

Reply to Ganaie et al.

Paul Turner^{1,2}, Raquel Sá-Leão³, Andrew Greenhill⁴, Amanda Leach⁵, 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, UK

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, The University of Melbourne, Parkville, Victoria, Australia

⁸Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and
Immunity, The University of Melbourne, Melbourne, Victoria, Australia

Corresponding author: Paul Turner (alternate: Catherine Satzke)

Phone: +855 89287059 (alternate: +61 (3) 8341 6438)

Email: pault@tropmedres.ac (alternate: catherine.satzke@mcri.edu.au)

Words: 467

Keywords: pneumococcus, carriage, identification, PCR

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, CDC *lytA* 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 paper, 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, (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.

Funding statement

No relevant funding to disclose.

Conflicts of Interest

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.

References

1. Satzke C, Turner P, Virolainen-Julkunen A, et al. Standard method for detecting upper respiratory carriage of *Streptococcus pneumoniae*: updated recommendations from the World Health Organization Pneumococcal Carriage Working Group. *Vaccine* **2013**; 32(1): 165-79.

2. Ganaie F, Branche AR, Peasley M, Rosch JW, Nahm MH. Oral streptococci expressing pneumococci-like cross-reactive capsule types can affect WHO recommended pneumococcal carriage procedure. Clin Infect Dis **2021**.
3. O'Brien KL, Nohynek H. Report from a WHO Working Group: standard method for detecting upper respiratory carriage of *Streptococcus pneumoniae*. Pediatr Infect Dis J **2003**; 22(2): e1-11.
4. Carvalho Mda G, Bigogo GM, Junghae M, et al. Potential nonpneumococcal confounding of PCR-based determination of serotype in carriage. J Clin Microbiol **2012**; 50(9): 3146-7.
5. Trzcinski K, Bogaert D, Wyllie A, et al. Superiority of trans-oral over trans-nasal sampling in detecting *Streptococcus pneumoniae* colonization in adults. PLoS One **2013**; 8(3): e60520.
6. Boelsen LK, Dunne EM, Gould KA, et al. The Challenges of Using Oropharyngeal Samples To Measure Pneumococcal Carriage in Adults. mSphere **2020**; 5(4).
7. Farrar JL, Odiembo H, Odoyo A, et al. Limited Added Value of Oropharyngeal Swabs for Detecting Pneumococcal Carriage in Adults. Open Forum Infect Dis **2020**; 7(9): ofaa368.
8. Almeida ST, Paulo AC, Froes F, de Lencastre H, Sa-Leao R. Dynamics of Pneumococcal Carriage in Adults: A New Look at an Old Paradigm. J Infect Dis **2021**; 223(9): 1590-600.

Tables

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 analysed 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.	<i>lytA</i> real-time PCR on DNA extracted from combined NP-OP samples.
	Insufficient evidence to recommend molecular testing, although <i>lytA</i> appears most useful target	

*NP: nasopharyngeal

†OP: oropharyngeal