

Serotype distribution and antimicrobial drugs susceptibility of *Streptococcus pneumoniae* in human immunodeficiency virus infected children

Dodi Safari • Eijkman Institute for Molecular Biology • Jakarta, Indonesia



Dodi Safari

Dr. Safari joined the Eijkman Institute for Molecular Biology, Jakarta, Indonesia in 2001 as a research assistant. In 2006, he left for Utrecht in the Netherlands as a PhD student in the Department of Medical Microbiology, University Medical Center Utrecht. He returned to Indonesia in 2010 and was eager to continue his research on molecular epidemiology of *Streptococcus pneumoniae* in the Indonesian population at the Eijkman Institute as a research fellow.

This research was supported with a grant from the International Society for Infectious Diseases (ISID).

Background

Streptococcus pneumoniae is a leading cause of bacterial pneumonia, meningitis, and sepsis worldwide. Incidence of invasive pneumococcal disease (IPD) varies substantially by age, socioeconomics, immune status, and geographical location [1]. Capsular polysaccharide is considered the ultimate virulence factor of *S. pneumoniae*. With over 90 serotypes identified based on the polysaccharide structure and immunogenicity, there is no universal pneumococcal vaccine and those available cover only selected serotypes. Knowledge on the exact serotypes distribution in disease is critical for making an informed decisions about vaccination policies, especially in high risk groups (like HIV-infected individuals) or in national immunization programs.

Nasopharyngeal colonisation is the obligatory first step in the pathogenesis of pneumococcal disease and therefore considered the most important risk factor for IPD. It also provides the basis for horizontal spread of pneumococci in the community, making it an important target for preventive measures [2]. In present study, a cross sectional study was performed to investigate *S. streptococcus* carriage in human immunodeficiency virus (HIV)-infected children in Jakarta, Indonesia. Currently, the publish data on *S. pneumoniae* carriage or invasive from Indonesian population was still limited. In 2001, Soewignjo et al was reported that prevalence of *S. pneumoniae* carriage in healthy children in Lombok Island, Indonesia was 48% [3].

Materials and Methods

A cross-sectional study was performed among human immunodeficiency virus (HIV)-infected children group from January to July 2012 in Jakarta, Indonesia in collaboration with Nia Kurniati, MD from the CiptoMangun Kusumo Hospital/Faculty of Medicine, University of Indonesia, Jakarta, Indonesia. During this study, we collected 116 of sample swabs and we confirmed that 90 of 116 swabs obtained from positive HIV-infected children based on medical information. Nasopharyngeal (NP) swabs were collected from children using a flexible nasopharyngeal floxed swab with deep nasopharyngeal swab as described previously [4]. These NP swabs were placed into 1.0 ml of skim milk tryptone glucose glycerol (STGG) transport medium and shipped on wet ice within 4 h to Microbiology Laboratory, Eijkman Institute. Samples of NP-STGG (20 µl) were plated onto a 5% sheep blood agar plate supplemented with 5 mg/L gentamicin and incubated at 35°C for 24–48 h with 5% CO₂. *S. pneumoniae* was identified based on strain susceptibility to optochin. All pneumococcal isolates were tested in PCR for presence of genes coding pneumococcal surface antigen A (psaA) and major pneumococcal autolysin (lytA) and capsule transcriptional regulator (wzg) [5–7]. Strains PCR-negative for wzg were tested for polymorphism within recA in order to determine the phylogenetic relation with *S. pneumoniae*. Serotyping was performed by a multiplex sequential PCR (SM-PCR) [5]. All *S. pneumoniae* isolates were tested for susceptibility to a set of antimicrobial drug discs: Ampicillin, amoxicillin, choramphenicol, cefixime, erythromycin, penicillin, sulfamethoxazole/ trimethoprim, cefotaxime, cefprozil, spiramycin, and clindamycin by use the disc diffusion method. *S. pneumoniae* strain (ATCC 49619) was also grown and used as a control test. Definition of non-susceptibility was interpreted based on the size of the zone inhibition.

continued on next page



ISID Report of Dodi Safari

Serotype distribution
and antimicrobial drugs
susceptibility of *Streptococcus
pneumoniae* in human
immunodeficiency virus
infected children

*This research was supported
with a grant from the
International Society for
Infectious Diseases (ISID).*

Results and Discussion

Nasopharyngeal samples from 42 children were culture-positive for *S. pneumoniae*. All pneumococcal strains were *pspA*-, *lytA*- and *wzg*-positive except for 5 strains negative for *wzg* alone. Eleven strains were untypeable in SM-PCR including all negative for *wzg*. The *recA* analysis revealed all but one of those to be *S. pseudopneumoniae*. It lowers the number of carriers to 41 (46%). Serotype 19F was identified as most common (8 isolates, 20% carriers) followed by 9A and 6A/B (4 carriers each), 23F (3 carriers), 9V, 35B, 11A (two carriers each) and serotypes 18C, 12F, 15B/C and 35F (single carrier each). Presence of *S. pneumoniae* in carriage correlated negatively with CD4 count. There was no correlation between carriage and patient age, previous antibiotic use, exposure to cigarette smoke or crowding at home. The coverage for conjugated pneumococcal vaccines immunogenic in children but targeting selected few serotype was between 62% to 74% for only serotyped strains isolated by us from carriage for 7- and 13-valent vaccines, respectively. We found that more than a half of isolates were non susceptible to cefixime and sulfamethoxazole/ trimethoprim and 12.5 % of isolates were non-susceptible to penicillin. Serotype 19F has more non susceptible to different antimicrobial drugs excepted to amoxycillin and cefuroxime. No significantly correlation between carriage of *S. pneumoniae* and risk factors of age, antibiotic use, exposure to cigarette, or number of family member, only risk factor of CD4 percentage negatively correlated with *S. pneumoniae* carriage.

Our study gives insight into population of *S. pneumoniae* strains circulating in carriage in patients at high risk for IPD due to age and comorbidity. We expect our results to be helpful in shaping preventive strategies targeting IPD in Indonesia both on national and on local level.

References

1. van der PT, Opal SM. Pathogenesis, treatment, and prevention of pneumococcal pneumonia. *Lancet*. 2009. 374(9700):1543–56.
2. Bogaert D, de Groot R, Hermans PWM. *Streptococcus pneumoniae* colonisation: The key to pneumococcal disease. *Lancet. Infect. Dis*. 2004. 4:144–54.
3. Soewignjo S, Gessner BD, Sutanto A, Steinhoff M, Prijanto M, Nelson C, Widjaya A, Arjoso S. *Streptococcus pneumoniae* nasopharyngeal carriage prevalence, serotype distribution, and resistance patterns among children on Lombok Island, Indonesia. *Clin. Infect. Dis*. 2001. 32(7):1039–43.
4. 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.
5. Pai R, Gertz RE, Beall B. Sequential multiplex PCR approach for determining capsular serotypes of *Streptococcus pneumoniae* isolates. *J Clin. Microbiol*. 2006. 44(1):124–31.
6. Carvalho MG, Tondella ML, McCaustland K, Weidlich L, McGee L, Mayer LW, Steigerwalt A, Whaley M, Facklam RR, Fields B, Carlone G, Ades EW, Dagan R, Sampson JS. Evaluation and improvement of realtime PCR assays targeting *lytA*, *ply*, and *psaA* genes for detection of pneumococcal DNA. *J Clin. Microbiol*. 2007. 45:2460–6.
7. Morrison KE, Lake D, Crook J, Carlone GM, Ades E, Facklam R, Sampson JS. Confirmation of *psaA* in all 90 serotypes of *Streptococcus pneumoniae* by PCR and potential of this assay for identification and diagnosis. *J Clin. Microbiol*. 2000. 38(1):434–7.