
Charles Darwin University

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Turner, Paul; Sá-Leão, Raquel; Greenhill, Andrew; Leach, Amanda; Satzke, Catherine

Published in:

Clinical infectious diseases : an official publication of the Infectious Diseases Society of America

DOI:

[10.1093/cid/ciac221](https://doi.org/10.1093/cid/ciac221)

Published: 01/09/2022

Document Version

Publisher's PDF, also known as Version of record

[Link to publication](#)

Citation for published version (APA):

Turner, P., Sá-Leão, R., Greenhill, A., Leach, A., & Satzke, C. (2022). World Health Organization (WHO) Standard Methods for Pneumococcal Carriage Studies. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 75(5), 924-925. <https://doi.org/10.1093/cid/ciac221>

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World Health Organization (WHO) Standard Methods for Pneumococcal Carriage Studies

TO THE EDITOR—As authors of the current World Health Organization (WHO) standard methods for pneumococcal carriage studies (“WHO guideline”) [1], we read with interest the article from Ganaie et al entitled “Oral streptococci expressing pneumococci-like cross-reactive capsule types can affect WHO recommended pneumococcal carriage procedure” [2].

Ganaie et al used molecular approaches for identification and serotyping of *Streptococcus pneumoniae* (the pneumococcus) from 2400 nasopharyngeal (NP) and oropharyngeal (OP) swabs from adult participants that were collected separately but then “combined” for testing. Following broth enrichment, DNA extraction, Centers for Disease Control and Prevention (CDC) *lytA* polymerase chain reaction (PCR), and a storage period, *lytA* positive broth cultures were sub-cultured onto solid media for formal identification and characterization of *S. pneumoniae*. Just over 10% (301/2400) of NP-OP broth cultures were *lytA* positive, and most were culture-negative: only 20 “probable pneumococcal-like colonies” were recovered from the 244 broth cultures available for downstream analysis. In the title and body of the article, the authors repeatedly state that they followed the WHO guideline and that their results

call this standard into question; we refute both of these claims.

The WHO guideline was originally published in 2003 to provide a set of minimum standards to those conducting pneumococcal carriage studies globally [3]. The guideline was updated in 2013 with a panel of experts from 15 countries who revised the literature and developed consensus standard methods accordingly [1]. The 2013 guideline has been applied in at least 238 published studies from 64 countries including in carriage surveys, vaccine trials, disease surveillance, assay development, and vaccine impact studies.

Several methods used in Ganaie et al are inconsistent with the WHO guideline. The guideline does not recommend: (i) testing of combined NP-OP swabs, (ii) broth culture enrichment, and (iii) molecular methods for direct testing of samples. A table clarifying the WHO recommendations and how they contrast with the methods used is included below (Table 1). We recognize that researchers may conduct additional or alternative methods to address specific research questions. However, in this case the use of NP-OP specimens, and perhaps culture-based enrichment, led to erroneous conclusions regarding the overall suitability of the WHO procedure.

Recognizing the limitations of the existing literature, the 2013 WHO standard

highlighted research gaps to inform future guidelines, including that there was scant evidence on the application of molecular approaches for pneumococcal identification and serotyping. Ganaie et al provide important data to the existing body of evidence that indicates that the use of molecular methods with combined NP-OP swabs leads to erroneous results. There are clear challenges for accurate pneumococcal identification and serotyping, particularly when using molecular methods from oropharyngeal or combined samples [4–8]. Studies that identify and address these challenges will be informative for the development of any future guideline. However, researchers should have confidence that the current WHO guideline, if followed correctly, will provide accurate and comparable data on pneumococcal carriage.

Notes

Potential conflicts of interest. A. L. reports MSD contract for pneumococcal carriage data analysis, funds paid to institution, outside of the submitted work. R. S.-L. reports funds directly paid to institution from Pfizer as investigator initiated research grant outside of the submitted work and received payment for lectures paid directly to self from Pfizer. C. S. reports being lead investigator on a Merck Investigator Studies Program grant funded by MSD on pneumococcal serotype epidemiology in children with empyema paid to institution and investigator on a clinical research collaboration on PVC vaccination in Mongolia from Pfizer paid to the institution, both outside of the

Table 1. Key Differences from WHO Guideline for Pneumococcal Carriage Studies

Relevant Section(s) in WHO Guideline [1]	Recommendation in WHO Guideline	Conducted in Ganaie et al [2].
Site of sample	NP and OP (collected and analyzed separately); if only one possible collect from the NP	Combined NP-OP
Culture-based broth enrichment of nasopharyngeal samples	Insufficient evidence to recommend broth enrichment	Culture-based enrichment in Lim broth
Identification of pneumococci (culture and non-culture based)	Only culture was recommended. The identification algorithm was for culture-based methods on pneumococcal isolates Insufficient evidence to recommend molecular testing, although <i>lytA</i> appears most useful target	<i>lytA</i> real-time PCR on DNA extracted from combined NP-OP samples

Abbreviations: NP, nasopharyngeal; OP, oropharyngeal; PCR, polymerase chain reaction; WHO, World Health Organization.

submitted work. P. T. reports PI of the ACORN AMR surveillance project (grant number 222156/Z/20/Z) from the Wellcome Trust outside of the submitted work. A. G. reports no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Paul Turner,^{1,2} Raquel Sá-Leão,³ Andrew Greenhill,⁴ Amanda Leach,⁵ and Catherine Satzke^{6,7,8}

¹Cambodia Oxford Medical Research Unit, Angkor Hospital for Children, Siem Reap, Cambodia; ²Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom; ³Molecular Microbiology of Human Pathogens Laboratory, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal; ⁴Life Sciences, School of Science, Psychology and Sport, Federation University, Churchill, Australia; ⁵Child Health Division, Menzies School of Health Research, Charles Darwin University, Darwin, Australia; ⁶Translational Microbiology Group, Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Victoria, Australia; ⁷Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia; and ⁸Department of

Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Victoria, Australia

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Correspondence: P. Turner, Cambodia Oxford Medical Research Unit, Angkor Hospital for Children, Siem Reap, Cambodia, Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK (pault@tropmedres.ac).

Clinical Infectious Diseases® **2022;75(5):924–5**

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