunoglobulin M complexes as novel biomarkers for hepatocellular carcinoma. *Cancer* 2005; **103**: 2558-2565 [PMID: 15887222 DOI: 10.1002/ncr.21106]
- 83 **Biassiolo A**, Chemello L, Quarta S, Cavalletto L, Bortolotti F, Bernardotto C, Beneduce L, Bernardinello E, Tono N, Fassina G, Gatta A, Pontisso P. Monitoring SCCA-IgM complexes in serum predicts liver disease progression in patients with chronic hepatitis. *J Viral Hepat* 2008; **15**: 246-249 [PMID: 18248333 DOI: 10.1111/j.1365-2893.2007.00935.x]
- 84 **Giannini EG**, Basso M, Bazzica M, Contini P, Marengo S, Savarino V, Picciotto A. Successful antiviral therapy determines a significant decrease in squamous cell carcinoma antigen-associated (SCCA) variants' serum levels in anti-HCV positive cirrhotic patients. *J Viral Hepat* 2010; **17**: 563-568 [PMID: 19840364 DOI: 10.1111/j.1365-2893.2009.01217.x]
- 85 **Fransvea E**, Trerotoli P, Sacco R, Bernabucci V, Milella M, Napoli N, Mazzocca A, Renna E, Quaranta M, Angarano G, Villa E, Antonaci S, Giannelli G. SCCA-IC serum levels are predictive of clinical response in HCV chronic hepatitis to antiviral therapy: a multicentric prospective study. *J Viral Hepat* 2012; **19**: 704-710 [PMID: 22967101 DOI: 10.1111/j.1365-2893.2012.01604.x]
- 86 **Martini A**, Fattovich G, Guido M, Bugianesi E, Biassiolo A, Ieluzzi D, Gallotta A, Fassina G, Merkel C, Gatta A, Negro F, Pontisso P. HCV genotype 3 and squamous cell carcinoma antigen (SCCA)-IgM are independently associated with histological features of NASH in HCV-infected patients. *J Viral Hepat* 2015; **22**: 800-808 [PMID: 25611978 DOI: 10.1111/jvh.12394]
- 87 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; **55**: 2005-2023 [PMID: 22488764 DOI: 10.1002/hep.25762]
- 88 **Pontisso P**, Quarta S, Caberlotto C, Beneduce L, Marino M, Bernardinello E, Tono N, Fassina G, Cavalletto L, Gatta A, Chemello L. Progressive increase of SCCA-IgM immune complexes in cirrhotic patients is associated with development of hepatocellular carcinoma. *Int J Cancer* 2006; **119**: 735-740 [PMID: 16550605 DOI: 10.1002/ijc.21908]
- 89 **Buccione D**, Fatti G, Gallotta A, Loggi E, Di Donato R, Testa L, Saitta C, Santi V, Di Micoli A, Erroi V, Frigerio M, Fazio V, Picciotto A, Biassiolo A, Degos F, Pontisso P, Raimondo G,



- Trevisani F. Serum Scca-IgM as a predictor of hepatocellular carcinoma in patients with liver cirrhosis. *OJGas* 2012; **2**: 56-61 [DOI: 10.4236/ojgas.2012.22012]
- 90 **Jang JY**, Shao RX, Lin W, Weinberg E, Chung WJ, Tsai WL, Zhao H, Goto K, Zhang L, Mendez-Navarro J, Jilg N, Peng LF, Brockman MA, Chung RT. HIV infection increases HCV-induced hepatocyte apoptosis. *J Hepatol* 2011; **54**: 612-620 [PMID: 21146890 DOI: 10.1016/j.jhep.2010.07.042]
- 91 **Welsch C**, Jesudian A, Zeuzem S, Jacobson I. New direct-acting antiviral agents for the treatment of hepatitis C virus infection and perspectives. *Gut* 2012; **61** Suppl 1: i36-i46 [PMID: 22504918 DOI: 10.1136/gutjnl-2012-302144]
- 92 **Lutchman G**, Kim WR. A glass half full: Implications of screening for hepatitis C virus in the era of highly effective antiviral therapy. *Hepatology* 2015; **61**: 1455-1458 [PMID: 25614010 DOI: 10.1002/hep.27718]

**P- Reviewer:** Guan YS, Valenti L  
**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Li D



## Interplay between rabies virus and the mammalian immune system

Nicholas Johnson, Adam F Cunningham

Nicholas Johnson, Animal and Plant Health Agency, Surrey KT15 3NB, United Kingdom

Adam F Cunningham, Institute of Microbiology and Infection, School of Immunity and Infection, University of Birmingham, Birmingham BT15 2TT, United Kingdom

Author contributions: Both authors wrote the paper.

Supported by The European Union Seventh Framework Programme through project ANTIGONE: Anticipating the global onset of novel epidemics, No. 278976.

Conflict-of-interest statement: The authors declare that no competing interest exists.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Nicholas Johnson, PhD, Animal and Plant Health Agency, Woodham Lane, Surrey KT15 3NB, United Kingdom. [nick.johnson@apha.gsi.gov.uk](mailto:nick.johnson@apha.gsi.gov.uk)  
Telephone: +44-19-32357724  
Fax: +44-19-32357237

Received: June 2, 2015

Peer-review started: June 3, 2015

First decision: August 8, 2015

Revised: September 23, 2015

Accepted: November 13, 2015

Article in press: November 17, 2015

Published online: November 25, 2015

### Abstract

Rabies is a disease caused following infection of the brain

by the rabies virus (RABV). The principle mechanism of transmission is through a bite wound. The virus infects peripheral nerves and moves to the central nervous system (CNS). There appears to be little involvement of other organ systems and little detectable immune stimulation prior to infection of the CNS. This failure of the mammalian immune system to respond to rabies virus infection leads, in the overwhelming majority of cases, to death of the host. To some extent, this failure is likely due to the exclusive replication of RABV in neurons and the limited ability to generate, sufficiently rapidly, an anti-viral antibody response *in situ*. This is reflected in the ability of post-exposure vaccination, when given early after infection, to prevent disease. The lack of immune stimulation during RABV infection preceding neural invasion is the Achilles heel of the immune response. Whilst many viruses infect the brain, causing encephalitis and neuronal deficit, none are as consistently fatal to the host as RABV. This is in part due to prior replication of many viruses in peripheral, non-neural tissue by other viruses that allows timely activation of the immune response before the host is overwhelmed. Our current understanding of the correlates of protection for rabies suggests that it is the action of neutralising antibodies that prevent infection and control spread of RABV. Furthermore, it tells us that the induction of immunity can protect and understanding how and why this happens is critical to controlling infection. However, the paradigm of antibody development suggests that antigen presentation overwhelmingly occurs in lymphoid tissue (germinal and non-germinal centres) and these are external to the CNS. In addition, the blood-brain-barrier may provide a block to the delivery of immune effectors (antibodies/plasma B-cells) entering where they are needed. Alternatively, there may be insufficient antigen exposure after natural infection to mount an effective response or the virus actively suppresses immune function. To improve our ability to treat this fatal infection it is imperative to understand how immunity to RABV develops and functions so that parameters of protection

are better defined.

**Key words:** Rabies virus; Immune stimulation; Central nervous system; Vaccination; Blood-brain-barrier

© **The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Rabies is a devastating disease in developing countries with a very high case-fatality rate. The delayed immune response to infection with rabies virus could be a defining factor in poor prognosis following infection. Understanding the reasons for this muted response and identifying ways to manipulate immune effectors may lead to new therapeutic approaches to the treatment of rabies. This article reviews the reasons for the apparent failure of the immune response and identifies areas for therapeutic development.

Johnson N, Cunningham AF. Interplay between rabies virus and the mammalian immune system. *World J Clin Infect Dis* 2015; 5(4): 67-76 Available from: URL: <http://www.wjgnet.com/2220-3176/full/v5/i4/67.htm> DOI: <http://dx.doi.org/10.5495/wjcid.v5.i4.67>

## INTRODUCTION

### Rabies virus infection

Rabies has been with humanity since antiquity<sup>[1]</sup> and is caused by viruses of the *Lyssavirus* genus within the family *Rhabdoviridae*. This genus includes the rabies virus (RABV) and a number of related viruses that consist of an enveloped virus particle with a negative-stranded RNA genome approximately 11000 base pairs in length. The genome of the virus is remarkably simple, coding for only 5 proteins in the order nucleoprotein (N), phosphoprotein (P), matrix (M), glycoprotein (G) and polymerase (L)<sup>[2]</sup>. All viruses within the genus have this basic genomic structure (Figure 1). Each protein is multifunctional and expression of these five proteins from the virus genome is sufficient to enable efficient replication of virus in susceptible cells.

The lyssaviruses are hypothesized to have evolved in bats, with RABV being present in many species of new world bats<sup>[3]</sup>. However, at some point, the virus made the jump from the order Chiroptera to Carnivora and became established in new reservoir species, and the translocation of the virus to regions where it is now endemic, mainly Africa and Asia<sup>[4]</sup>. The principal reservoir is the domestic dog. Indeed, virtually all human infections are due to dog bites<sup>[5]</sup>. Bat bites are also a source of infection but are responsible for a small number of cases. This means that control of rabies is technically simple, control dog rabies. This unfortunately is not the case in parts of the world where public health is under-resourced and uncoordinated. However, due to the relatively long incubation period between exposure to virus following a

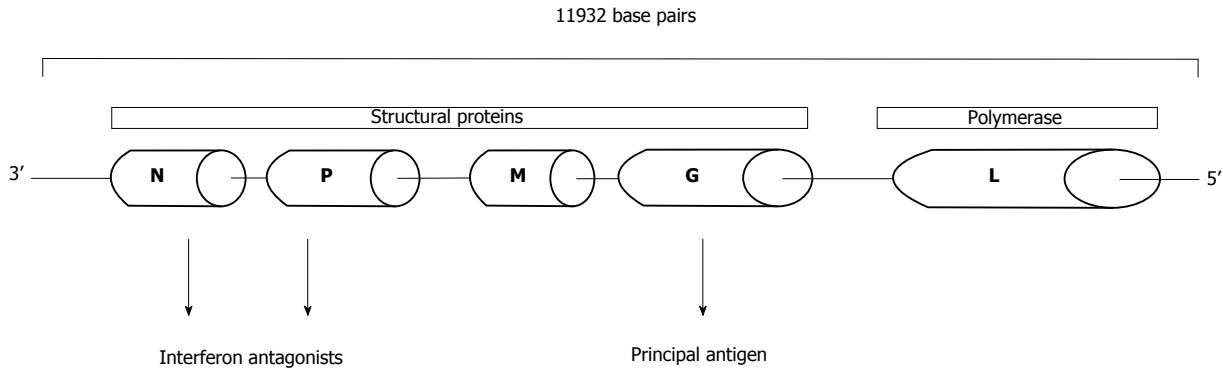
bite, and development of clinical disease, which is often measured in months, post-exposure vaccination, as pioneered over one hundred years ago by Louis Pasteur, is effective. Therefore, no one needs to die of rabies and yet they continue to do so in their thousands every year<sup>[6]</sup>. Only mammals appear to be infected naturally. This includes livestock that cause further threat to public health and economic liabilities for developing countries<sup>[7]</sup>.

Once RABV infects peripheral nerves it proceeds rapidly by axonal transport to the spinal cord and then onwards to the brain. This is where virus replication accelerates, symptoms of infection develop and the host is almost certain to die. A number of survivors have been documented but in most cases there was evidence of prior immunization<sup>[8]</sup>. Survivors have also suffered persistent severe neurological sequelae following acute disease.

The reasons for the high mortality observed following RABV infection are unclear and almost unique for an infectious disease of mammals. It is still uncertain whether there is any replication before RABV infects the nervous system. There is some evidence for low-level replication in muscles, and the nerve-muscle junction is one possible route of entry to the peripheral nervous system<sup>[9]</sup>. There is also evidence that cells of the monocyte/macrophage lineage can be infected<sup>[10]</sup>. However, post-mortem investigation of the tissue distribution of RABV inevitably finds the virus exclusively associated with nervous tissue. This may have serious implications for generating protective immunity to RABV infection if replication only occurs in the central nervous system (CNS), with negligible extracellular antigen availability, there may be insufficient antigen to initiate T and B cell responses. If virus replication and amplification occurs exclusively in the CNS, the consequences for the host are profound.

### Immune responses to RABV vaccination

What can we learn from the effectiveness of vaccination in controlling RABV infection? The earliest vaccines were crude nervous tissue preparations with virus inactivated by desiccation or subsequently, by chemical means. Current human vaccines are cell-culture expressed virus that is inactivated with beta-propiolactone (extensively reviewed by Wu *et al.*<sup>[11]</sup>). The development of cell-culture vaccines was a critical step in eliminating myelin, present in all neuronal tissue, from human vaccines and reducing the risk of adverse autoimmune reactions from vaccination. A range of vaccination protocols are effective, with induction of neutralizing antibodies (VNAs) being considered the correlate of immunity whether the vaccine is given pre- or post-exposure<sup>[12,13]</sup>. In both cases, the mechanism of protection is the prevention of virus infecting neuronal cells by neutralising antibodies directed against the principal virus antigen, the glycoprotein. A number of studies have investigated the longevity of the VNA response following anti-rabies vaccination. This appears to be highly variable



**Figure 1 Schematic of the rabies virus genome.** Coding regions are shown as cylinders and intergenic regions are shown as single lines. The genome consists of the nucleoprotein (N), phosphoprotein (P), matrix (M), glycoprotein (G) and RNA dependent RNA polymerase (L). Attenuation of RABV has been achieved through the addition of point mutations within the G protein, specifically at amino acid position 333 in the SAG2 vaccine strain. Virus attenuation has also been achieved through inclusion of multiple copies of the G protein or by addition of mammalian immunity genes<sup>[41]</sup>. RABV: Rabies virus.

and with clear inter-individual dependence. However, serum levels of VNA generally decline over time and a booster vaccination is recommended to raise the level of VNA<sup>[14-16]</sup>. Perhaps because of its effectiveness over many decades, there has been little incentive to study the immune stimulation that follows inoculation with inactivated virus. A single study has demonstrated peak levels of blood IgG plasma cells 10 d following primary vaccination with inactivated RABV vaccine<sup>[17]</sup>. This appears earlier and to higher levels in those individuals who have previously been vaccinated, consistent with a classical T-dependent recall response. However, the long term fate of these plasma cells is not known. Some form of T-cell stimulation would be expected after vaccination although this has not been investigated. Natural killer cell responses have been reported<sup>[18]</sup> although their long-term persistence or role in preventing infection is unclear.

More recent work has focused on the immune response to the next generation of rabies vaccines. These are based on live-attenuated viruses that either lack one of the virus proteins or have an immune response gene inserted into the genome region between the glycoprotein and polymerase coding sequences (Figure 1). These studies are discussed later in this review.

### Immunity in the central nervous system

Until recently the CNS was considered an immune-privileged site within the body<sup>[19]</sup>. This was primarily due to the absence of immune cells in the normal brain and the presence of the blood-brain-barrier (BBB). The BBB is an impediment to free movement of molecules, proteins and cells between the blood supply to the brain and spinal cord, and the structures of the CNS<sup>[20]</sup>. A key feature is the endothelial layer on the luminal side of the vasculature. This expresses low levels of adhesion molecules and there are tight junctions that form between endothelial cells to exclude movement of cells across the vessel wall. However, immune privilege does not mean an absence of immune surveillance. There

appears to be little antigen presentation within the CNS itself and this appears to be limited to the microglial cell component that can present antigen following stimulation<sup>[21]</sup>. In addition, there is lymphatic drainage from the brain<sup>[22]</sup> that enables movement of soluble antigens to the deep cervical lymph nodes<sup>[23]</sup>. Activated CD4<sup>+</sup> T cells also appear to move between the brain and cervical lymph nodes<sup>[24]</sup>. Despite this limited immune footprint in the brain during the steady-state, diseases such as multiple sclerosis show that immune cells can, and do, invade the brain under certain circumstances.

The alternative focus in recent years has been the stimulation and action of the innate immune response within the infected brain. RABV, principally through a range of functions of the phosphoprotein, can prevent type 1 interferon activation<sup>[25-27]</sup>, a property shared by all members of the genus<sup>[28]</sup>. More recent work has suggested that the nucleoprotein also inhibits type 1 interferons and that this function has been mapped to a specific region of the protein<sup>[29,30]</sup>. However, the mechanism through which this defined region of the nucleoprotein inhibits immune responses has not been identified. Interferon inhibition appears to be an early effect as numerous studies have shown substantial innate immune stimulation within the brain of an infected host during the clinical phase of infection. Stimulation of interferons<sup>[31]</sup>, toll-like receptors<sup>[32,33]</sup> and chemokines<sup>[34-36]</sup> have all been reported but these may equally cause tissue damage in addition to restricting virus replication<sup>[37]</sup>.

A key model for understanding the immune response to infection with RABV has been infection of mice with attenuated RABVs. Early studies used the attenuated strain CVS-F3 that has an arginine-to-glutamine substitution at position 333 within the glycoprotein gene<sup>[38]</sup>. Peripheral inoculation of mice with a virulent RABV leads to neuroinvasion and death within 10-20 d. Infection with CVS-F3 leads to transient weight loss and neuroinvasion. However, the virus is rapidly cleared and the mice survive. Mice that are unable to respond to interferon or produce T cells also survive infection with



CVS-F3. However, mice unable to generate antibody develop disease in a similar manner to infection with virulent virus, confirming the central role of antibody in controlling RABV infection<sup>[38]</sup>. Direct comparison of infection with isolates derived from a silver-haired bat, a representative virulent virus, and an attenuated virus (CVS B2C) demonstrate clear differences in the immune response to both viruses<sup>[39]</sup>. In the former, immune responses are muted and the outcome of infection is poor, whereas replication of the attenuated strain is extensive but controlled by the immune response. This may be important as the widespread nature of the attenuated virus infection may mean increased antigen availability resulting in an enhanced immune response to the virus.

Further studies have used attenuated viruses that have been derived through the ability to mutate the virus genome and rescue recombinant RABV<sup>[40]</sup>. Such viruses could form the basis of future vaccines for rabies (reviewed by Hicks *et al.*<sup>[41]</sup>) and one variant has been trialled as an oral vaccine for wildlife<sup>[42]</sup>. The method of attenuation falls into two categories. The first are those constructs that have a gene deletion, usually the phosphoprotein<sup>[43]</sup> or matrix<sup>[44]</sup>. The second are those that have an immunity gene, for example a chemokine such as CCL3<sup>[45]</sup>, inserted into an intergenic region. The investigation of immune responses to these viruses has resulted in important advances in understanding immune stimulation in response to infection<sup>[46]</sup>. Single-dose vaccination with a matrix-deficient recombinant RABV induces germinal centre-independent B-cell development and antibody secretion more rapidly than a preparation of inactivated RABV<sup>[44]</sup>.

## BARRIERS TO THE IMMUNE RESPONSE TO RABV IN THE CNS

A number of barriers need to be overcome in order for the immune response to react effectively to RABV infection in the CNS. This is summarised in Figure 2 and can be grouped into three categories.

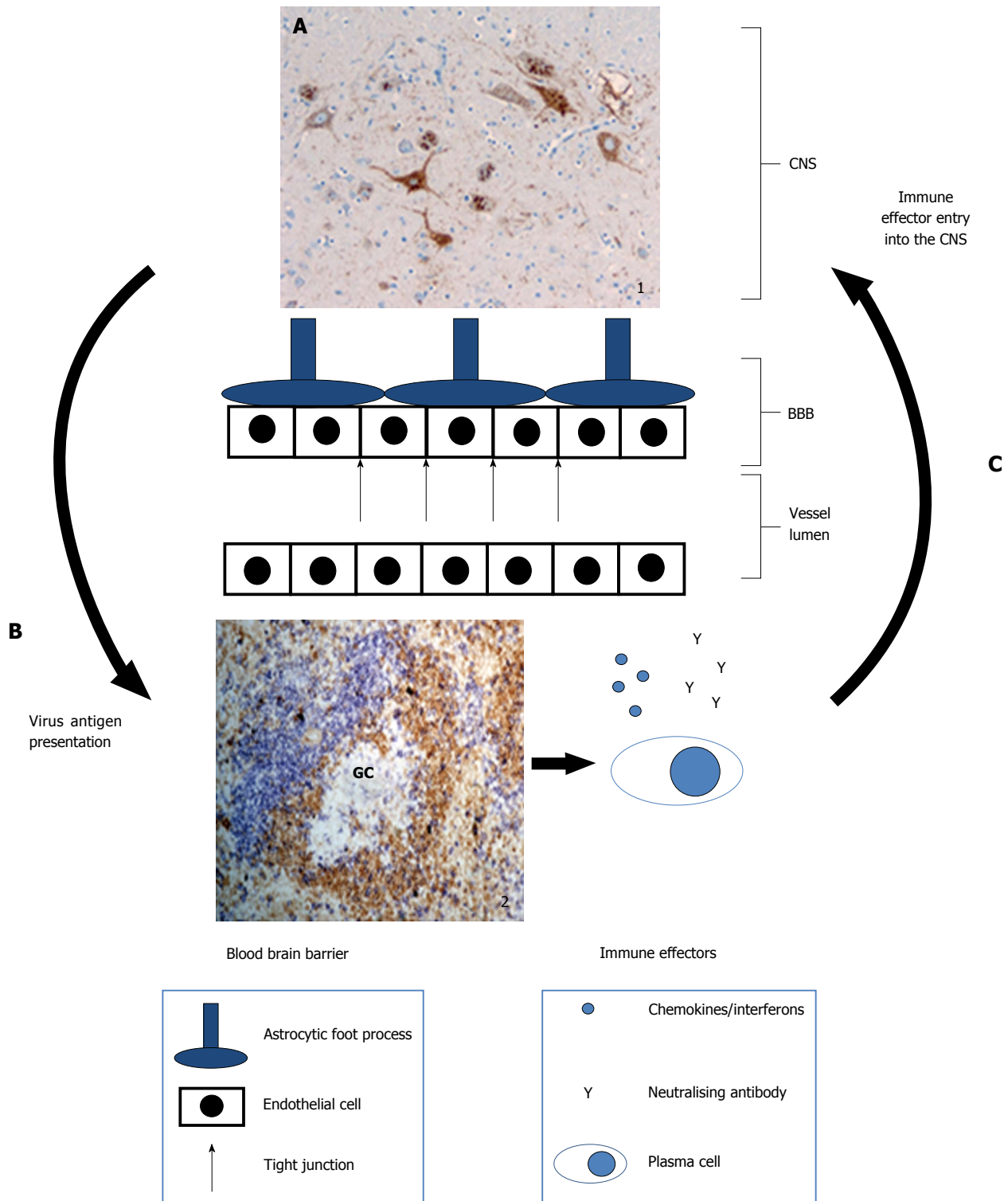
### Detection

The first is timely detection of infection. All evidence suggests that the first significant replication of RABV occurs in the CNS. Mammalian cells detect infection with negative stranded viruses through detection of non-self-pattern, structures associated with the virus or virus intermediates generated during the replicative process. This is mediated by proteins including retinoic acid-inducible gene I (RIG-I)-like receptors or melanoma differentiation associated gene 5 proteins<sup>[47]</sup>. In the case of RABV, RIG-I detects the 5'-triphosphate RNA and leads to activation of interferon and interferon-inducible genes<sup>[48]</sup>. However, as discussed above, negative-stranded virus actively suppresses this response at the cellular level<sup>[49]</sup>, and rabies is no exception. This early suppression of the interferon response could lead to a

failure to fully orchestrate the immune response required to limit virus spread. In comparison with attenuated strains, the early chemokine response to virulent RABV appears suppressed, possibly as a result of inhibition of transcriptional activators<sup>[39]</sup>. This delay could have profound consequences for the host.

### Temporal

A known, critical barrier to an effective response is time. This takes two forms: The time from virus detection to the influx of immune effectors into the CNS that can control infection and second, the time from infection to the generation of a high quality antibody response. Pathogens can induce characteristic responses, whereby they vary in the kinetics of induction of antibody responses and the sequelae of their induction, such as the persistence of memory for example *Salmonella* infections and helminth infections in mice<sup>[50]</sup>. Typically, after primary viral infections, immunoglobulin M (IgM) appears in the first days post-infection, followed by a gradual rise in IgG titres in subsequent weeks and months<sup>[51]</sup>. This parallels the long-term dominance of antibody derived plasma cells selected through the germinal centre, compared to the extrafollicular response alone<sup>[52]</sup>. A key consideration is that antibody responses typically develop in secondary lymphoid tissues and although some long-term plasma cells can reside in sites such as the spleen, they more typically localise to the bone marrow. From these sites antibody can be readily released into the blood, but this does not necessarily mean the antibody can cross into the cerebrospinal fluid (CSF) or brain parenchyma as antibody is blocked by an intact BBB. Therefore measurement of serum antibody titres may not reflect titres in the CSF and furthermore it is unclear whether the IgG isotypes present in serum correlate with those in CSF. Close examination of the antibody response to neurotropic viruses reveals layers of complexity when making direct comparisons between serological and intrathecal responses<sup>[52]</sup>. Virus-specific antibody secreting cell (ASC) numbers can peak in peripheral lymphoid tissue prior to their accumulation in the CNS and following inoculation with high doses of RABV, a neutralising antibody response can be detected within 4-5 d (APHA, unpublished data). However, this is unrepresentative of a normal infection where virus appears to enter the CNS by stealth before replication and detection can occur. Such a dichotomy of responses to the same antigen raises a question as to whether this reflects impairment in the development of antibodies to viral antigens. Engagement of the B cell receptor is the initiating event for an antibody response and this requires exposed antigen. If the virus is restricted to an intracellular location during the early stages of infection then antigen presentation will be delayed. There is a clear precedent for a lack of antigen availability resulting in a failure to generate a productive antibody response. This is the observation that many non-vaccinated individuals infected with *Corynebacterium*



**Figure 2** Model showing the movement of rabies virus antigen from the central nervous system to lymphoid tissue and then immune effectors (chemokines, antibodies and plasma cells) return to the central nervous system. Inset 1 shows a section of RABV infected brain stained with anti-nucleoprotein antibodies. Brown staining shows accumulation of nucleoprotein within neuronal cells. Inset 2 shows a murine spleen section stained with anti-CD3 for T cells (blue staining) and anti-IgD for naïve B cells (brown staining). The location of a GC is indicated. The arrows indicate the movement of antigen to lymphoid tissue and immune effectors returning to the CNS. The exact route of these is uncertain in the context of RABV infection. Letters signify areas for future research: A: Identification of therapeutics that restrict the replication and spread of RABV between neurons. Critically, such antiviral strategies must work within the CNS and without toxicity; B: Investigate the mechanisms by which RABV antigens reach antigen presenting cells either in situ or through exit of the CNS. Therapies that accelerate this process should assist the development of immune responses to infection; C: Development of methods that improves access of antivirals and immune effectors (cytokines, antibodies and cells) into the CNS. RABV: Rabies virus; CNS: Central nervous system; GC: Germinal centre.

*diphtheriae*, the causative agent of diphtheria, fail to generate protection against reinfection. This is because

the level of toxin required to induce disease is so low as to not consistently induce an antibody response. In

**Table 1 United Kingdom cases of rabies 2001-2012**

Yr	Virus	Source	Country of origin	Findings of tests to detect neutralizing antibody in cerebrospinal fluid	Ref.
2001	RABV	Dog	Nigeria	Not tested	[75]
2001	RABV	Dog	Philippines	None detected on hospital admission	[76]
2002	EBLV-2 <sup>1</sup>	Bat	Britain	None detected in any sample submitted	[77]
2005	RABV	Dog	India	Not tested	[78]
2008	RABV	Dog	South Africa	None detected on hospital admission. Evidence for low levels after 5 d of hospitalization	[73]
2012	RABV	Dog	India	None detected in any sample submitted	[79]

<sup>1</sup>European bat lyssavirus 2. RABV: Rabies virus.

this instance, the extracellular toxin is produced by an extracellular organism and acts through binding of the host cell surface and yet even under such circumstances it is still not sufficient to elicit protective B cell responses. The limited antibody response observed early after RABV contrast with events at later times after infection. Then, a combination of increased antigen availability due to a higher viral load, host cell death and pathology resulting in BBB perturbation can result in measurable antibody responses. This is observed in the late stages of infection in experimental models where evidence for lymphocyte infiltration and antibody in the CSF is clear. The proportion of infiltrating cells that are B-cells is low, often approximately 1% of labelled lymphocytes<sup>[53]</sup>, and these may have effector functions other than antibody secretion. It is not clear where at these later time points B cells and antibody is derived from and this may be important in understanding how best to accelerate the induction of protective responses after infection with RABV.

### Physical

Finally there is the barrier presented by the BBB itself. The BBB is the interface between the CNS and the blood supply. It is composed of both physical and physiological barriers that control entry of cells, proteins and large molecular weight molecules into the CNS (described in more detail below). Studies by Hooper *et al.*<sup>[54]</sup> identified opening of the BBB as a critical factor in enabling access of ASCs that might contribute to control of RABV infection. They further deduced that opening of the BBB could lead to successful resolution of infection with a virulent RABV<sup>[55]</sup>. This provides a new avenue in therapy for RABV for both immune and antiviral compounds that could lead to successful treatment for infection.

## INTERVENTION IN HUMAN RABIES

Treatment of human rabies is confounded by a range of factors. Firstly, the majority of cases occur in developing countries that have a poor health infrastructure, low ratios of doctors to the population, little access to pharmacological agents or the ability to deliver intensive medical care<sup>[8,56]</sup>. This means that prevention of rabies in reservoir populations, particularly the domestic dog, is both a cost-efficient and effective approach to reducing

human rabies<sup>[57]</sup>. A second problem, even encountered in developed countries, is that once a case of rabies has been confirmed, the patient is in an advanced stage of disease. If contact with a rabid animal is not recognised or appropriate post-exposure prophylaxis is not sought, the first indications of neurological disease are usually a sign that the virus has reached the brain. This makes treatment of rabies extremely challenging and identifying potential therapies impossible. Although the Milwaukee protocol or therapeutic coma<sup>[58]</sup> has been hailed as a possible breakthrough, its successes have been sporadic and has been criticised as it lacks a firm mechanistic basis<sup>[8]</sup>. Here again, the lack of an experimental model prevents progress in treatment development. Notwithstanding these problems, based on what is known it is possible to speculate on what a potential treatment regimen might include. It will not be simple or based on a single drug.

### The role of anti-rabies antibodies in cerebrospinal fluid

The above points highlight the complexity of understanding and interpreting the immune response to rabies as there are multiple variables such as the involvement of multiple anatomical sites and differing levels of viral burden at distinct times during infection. One potential measure of immune response within the CNS is the presence of antibody in CSF. Here the picture is mixed. Some authors report that the presence of immune complexes in the CSF is a valid ante-mortem diagnostic marker of infection<sup>[59]</sup>. The experience of patients admitted in the United Kingdom with rabies, whilst modest, suggests that CSF antibody is detected at a relatively late stage in the course of infection (Table 1) and all of these patients died as a result of infection. One striking feature of the first survivor to receive therapeutic coma as a treatment for rabies infection was the high levels of neutralising antibody, both IgM and IgG, in both serum and CSF<sup>[60]</sup>.

More recent evidence from animal models of infection suggest that the early appearance of antibody in the CSF is a good indicator of survival<sup>[61,62]</sup> although these experiments are problematic as intermediate events such as neuroinvasion are not tightly controlled nor is the point at which antibody is detected in the CSF. Also the source of this antibody is not known. Is it produced *in situ* or in the periphery and if this is the case, is it accessing the parenchyma of the CNS where it is needed

to neutralise virus or is it restricted to the CSF? However, the presumption is that controlled opening of the BBB combined with an early antibody response are critical steps required to control infection with RABV. When this does not happen, as in the case of the vast majority of infections, the outcome is death of the host.

#### ***Perturbations of the BBB and access of immune mediators and antiviral drugs to the site of infection***

The BBB forms the interface between the circulation and the brain parenchyma and is critical to maintaining stable homeostatic control within the CNS. It is formed by endothelial cells on the vasculature of the brain, which are in turn surrounded by astrocyte end-feet<sup>[63]</sup>. Neuronal cells are also an integral component of the structure. Tight junctions form between endothelial cells that exclude large molecular weight molecules, proteins and cells. In addition, transporter proteins actively reduce molecule concentrations in the brain interstitial spaces. Attempts to overcome the BBB have been extensively studied for the delivery of anti-tumour therapies<sup>[64,65]</sup>. A range of methods have been developed to open tight junctions ranging from drug mediated effectors to osmotic disruption<sup>[66]</sup>. Many of these approaches have been used in human medicine and so could be adapted to therapy of acute viral infection.

An antiviral that would stop virus replication and spread within the CNS is the Holy Grail for the effective treatment of clinical rabies. Numerous candidates have been proposed as potential anti-rabies therapeutics (extensively reviewed by Dacheux *et al.*<sup>[67]</sup>). In virtually all cases, promising results in tissue culture systems are not matched by findings in animal models. Partly, this may be due to the difficulties of accessing the CNS and would be alleviated by administration in a coordinated way with BBB opening compounds. However, successful demonstration of this within an appropriate model is challenging.

#### ***Immune stimulation***

As discussed above, the adaptive immune response to infection appears delayed. Stimulation of immune responses may assist the patient, particularly in clearing the virus from the CNS where antibodies are a key component<sup>[38]</sup>. Here, live attenuated vaccines may have a role to play in the stimulation of antibody responses. A matrix gene-deleted RABV construct inoculated into mice efficiently stimulated antibody production within 7 d<sup>[68]</sup>. If this can be accelerated further it may support the patient's ongoing adaptive responses. The major difficulty in achieving this is that RABV-specific B cells, as found for other antigens, will only be present in naïve subjects at a low frequency. This suggests that to achieve an accelerated enhancement of antibody levels a range of methods might be needed including vaccination, passive transfer of antibody or by immunizing with high copy number protective epitopes to engage as many specific B cells as rapidly as possible. An additional unknown is the contribution CD8 T cells

make to protection. With the prolonged nature of the infection and its near 100% fatality rate it suggests that they contribute little, and of course adaptive immunity can always exacerbate infection. It is possible that CD8 T cells cannot access the brain parenchyma because of the BBB, which is a key stumbling block to all interventions. Both T and B cells destined for entry into the CNS need to express a range of surface molecules that allow adhesion to the brain vascular endothelium and promote transit across the physical structures of the BBB<sup>[69,70]</sup>. It is also clear that CD8 T cell responses generated after vaccination in the absence of B cells are not protective in experimental models of infection<sup>[38]</sup>. However, T cells are the major lymphocyte population forming perivascular cuffs and accessing the brain parenchyma during the late stages of infection with RABV<sup>[53]</sup>. The role of these cells, either positive or negative, in response to infection should be defined.

#### ***Neuroprotection***

When patients come to the attention of clinicians, the disease is already well advanced in the CNS. Some neuronal deficit would be expected even if treatment was applied early and was effective. One of the unexpected benefits of the therapeutic coma approach has been an increase in understanding of the metabolic effects of rabies virus infection on the human brain<sup>[71]</sup>. Using this knowledge it may be possible to develop neurosupportive therapeutic strategies to assist in recovery from infection. Here again, recombinant rabies viruses could play a role in delivering neurotrophins, proteins that act as survival and growth factors for nerves, into the CNS<sup>[72]</sup>.

A major practical consideration is how to monitor patient progress through the treatment period. In past studies where therapeutic coma has been used there have been few indicators of patient progress. Serum levels of neutralising antibodies have been used although this can be a slow, crude measure of the patient's response to infection<sup>[73]</sup>. Potentially, development of virus-specific antibody in the CSF may provide a more appropriate measure although this would be a harder sample to take at regular intervals. Such a measure might also be an indicator of when treatment has failed.

## **CONCLUSION**

Neutralising antibodies are the main correlate of protection for vaccination against rabies<sup>[38]</sup> and it is likely that they could play a role in limiting the spread of RABV within the CNS. The absence of antibody in patient serum or CSF coincident with disease onset suggests that there is a delay in development of B cell responses as a result of poor antigen availability. This in turn implies that RABV replicates exclusively in the CNS and that seropositive animals without disease occur either because there has been exposure to antigen in the absence of replication, for example aerosol exposure in bats<sup>[74]</sup>, or RABV replication in the periphery that has



stimulated antibody and then controlled infection. In the latter case such individuals, either animal or human, do not come to the attention of veterinarians or public health professionals. Even when immune effectors are stimulated, limited accessibility of antibody and lymphocytes to the CNS appears to nullify the ability of the adaptive immune response to control rabies. Further investigation is needed to identify ways of accelerating patient immune response and enabling its entry into the CNS. This first requires a suitable model. Most studies use rodents, particularly mice, as they are convenient, but infection with RABV in this model can be unpredictable, result in a short incubation period (< 5 d) and follow a rapid disease course (1-2 d). Rodents are also unsuitable for use in assessing human therapies. Alternative models for rabies pathogenesis and vaccine response include ferrets<sup>[80]</sup> and non-human primates<sup>[81,82]</sup>. These are also problematic for similar reasons to those seen in rodents and are considerably more costly. Until a suitable model can be resolved, progress in understanding the complex processes associated with disease pathogenesis and immune response will be slow.

It is well over one hundred years since Louis Pasteur pioneered therapeutic vaccination for rabies. Despite this success, or possibly because of it, it is still not clear why rabies is overwhelmingly fatal to all mammals and continues to affect thousands of people every year<sup>[83]</sup>. To date no antiviral or therapeutic approach has been identified that improves outcome<sup>[8]</sup>. This suggests that a range of measures will be needed to treat infected patients and that a single mode of intervention will be insufficient. Advances in the development of research tools to investigate the disease process such as recombinant rabies viruses and other viruses expressing RABV proteins<sup>[84]</sup> should identify ways to rationally target inhibition of virus replication, stimulate the immune response and provide supportive treatment that will enhance delivery of such inhibitors.

## REFERENCES

- Neville J. Rabies in the Ancient World. In: Ed King AA, Fooks AR, Aubert M, Wandler AI. Pub. Historical perspective of rabies in Europe and the Mediterranean Basin. OIE (World organisation for animal health), 2004: 1-13
- Tordo N, Kouknetzoff A. The rabies virus genome: an overview. *Onderstepoort J Vet Res* 1993; **60**: 263-269 [PMID: 7777310]
- Banyard AC, Hayman D, Johnson N, McElhinney L, Fooks AR. Bats and lyssaviruses. *Adv Virus Res* 2011; **79**: 239-289 [PMID: 21601050 DOI: 10.1016/B978-0-12-387040-7.00012-13]
- Badrane H, Tordo N. Host switching in Lyssavirus history from the Chiroptera to the Carnivora orders. *J Virol* 2001; **75**: 8096-8104 [PMID: 11483755 DOI: 10.1128/JVI.75.17.8096-8104.2001]
- Lankster F, Hampson K, Lembo T, Palmer G, Taylor L, Cleaveland S. Infectious Disease. Implementing Pasteur's vision for rabies elimination. *Science* 2014; **345**: 1562-1564 [PMID: 25258065 DOI: 10.1126/science.1256306]
- Hampson K, Coudeville L, Lembo T, Sambo M, Kieffer A, Attlan M, Barrat J, Blanton JD, Briggs DJ, Cleaveland S, Costa P, Freuling CM, Hiby E, Knopf L, Leanes F, Meslin FX, Metlin A, Miranda ME, Müller T, Nel LH, Recuenco S, Rupprecht CE, Schumacher C, Taylor L, Vigilato MA, Zinsstag J, Dushoff J. Estimating the global burden of endemic canine rabies. *PLoS Negl Trop Dis* 2015; **9**: e0003709 [PMID: 25881058 DOI: 10.1371/journal.pntd.003709]
- Vos A, Un H, Hampson K, De Balogh K, Aylan O, Freuling CM, Müller T, Fooks AR, Johnson N. Bovine rabies in Turkey: patterns of infection and implications for costs and control. *Epidemiol Infect* 2014; **142**: 1925-1933 [PMID: 24280252 DOI: 10.1017/S0950268813002811]
- Jackson AC. Current and future approaches to the therapy of human rabies. *Antiviral Res* 2013; **99**: 61-67 [PMID: 23369672 DOI: 10.1016/j.antiviral.2013.01.003]
- Burroughs TG, Tignor GH, Smith AL. Rabies virus binding at neuromuscular junctions. *Virus Res* 1985; **2**: 273-289 [PMID: 3890406]
- Nakamichi K, Inoue S, Takasaki T, Morimoto K, Kurane I. Rabies virus stimulates nitric oxide production and CXC chemokine ligand 10 expression in macrophages through activation of extracellular signal-regulated kinases 1 and 2. *J Virol* 2004; **78**: 9376-9388 [PMID: 15308732 DOI: 10.1128/JVI.78.17.9376-9388.2004]
- Wu X, Smith TG, Rupprecht CE. From brain passage to cell adaptation: the road of human rabies vaccine development. *Expert Rev Vaccines* 2011; **10**: 1597-1608 [PMID: 22043958 DOI: 10.1586/erv.11.140]
- Warrell MJ. Current rabies vaccines and prophylaxis schedules: preventing rabies before and after exposure. *Travel Med Infect Dis* 2012; **10**: 1-15 [PMID: 22342356 DOI: 10.1016/j.tmaid.2011.12.005]
- Malerczyk C, Vakil HB, Bender W. Rabies pre-exposure vaccination of children with purified chick embryo cell vaccine (PCECV). *Hum Vaccin Immunother* 2013; **9**: 1454-1459 [PMID: 23571224 DOI: 10.4161/hv.24502]
- Nicholson KG, Turner GS, Aoki FY. Immunization with a human diploid cell strain of rabies virus vaccine: two-year results. *J Infect Dis* 1978; **137**: 783-788 [PMID: 659922]
- Briggs DJ, Schwenke JR. Longevity of rabies antibody titre in recipients of human diploid cell rabies vaccine. *Vaccine* 1992; **10**: 125-129 [PMID: 1539465]
- Morris J, Crowcroft NS, Fooks AR, Brookes SM, Andrews N. Rabies antibody levels in bat handlers in the United Kingdom: immune response before and after purified chick embryo cell rabies booster vaccination. *Hum Vaccin* 2007; **3**: 165-170 [PMID: 17786037 DOI: 10.4161/hv.3.5.4216]
- Blanchard-Rohner G, Pulickal AS, Jol-van der Zijde CM, Snape MD, Pollard AJ. Appearance of peripheral blood plasma cells and memory B cells in a primary and secondary immune response in humans. *Blood* 2009; **114**: 4998-5002 [PMID: 19843885 DOI: 10.1182/blood-2009-03-211052]
- Horowitz A, Behrens RH, Okell L, Fooks AR, Riley EM. NK cells as effectors of acquired immune responses: effector CD4+ T cell-dependent activation of NK cells following vaccination. *J Immunol* 2010; **185**: 2808-2818 [PMID: 20679529 DOI: 10.4049/jimmunol.1000844]
- Bailey SL, Carpentier PA, McMahon EJ, Begolka WS, Miller SD. Innate and adaptive immune responses of the central nervous system. *Crit Rev Immunol* 2006; **26**: 149-188 [PMID: 16700651 DOI: 10.1615/CritRevImmunol.v26.i2.40]
- Hendricks BK, Cohen-Gadol AA, Miller JC. Novel delivery methods bypassing the blood-brain and blood-tumor barriers. *Neurosurg Focus* 2015; **38**: E10 [PMID: 25727219 DOI: 10.3171/2015.1.FOCUS14767]
- Fischer HG, Reichmann G. Brain dendritic cells and macrophages/microglia in central nervous system inflammation. *J Immunol* 2001; **166**: 2717-2726 [PMID: 11160337 DOI: 10.4049/jimmunol.166.4.2717]
- Cserr HF, Knopf PM. Cervical lymphatics, the blood-brain barrier and the immunoreactivity of the brain: a new view. *Immunol Today* 1992; **13**: 507-512 [PMID: 1463583 DOI: 10.1016-5699(92)90027-5]
- Yamada S, DePasquale M, Patlak CS, Cserr HF. Albumin outflow into deep cervical lymph from different regions of rabbit brain. *Am J Physiol* 1991; **261**: H1197-H1204 [PMID: 1928403]
- Goldmann J, Kwizdzinski E, Brandt C, Mahlo J, Richter D,

- Bechmann I. T cells traffic from brain to cervical lymph nodes via the cribroid plate and the nasal mucosa. *J Leukoc Biol* 2006; **80**: 797-801 [PMID: 16885505 DOI: 10.1189/jlb.0306176]
- 25 **Brzózka K**, Finke S, Conzelmann KK. Identification of the rabies virus alpha/beta interferon antagonist: phosphoprotein P interferes with phosphorylation of interferon regulatory factor 3. *J Virol* 2005; **79**: 7673-7681 [PMID: 15919920 DOI: 10.1128/JVI.79.12.7673-7681.2005]
  - 26 **Chelbi-Alix MK**, Vidy A, El Bougrini J, Blondel D. Rabies viral mechanisms to escape the IFN system: the viral protein P interferes with IRF-3, Stat1, and PML nuclear bodies. *J Interferon Cytokine Res* 2006; **26**: 271-280 [PMID: 16689655 DOI: 10.1089/jir.2006.26.271]
  - 27 **Schnell MJ**, McGettigan JP, Wirblich C, Papaneri A. The cell biology of rabies virus: using stealth to reach the brain. *Nat Rev Microbiol* 2010; **8**: 51-61 [PMID: 19946287 DOI: 10.1038/nrmicro2260]
  - 28 **Wiltzer L**, Larrous F, Oksayan S, Ito N, Marsh GA, Wang LF, Blondel D, Bourhy H, Jans DA, Moseley GW. Conservation of a unique mechanism of immune evasion across the *Lyssavirus* genus. *J Virol* 2012; **86**: 10194-10199 [PMID: 22740405 DOI: 10.1128/JVI.01249-12]
  - 29 **Masatani T**, Ito N, Shimizu K, Ito Y, Nakagawa K, Abe M, Yamaoka S, Sugiyama M. Amino acids at positions 273 and 394 in rabies virus nucleoprotein are important for both evasion of host RIG-I-mediated antiviral response and pathogenicity. *Virus Res* 2011; **155**: 168-174 [PMID: 20875468 DOI: 10.1016/j.virusres.2010.09.016]
  - 30 **Masatani T**, Ito N, Ito Y, Nakagawa K, Abe M, Yamaoka S, Okadera K, Sugiyama M. Importance of rabies virus nucleoprotein in viral evasion of interferon response in the brain. *Microbiol Immunol* 2013; **57**: 511-517 [PMID: 23607781 DOI: 10.1111/1348-0421.12058]
  - 31 **Johnson N**, McKimmie CS, Mansfield KL, Wakeley PR, Brookes SM, Fazakerley JK, Fooks AR. *Lyssavirus* infection activates interferon gene expression in the brain. *J Gen Virol* 2006; **87**: 2663-2667 [PMID: 16894206 DOI: 10.1099/vir.0.82024]
  - 32 **McKimmie CS**, Johnson N, Fooks AR, Fazakerley JK. Viruses selectively upregulate Toll-like receptors in the central nervous system. *Biochem Biophys Res Commun* 2005; **336**: 925-933 [PMID: 16157304 DOI: 10.1016/j.bbrc.2005.08.209]
  - 33 **Préhaud C**, Mégret F, Lafage M, Lafon M. Virus infection switches TLR-3-positive human neurons to become strong producers of beta interferon. *J Virol* 2005; **79**: 12893-12904 [PMID: 16188991 DOI: 10.1128/JIV.79.20.12893-12904.2005]
  - 34 **Johnson N**, Mansfield KL, Hicks D, Nunez A, Healy DM, Brookes SM, McKimmie C, Fazakerley JK, Fooks AR. Inflammatory responses in the nervous system of mice infected with a street isolate of rabies virus. *Dev Biol (Basel)* 2008; **131**: 65-72 [PMID: 18634467]
  - 35 **Niu X**, Wang H, Fu ZF. Role of chemokines in rabies pathogenesis and protection. *Adv Virus Res* 2011; **79**: 73-89 [PMID: 21601043 DOI: 10.1016/B798-0-12-387040-7.00005-6]
  - 36 **Hicks DJ**, Núñez A, Banyard AC, Williams A, Ortiz-Pelaez A, Fooks AR, Johnson N. Differential chemokine responses in the murine brain following *lyssavirus* infection. *J Comp Pathol* 2013; **149**: 446-462 [PMID: 23746482 DOI: 10.1016/j.jcpa.2013.04.001]
  - 37 **Hooper DC**, Sauder C, Scott GS, Dietzschold B, Richt JA. Immunopathology and immunoprotection in CNS virus infections: mechanisms of virus clearance from the CNS. *Curr Top Microbiol Immunol* 2002; **265**: 163-182 [PMID: 12014188]
  - 38 **Hooper DC**, Morimoto K, Bette M, Weihe E, Koprowski H, Dietzschold B. Collaboration of antibody and inflammation in clearance of rabies virus from the central nervous system. *J Virol* 1998; **72**: 3711-3719 [PMID: 9557653]
  - 39 **Wang ZW**, Sarmiento L, Wang Y, Li XQ, Dhingra V, Tseggei T, Jiang B, Fu ZF. Attenuated rabies virus activates, while pathogenic rabies virus evades, the host innate immune responses in the central nervous system. *J Virol* 2005; **79**: 12554-12565 [PMID: 16160183 DOI: 10.1128/JVI.19.12554-12565.2005]
  - 40 **Schnell MJ**, Mebatsion T, Conzelmann KK. Infectious rabies viruses from cloned cDNA. *EMBO J* 1994; **13**: 4195-4203 [PMID: 7925265]
  - 41 **Hicks DJ**, Fooks AR, Johnson N. Developments in rabies vaccines. *Clin Exp Immunol* 2012; **169**: 199-204 [PMID: 22861358 DOI: 10.1111/j.1365-2249.2012.04592.x]
  - 42 **Vos A**, Conzelmann KK, Finke S, Müller T, Teifke J, Fooks AR, Neubert A. Immunogenicity studies in carnivores using a rabies virus construct with a site-directed deletion in the phosphoprotein. *Adv Prev Med* 2011; **2011**: 898171 [PMID: 21991446 DOI: 10.4061/2011/898171]
  - 43 **Rieder M**, Brzózka K, Pfäler CK, Cox JH, Stitz L, Conzelmann KK. Genetic dissection of interferon-antagonistic functions of rabies virus phosphoprotein: inhibition of interferon regulatory factor 3 activation is important for pathogenicity. *J Virol* 2011; **85**: 842-852 [PMID: 21084487 DOI: 10.1128/JVI.01427-10]
  - 44 **Dorfmeier CL**, Lytle AG, Dunkel AL, Gatt A, McGettigan JP. Protective vaccine-induced CD4(+) T cell-independent B cell responses against rabies infection. *J Virol* 2012; **86**: 11533-11540 [PMID: 22896601 DOI: 10.1128/JVI.00615-12]
  - 45 **Zhao L**, Toriumi H, Wang H, Kuang Y, Guo X, Morimoto K, Fu ZF. Expression of MIP-1alpha (CCL3) by a recombinant rabies virus enhances its immunogenicity by inducing innate immunity and recruiting dendritic cells and B cells. *J Virol* 2010; **84**: 9642-9648 [PMID: 20592092 DOI: 10.1128/JVI.00326-10]
  - 46 **McGettigan JP**. Experimental rabies vaccines for humans. *Expert Rev Vaccines* 2010; **9**: 1177-1186 [PMID: 20923268 DOI: 10.1586/erv.10.105]
  - 47 **Yoneyama M**, Onomoto K, Jogi M, Akaboshi T, Fujita T. Viral RNA detection by RIG-I-like receptors. *Curr Opin Immunol* 2015; **32**: 48-53 [PMID: 25594890 DOI: 10.1016/j.coi.2014.12.012]
  - 48 **Hornung V**, Ellegast J, Kim S, Brzózka K, Jung A, Kato H, Poeck H, Akira S, Conzelmann KK, Schlee M, Endres S, Hartmann G. 5'-Triphosphate RNA is the ligand for RIG-I. *Science* 2006; **314**: 994-997 [PMID: 17038590 DOI: 10.1126/science.1132505]
  - 49 **Randall RE**, Goodbourn S. Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. *J Gen Virol* 2008; **89**: 1-47 [PMID: 18089727 DOI: 10.1099/vir.0.83391-0]
  - 50 **Bobat S**, Darby M, Mrdjen D, Cook C, Logan E, Auret J, Jones E, Schnoeller C, Flores-Langarica A, Ross EA, Vira A, López-Macías C, Henderson IR, Alexander J, Brombacher F, Horsnell WG, Cunningham AF. Natural and vaccine-mediated immunity to *Salmonella Typhimurium* is impaired by the helminth *Nippostrongylus brasiliensis*. *PLoS Negl Trop Dis* 2014; **8**: e3341 [PMID: 25474738 DOI: 10.1371/journal.pntd.0003341]
  - 51 **Libbey JE**, Fujinami RS. Adaptive immune response to viral infections in the central nervous system. *Handb Clin Neurol* 2014; **123**: 225-247 [PMID: 25015488 DOI: 10.1016/B978-0-444-0.00010-9]
  - 52 **Phares TW**, Stohlman SA, Bergmann CC. Intrathecal humoral immunity to encephalitic RNA viruses. *Viruses* 2013; **5**: 732-752 [PMID: 23435240 DOI: 10.3390/v5020732]
  - 53 **Hicks DJ**, Núñez A, Healy DM, Brookes SM, Johnson N, Fooks AR. Comparative pathological study of the murine brain after experimental infection with classical rabies virus and European bat *lyssaviruses*. *J Comp Pathol* 2007; **140**: 113-126 [PMID: 19111840 DOI: 10.1016/j.jcpa.2008.09.001]
  - 54 **Roy A**, Phares TW, Koprowski H, Hooper DC. Failure to open the blood-brain barrier and deliver immune effectors to central nervous system tissues leads to the lethal outcome of silver-haired bat rabies virus infection. *J Virol* 2007; **81**: 1110-1118 [PMID: 17108029 DOI: 10.1128/JVI.01964-06]
  - 55 **Roy A**, Hooper DC. Lethal silver-haired bat rabies virus infection can be prevented by opening the blood-brain barrier. *J Virol* 2007; **81**: 7993-7998 [PMID: 17507463 DOI: 10.1128/JVI.00710-07]
  - 56 **Hampson K**, Cleaveland S, Briggs D. Evaluation of cost-effective strategies for rabies post-exposure vaccination in low-income countries. *PLoS Negl Trop Dis* 2011; **5**: e982 [PMID: 21408121 DOI: 10.1371/journal.pntd.0000982]

- 57 **Lembo T**, Hampson K, Kaare MT, Ernest E, Knobel D, Kazwala RR, Haydon DT, Cleaveland S. The feasibility of canine rabies elimination in Africa: dispelling doubts with data. *PLoS Negl Trop Dis* 2010; **4**: e626 [PMID: 20186330 DOI: 10.1371/journal.pntd.0000626]
- 58 **Rubin J**, David D, Willoughby RE, Rupprecht CE, Garcia C, Guarda DC, Zohar Z, Stamler A. Applying the Milwaukee protocol to treat canine rabies in Equatorial Guinea. *Scand J Infect Dis* 2009; **41**: 372-375 [PMID: 19263274 DOI: 10.1080/00365540902798333]
- 59 **Muhamuda K**, Madhusudana SN, Ravi V, Desai A. Presence of rabies specific immune complexes in cerebro-spinal fluid can help in ante-mortem diagnosis of human paralytic rabies. *J Clin Virol* 2006; **37**: 162-167 [PMID: 16931137 DOI: 10.1016/j.jcv.2006.06.010]
- 60 **Willoughby RE**, Tieves KS, Hoffman GM, Ghanayem NS, Amlie-Lefond CM, Schwabe MJ, Chusid MJ, Rupprecht CE. Survival after treatment of rabies with induction of coma. *N Engl J Med* 2005; **352**: 2508-2514 [PMID: 15958806 DOI: 10.1056/NEJMoa050382]
- 61 **Liao PH**, Yang HH, Chou PT, Wang MH, Chu PC, Liu HL, Chen LK. Sufficient virus-neutralizing antibody in the central nerve system improves the survival of rabid rats. *J Biomed Sci* 2012; **19**: 61 [PMID: 22734518 DOI: 10.1186/1423-0127-19-61]
- 62 **Gnanadurai CW**, Zhou M, He W, Leyson CM, Huang CT, Salyards G, Harvey SB, Chen Z, He B, Yang Y, Hooper DC, Dietzchold B, Fu ZF. Presence of virus neutralizing antibodies in cerebral spinal fluid correlates with non-lethal rabies in dogs. *PLoS Negl Trop Dis* 2013; **7**: e2375 [PMID: 24069466 DOI: 10.1371/journal.pntd.0002375]
- 63 **Serlin Y**, Shelef I, Knyazer B, Friedman A. Anatomy and physiology of the blood-brain barrier. *Semin Cell Dev Biol* 2015; **38**: 2-6 [PMID: 25681530 DOI: 10.1016/j.semedb.2015.01.002]
- 64 **Bellavance MA**, Blanchette M, Fortin D. Recent advances in blood-brain barrier disruption as a CNS delivery strategy. *AAPS J* 2008; **10**: 166-177 [PMID: 18446517 DOI: 10.1208/s12248-008-9018-7]
- 65 **Cooper I**, Last D, Guez D, Sharabi S, Elhaik Goldman S, Lubitz I, Daniels D, Salomon S, Tamar G, Tamir T, Mardor R, Fridkin M, Shechter Y, Mardor Y. Combined local blood-brain barrier opening and systemic methotrexate for the treatment of brain tumors. *J Cereb Blood Flow Metab* 2015; **35**: 967-976 [PMID: 25669901 DOI: 10.1038/jcbfm.2015.6]
- 66 **Misra A**, Ganesh S, Shahiwala A, Shah SP. Drug delivery to the central nervous system: a review. *J Pharm Pharm Sci* 2003; **6**: 252-273 [PMID: 12935438]
- 67 **Dacheux L**, Delmas O, Bourhy H. Human rabies encephalitis prevention and treatment: progress since Pasteur's discovery. *Infect Disord Drug Targets* 2011; **11**: 251-299 [PMID: 21488832 DOI: 10.2174/187152611795768079]
- 68 **Lytle AG**, Shen S, McGettigan JP. Lymph node but not intradermal injection site macrophages are critical for germinal centre formation and antibody responses to rabies vaccination. *J Virol* 2015; **89**: 2842-2848 [PMID: 25540370 DOI: 10.1128/JVI.3409-14]
- 69 **Laschinger M**, Engelhardt B. Interaction of alpha4-integrin with VCAM-1 is involved in adhesion of encephalitogenic T cell blasts to brain endothelium but not in their transendothelial migration in vitro. *J Neuroimmunol* 2000; **102**: 32-43 [PMID: 10626664]
- 70 **Lehmann-Horn K**, Sagan SA, Bernard CC, Sobel RA, Zamvil SS. B-cell very late antigen-4 deficiency reduces leukocyte recruitment and susceptibility to central nervous system autoimmunity. *Ann Neurol* 2015; **77**: 902-908 [PMID: 25712734 DOI: 10.1002/ana.24387]
- 71 **O'Sullivan A**, Willoughby RE, Mishchuk D, Alcarraz B, Cabezas-Sanchez C, Condori RE, David D, Encarnacion R, Fatteh N, Fernandez J, Franka R, Hedderwick S, McCaughey C, Ondrush J, Paez-Martinez A, Rupprecht C, Velasco-Villa A, Slupsky CM. Metabolomics of cerebrospinal fluid from humans treated for rabies. *J Proteome Res* 2013; **12**: 481-490 [PMID: 23163834 DOI: 10.1021/pr3009176]
- 72 **Gnanadurai CW**, Huang CR, Kumar D, Fu ZF. Novel approaches to the prevention and treatment of rabies. *Int J Virol Stud Res* 2015; **3**: 8-16
- 73 **Hunter M**, Johnson N, Hedderwick S, McCaughey C, Lowry K, McConville J, Herron B, McQuaid S, Marston D, Goddard T, Harkess G, Goharriz H, Voller K, Solomon T, Willoughby RE, Fooks AR. Immunovirological correlates in human rabies treated with therapeutic coma. *J Med Virol* 2010; **82**: 1255-1265 [PMID: 20513093 DOI: 10.1002/jmv.21785]
- 74 **Davis AD**, Rudd RJ, Bowen RA. Effects of aerosolized rabies virus exposure on bats and mice. *J Infect Dis* 2007; **195**: 1144-1150 [PMID: 17357050 DOI: 10.1086/512616]
- 75 **Johnson N**, Lipscomb DW, Stott R, Gopal Rao G, Mansfield K, Smith J, McElhinney L, Fooks AR. Investigation of a human case of rabies in the United Kingdom. *J Clin Virol* 2002; **25**: 351-356 [PMID: 12423699 DOI: 10.1016/S1386-6532(02)00131-2]
- 76 **Smith J**, McElhinney L, Parsons G, Brink N, Doherty T, Agranoff D, Miranda ME, Fooks AR. Case report: rapid ante-mortem diagnosis of a human case of rabies imported into the UK from the Philippines. *J Med Virol* 2003; **69**: 150-155 [PMID: 12436491 DOI: 10.1002/jmv.10253]
- 77 **Fooks AR**, McElhinney LM, Pounder DJ, Finnegan CJ, Mansfield K, Johnson N, Brookes SM, Parsons G, White K, McIntyre PG, Nathwani D. Case report: isolation of a European bat lyssavirus type 2a from a fatal human case of rabies encephalitis. *J Med Virol* 2003; **71**: 281-289 [PMID: 12938204 DOI: 10.1002/jmv.10481]
- 78 **Solomon T**, Marston D, Mallewa M, Felton T, Shaw S, McElhinney LM, Das K, Mansfield K, Wainwright J, Kwong GN, Fooks AR. Paralytic rabies after a two week holiday in India. *BMJ* 2005; **331**: 501-503 [PMID: 16141158 DOI: 10.1136/bmj.331.7515.501]
- 79 **Pathak S**, Horton DL, Lucas S, Brown D, Quaderi S, Polhill S, Walker D, Nastouli E, Núñez A, Wise EL, Fooks AR, Brown M. Diagnosis, management and post-mortem findings of a human case of rabies imported into the United Kingdom from India: a case report. *Virol J* 2014; **11**: 63 [PMID: 24708671 DOI: 10.1186/174-422X-11-63]
- 80 **Hamir AN**, Niezgoda M, Rupprecht CE. Recovery from and clearance of rabies virus in a domestic ferret. *J Am Assoc Lab Anim Sci* 2011; **50**: 248-251 [PMID: 21439220]
- 81 **Lodmell DL**, Parnell MJ, Bailey JR, Ewalt LC, Hanlon CA. One-time gene gun or intramuscular rabies DNA vaccination of non-human primates: comparison of neutralizing antibody responses and protection against rabies virus 1 year after vaccination. *Vaccine* 2001; **20**: 838-844 [PMID: 11738747 DOI: 10.1016/S0204-410X(01)00392-9]
- 82 **Xiang ZQ**, Greenberg L, Ertl HC, Rupprecht CE. Protection of non-human primates against rabies with an adenovirus recombinant vaccine. *Virology* 2014; **450-451**: 243-249 [PMID: 24503087 DOI: 10.1016/j.virol.2013.12.029]
- 83 **Fooks AR**, Banyard AC, Horton DL, Johnson N, McElhinney LM, Jackson AC. Current status of rabies and prospects for elimination. *Lancet* 2014; **384**: 1389-1399 [PMID: 24828901 DOI: 10.1016/S0140-6736(13)62707-5]
- 84 **Huang Y**, Chen Z, Huang J, Fu Z, He B. Parainfluenza virus 5 expressing the G protein of rabies virus protects mice after rabies virus infection. *J Virol* 2015; **89**: 3427-3429 [PMID: 25552723 DOI: 10.1128/JVI.03656-14]

P- Reviewer: Gadi B, Toshi N S- Editor: Qiu S

L- Editor: A E- Editor: Li D





## Epidemiological perspective of drug resistant extrapulmonary tuberculosis

Pravin Kumar Singh, Amita Jain

Pravin Kumar Singh, Amita Jain, Department of Microbiology, King George's Medical University, Lucknow, Uttar Pradesh 226003, India

**Author contributions:** Both the authors collected the relevant information and wrote the paper.

**Conflict-of-interest statement:** Pravin Kumar Singh is employed by Foundation for Innovative New Diagnostic, however author alone is responsible for the views expressed in this paper.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Amita Jain, Professor, Department of Microbiology, King George's Medical University, Shah Mina Road, Chowk, Lucknow, Uttar Pradesh 226003, India. [amita602002@yahoo.com](mailto:amita602002@yahoo.com)  
Telephone: +91-522-2258633  
Fax: +91-522-2258633

Received: January 15, 2015  
Peer-review started: January 16, 2015  
First decision: March 20, 2015  
Revised: August 11, 2015  
Accepted: September 7, 2015  
Article in press: September 8, 2015  
Published online: November 25, 2015

### Abstract

Tuberculosis (TB) remains one of the leading infectious diseases causing significant morbidity and mortality worldwide. Although, pulmonary TB is the most common presentation and is the main transmissible form of the disease, extrapulmonary TB

also significantly contributes to the burden of disease and can cause severe complications and disabilities. At present, the most serious issue with TB control programme is emergence of multi and extensively drug resistant *Mycobacterium tuberculosis* strain worldwide. As the number of drug resistant pulmonary TB is increasing around the world, the number of drug resistant TB with extrapulmonary manifestations are also on rise. However, there is surprisingly scant information in medical literatures on prevalence and impact of extrapulmonary drug-resistant TB. Here, we appraise the recent epidemiological studies that underpin the status and impact of drug resistance in TB cases with extrapulmonary manifestations.

**Key words:** Extrapulmonary tuberculosis; Tuberculosis; Epidemiology; *Mycobacterium tuberculosis*; Drug resistance

© The Author(s) 2015. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The emergence of highly drug resistant (DR) *Mycobacterium tuberculosis* and human immunodeficiency virus epidemic has paved the way for resurgence of tuberculosis (TB). Extrapulmonary tuberculosis (EPTB), accounts for a significant proportion of all notified TB cases, is a persistent global health issue. Although DR-EPTB is uncommon, but the increasing rate of DR-pulmonary TB around the world has heightened our concern for DR-EPTB too. Unfortunately, systematic surveillance data on DR-EPTB is lacking, however, sporadic information from different countries has begun to accumulate. Here, we aim to provide current understanding on epidemiological scenario of DR-EPTB and also to address some of the key challenges associated with diagnosis, control and management of DR-EPTB.

Singh PK, Jain A. Epidemiological perspective of drug



resistant extrapulmonary tuberculosis. *World J Clin Infect Dis* 2015; 5(4): 77-85 Available from: URL: <http://www.wjgnet.com/2220-3176/full/v5/i4/77.htm> DOI: <http://dx.doi.org/10.5495/wjcid.v5.i4.77>

## INTRODUCTION

Despite the availability of effective treatment for many decades, tuberculosis (TB) remains an enormous global health problem, responsible for about 1.5 million deaths annually<sup>[1]</sup>. *Mycobacterium tuberculosis* (MTB), the causative agent, usually affects the lungs [pulmonary TB (PTB)], however it may spread through lymphatic or hematogenous dissemination to virtually any organs and tissues of body resulting in the development of extrapulmonary TB (EPTB). In last few decades, extensive information has been gathered describing the clinical pictures of different common and rare forms of EPTB. The distribution of various types of EPTB may vary among different populations and countries. However, the most common sites of EPTB includes peripheral lymph nodes, pleura, genitourinary sites, bones and joints, abdomen (peritoneum and gastrointestinal tract), and the central nervous system<sup>[2,3]</sup>. A detailed discussion on clinical aspect of each form of EPTB is beyond the scope of this review but can be found elsewhere<sup>[2,3]</sup>.

The existence of EPTB is centuries old however the prime attention of global TB control programme has been principally focused on contagious pulmonary TB. Indeed, EPTB is milder and less contagious as compared to PTB it cannot be overlooked as it constitutes about 20% of all form of TB among immuno-competent adults<sup>[2]</sup>. Moreover, EPTB result in significant morbidity and mortality dependent on the organs affected (like central nervous system) and also due to various difficulties encountered in achieving a timely and definite diagnosis<sup>[4]</sup>. EPTB became more common worldwide since the advent of human immunodeficiency virus (HIV) infection. Consequently, a significantly high predisposition to the development of EPTB can be found in patients with concurrent acquired immune deficiency syndrome and TB<sup>[3]</sup>. Younger age is another potential risk factor for developing EPTB and even some aggressive form of TB like tubercular meningitis or miliary TB are commonly reported in children<sup>[5,6]</sup>. Such mounting significance of EPTB is suggestive to consider EPTB as much a public health priority as pulmonary TB (Table 1).

At present, the global emergence of multi-drug resistant (MDR) and even extensively drug resistant (XDR) TB is a great challenge to success of global TB control efforts. MDR-TB is usually caused by spontaneous genetic mutation in MTB that confers resistance to the two main first line antimicrobials rifampicin (RIF) and isoniazid (INH). Mutation in a hotspot region (81-bp) of *rpoB* gene (encoding beta-subunit of DNA dependent RNA polymerase) is responsible for rifampicin resistance

**Table 1 Drug resistant-extrapulmonary tuberculosis: Reasons to worry**

Drug resistance (including MDR) in cases of EPTB is increasing and now it cannot be considered as rare  
Accurate and timely diagnosis and drug susceptibility testing are very difficult and may result into high morbidity and mortality  
DR-EPTB is often difficult to treat due to poor penetration of some key anti-tubercular drugs into extra-pulmonary sites (especially in CSF)  
HIV and young age are independent key risk factors  
Although not contagious but it may co-exist with highly contagious pulmonary manifestation

MDR: Multi-drug resistant; DR-EPTB: Drug resistant-extrapulmonary tuberculosis; CSF: Cerebrospinal fluid; HIV: Human immunodeficiency virus.

while INH resistance is largely conferred by mutations in *katG* gene and *inhA* gene<sup>[7]</sup>. When MDR MTB develops additional resistance to a fluoroquinolone and a second line injectable drug (*i.e.*, amikacin, kanamycin or capreomycin), it is termed as XDR. Although, drug resistance in PTB has been extensively studied on various aspects, status of drug resistant (DR) EPTB is not clear. While some presentations of EPTB may be life threatening, the involvement of drug resistant MTB strain may amplify the risk of mortality. Thus, in the present era of HIV pandemic coupled with global emergence of MDR and XDR MTB, DR-EPTB represents as a real and new significant challenge to public health that is yet to receive serious attention. In this review, we aim to provide an epidemiological overview of drug resistant EPTB and to discuss some of key challenges associated with diagnosis and control of disease.

## EPIDEMIOLOGICAL SCENARIO

As many population based TB surveys of different countries are more confined to smear positive PTB especially in adult populations, the knowledge of the global epidemiology of EPTB is rather limited. Despite this, surveys from different parts of world have accumulated that demonstrate substantial increase of global burden of EPTB in both developed and developing countries, particularly in regions where the prevalence of HIV infection is high. In the areas where adequate diagnostic and reporting systems are available, EPTB accounts for 20%-25% of reported cases<sup>[8]</sup>. Recent estimate of WHO showed that among 5.4 million new TB cases, 0.8 million had EPTB of which a significant proportion (about 70%) were localized into Southeast and African regions<sup>[1]</sup>. In developed countries, 10%-15% of TB cases have extra-pulmonary involvement, but this rate is much higher in patients belonging to high TB burden countries<sup>[3]</sup>. India ranks first in having maximum number (about 0.35 million) of EPTB among new cases<sup>[1]</sup>. The successful TB control program in many developed countries resulted into a declining trend of incidence of pulmonary TB; however the rate of EPTB has remained constant or increased

Table 2 Drug resistant extrapulmonary tuberculosis in different parts of world

Region	Type of EPTB	Study period	Culture positive cases/total cases analyzed	Prevalence of drug resistant MTB	DST method	Concomitant Pulmonary TB cases/ total cases analyzed	HIV positive cases/ total cases tested <sup>1</sup>	Treatment outcomes	Ref.
Argentina	TBM	1996-2004	101	Any DR: 51.5% MDR: 41.6%	E	Not stated	101/101	64/101 died during hospitalization	Cecchini <i>et al</i> <sup>[49]</sup>
Australia	Various sites (including pulmonary)	2007	871 <sup>2</sup> (culture proven PTB/EPTB cases with available DST result)	MDR: 25% (6/24) of all MDR-TB cases	E	Not stated	Not stated	Not stated	Lumb <i>et al</i> <sup>[50]</sup>
Bangladesh	Various sites	2011	152 (culture proven TB cases)	Any DR: 45.4% MDR: 11.2 %	C	Not stated	Not stated	All cured	Afroz <i>et al</i> <sup>[51]</sup>
Brazil	TBM	1999-2007	108 (DST result available for 90 cases)	Any DR: 40% MDR: 9%	D	Not stated	108/108	31/108 died during hospitalization	Croda <i>et al</i> <sup>[56]</sup>
Canada	Various sites	1995-2002	126/214	Any DR: 19% INH-R: 7.9%	G	0/214	4/126	Not stated	Yang <i>et al</i> <sup>[52]</sup>
China	Spinal TB	2005-2010	127/249 (35 DR cases studied in detail)	Any DR: 30.7%	A	9/35	0/35	No death; 33/35 cases cured	Li <i>et al</i> <sup>[47]</sup>
	Spinal TB	2006-2011	76/152 (19 DR cases studied in detail)	Any DR: 30.3%	A	Not stated	Not stated	No death; 19/19 cases cured	Xu <i>et al</i> <sup>[53]</sup>
	TBM	2009-2010	30 (culture proven TB cases)	Any DR: 66.7% MDR: 32.1%	B	20/30	0/30	Not stated	Duo <i>et al</i> <sup>[54]</sup>
Denmark	TBM	2000-2008	41/50	INH-R: 4% MDR: 2%	D	23/50	5/50	9/50 cases died	Christensen <i>et al</i> <sup>[55]</sup>
Ethiopia	Tuberculosis lymphadenitis	2012	225/437	Any DR: 6.7% MDR: 1.3%	D	Not stated	Not stated	Not stated	Biadlegne <i>et al</i> <sup>[56]</sup>
France	Various sites (including pulmonary)	1992-1999	264 MDR-TB cases (207 PTB cases, 19 EPTB cases and 38 EPTB cases with concomitant PTB)	Any DR: 6.7% MDR: 1.3%	D	38/264	55/224	Not stated	Robert <i>et al</i> <sup>[16]</sup>
India	Various EP sites	2007-2010	227/756 (165 isolates confirmed as MTB)	Any DR: 39.9% MDR: 13.5%	C	Not stated	3/165	Not stated	Maurya <i>et al</i> <sup>[18]</sup>
	TBM	Not stated	51/370	Any DR: 33.3% MDR: 1.9%	D	Not stated	Not stated	Not stated	Jain <i>et al</i> <sup>[57]</sup>
	Abdominal TB	2008-2013	31/61 (DST analyzed for 18 isolates)	Any DR: 14.3% MDR: 5.4 %	A	0/61	0/61	Not stated	Samant <i>et al</i> <sup>[58]</sup>
	Various EP sites	2010	150/547 (14 cases excluded)	MDR: 33%	A	Not stated	16/547	Not stated	Vadwai <i>et al</i> <sup>[59]</sup>
	Various EP sites	2007-2011	125/419	Any DR: 20.8% MDR: 12%	A	Not stated	7/125	Not stated	Sankar <i>et al</i> <sup>[62]</sup>
	Various EP sites	2002-2006	338 (culture proven TB cases)	Any DR: 52.7% MDR: 11.8	D	Not stated	Not stated	Not stated	Sethi <i>et al</i> <sup>[19]</sup>
	Various EP sites	2010-2011	18 (culture proven TB cases)	MDR: 5%	D	Not stated	0/18	Not stated	Desikan <i>et al</i> <sup>[60]</sup>
	TBM	2004-2005	22/100	MDR: 18.2%	C	Not stated	1/4 MDR cases	All 4 MDR cases died	Baveja <i>et al</i> <sup>[61]</sup>
	TBM	2000-2003	256/2325 (DST analyzed for 205 isolates)	Any DR: 19% MDR: 1.5%	C	Not stated	Not stated	Not stated	Venkataswamy <i>et al</i> <sup>[22]</sup>
	TBM	2001-2005	366 (culture proven TB cases)	Any DR: 17.8% MDR: 2.4 %	C	Not Stated	48/107	Not Stated	Nagarathna <i>et al</i> <sup>[62]</sup>
	Spine TB	2004-2007	25 (culture proven MDR-TB cases)		E	Not Stated	2/25 MDR cases	19/25 MDR-EPTB cases cured; rest 6 cases not concluded	Pawar <i>et al</i> <sup>[63]</sup>

Kazakhstan	Osteoarticular TB	2007-2009	76/285	MDR: 54.4 %	I	Not stated	Not stated	Tutkysbaev and Amanzholova <sup>[64]</sup> Cho <i>et al</i> <sup>[65]</sup>
Korea	Various sites	2008-2010	168 (culture proven TB cases)	Any DR: 8.9% MDR: 1.8%	E	52/168 (5 cases had DR-TB; 43 cases had disseminated TB)	4/168	Not stated
Nepal	Various sites	2004	54/513 (48 isolates confirmed as MTB)	Any DR: 62.9% MDR: 12.6%	F	Not stated	Not stated	Gurung <i>et al</i> <sup>[66]</sup>
Pakistan	Various sites	2000-2002	98/460 (88 isolates confirmed as MTB)	MDR: 21.4%	H	Not stated	Not stated	Butt <i>et al</i> <sup>[67]</sup>
Russia	Tuberculous spondylitis	2008-2011	107 (culture proven TB cases)	DR: 75.7% MDR: 69.1%	I	66/107	25/107 (15 HIV cases had MDR)	Not stated
South Africa	TBM (in children)	1992-2003	362/6781	Any DR: 11.6% MDR: 2%	E	Not stated	6/8 MDR cases	Vyazovaya <i>et al</i> <sup>[30]</sup> Padayatchi <i>et al</i> <sup>[68]</sup>
Taiwan	TBM	1999-2002	350/6762 (Only MDR cases studied)	MDR: 8.6%	E	14/30	18/30 MDR cases	Patel <i>et al</i> <sup>[20]</sup>
	Various sites	2000-2010	798 (culture proven TB cases)	Any DR: 15.5% INH-R: 9.4%	D	Not stated	17/30 MDR cases died (rest cases survived with disability)	Lai <i>et al</i> <sup>[17]</sup>
Turkey	Various EP sites	2001-2007	103 (culture proven TB cases)	MDR: 2.5%	A/C	Not stated	Not stated	Gunal <i>et al</i> <sup>[69]</sup>
United States	Various sites	1993-2006	31633/47293	Any DR: 25.2% MDR: 0.9%	E	Not stated	Unknown	Peto <i>et al</i> <sup>[13]</sup>
	Pleural TB	1993-2003	4215/7549	Any DR: 9.9% INH-R: 6%; MDR: 1%	E	264/7549 (sputum positive by culture)	305/1378	Baumann <i>et al</i> <sup>[15]</sup>
	Various sites	1993-2003	197/239 (in an ethnic group)	Any DR: 18% MDR: 3%	D	41/239	2/175	Rock <i>et al</i> <sup>[23]</sup>
	TBM	1993-2005	1614/1896 (from CSF samples)	INH-R: 6%	E	468/777 (sputum positive by culture)	404/989	Vinnard <i>et al</i> <sup>[24]</sup>
Vietnam	TBM	2004-2005	51/58 (DST result available for 46 cases)	Any DR: 54.3% MDR: 8.7%	D	Not stated	36/55	Torok <i>et al</i> <sup>[27]</sup>
	TBM	2001-2003	222 (culture proven TB cases)	Any DR: 35.1% MDR: 4.1%	D	Not stated	35/222 (17 HIV cases had DR-TB)	Caws <i>et al</i> <sup>[28]</sup>
	TBM	2000-2003	180 (culture proven TB cases)	Any DR: 40% MDR: 5.6%	A	Not stated	40/178 (21 HIV cases had DR-TB)	Thwaites <i>et al</i> <sup>[20]</sup>

Besides the reports tabulated here, some case studies as well as studies with very low sample size have also been reported from different countries but could not be addressed in this review.<sup>1</sup> Cases with unknown HIV status are excluded from total number of cases tested; <sup>2</sup> Isolates recovered from both pulmonary and extra-pulmonary sites. DST methods: A: BACTEC MGIT 960 system; B: PCR and Genotype MTBDRplus line-probe assay; C: BACTEC 460 TB system; D: Conventional DST using proportion method; E: Method not specified; F: Conventional DST using minimum inhibitory concentration method; G: Middlebrook 7H10 agar using proportion method; H: BacT/Alert 3D system; I: Conventional DST using absolute concentration method. DR: Drug resistance to any one first line anti-tuberculosis drugs; MDR: Multi-drug resistance; DST: Drug susceptibility test; MGIT: Mycobacterial growth indicator tube; MTB: *Mycobacterium tuberculosis*; EPTB: Extra-pulmonary tuberculosis; PTB: Pulmonary tuberculosis; INH: Isoniazid; HIV: Human immunodeficiency virus; R: Resistant; CSF: Cerebrospinal fluid; TBM: Tubercular meningitis; TB: Tuberculosis.

substantially. In USA, EPTB ratio has increased from 8% to 17.5% during year 1964 to 1986<sup>[9]</sup>. Similarly, an increase in prevalence of EPTB had been reported as 21% in Western Europe and 10% in Eastern Europe<sup>[10]</sup>. Recently, the incidence of EPTB is reported as 22% and about 50% of all diagnosed TB cases in United States and the United Kingdom, respectively<sup>[11,12]</sup>.

Unlike pulmonary TB, systematic drug resistant surveillance for EPTB has not been conducted. Furthermore, drug susceptibility testing for EPTB is not routinely

undertaken in many resource-limited countries, thus no authentic estimates on prevalence of drug resistant EPTB could be made available. However, sporadic information mostly derived either from retrospective cohort studies or TB surveillance data is now available from different parts of world and is summarized in Table 2. As per data accrued thus from different countries, the prevalence of MDR may lie between 1%-69% of total of EPTB cases; whereas, the proportion of resistant cases to any one anti-tuberculosis drug is about 10%-75% (Table 2). The wide variation in proportion of drug resistant EPTB among different studies is probably due to variation in study settings, burden of MDR-TB and quality of medical services in particular region, demographic characteristic and HIV status of patients, types of EPTB cases investigated, sample size and its selection criteria *etc.*. The comprehensive public health surveillance data of United States showed that about only 1% of total EPTB cases studied during 1993-2006 were MDR-EPTB<sup>[13]</sup>. On the other hand, some studies were published from high TB burden countries with much higher rate of DR-EPTB (Table 2). However in most of these studies data were collected from a cohort in a specific programmatic setting, mostly tertiary health care facility/hospitals that serves underprivileged population. In addition to this, the prevalence estimates in most of the tabulated studies collected from only a subset cases *i.e.*, culture confirmed EPTB cases. Looking at these potential limitations the prevalence figures may not be truly representative to that particular region or country; nonetheless, these studies are definitely concerning. Further, it is worth-while to note that the deadly combination of drug resistance among cases of tubercular meningitis (TBM) has been reported increasingly in many countries (Table 2). Recently, the rare possibility for the presence of XDR strain in extrapulmonary site has also been documented in India<sup>[14]</sup>.

Although, the behavior of the mycobacterium does not differ from site to site, drug resistance including MDR-TB is less common in cases of EPTB as compared to PTB. Studies from different countries have compared the drug resistance profiles between MTB isolates recovered from pulmonary and extra-pulmonary sites and showed negative association of EPTB with anti-TB drug resistance<sup>[14-17]</sup>. This is due to the fact that the selective multiplication of resistant mutants of MTB in caseous focal lesions of extrapulmonary sites is much less than in cavitary lesions of pulmonary sites. The development of DR-EPTB is mainly acquired through previous improper anti-tuberculosis regimen, poor patient compliance, a prolonged diagnosis of drug resistance and the spreading of drug-resistant strains. Drug resistance in EPTB is more common in previously treated patients<sup>[18]</sup>. Recently, a high level drug resistance among treatment failure PTB (48.1%) and EPTB cases (52.7%) from north India was reported<sup>[19]</sup>. Similarly, in another study, about 73% patients with drug resistant

TBM had a history of prior exposure to anti-tuberculosis drugs<sup>[20]</sup>.

The heterogeneous nature of extrapulmonary samples is another factor that not only contributes the variability in sensitivity values of various diagnostics but may also contribute to variation in drug resistance profile. In a study conducted in Taiwan, drug resistance profile of MTB isolates in specimens derived from various extrapulmonary sites was compared. In this study, isoniazid-resistant (or resistant to any first line drugs) MTB was more common in genitourinary and pleural sites followed by skin and soft tissue, peritoneum and lymphnode<sup>[17]</sup>. Cerebrospinal fluid, a preferred sample for diagnosis of TBM was not included in this study but drug resistance among TBM cases have been reported relatively high in different countries. Different Indian studies investigated the cerebrospinal fluid (CSF) collected from the cases of TBM and a high prevalence of drug resistant strain (MDR: about 2%; Resistance to any one first line drugs: 18%-33%) were found. Previous studies from different parts of the world showed a predominant resistance to INH in EPTB cases especially in TBM<sup>[17,21-23]</sup>. Among the first line drugs, INH is the only bactericidal agent that easily crosses the blood-brain barrier and is known to penetrate freely into the CSF. Therefore, INH resistance is a potential threat to the successful treatment and causes significant mortality in EPTB especially when meningeal involvement is present<sup>[24,25]</sup>.

EPTB is more common in HIV infected individuals and it may be undiagnosed until advance stages of the disease<sup>[26]</sup>. In addition to this, recently it is found that HIV status may also influence the incidence of drug resistance among cases of EPTB especially in TBM. Studies from Vietnam demonstrated that a significantly higher proportion (> 50%) of CSF mycobacterial isolates from TBM patients were resistant to one or more first-line anti-tuberculous drugs<sup>[27,28]</sup>. In contrast, no significant association between HIV infection and drug resistance among TBM cases were seen in some other studies<sup>[21,24]</sup>.

Recent studies suggest that different lineages of MTB may have different clinical manifestation. Indo-Oceanic and East-African Indian lineages were associated with exclusively extrapulmonary tuberculosis disease<sup>[29]</sup>. Interestingly, a strong association between "Beijing genotype" lineage, drug resistance, and HIV infection in a cohort of TBM patients has been shown<sup>[28]</sup>. Similarly, a Russian study also observed prominent association of Beijing genotype with multi-drug resistance (out of 80 Beijing genotype isolates, 90.5% were multi-drug resistant) in cases of tuberculous spondylitis<sup>[30]</sup>. However, it seems that such association may be influenced by variable distributions of MTB lineages in different parts of world. Despite this possibility, the Beijing family of MTB is considered highly virulent and associated with drug resistance in several settings<sup>[31,32]</sup>.



## CHALLENGES IN DIAGNOSIS, TREATMENT AND MANAGEMENT

Respiratory tract infections caused by pathogens other than *Mycobacteria* is often implicated by physician. But the treatment failure and recurrence or persistence of symptoms led to suspicion and investigation of PTB. Despite this initial delay, the definite diagnosis and effective treatment management of PTB is feasible. Unlike PTB, the diagnosis of EPTB is a major challenge for clinician. As the clinical manifestation of EPTB is highly heterogeneous, many of patients are examined by different specialists with little awareness in TB diagnosis thus may cause significant delay in including the EPTB into differential diagnosis panel. Data from many countries shows that 20%-50% cases of EPTB are diagnosed post-mortem<sup>[33]</sup>, which not only highlights our limited ability to diagnose the EPTB but also provide a possible reason for lower incidence of EPTB in many countries. Lack of strong laboratory backup at peripheral health facility level in many high TB burden countries also contribute significantly for excess delay and difficulty to establish EPTB.

It is believed that a small amount of tubercle bacilli even sensitive to anti-tubercular drugs often cause great damage in some aggressive form of TB like TBM. Therefore, diagnostic delay in EPTB poses great risk of mortality. Previous studies demonstrated, a delay of 3 d between TBM presentation and initiation of anti-tuberculous therapy among pediatric as well as adult cases is associated with increased risk of death<sup>[34,35]</sup>. Further, drug resistance may severely complicate the situation because a twofold increase in mortality was observed recently among the isoniazid drug resistant TBM cases<sup>[36]</sup>. The various hurdles making the diagnosis of DR-EPTB an issue are highlighted in Table 3.

Although, EPTB is not considered as contagious but concomitant contagious PTB may spread the infection to others. European TB surveillance report<sup>[37]</sup> showed that about 1/4<sup>th</sup> of all EPTB cases had also pulmonary involvement. Thus the presence of EPTB does not exclude the pulmonary involvement, even though such cases are nevertheless classified as contagious PTB<sup>[1]</sup>. Recent studies have found that even with a normal chest radiograph, the concomitant pulmonary involvement may be present in considerable proportion (up to 18%) of patients<sup>[38,39]</sup>. However still in routine practice, the existence of PTB in cases of EPTB is not generally ruled out.

For a definite diagnosis of EPTB, WHO recommends that it should be on the basis of culture-positive specimen and/or positive histology and/or strong clinical evidence consistent with active EPTB<sup>[1]</sup>. A cautious investigation of clinical, radiological and histopathological representations in different form of EPTB may provide some clues to achieve the diagnosis even than seeking the laboratory based diagnosis is important to establish the disease. Although laboratory testing for EPTB follows the same principles as for PTB, but it is very difficult and

negative test result does not rule out the EPTB.

The multiplication of tubercle bacilli is generally hindered within the caseous foci lesion/tissues of extrapulmonary sites due to low availability of oxygen as well as acidic pH, and the presence of toxic fatty acids<sup>[40]</sup>. Therefore under these stress condition, EPTB is generally found in pauci-bacillary that may be skipped to be detected by conventional diagnostic tests. Direct visualization of acid-fast bacteria is still the first and most preferred microbiological test but it is far from being sensitive to diagnose EPTB<sup>[41]</sup>. At present, microbiological confirmation (either by solid or liquid culture method) of MTB in samples from the affected sites of EPTB is considered as gold standard which also gives the opportunity to identify the *Mycobacterium* species and to perform phenotypic drug-susceptibility tests as well as genotyping for molecular epidemiology studies. However, the main drawback of culture is long turn-around time and compromised sensitivity due to lower and non-uniform distribution of bacillary load at extrapulmonary sites as well as compromised quality and quantity of specimen. It has been demonstrated that concentration of large volumes of sampled fluid (CSF, ascites, etc.) and repeated analyses can increase the diagnostic yield<sup>[42]</sup>. However, it is often too difficult to get additional and adequate amount of samples from the patients.

As discussed above, delay in diagnosis may not be acceptable in the management of aggressive TB cases like TBM or HIV-associated EPTB, a rapid and sensitive diagnosis and DST method is always pre-requisite. In the same regard, several nucleic-acid amplification tests (NAAT) are now available that can detect MTB and can also determine the drug resistance to some key drugs. Of the various NAAT, Xpert MTB/RIF seems most promising as it is fully automated cartridge-based real-time PCR based test that efficiently detects both TB and resistance to rifampicin in less than 2 h. WHO reviewed a set of studies and concluded into an excellent sensitivity and specificity of Xpert MTB/RIF from pooled data on samples collected from various extrapulmonary sites (CSFs, gastric fluids and biopsies)<sup>[43]</sup>. Unfortunately, this system does not determine the isoniazid resistance which is known to be significantly associated with mortality among TBM cases. The cost of the test, dependency on electric supply, the cartridge supply and storage conditions and the difficulty to carry and implement the system in limited resource settings are some other challenges. Nevertheless, Xpert MTB/RIF assay coupled with its speed, simplicity and less biohazard problems could play an important routine role in diagnosis of EPTB.

Like diagnosis, the treatment and management of EPTB is also full of challenges and recently a meta-analysis showed that treatment outcomes were poor in patients with DR-TB<sup>[44]</sup>. This is especially due to poor drug penetration to the affected tissue and the lack of accessibility of tissue/sample for assessing the treatment response by serial cultures. Further, any degree of drug

**Table 3** Key issues in diagnosis of drug resistant-extrapulmonary tuberculosis

Issues in laboratory diagnosis
In general, the sensitivity of laboratory tests is often compromised
Due to pauci-bacillary nature of EPTB
Due to difficulty in obtaining an adequate sample
Risk associated with the sampling procedure ( <i>e.g.</i> , lumbar puncture, biopsy of deep lymph nodes, <i>etc.</i> )
Lack of accessibility of serial samples for monitoring the treatment response
Un-availability of reliable host biomarkers that can be analyzed in easily attainable specimens
Xpert-MTB-RIF test seems promising diagnostic tool but the negative test result does not rule out EPTB and it can only determine the resistance to rifampicin (not other crucial drugs)
Issues at programmatic/administrative level
Lack of focused programme (like pulmonary TB) in many high TB burden countries
Lack of reliable estimates on impact and magnitude of DR-EPTB
Although seeking microbiological, histopathological diagnosis and drug susceptibility testing is crucial, it is however not in routine practice (in many high TB burden countries)
Rapid molecular based diagnostic (Xpert-MTB-RIF) is still away from its accessibility at peripheral health care centers in many resource limited countries
Wide variation in diagnostic and treatment practices among health service providers (as reported in private sectors of India) that often do not comply with national or international standards

DR-EPTB: Drug resistant-extrapulmonary tuberculosis; MTB: *Mycobacterium tuberculosis*; RIF: Rifampicin; TB: Tuberculosis; EPTB: Extrapulmonary tuberculosis.

resistance hinders the treatment and may results into poor outcomes especially in severe form of disease. The problem gets compounded among HIV infected individual as it has been shown that MDR-TB at treatment initiation, positive HIV status among EPTB cases are significantly associated with mortality<sup>[24,45]</sup>. WHO recommended the treatment of MDR-EPTB with the same strategy involving the same regimen and duration as pulmonary MDR-TB. However in severe complication, individualized chemotherapy and some other means like surgery, adjunctive corticosteroids, immune-modulators may result into good treatment outcomes<sup>[46,47]</sup>. Owing to increasing incidence of drug resistance and severity of some kinds of EPTB, there is an urgent need for effective short-term regimens with newer drugs having better penetration at various sites in body.

At programmatic management level, EPTB also deserves special attention to ensure access of quality diagnosis, drug susceptibility testing and prompt initiation of appropriate therapy. The political commitment and support in this regard is utmost important. In many high TB burden countries, the peripheral health centers are still devoid of strong laboratory back-up and sensitized medical staff. In India, EPTB is predominantly managed in the private sector and these cases are rarely notified to government agencies. Additionally, in private sector the diagnostic and treatment practices is not firmly followed as per national or international standards and usually treatment is started without having culture confirmation and drug susceptibility testing<sup>[48]</sup>. Taking all these challenges into consideration, it is anticipated that MDR-TB affecting extrapulmonary site may continue to increase.

## CONCLUSION

Drug resistance among cases of EPTB is a definitely

rising problem in most of the countries where ever it has been investigated. Since survey study on similar line begun to accumulate only recently, a continued effort in future will be of exquisite importance to achieve reliable estimates of the incidence in different parts of world. Such information will be imperative in establishment of strategic frameworks for intensified cases finding, effective treatment management and also to garner the resources necessary for the prevention of associated high morbidity and mortality. Further, there is an urgent need for increasing awareness of the clinician to rising incidence drug resistant EPTB and to consider the drug resistance testing before start of therapy for better treatment outcomes. We hope this preliminary review will encourage the future systematic studies to precisely define the epidemiological picture of drug-resistance EPTB.

## REFERENCES

- 1 **World Health Organization.** Global Tuberculosis Report 2013. Geneva: WHO, 2013. [accessed 2015 Jan 5]. Available from: URL: [http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656\\_eng.pdf](http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf)
- 2 **Sharma SK, Mohan A.** Extrapulmonary tuberculosis. *Indian J Med Res* 2004; **120**: 316-353 [PMID: 15520485]
- 3 **Golden MP, Vikram HR.** Extrapulmonary tuberculosis: an overview. *Am Fam Physician* 2005; **72**: 1761-1768 [PMID: 16300038]
- 4 **Rossato Silva D, Müller AM, Dalcin Pde T.** Factors associated with delayed diagnosis of tuberculosis in hospitalized patients in a high TB and HIV burden setting: a cross-sectional study. *BMC Infect Dis* 2012; **12**: 57 [PMID: 22420509 DOI: 10.1186/1471-2334-12-57]
- 5 **Nelson LJ, Wells CD.** Global epidemiology of childhood tuberculosis. *Int J Tuberc Lung Dis* 2004; **8**: 636-647 [PMID: 15137548]
- 6 **Carrol ED, Clark JE, Cant AJ.** Non-pulmonary tuberculosis. *Paediatr Respir Rev* 2001; **2**: 113-119 [PMID: 12531057 DOI: 10.1053/prrv.2000.0118]
- 7 **Zhang Y, Yew WW.** Mechanisms of drug resistance in *Mycobacterium tuberculosis*. *Int J Tuberc Lung Dis* 2009; **13**: 1320-1330 [PMID: 19861002]
- 8 **World Health Organization.** Treatment of tuberculosis:

- Guidelines. 4th ed. Available from: URL: [http://apps.who.int/iris/bitstream/10665/44165/1/9789241547833\\_eng.pdf](http://apps.who.int/iris/bitstream/10665/44165/1/9789241547833_eng.pdf)
- 9 **Gonzalez OY**, Adams G, Teeter LD, Bui TT, Musser JM, Graviss EA. Extra-pulmonary manifestations in a large metropolitan area with a low incidence of tuberculosis. *Int J Tuberc Lung Dis* 2003; **7**: 1178-1185 [PMID: 14677893]
  - 10 **Euro TB**. (InVS/KNCV) and the national coordinators for tuberculosis surveillance in the WHO European Region. Surveillance of tuberculosis in Europe. Report on tuberculosis cases notified in 1998. 2001. [accessed 2015 Jan 5]. Available from: URL: [http://opac.invs.sante.fr/doc\\_num.php?explnum\\_id=5656](http://opac.invs.sante.fr/doc_num.php?explnum_id=5656)
  - 11 **Centers for Disease Control and Prevention**. Reported Tuberculosis in the United States, 2010. Atlanta, GA: CDC, 2011
  - 12 **Health Protection Agency**. Tuberculosis in the UK: Annual report on tuberculosis surveillance in the UK, 2012. Available from: URL: [https://www.gov.uk/government/uploads/system/uploads/attachment\\_data/file/332560/TB\\_Annual\\_Report\\_2012.pdf](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/332560/TB_Annual_Report_2012.pdf)
  - 13 **Peto HM**, Pratt RH, Harrington TA, LoBue PA, Armstrong LR. Epidemiology of extrapulmonary tuberculosis in the United States, 1993-2006. *Clin Infect Dis* 2009; **49**: 1350-1357 [PMID: 19793000 DOI: 10.1086/605559]
  - 14 **Balaji V**, Daley P, Anand AA, Sudarsanam T, Michael JS, Sahni RD, Chordia P, George IA, Thomas K, Ganesh A, John KR, Mathai D. Risk factors for MDR and XDR-TB in a tertiary referral hospital in India. *PLoS One* 2010; **5**: e9527 [PMID: 20209106 DOI: 10.1371/journal.pone.0009527]
  - 15 **Baumann MH**, Nolan R, Petrini M, Lee YC, Light RW, Schneider E. Pleural tuberculosis in the United States: incidence and drug resistance. *Chest* 2007; **131**: 1125-1132 [PMID: 17426219 DOI: 10.1378/chest.06-2352]
  - 16 **Robert J**, Trystram D, Truffot-Pernot C, Jarlier V. Multidrug-resistant tuberculosis: eight years of surveillance in France. *Eur Respir J* 2003; **22**: 833-837 [PMID: 14621093 DOI: 10.1183/09031936.03.00014103]
  - 17 **Lai CC**, Liu WL, Tan CK, Huang YC, Chung KP, Lee MR, Hsueh PR. Differences in drug resistance profiles of Mycobacterium tuberculosis isolates causing pulmonary and extrapulmonary tuberculosis in a medical centre in Taiwan, 2000-2010. *Int J Antimicrob Agents* 2011; **38**: 125-129 [PMID: 21592735 DOI: 10.1016/j.ijantimicag.2011.03.016]
  - 18 **Maurya AK**, Kant S, Nag VL, Kushwaha R, Dhole TN. Detection of 123 bp fragment of insertion element IS6110 Mycobacterium tuberculosis for diagnosis of extrapulmonary tuberculosis. *Indian J Med Microbiol* 2012; **30**: 182-186 [PMID: 22664434 DOI: 10.4103/0255-0857.96688]
  - 19 **Sethi S**, Biswal M, Chatterjee SS, Mewara A, Gupta D, Kumar S, Sharma M. Susceptibility pattern among pulmonary and extrapulmonary isolates of Mycobacterium tuberculosis in north India. *African J Microbiol Res* 2012; **6**: 3696-3699
  - 20 **Patel VB**, Padayatchi N, Bhigjee AI, Allen J, Bhagwan B, Moodley AA, Mthiyane T. Multidrug-resistant tuberculous meningitis in KwaZulu-Natal, South Africa. *Clin Infect Dis* 2004; **38**: 851-856 [PMID: 14999630]
  - 21 **Vinnard C**, Winston CA, Wileyto EP, MacGregor RR, Bisson GP. Isoniazid-resistant tuberculous meningitis, United States, 1993-2005. *Emerg Infect Dis* 2011; **17**: 539-542 [PMID: 21392454 DOI: 10.3201/eid1703.101715]
  - 22 **Venkataswamy MM**, Rafi W, Nagarathna S, Ravi V, Chandramuki A. Comparative evaluation of BACTEC 460TB system and Lowenstein-Jensen medium for the isolation of MTB from cerebrospinal fluid samples of tuberculous meningitis patients. *Indian J Med Microbiol* 2007; **25**: 236-240 [PMID: 17901641]
  - 23 **Rock RB**, Sutherland WM, Baker C, Williams DN. Extrapulmonary Tuberculosis among Somalis in Minnesota. *Emerg Infect Dis* 2006; **12**: 1434-1436 [PMID: 17073097]
  - 24 **Vinnard C**, Winston CA, Wileyto EP, Macgregor RR, Bisson GP. Isoniazid resistance and death in patients with tuberculous meningitis: retrospective cohort study. *BMJ* 2010; **341**: c4451 [PMID: 20819874 DOI: 10.1136/bmj.c4451]
  - 25 **Daikos GL**, Cleary T, Rodriguez A, Fischl MA. Multidrug-resistant tuberculous meningitis in patients with AIDS. *Int J Tuberc Lung Dis* 2003; **7**: 394-398 [PMID: 12729347]
  - 26 **Centers for Diseases Control and Prevention**. CDC grand rounds: the TB/HIV syndemic. *MMWR* 2012; **61**: 484-489. [accessed 2015 Jan 5]. Available from: URL: <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6126a3.htm>
  - 27 **Torok ME**, Chau TT, Mai PP, Phong ND, Dung NT, Chuong LV, Lee SJ, Caws M, de Jong MD, Hien TT, Farrar JJ. Clinical and microbiological features of HIV-associated tuberculous meningitis in Vietnamese adults. *PLoS One* 2008; **3**: e1772 [PMID: 18350135 DOI: 10.1371/journal.pone.0001772]
  - 28 **Caws M**, Thwaites G, Stepniewska K, Nguyen TN, Nguyen TH, Nguyen TP, Mai NT, Phan MD, Tran HL, Tran TH, van Soolingen D, Kremer K, Nguyen VV, Nguyen TC, Farrar J. Beijing genotype of Mycobacterium tuberculosis is significantly associated with human immunodeficiency virus infection and multidrug resistance in cases of tuberculous meningitis. *J Clin Microbiol* 2006; **44**: 3934-3939 [PMID: 16971650]
  - 29 **Click ES**, Moonan PK, Winston CA, Cowan LS, Oeltmann JE. Relationship between Mycobacterium tuberculosis phylogenetic lineage and clinical site of tuberculosis. *Clin Infect Dis* 2012; **54**: 211-219 [PMID: 22198989 DOI: 10.1093/cid/cir788]
  - 30 **Vyazovaya A**, Mokrousov I, Solovieva N, Mushkin A, Manicheva O, Vishnevsky B, Zhuravlev V, Narvskaya O. Tuberculous spondylitis in Russia and prominent role of multidrug-resistant clone Mycobacterium tuberculosis Beijing B0/W148. *Antimicrob Agents Chemother* 2015; **59**: 2349-2357 [PMID: 25645851 DOI: 10.1128/AAC.04221-14]
  - 31 **European Concerted Action on New Generation Genetic Markers and Techniques for the Epidemiology and Control of Tuberculosis**. Beijing/W genotype Mycobacterium tuberculosis and drug resistance. *Emerg Infect Dis* 2006; **12**: 736-743 [PMID: 16704829]
  - 32 **Sankar MM**, Singh J, Diana SC, Singh S. Molecular characterization of Mycobacterium tuberculosis isolates from North Indian patients with extrapulmonary tuberculosis. *Tuberculosis (Edinb)* 2013; **93**: 75-83 [PMID: 23140853 DOI: 10.1016/j.tube.2012.10.005]
  - 33 **Rowińska-Zakrzewska E**. [Extrapulmonary tuberculosis, risk factors and incidence]. *Pneumonol Alergol Pol* 2011; **79**: 377-378 [PMID: 22028114]
  - 34 **Delage G**, Dusseault M. Tuberculous meningitis in children: a retrospective study of 79 patients, with an analysis of prognostic factors. *Can Med Assoc J* 1979; **120**: 305-309 [PMID: 427668]
  - 35 **Verdon R**, Chevret S, Laissy JP, Wolff M. Tuberculous meningitis in adults: review of 48 cases. *Clin Infect Dis* 1996; **22**: 982-988 [PMID: 8783697]
  - 36 **Marx GE**, Chan ED. Tuberculous meningitis: diagnosis and treatment overview. *Tuberc Res Treat* 2011; **2011**: 798764 [PMID: 22567269 DOI: 10.1155/2011/798764]
  - 37 **World Health Organization (Regional Office for Europe)**. Surveillance report: Tuberculosis surveillance and monitoring in Europe 2012. [accessed 2015 Jan 5]. Available from: URL: <http://ecdc.europa.eu/en/publications/Publications/1203-Annual-TB-Report.pdf>
  - 38 **Parimon T**, Spitters CE, Muangman N, Euathrongchit J, Oren E, Narita M. Unexpected pulmonary involvement in extrapulmonary tuberculosis patients. *Chest* 2008; **134**: 589-594 [PMID: 18641092 DOI: 10.1378/chest.08-0319]
  - 39 **Herath S**, Lewis C. Pulmonary involvement in patients presenting with extra-pulmonary tuberculosis: thinking beyond a normal chest x-ray. *J Prim Health Care* 2014; **6**: 64-68 [PMID: 24624413]
  - 40 **Smith I**. Mycobacterium tuberculosis pathogenesis and molecular determinants of virulence. *Clin Microbiol Rev* 2003; **16**: 463-496 [PMID: 12857778]
  - 41 **World Health Organization (Country Office for India)**. Standards for TB Care in India. [accessed 2015 Jan 5]. Available from: URL: [http://www.tbcindia.nic.in/pdfs/stci%20Book\\_Final%20%20060514.pdf](http://www.tbcindia.nic.in/pdfs/stci%20Book_Final%20%20060514.pdf)
  - 42 **Norbis L**, Alagna R, Tortoli E, Codecasa LR, Migliori GB, Cirillo



- DM. Challenges and perspectives in the diagnosis of extrapulmonary tuberculosis. *Expert Rev Anti Infect Ther* 2014; **12**: 633-647 [PMID: 24717112 DOI: 10.1586/14787210.2014.899900]
- 43 **Thwaites G**, Fisher M, Hemingway C, Scott G, Solomon T, Innes J. British Infection Society guidelines for the diagnosis and treatment of tuberculosis of the central nervous system in adults and children. *J Infect* 2009; **59**: 167-187 [PMID: 19643501 DOI: 10.1016/j.jinf.2009.06.011]
- 44 **World Health Organization**. Policy update: Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system for the diagnosis of pulmonary and extrapulmonary TB in adults and children. [accessed 2015 Jan 5]. Available from: URL: [http://apps.who.int/iris/bitstream/10665/112472/1/9789241506335\\_eng.pdf](http://apps.who.int/iris/bitstream/10665/112472/1/9789241506335_eng.pdf)
- 45 **Lew W**, Pai M, Oxlade O, Martin D, Menzies D. Initial drug resistance and tuberculosis treatment outcomes: systematic review and meta-analysis. *Ann Intern Med* 2008; **149**: 123-134 [PMID: 18626051]
- 46 **Croda MG**, Vidal JE, Hernández AV, Dal Molin T, Gualberto FA, de Oliveira AC. Tuberculous meningitis in HIV-infected patients in Brazil: clinical and laboratory characteristics and factors associated with mortality. *Int J Infect Dis* 2010; **14**: e586-e591 [PMID: 20005759 DOI: 10.1016/j.ijid.2009.08.012]
- 47 **Li L**, Zhang Z, Luo F, Xu J, Cheng P, Wu Z, Zhou Q, He Q, Dai F, Wang J, Zhang J. Management of drug-resistant spinal tuberculosis with a combination of surgery and individualised chemotherapy: a retrospective analysis of thirty-five patients. *Int Orthop* 2012; **36**: 277-283 [PMID: 22065055 DOI: 10.1007/s00264-011-1398-0]
- 48 **Pai M**, Nathavitharana R. Extrapulmonary tuberculosis: new diagnostics and new policies. *Indian J Chest Dis Allied Sci* 2014; **56**: 71-73 [PMID: 25230546]
- 49 **Cecchini D**, Ambrosioni J, Brezzo C, Corti M, Rybko A, Perez M, Poggi S, Ambroggi M. Tuberculous meningitis in HIV-infected patients: drug susceptibility and clinical outcome. *AIDS* 2007; **21**: 373-374 [PMID: 17255747 DOI: 10.1097/QAD.0b013e328012b84d]
- 50 **Lumb R**, Bastion I, Carter R, Jelfs P, Keehner T, Sievers A. Tuberculosis in Australia: bacteriologically confirmed cases and drug resistance, 2007. A report of the Australian Mycobacterium Reference Laboratory Network. *Commun Dis Intell Q Rep* 2009; **33**: 298-303 [PMID: 20043600]
- 51 **Afroz H**, Ali MA, Faruddin M, Kamrunnahar, Datta S. Prevalence and treatment follow-up of drug-resistant extra-pulmonary tuberculosis in rural communities in Narshingdi, Bangladesh. *Int J Advan Med* 2014; **1**: 71-77 [DOI: 10.5455/2349-3933.ijam20140801]
- 52 **Yang H**, Field SK, Fisher DA, Cowie RL. Tuberculosis in Calgary, Canada, 1995-2002: site of disease and drug susceptibility. *Int J Tuberc Lung Dis* 2005; **9**: 288-293 [PMID: 15786892]
- 53 **Xu L**, Jian-Zhong X, Xue-Mei L, Bao-Feng G. Drug susceptibility testing guided treatment for drug-resistant spinal tuberculosis: a retrospective analysis of 19 patients. *Int Surg* 2013; **98**: 175-180 [PMID: 23701156 DOI: 10.9738/INTSURG-D-12-00004.1]
- 54 **Duo L**, Ying B, Song X, Lu X, Ye Y, Fan H, Xin J, Wang L. Molecular profile of drug resistance in tuberculous meningitis from southwest china. *Clin Infect Dis* 2011; **53**: 1067-1073 [PMID: 22021920 DOI: 10.1093/cid/cir663]
- 55 **Christensen AS**, Andersen AB, Thomsen VO, Andersen PH, Johansen IS. Tuberculous meningitis in Denmark: a review of 50 cases. *BMC Infect Dis* 2011; **11**: 47 [PMID: 21342524 DOI: 10.1186/1471-2334-11-47]
- 56 **Biadlegne F**, Tessema B, Sack U, Rodloff AC. Drug resistance of Mycobacterium tuberculosis isolates from tuberculosis lymphadenitis patients in Ethiopia. *Indian J Med Res* 2014; **140**: 116-122 [PMID: 25222786]
- 57 **Jain A**, Dixit P, Jaiswal I, Garg RK, Kumar R. Drug resistance in mycobacterial isolates from meningitis cases. *Pediatr Infect Dis J* 2012; **31**: 1317 [PMID: 23188103 DOI: 10.1097/INF.0b013e3182717f25]
- 58 **Samant H**, Desai D, Abraham P, Joshi A, Gupta T, Rodrigues C, George S. Acid-fast bacilli culture positivity and drug resistance in abdominal tuberculosis in Mumbai, India. *Indian J Gastroenterol* 2014; **33**: 414-419 [PMID: 24927950 DOI: 10.1007/s12664-014-0467-x]
- 59 **Vadwai V**, Boehme C, Nabeta P, Shetty A, Alland D, Rodrigues C. Xpert MTB/RIF: a new pillar in diagnosis of extrapulmonary tuberculosis? *J Clin Microbiol* 2011; **49**: 2540-2545 [PMID: 21593262 DOI: 10.1128/JCM.02319-10]
- 60 **Desikan P**, Chauhan DS, Sharma P, Panwalkar N, Yadav P, Ohri BS. Clonal diversity and drug resistance in Mycobacterium tuberculosis isolated from extra-pulmonary samples in central India--a pilot study. *Indian J Med Microbiol* 2014; **32**: 434-437 [PMID: 25297032 DOI: 10.4103/0255-0857.142255]
- 61 **Baveja CP**, Gumma V, Jain M, Chaudhary M, Talukdar B, Sharma VK. Multi drug resistant tuberculous meningitis in pediatric age group. *Iranian J Pediatr* 2008; **18**: 309-314
- 62 **Nagarathna S**, Rafi W, Veenakumari HB, Mani R, Satishchandra P, Chandramuki A. Drug susceptibility profiling of tuberculous meningitis. *Int J Tuberc Lung Dis* 2008; **12**: 105-107 [PMID: 18173886]
- 63 **Pawar UM**, Kundnani V, Agashe V, Nene A, Nene A. Multidrug-resistant tuberculosis of the spine--is it the beginning of the end? A study of twenty-five culture proven multidrug-resistant tuberculosis spine patients. *Spine (Phila Pa 1976)* 2009; **34**: E806-E810 [PMID: 19829244 DOI: 10.1097/BRS.0b013e3181af7797]
- 64 **Tutkysbbaev S**, Amanzholova L. Multidrug resistance in patients with osteoarticular tuberculosis. *Med Health Sci J* 2011; **6**: 85-87 [DOI: 10.15208/mhsj.2010.114]
- 65 **Cho OH**, Park KH, Park SY, Moon SM, Chong YP, Kim MN, Lee SO, Choi SH, Woo JH, Kim YS, Kim SH. Drug-resistant extrapulmonary tuberculosis. *Infect Chemother* 2011; **43**: 258-261 [DOI: 10.3947/ic.2011.43.3.258]
- 66 **Gurung R**, Bhattacharya SK, Pradhan B, Gurung S, Singh YI. Phenotypic characterisation and drug sensitivity testing of mycobacteria isolated from extra-pulmonary tuberculosis. *Kathmandu Univ Med J (KUMJ)* 2010; **8**: 57-61 [PMID: 21209509 DOI: 10.3126/kumj.v8i1.3223]
- 67 **Butt T**, Kazmi SY, Ahmad RN, Mahmood A, Karamat KA, Anwar M. Frequency and antibiotic susceptibility pattern of mycobacterial isolates from extra-pulmonary tuberculosis cases. *J Pak Med Assoc* 2003; **53**: 328-332 [PMID: 14558735]
- 68 **Padayatchi N**, Bamber S, Dawood H, Bobat R. Multidrug-resistant tuberculous meningitis in children in Durban, South Africa. *Pediatr Infect Dis J* 2006; **25**: 147-150 [PMID: 16462292 DOI: 10.1097/01.inf.0000199314.88063.4c]
- 69 **Gunel S**, Yang Z, Agarwal M, Koroglu M, Arıcı ZK, Durmaz R. Demographic and microbial characteristics of extrapulmonary tuberculosis cases diagnosed in Malatya, Turkey, 2001-2007. *BMC Public Health* 2011; **11**: 154 [PMID: 21385458 DOI: 10.1186/1471-2458-11-154]
- 70 **Thwaites GE**, Lan NT, Dung NH, Quy HT, Oanh DT, Thoa NT, Hien NQ, Thuc NT, Hai NN, Bang ND, Lan NN, Duc NH, Tuan VN, Hiep CH, Chau TT, Mai PP, Dung NT, Stepniowska K, White NJ, Hien TT, Farrar JJ. Effect of antituberculosis drug resistance on response to treatment and outcome in adults with tuberculous meningitis. *J Infect Dis* 2005; **192**: 79-88 [PMID: 15942897 DOI: 10.1086/430616]

P- Reviewer: Boonsarngsuk V, Garcia-Elorriaga G  
S- Editor: Gong XM L- Editor: A E- Editor: Li D





## Basic Study

***Tuf* mRNA rather than 16S rRNA is associated with culturable *Staphylococcus aureus***

Anne JM Loonen, Petra FG Wolffs, Maikel de Bresser, Maurice Habraken, Cathrien A Bruggeman, Mirjam HA Hermans, Adriaan JC van den Brule

Anne JM Loonen, Maikel de Bresser, Maurice Habraken, Mirjam HA Hermans, Adriaan JC van den Brule, Laboratory for Molecular Diagnostics, Department of Medical Microbiology and Pathology, Jeroen Bosch Hospital, 5200 ME 's-Hertogenbosch, The Netherlands

Anne JM Loonen, Maikel de Bresser, Maurice Habraken, Department of Medical Molecular Diagnostics, Fontys University of Applied Sciences, 5600 AH Eindhoven, The Netherlands

Anne JM Loonen, Petra FG Wolffs, Cathrien A Bruggeman, Department of Medical Microbiology, CAPHRI, Maastricht University Medical Centre, 6229 HX Maastricht, The Netherlands

**Author contributions:** Loonen AJM, Wolffs PFG and van den Brule AJC designed the research; Loonen AJM, de Bresser M and Habraken M performed the research; Loonen AJM, Wolffs PFG, Bruggeman CA, Hermans MHA and van den Brule AJC analyzed the data and wrote the paper.

**Institutional review board statement:** No human subjects were used for this study and therefore no ethical approval was required.

**Institutional animal care and use committee statement:** No animals were used for this study.

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

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Adriaan JC van den Brule, PhD,

Laboratory for Molecular Diagnostics, Department of Medical Microbiology and Pathology, Jeroen Bosch Hospital, Box 90153, 5200 ME 's-Hertogenbosch, The Netherlands. [a.v.d.brule@jzbz.nl](mailto:a.v.d.brule@jzbz.nl)  
Telephone: +31-73-5538480  
Fax: +31-73-5532136

Received: February 9, 2015

Peer-review started: February 10, 2015

First decision: March 6, 2015

Revised: May 11, 2015

Accepted: June 9, 2015

Article in press: June 11, 2015

Published online: November 25, 2015

**Abstract**

**AIM:** To study the presence of various nucleic acids targets of *Staphylococcus aureus* (*S. aureus*) during bacterial growth and antibiotic induced killing in relation to viability.

**METHODS:** *S. aureus* was cultured to log phase and spiked in Todd Hewitt (TH) broth and whole blood of healthy human volunteers. Viability of *S. aureus* after flucloxacillin treatment (0, 1, 3 and 6 d) was assessed by culture on bloodagar plates. DNA and RNA were isolated from 200 µL. cDNA synthesis was performed by using random primers. The presence of *S. aureus* DNA, rRNA, and mRNA were determined by real-time polymerase chain reaction of the 16S rDNA and *tuf* gene (elongation factor Tu).

**RESULTS:** *S. aureus* spiked in TH broth without antibiotics grew from day 0-6 and DNA (*tuf* and 16S), and 16S rRNA remained detectable during this whole period. During flucloxacillin treatment *S. aureus* lost viability from day 3 onwards, while the 16S rRNA-gene and its RNA transcripts remained detectable. DNA and

rRNA can be detected in flucloxacillin treated *S. aureus* cultures that do not further contain culturable bacteria. However, *tuf* mRNA became undetectable from day 3 onwards. *Tuf* mRNA can only be detected from samples with culturable bacteria. When spiking *S. aureus* in whole blood instead of broth no bacterial growth was seen, neither in the absence nor in the presence of flucloxacillin. Accordingly, no increase in DNA and RNA levels of both 16S rDNA and the *tuf* gene were detected.

**CONCLUSION:** *Tuf* mRNA expression is associated with culturable *S. aureus* and might be used to monitor antibiotic effects.

**Key words:** Bloodstream infection; *Staphylococcus aureus*; Viability; mRNA; Polymerase chain reaction; Sepsis; Molecular diagnostics; Blood

© The Author(s) 2015. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** We report our first results from a proof-of-principle study where we show that *tuf* mRNA expression seems to correlate with active *Staphylococcus aureus* (*S. aureus*) infection. The commonly used target, 16S rRNA, seems unsuitable for viability measurements as it can be detected from samples containing unculturable bacteria. This study indicates that *tuf* mRNA expression is associated with viable *S. aureus*, as determined by culture.

Loonen AJM, Wolffs PFG, de Bresser M, Habraken M, Bruggeman CA, Hermans MHA, van den Brule AJC. *Tuf* mRNA rather than 16S rRNA is associated with culturable *Staphylococcus aureus*. *World J Clin Infect Dis* 2015; 5(4): 86-93 Available from: URL: <http://www.wjgnet.com/2220-3176/full/v5/i4/86.htm> DOI: <http://dx.doi.org/10.5495/wjcid.v5.i4.86>

## INTRODUCTION

Bacteremia is defined as the presence of viable bacteria in the bloodstream<sup>[1]</sup>. The current gold standard method for the detection of microorganisms in the bloodstream is blood culture and subsequent identification of the bacteria by conventional (sometimes automated) biochemical techniques or MALDI-TOF MS<sup>[2-4]</sup>. An important advantage of this method is that only viable microorganisms are detected. A major disadvantage of the method is that the time-to-results is long (24-72 h) due to the involvement of culturing steps. Because fast and accurate diagnosis is of crucial importance for patients suffering from bloodstream infection (BSI), molecular (real-time) polymerase chain reaction (PCR) applications are increasingly being applied to decrease time to pathogen identification, thereby improving patient outcome<sup>[5-8]</sup>. However, all commercially available sepsis tests [SeptiFAST (Roche), SepsiTtest (Molzym),

and MagicPlex Sepsis Test (Seegene)] are based on DNA detection. DNA is a stable molecule and the presence of DNA of a certain pathogen does not provide information about the viability status of that pathogen as the DNA can originate from either living or dead pathogens<sup>[9-12]</sup>. In contrast to DNA, bacterial messenger RNA molecules have a half-life of only minutes<sup>[13]</sup>. For that reason, several studies have evaluated the detection of mRNA as a marker for the presence of actively growing bacteria<sup>[9,11,14-18]</sup>. Some of these studies have focused on detection of viable pathogens from food and environmental samples<sup>[14]</sup>, while other studies focused on human disease and viability of pathogens from spiked culture broths (*i.e.*, *Borrelia burgdorferi*, *Escherichia coli*, *Salmonella typhi*, *Shigella sonnei*, *Mycobacterium smegmatis*)<sup>[10,11,17,19]</sup>. Few studies used clinical specimens (*Mycobacterium tuberculosis* from sputum samples, *Aspergillus* spp. from blood samples, *Chlamydia trachomatis* from cervical smears and urine)<sup>[12,15,16]</sup>. If RNA markers can be used to assess pathogen viability for BSI, the application of PCR based methods on RNA (cDNA) would be of great significance.

BSI can be caused by numerous pathogens<sup>[20]</sup>. In this study, the most commonly detected Gram-positive bacterium; *Staphylococcus aureus* (*S. aureus*) was chosen for reconstruction experiments. To investigate which nucleic acid molecule most favourably correlates to the viability status of *S. aureus*, DNA and rRNA of 16S rRNA gene, and DNA and mRNA levels of the *tuf* gene (elongation factor Tu) were measured in response to antibiotic therapy. Both the 16S rRNA and *tuf* gene are household genes with relatively high expression levels and therefore indicative of protein expression and thus most likely bacterial viability<sup>[21]</sup>. The aim of this work was to find a suitable marker for *S. aureus* viability to be able to improve BSI diagnostics.

## MATERIALS AND METHODS

### Bacterial strain and growth conditions

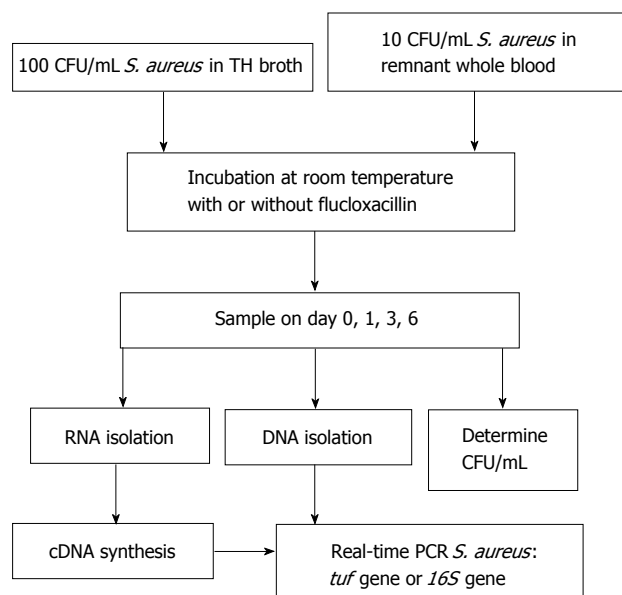
*S. aureus* (ATCC 25923) was used for reconstruction (spiking) experiments. Todd Hewitt (TH) broth was inoculated with *S. aureus* and cultured overnight at 35 °C. Subsequently, a 1:100 dilution was made in fresh TH broth (5 mL) for additional culturing to exponential phase (optical density 0.2 at 600 nm; approximately 1 x 10<sup>7</sup> cells/mL).

### Spiking of *S. aureus* in TH broth and whole blood

See Figure 1 for an overview of the experimental setup. *S. aureus* bacteria grown in exponential phase were diluted in either TH broth or pooled (of similar blood type, *i.e.*, O+), 1 d old, residual whole blood from healthy volunteers. The 100 and 10 colony forming units (CFU)/mL dilutions were made in 2 tubes with an end volume of 10 mL TH broth or whole blood, respectively. To one tube an overdose of flucloxacillin (floxapen 5 µg/mL, 1 mL, Actavis, Baarn, the Netherlands) was added (to kill the bacteria) and to the other 1 mL physiological

**Table 1** Primers and probes used in this study

Gene	Forward primer 5'-3'	Reverse primer 5'-3'	Probe 5'-3'	Ref.
<i>tuf</i>	tcctggttcaattaccacacatactg	ggaaatagaattgtggacgatagttga	FAM-tgataatacrtawacttctgc-BHQ1	[24]
16S	acggctctgctgcactta	tacacatatgttcttcctaataa	VIC-gtaacggcttaccaggc-BHQ1	[22]

**Figure 1** Overview of the experimental setup. CFU: Colony forming unit; *S. aureus*: *Staphylococcus aureus*; TH: Todd Hewitt; PCR: Polymerase chain reaction.

salt solution, this was used as a control. Both tubes were placed on a shaker at room temperature. On days 0, 1, 3, and 6, 200  $\mu$ L samples were taken from both tubes (flucloxacillin treated and untreated) for DNA and RNA isolation. Additionally, 100  $\mu$ L was taken to determine CFU/mL on blood agar plates (Tryptone Soya Agar with sheep blood, Oxoid Deutschland GmbH, Wesel, Germany). Bacterial death was defined as the inability of producing colonies on bloodagar.

### DNA and RNA isolation

The obtained 200  $\mu$ L samples (TH broth and whole blood) were centrifuged at 14000 rpm for 2 min. The supernatant was removed and the pellet was washed once with 200  $\mu$ L ultra-pure water and centrifuged for 2 min at 14000 rpm. The obtained pellet was resuspended in 20  $\mu$ L lysozym (12.5%) and 75  $\mu$ L lysostaphin (100  $\mu$ g/mL) and incubated for 30 min at 37  $^{\circ}$ C while shaking (1000 rpm). RLT buffer with  $\beta$ -mercaptoethanol (1:100) (Qiagen RNA blood mini kit) was added and the samples were stored at -80  $^{\circ}$ C until all time points were collected. The EasyMAG (BioMérieux, Marcy L'Etoile, France) was used for DNA isolation by using the specific B protocol. RNA was isolated by using the RNA blood mini kit (Qiagen), according to manufacturer's instructions. DNase treatment was performed as described in the manual provided (Qiagen RNA blood mini kit) using columns to degrade the DNA in the samples.

### cDNA synthesis with random primers

Reverse transcription was performed on RNA samples using the SuperScript<sup>TM</sup> II First-Strand Synthesis System for real-time-PCR (Invitrogen, Carlsbad, CA, United States, according to manufacturer's protocol). Each sample was split in two for the plus and minus reverse transcriptase reaction to check DNA degradation (DNase treatment on column).

### Real-time PCR for *tuf* and 16S rRNA

Table 1 for an overview of the used primers and probes (*tuf* and 16S rDNA). The 16S rDNA primers, specific for most clinically relevant staphylococci, were described by Matsuda *et al.*<sup>[22]</sup>. However, the 16S rDNA forward primer was slightly modified to adapt to proper annealing temperature. An XS- probe (Biolegio, Nijmegen, The Netherlands) for *Staphylococcus* spp. detection based on 16S was specifically designed. The PCR mix used has been described previously<sup>[23]</sup>. Additionally, *tuf* or 16S primers (900 nmol/L), *tuf* or 16S probe (200 nmol/L), and 5  $\mu$ L sample were added to obtain an final volume of 20  $\mu$ L. A positive and negative control (nuclease free water) were added in each PCR run. The *tuf* PCR and program used were described previously<sup>[24]</sup>. Both PCRs were run in white plates on the LightCycler 480 II (Roche Diagnostics).

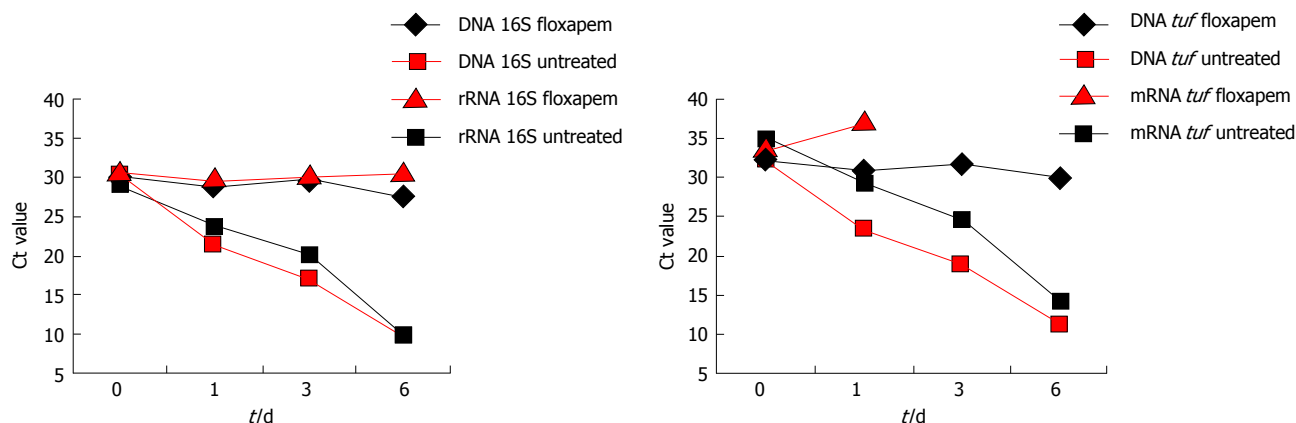
## RESULTS

### Detection of *S. aureus* DNA, rRNA, and mRNA after antibiotic treatment from TH broth

In absence of flucloxacillin the *S. aureus* bacteria continued to grow. At day 0, on average  $330 \pm 28$  (average  $\pm$  SD) CFU/mL were detected on bloodagar plates (Table 2). At days 1, 3, and 6 the CFU/mL increased to > 1000 CFU/mL. In contrast, bacterial growth was arrested in flucloxacillin (antibiotic) treated samples and no colonies were detected on bloodagar at days 3 and 6.

Simultaneously, samples were taken for DNA and RNA isolation. In the absence of flucloxacillin, Ct values of both 16S (DNA and rRNA) and *tuf* (DNA and mRNA) decreased in time (Figure 2). In the presence of flucloxacillin, DNA of the 16S rDNA gene and the *tuf* gene were detected until day 6, while bloodagar plates indicated absence of culturable *S. aureus* on day 3. 16S rRNA also remained detectable up to 6 d of treatment. However, *tuf* mRNA could not be detected on days 3 and 6. The data indicate that *S. aureus* DNA and rRNA can still persist in the absence of viable bacteria as demonstrated using culture.

The Ct values obtained on day zero are similar for



flucloxacillin	Yes		No		Yes		No		Yes		No		Yes		No	
(floxapen)	DNA 16S		DNA 16S		rRNA 16S		rRNA 16S		DNA <i>Tuf</i>		DNA <i>Tuf</i>		mRNA <i>Tuf</i>		mRNA <i>Tuf</i>	
Days	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
0	30.09	0.34	30.57	1.65	30.64	4.49	29.09	3.87	32.01	0.42	32.41	0.98	33.41	3.66	34.95	5.48
1	28.61	0.40	21.46	0.65	29.73	0.30	23.84	1.72	30.80	0.59	23.39	0.99	37.02	0	29.42	1.71
3	29.62	0.62	16.95	0.04	30.00	0.71	20.17	0.93	31.72	0.46	18.95	0.29			24.77	2.95
6	27.72	1.11	9.62	0.16	30.49	2.08	10.00	0.00	29.83	1.38	11.31	0.46			14.35	0.06

**Figure 2** *Staphylococcus aureus* polymerase chain reaction results with and without flucloxacillin treatment in Todd Hewitt broth. *S. aureus* bacteria (100 CFU/mL) continue to grow in absence of flucloxacillin and as a result DNA and RNA levels of both *tuf* and 16S increase in time (Ct values decrease). When flucloxacillin is added 16S DNA, 16S rRNA, and *tuf* DNA levels remain relatively stable in time. Whereas *tuf* DNA is still detectable, *tuf* mRNA is not detectable on day 3 and 6 after flucloxacillin treatment. The experiment was performed twice. Ct values are depicted. CFU: Colony forming unit; *S. aureus*: *Staphylococcus aureus*.

**Table 2** Plate counts of *Staphylococcus aureus* samples with or without flucloxacillin treatment from Todd Hewitt broth

Flucloxacillin		Yes		No	
		Average	SD	Average	SD
Days	0	290	14	330	28
	1	255	7	Infinity	ND
	3	0	0	Infinity	ND
	6	0	0	Infinity	ND

Numbers represent colony forming units/mL. Infinity: Uncountable plate due to large amount of colonies; ND: Not determined.

both 16S DNA and rRNA with(out) flucloxacillin. This is not true for *tuf* DNA and mRNA. Ct values obtained for *tuf* mRNA are on average 2 Ct higher as compared to *tuf* DNA (day 0).

These results demonstrate that *tuf* mRNA is the nucleic acid target that could only be detected from samples which contain culturable bacteria. DNA and rRNA targets could be detected in flucloxacillin treated *S. aureus* cultures that do not further contain culturable bacteria. This experiment was performed twice on independent days, and showed similar results.

### *S. aureus* viability measurements from spiked whole blood

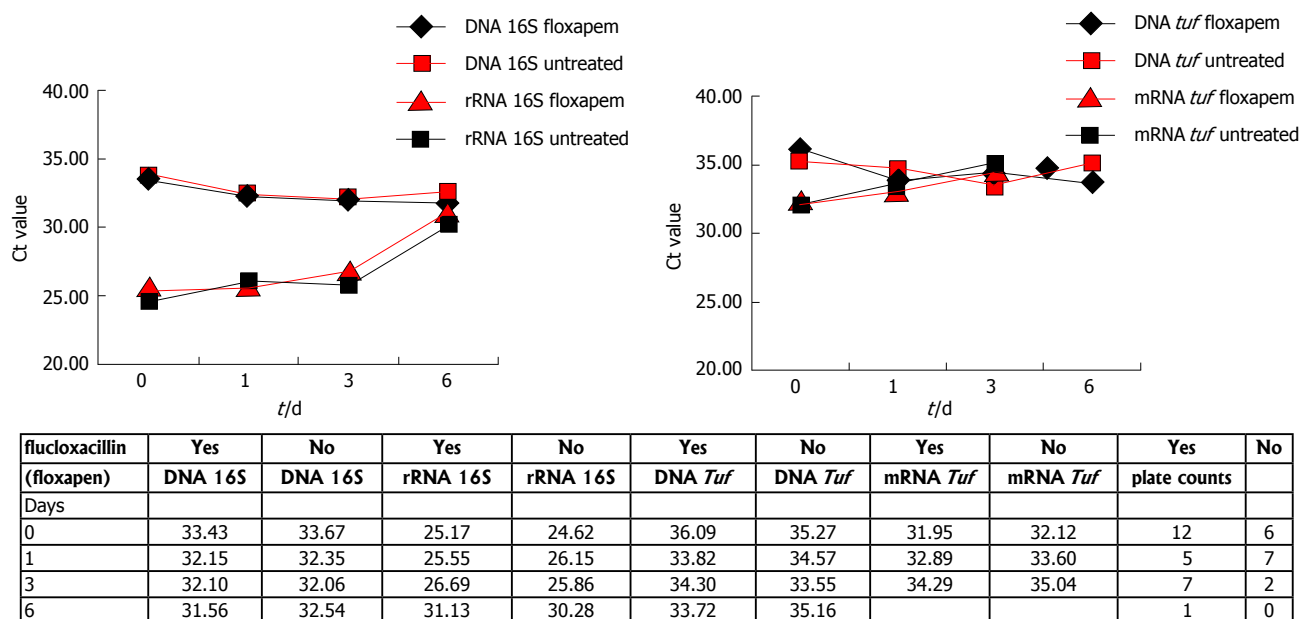
In order to mimic a bloodstream infection, whole blood samples instead of TH broth were spiked with log phase *S. aureus* and growth was measured both in presence and absence of flucloxacillin. Furthermore, a more clinical significant initial bacterial load was used of 10 CFU/mL (instead of 100 CFU/mL). The plate counts

(Figure 3) show that in both presence and absence of flucloxacillin bacterial numbers decrease. DNA and RNA measurements of 16S and *tuf* also showed growth arrest, both in presence and absence of flucloxacillin, as no decrease in Ct values was observed in time (0-6 d) (Figure 3), this in contrast to the results obtained by using spiked TH broth in absence of flucloxacillin (Figure 2).

The Ct values obtained by detecting 16S DNA and rRNA are lower as compared to the Ct values for *tuf* DNA and mRNA. A difference of at least 6 Ct was observed when comparing 16S rRNA levels to *tuf* mRNA levels. When comparing Ct values for DNA detection of both genes the differences were less pronounced, but still significant (approximately 3 Ct). A clear difference was observed between the spiked TH broth samples (Figure 2) and the spiked whole blood samples at day zero (Figure 3). Ct values for 16S DNA and rRNA were comparable in TH broth (day 0), but not in whole blood. For *tuf* DNA and mRNA this phenomenon was also observed, in whole blood the Ct values for *tuf* mRNA were lower than for *tuf* DNA (day 0). Furthermore, there seemed to be a trend towards higher Ct values from day 0-6, independent of flucloxacillin, for both 16S rRNA and *tuf* mRNA in whole blood. This confirms the culture results obtained from whole blood.

In addition, it was investigated whether fresh (max 1 h) and residual (1 d old) whole blood differed in their performances as medium for bacterial culture. TH broth was used as a control: In the absence of flucloxacillin large amounts of colonies were detected up to 6 d of culture, while in the presence of flucloxacillin colonies were detected on days 0 and 1, and no colonies were





**Figure 3** *Staphylococcus aureus* polymerase chain reaction results with and without flucloxacillin treatment in whole blood. Both 16S and *tuf* DNA levels remain relatively stable in time in presence and absence of flucloxacillin. 16S rRNA and *tuf* mRNA show an increase in Ct values (reduction in RNA level). *Tuf* mRNA was not detectable on day 6. Ct values and CFU/mL (plate counts) are depicted.

**Table 3** Plate counts of *Staphylococcus aureus* with or without flucloxacillin treatment from Todd Hewitt broth, fresh whole blood (1 h) and remnant whole blood (1 d)

Flucloxacillin	Days	TH broth		Fresh whole blood		Remnant whole blood	
		Yes	No	Yes	No	Yes	No
	0	20	22	10	4	3	6
	1	14	Infinity	2	3	1	1
	3	0	Infinity	2	1	0	3
	6	0	Infinity	1	0	0	0

Numbers represent colony forming units/mL. Infinity: Uncountable plate due to large amount of colonies; TH: Todd Hewitt.

detected on bloodagar plates on days 3 and 6 (Table 3). Plate counts from spiked blood samples did not resemble those obtained from spiked TH broth samples. The initial plate count on day 0, while initiated from the same log phase culture, was half or less when compared to the count on day 0 for TH broth samples. However, no significant differences were observed between fresh and residual blood samples both in presence or absence of flucloxacillin.

## DISCUSSION

For patient survival it is important to provide fast and accurate identification of BSI causing pathogens. Only viable microorganisms can be cultured, and fastidious or damaged organisms can be present in whole blood but often remain culture negative. Molecular diagnostics might provide solutions for these problems, as pathogens in antibiotic treated patients who remain culture negative (due to presence of antibiotics in the bloodstream) can be identified by PCR<sup>[25,26]</sup>. As BSI

is defined as the presence of viable pathogens in the bloodstream, it might be important for a molecular assay to allow pathogen viability measurements from whole blood.

The results that were obtained after spiking *S. aureus* in TH broth, with and without flucloxacillin, indicated that *tuf* mRNA might be a more promising marker to measure viability than DNA and rRNA. *Tuf* mRNA levels correlated with the culture results from both TH broth and whole blood, whereas 16S DNA, 16S rRNA and *tuf* DNA levels did not. These data confirm results from previous studies<sup>[9,10,15,18]</sup>.

The *S. aureus* bacteria used in this study seemed to die in whole blood (growth reduction on agar plates in absence of flucloxacillin) or enter a state in which they are viable but non-culturable (VBNC)<sup>[27-29]</sup>. Bacteria enter the VBNC state in response to stress, such as starvation, incubation outside the growth temperature range, or oxygen concentration<sup>[29]</sup>. In this study, several stressful conditions might have been present. *S. aureus* was cultured in whole blood in which white cells might inhibit bacterial growth<sup>[30]</sup>. Additionally, incubation took place at room temperature (RT) for 6 d, and waste products were not removed from the culture tube. In future studies, it might be useful to remove the white blood cells from whole blood before spiking (buffy coat), and incubate the samples at 35 °C instead of RT to create better growth conditions. Furthermore, different *S. aureus* strains need to be tested to confirm our results.

In this study, bacteria were considered dead when they were unable to produce colonies on bloodagar. However, as mentioned before, bacteria can enter a VBNC under stressful conditions<sup>[27,31]</sup>. Bacteria that are not culturable can potentially still be viable and infective.

A limitation of this study is that bacterial viability was only measured by colony formation on bloodagar plates. In future studies additional methods to assess bacterial viability might be included, for instance the Live/Dead BacLight Bacterial Viability Kit (Invitrogen). This kit provides two nucleic acid stains [green-fluorescent SYTO 9 dye and red-fluorescent propidium iodide (PI)] to be able distinguish live bacteria (intact membranes) from dead bacteria (compromised membranes). PI is a cell membrane impermeable dye and can only enter compromised pathogens<sup>[32]</sup>. Another option to differentiate live from dead pathogens is exposure to the dye propidium monoazide (PMA) followed by real-time PCR. PMA cannot penetrate viable cells with intact cytoplasmic membranes<sup>[33]</sup>. The PMA dye can enter dead pathogens and bind DNA, thereby inhibiting PCR amplification.

The Ct values obtained for *S. aureus* spiked in TH broth are different from those in the whole blood. Because a lower amount of *S. aureus* bacteria (10 CFU/mL) was spiked in whole blood, as compared to TH broth (100 CFU/mL), one would expect the Ct value to be 3,3 (1 log) higher in whole blood samples. This difference of approximately 3 Ct was seen in whole blood as compared to TH broth for DNA (both 16S and *tuf*). However, the Ct values obtained for *tuf* mRNA and 16S rRNA ( $t = 0$ ) were higher in TH broth as compared to whole blood (approximately 2 and 5 Ct, respectively). Both RNA targets (*tuf* and 16S) seem to be expressed at a higher level in whole blood. This unexpected phenomenon might be a result of the difference in environment (blood vs broth). This confirms findings reported by Cenciarini *et al.*<sup>[14]</sup> who showed that it is difficult to compare RNA viability markers for one pathogen kept in different conditions.

In this study, detection of mRNA and rRNA was performed by using reverse-transcription real-time PCR. Birch *et al.*<sup>[34]</sup> investigated the use of PCR, real-time-PCR and nucleic acid sequence based amplification (NASBA) for assessment of bacterial viability. They found that NASBA offered the highest sensitivity of the three methods tested. However, presence of residual *fliC* DNA and mRNA could be detected by NASBA 30 h post-death (culture negative). Other studies have shown that RNA detection by NASBA could be used to monitor infections after antibiotic treatment<sup>[12,19]</sup>. These contradictory findings again demonstrate that it is important to thoroughly investigate which RNA target is suitable for viability measurement of a certain pathogen.

In this study, results were obtained from as little as 200  $\mu$ L whole blood. Larger volumes of blood are needed to be able to detect clinical relevant bacterial loads<sup>[35]</sup>. As bacterial enrichment is a prerequisite to be able to detect bacteria from whole blood, RNA isolation methods should include such an approach. Both Polaris (Biocartis, Mechelen, Belgium) and MoLYsis (Molzys GmbH, Bremen, Germany) have developed suitable techniques for pathogen DNA enrichment from large volumes of

blood<sup>[35]</sup>. However, these enrichment strategies are not suitable for RNA isolation. A small pilot study indicated that the first steps, of both the MoLYsis and the Polaris pathogen enrichment methods, in which human cells and DNA were removed, did not kill the pathogens present in the whole blood samples as shown by positive cultures (data not shown).

In conclusion, this study clearly demonstrated that detection of *S. aureus tuf* mRNA, in contrast to DNA and rRNA, correlates to bacterial viability status as determined by culture. Therefore, *tuf* mRNA might be a promising marker to measure active *S. aureus* bloodstream infection. After development of RNA isolation procedures from large volumes of whole blood, future clinical studies are needed to validate the preliminary findings obtained in this study.

## COMMENTS

### Background

Bloodstream infections (BSIs) are characterized by high morbidity and mortality and can be caused by a broad variety of microorganisms. Bacteremia is defined as the presence of viable bacteria in the bloodstream. Currently, blood cultures are still the gold standard to detect pathogens from the bloodstream. However, cultures are very time-consuming (24-72 h) and patients need to be treated immediately. Molecular assays (detection of pathogen DNA) can provide results within hours, but the clinical value of DNA detection is still unclear. It might be useful to be able to assess viability of the bacteria in blood samples of patients. Molecular assays that detect the presence of DNA of a specific pathogen can be positive even after viable organisms have been eradicated. The clinical value of pathogen DNA, rRNA and mRNA detection from whole blood needs further investigation.

### Research frontiers

Molecular diagnostics can provide improved detection and identification of pathogens causing BSI. Implementation of these methods reduces time-to-results, offers high sensitivity and specificity, and overall improve the laboratory process for BSI. Detection of DNA by polymerase chain reaction does not provide information about the viability status of a pathogen as the DNA can originate from either living or dead pathogens. The authors attempted to find a marker that enabled their to measure *Staphylococcus aureus* (*S. aureus*) viability to further improve BSI diagnostics.

### Innovations and breakthroughs

The authors describe a novel approach in molecular diagnostics based on the need to assess bacterial viability and not only detect the presence of bacterial DNA. Clinical relevance of bacterial DNA detection can be limited due to the longer half life of DNA. Antibiotic treatment does not result in the break-down of pathogen DNA. It is important to note that the frequently used 16S rDNA/rRNA target cannot be used to monitor viability of *S. aureus* bacteria. More research is needed to confirm them data and to find suitable mRNA targets to be able to detect the broad variety of bacteria that are commonly detected in patients.

### Applications

The study results suggest that *Tuf* mRNA may represent a suitable marker for the detection of viable *S. aureus*.

### Peer-review

This manuscript describes a novel approach in molecular diagnostics based on the need to monitor viability and not only DNA presence of a microorganism.

## REFERENCES

- 1 Spraycar M. Stedman's Medical Dictionary. Williams and

- Wilkins, 1995
- 2 **Loonen AJ**, Jansz AR, Stalpers J, Wolffs PF, van den Brule AJ. An evaluation of three processing methods and the effect of reduced culture times for faster direct identification of pathogens from BacT/ALERT blood cultures by MALDI-TOF MS. *Eur J Clin Microbiol Infect Dis* 2012; **31**: 1575-1583 [PMID: 22080416 DOI: 10.1007/s10096-011-1480-y]
- 3 **Riedel S**, Carroll KC. Blood cultures: key elements for best practices and future directions. *J Infect Chemother* 2010; **16**: 301-316 [PMID: 20490596 DOI: 10.1007/s10156-010-0069-1]
- 4 **van Veen SQ**, Claas EC, Kuijper EJ. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. *J Clin Microbiol* 2010; **48**: 900-907 [PMID: 20053859 DOI: 10.1128/JCM.02071-09]
- 5 **Fraser A**, Paul M, Almanasreh N, Tacconelli E, Frank U, Cauda R, Borok S, Cohen M, Andreassen S, Nielsen AD, Leibovici L. Benefit of appropriate empirical antibiotic treatment: thirty-day mortality and duration of hospital stay. *Am J Med* 2006; **119**: 970-976 [PMID: 17071166 DOI: 10.1016/j.amjmed.2006.03.034]
- 6 **Gaibani P**, Rossini G, Ambretti S, Gelsomino F, Pierro AM, Varani S, Paolucci M, Landini MP, Sambri V. Blood culture systems: rapid detection--how and why? *Int J Antimicrob Agents* 2009; **34** Suppl 4: S13-S15 [PMID: 19931809 DOI: 10.1016/S0924-8579(09)70559-X]
- 7 **Wallet F**, Nseir S, Baumann L, Herwegh S, Sendid B, Boulo M, Roussel-Delvallez M, Durocher AV, Courcol RJ. Preliminary clinical study using a multiplex real-time PCR test for the detection of bacterial and fungal DNA directly in blood. *Clin Microbiol Infect* 2010; **16**: 774-779 [PMID: 19689465 DOI: 10.1111/j.1469-0691.2009.02940.x]
- 8 **Yanagihara K**, Kitagawa Y, Tomonaga M, Tsukasaki K, Kohno S, Seki M, Sugimoto H, Shimazu T, Tasaki O, Matsushima A, Ikeda Y, Okamoto S, Aikawa N, Hori S, Obara H, Ishizaka A, Hasegawa N, Takeda J, Kamihira S, Sugahara K, Asari S, Murata M, Kobayashi Y, Ginba H, Sumiyama Y, Kitajima M. Evaluation of pathogen detection from clinical samples by real-time polymerase chain reaction using a sepsis pathogen DNA detection kit. *Crit Care* 2010; **14**: R159 [PMID: 20731880 DOI: 10.1186/cc9234]
- 9 **Hellyer TJ**, DesJardin LE, Teixeira L, Perkins MD, Cave MD, Eisenach KD. Detection of viable *Mycobacterium tuberculosis* by reverse transcriptase-strand displacement amplification of mRNA. *J Clin Microbiol* 1999; **37**: 518-523 [PMID: 9986805]
- 10 **Iyer R**, Mukherjee P, Wang K, Simons J, Wormser GP, Schwartz I. Detection of *Borrelia burgdorferi* nucleic acids after antibiotic treatment does not confirm viability. *J Clin Microbiol* 2013; **51**: 857-862 [PMID: 23269733 DOI: 10.1128/JCM.02785-12]
- 11 **Josephson KL**, Gerba CP, Pepper IL. Polymerase chain reaction detection of nonviable bacterial pathogens. *Appl Environ Microbiol* 1993; **59**: 3513-3515 [PMID: 8250575]
- 12 **Morré SA**, Sillekens PT, Jacobs MV, de Blok S, Ossewaarde JM, van Aarle P, van Gemen B, Walboomers JM, Meijer CJ, van den Brule AJ. Monitoring of *Chlamydia trachomatis* infections after antibiotic treatment using RNA detection by nucleic acid sequence based amplification. *Mol Pathol* 1998; **51**: 149-154 [PMID: 9850338 DOI: 10.1136/mp.51.3.149]
- 13 **Arraiano CM**, Yancey SD, Kushner SR. Stabilization of discrete mRNA breakdown products in ams pnp rnb multiple mutants of *Escherichia coli* K-12. *J Bacteriol* 1988; **170**: 4625-4633 [PMID: 2459106]
- 14 **Cenciari C**, Courtois S, Raoult D, La Scola B. Influence of long time storage in mineral water on RNA stability of *Pseudomonas aeruginosa* and *Escherichia coli* after heat inactivation. *PLoS One* 2008; **3**: e3443 [PMID: 18941615 DOI: 10.1371/journal.pone.0003443]
- 15 **Jou NT**, Yoshimori RB, Mason GR, Louie JS, Liebling MR. Single-tube, nested, reverse transcriptase PCR for detection of viable *Mycobacterium tuberculosis*. *J Clin Microbiol* 1997; **35**: 1161-1165 [PMID: 9114400]
- 16 **Loeffler J**, Hebart H, Cox P, Flues N, Schumacher U, Einsele H. Nucleic acid sequence-based amplification of *Aspergillus* RNA in blood samples. *J Clin Microbiol* 2001; **39**: 1626-1629 [PMID: 11283102 DOI: 10.1128/JCM.39.4.1626-1629.2001]
- 17 **Sheridan GE**, Masters CI, Shallcross JA, MacKey BM. Detection of mRNA by reverse transcription-PCR as an indicator of viability in *Escherichia coli* cells. *Appl Environ Microbiol* 1998; **64**: 1313-1318 [PMID: 9546166]
- 18 **Simpkins SA**, Chan AB, Hays J, Pöpping B, Cook N. An RNA transcription-based amplification technique (NASBA) for the detection of viable *Salmonella enterica*. *Lett Appl Microbiol* 2000; **30**: 75-79 [PMID: 10728566 DOI: 10.1046/j.1472-765x.2000.00670.x]
- 19 **van der Vliet GM**, Schepers P, Schukink RA, van Gemen B, Klatser PR. Assessment of mycobacterial viability by RNA amplification. *Antimicrob Agents Chemother* 1994; **38**: 1959-1965 [PMID: 7529012 DOI: 10.1128/AAC.38.9.1959]
- 20 **Wisplinghoff H**, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis* 2004; **39**: 309-317 [PMID: 15306996 DOI: 10.1086/421946]
- 21 **Chaffin DO**, Taylor D, Skerrett SJ, Rubens CE. Changes in the *Staphylococcus aureus* transcriptome during early adaptation to the lung. *PLoS One* 2012; **7**: e41329 [PMID: 22876285 DOI: 10.1371/journal.pone.0041329]
- 22 **Matsuda K**, Tsuji H, Asahara T, Kado Y, Nomoto K. Sensitive quantitative detection of commensal bacteria by rRNA-targeted reverse transcription-PCR. *Appl Environ Microbiol* 2007; **73**: 32-39 [PMID: 17071791 DOI: 10.1128/AEM.01224-06]
- 23 **Huijsmans CJ**, Damen J, van der Linden JC, Savelkoul PH, Hermans MH. Comparative analysis of four methods to extract DNA from paraffin-embedded tissues: effect on downstream molecular applications. *BMC Res Notes* 2010; **3**: 239 [PMID: 20840759 DOI: 10.1186/1756-0500-3-239]
- 24 **Loonen AJ**, Jansz AR, Kreeftenberg H, Bruggeman CA, Wolffs PF, van den Brule AJ. Acceleration of the direct identification of *Staphylococcus aureus* versus coagulase-negative staphylococci from blood culture material: a comparison of six bacterial DNA extraction methods. *Eur J Clin Microbiol Infect Dis* 2011; **30**: 337-342 [PMID: 20972809 DOI: 10.1007/s10096-010-1090-0]
- 25 **Mauro MV**, Cavalcanti P, Perugini D, Noto A, Sperli D, Giraldo C. Diagnostic utility of LightCycler SeptiFast and procalcitonin assays in the diagnosis of bloodstream infection in immunocompromised patients. *Diagn Microbiol Infect Dis* 2012; **73**: 308-311 [PMID: 22626731 DOI: 10.1016/j.diagmicrobio.2012.04.006]
- 26 **Wellinghausen N**, Kochem AJ, Disqué C, Mühl H, Gebert S, Winter J, Matten J, Sakka SG. Diagnosis of bacteremia in whole-blood samples by use of a commercial universal 16S rRNA gene-based PCR and sequence analysis. *J Clin Microbiol* 2009; **47**: 2759-2765 [PMID: 19571030 DOI: 10.1128/JCM.00567-09]
- 27 **Davey HM**. Life, death, and in-between: meanings and methods in microbiology. *Appl Environ Microbiol* 2011; **77**: 5571-5576 [PMID: 21705550 DOI: 10.1128/AEM.00744-11]
- 28 **Keer JT**, Birch L. Molecular methods for the assessment of bacterial viability. *J Microbiol Methods* 2003; **53**: 175-183 [PMID: 12654489 DOI: 10.1016/S0167-7012(03)00025-3]
- 29 **Oliver JD**. The viable but nonculturable state in bacteria. *J Microbiol* 2005; **43** Spec No: 93-100 [PMID: 15765062]
- 30 **Högmán CF**, Gong J, Eriksson L, Hambræus A, Johansson CS. White cells protect donor blood against bacterial contamination. *Transfusion* 1991; **31**: 620-626 [PMID: 1909820 DOI: 10.1046/j.1537-2995.1991.31791368338.x]
- 31 **Trevors JT**. Can dead bacterial cells be defined and are genes expressed after cell death? *J Microbiol Methods* 2012; **90**: 25-28 [PMID: 22534140 DOI: 10.1016/j.mimet.2012.04.004]
- 32 **Deligeorgiev TG**, Kaloyanova S, Vaquero JJ. Intercalating Cyanine Dyes for Nucleic Acid Detection. Recent Patents on Materials Science, 2009: 1-26 [DOI: 10.2174/1874464810902010001]
- 33 **Nocker A**, Cheung CY, Camper AK. Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells.

- J Microbiol Methods* 2006; **67**: 310-320 [PMID: 16753236 DOI: 10.1016/j.mimet.2006.04.015]
- 34 **Birch L**, Dawson CE, Cornett JH, Keer JT. A comparison of nucleic acid amplification techniques for the assessment of bacterial viability. *Lett Appl Microbiol* 2001; **33**: 296-301 [PMID: 11559404 DOI: 10.1046/j.1472-765X.2001.00999.x]
- 35 **Loonen AJ**, Bos MP, van Meerbergen B, Neerken S, Catsburg A, Dobbelaer I, Penterman R, Maertens G, van de Wiel P, Savelkoul P, van den Brule AJ. Comparison of pathogen DNA isolation methods from large volumes of whole blood to improve molecular diagnosis of bloodstream infections. *PLoS One* 2013; **8**: e72349 [PMID: 23977288 DOI: 10.1371/journal.pone.0072349]
- P- Reviewer:** Fukuda S, Giamarellos-Bourboulis EJ, Schwan WR  
**S- Editor:** Song XX **L- Editor:** A **E- Editor:** Liu SQ







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>

