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Serotypes and antibiotic susceptibility profile of *Streptococcus pneumoniae* isolated from nasopharynges of children infected with HIV in Jakarta, Indonesia, pre- and post-pneumococcal vaccination

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Abstract

The aim of this prospective study was to investigate the serotypes and antibiotic susceptibility of *S. pneumoniae* carried by children infected with HIV before and after vaccination with the seven-valent pneumococcal conjugate vaccine in Jakarta, Indonesia in 2013. We collected nasopharyngeal swab specimens from 52 children pre-vaccination and 6 months post-vaccination. Serotyping was performed by conventional multiplex polymerase chain reaction and Quellung reaction. The antibiotic susceptibility profile was obtained by disc diffusion. We determined that 27 (52%) and 24 (46%) of the 52 children carried *S. pneumoniae* during pre- and post-vaccination periods, respectively with the majority of the isolates being non-vaccine type strains (85% pre-vaccination and 75% post-vaccination). Serotypes 34, 6C, and 16F (two strains each) were the most commonly identified serotypes at pre-vaccination. Serotypes 23A (three strains) and 19F (two strains) were the most commonly identified serotypes post-vaccination. In general, isolates were most commonly susceptible to chloramphenicol (88%) and clindamycin (88%), followed by erythromycin (84%), trimethoprim-sulphamethoxazole (69%), tetracycline (61%), and penicillin (59%). In conclusion, serotypes of *S. pneumoniae* isolated from the nasopharynges of children infected with HIV varied and were more likely to be non-vaccine type strains both before and after vaccination.

INTRODUCTION

Streptococcus pneumoniae (pneumococcus) is a major cause of meningitis, bacteremia, and pneumonia, as well as sinusitis and otitis media [1]. Immunocompromised patients are at an increased risk of contracting invasive pneumococcal disease, particularly those with human immunodeficiency virus (HIV) infection and those who undergo transplantation [2]. Children with HIV have a significantly increased risk of pneumococcal disease compared with uninfected children; therefore, administration of the pneumococcal conjugate vaccine (PCV) should be considered as an important intervention in order to improve the lives of children infected with HIV [3]. Routine use of pneumococcal conjugate vaccines has dramatically reduced the prevalence of invasive pneumococcal disease (IPD) attributable to vaccine serotypes in many countries. However, currently, the number of infections caused by non-vaccine serotypes has increased [4].

Currently, pneumococcal vaccination is not part of Indonesia's national immunization programme for children. Pneumococcal vaccines are available in Indonesia at a commercial price and the use of pneumococcal vaccines is not monitored in any systemic way [5]. In 2017, the Ministry of Health of the Republic of Indonesia introduced the pneumococcal conjugate vaccine programme in Lombok Island, West Nusa Tenggara, Indonesia. In this prospective study, we investigated the serotype distribution and antibiotic susceptibility of *S. pneumoniae* isolated from nasopharynges of children infected with HIV during pre- and post-vaccination periods with the seven-valent pneumococcal conjugate vaccine (PCV7) in Jakarta, Indonesia in 2013. We hypothesized that the finding of the present study would provide the preliminary data on the impact of pneumococcal vaccination on S. pneumoniae colonization among high risk group population in Indonesia.

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Keywords: *Streptococcus pneumoniae* carriage; children infected with HIV; pneumococcal vaccination.

Abbreviations: HIV, human immunodeficiency virus; IPD, invasive pneumococcal disease; PCR, polymerase chain reaction; PCV7, the seven-valent pneumococcal conjugate vaccine; PCV, The pneumococcal conjugate vaccine; STGG, skim milk tryptone glucose glycerol.

Table 1. Prevalence of *Streptococcus pneumoniae* in children infected with HIV pre- and post-vaccination

***Streptococcus pneumoniae* colonization**		**Post-vaccination, n (%)**		**Total Pre-Vaccination, n (%)**
		Positive	**Negative**	
Pre-vaccination, n (%)	Positive	12 (23)	15 (29)	27 (52)
	Negative	12 (23)	13 (25)	25 (48)
Total Post-vaccination, n (%)		24 (46)	28 (54)	52 (100)

METHODS

Nasopharyngeal swab specimen collection

This was a prospective study to determine the prevalence, serotype distribution, and antibiotics susceptibility profile of *S. pneumoniae* in children infected with HIV. We gathered data at two time points related to pneumococcal vaccination: pre-vaccination (before the participants received the PCV7 vaccine, on the same day) and post-vaccination (6 months after the PCV7 vaccination) in Jakarta, Indonesia, from February to December 2013. This study design was reviewed and approved by the ethical committee of the Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia (Number: 126/H2.F1/ETIK/2013). An informed parental/guardian (grandfather/grandmother) permission form was completed prior to enrollment. A total of 52 children with HIV infection aged 12 to 152 months (mean age, 56.5 months) who had been treated with antiretroviral therapy for at least 6 months were included in this study. Patients were excluded based on the following criteria: opportunistic infections, previous receipt of the pneumococcal vaccine, intake of oral steroids or other immunosuppressive drugs, and parent/legal guardian refusal to participate in the study. Children with aged 12 to 71 months (n=33 children; 63.5%) received two doses of the PCV7 vaccine (Pfizer Inc) at 8 week intervals, and children aged >71 months (n=19 children; 36.5%) received one dose of the PCV7 vaccine (Unpublished data) [1, 6]. Nasopharyngeal (NP) swab specimens were collected pre- and post-vaccination by a well-trained medical staff using a flexible nasopharyngeal flocked swab (Copan, Italy no 503SC01). The swabs were each placed into 1.0 ml of skim milk tryptone glucose glycerol (STGG) transport medium, and the NP-STGG specimens were shipped with ice pack to the Bacteriology laboratory, Eijkman Institute for Molecular Biology, Jakarta, Indonesia, and stored at −80 °C within 4 h [5].

Streptococcus pneumoniae culture and identification

Overall, 20 µl of each NP-STGG sample was plated onto 5% sheep blood agar supplemented with 5 mg l^{-1} gentamicin and incubated at 35 °C with 5% CO_2 for 18–20 h. In the case of alpha-hemolytic colonies growth on the plate, a single colony was subcultured and tested by Gram-staining, and optochin (ethylhydrocupreine hydrochloride) susceptibility [6]. Gram-positive, optochin-sensitive isolates (designated by a zone of inhibition of 14 mm or greater) isolates were stored in STGG at −80 °C for further analysis. Bacterial DNA extraction and serotype determination by conventional multiplex polymerase chain reaction (PCR) were performed as described previously [5, 7]. Isolates that had negative serotypes by PCR were confirmed by Quellung reaction.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed according to Clinical and Laboratory Standards Institute guidelines using Mueller-Hinton agar supplemented with 5% sheep blood. The antimicrobial discs used were oxacillin, chloramphenicol, clindamycin, trimethoprim-sulfamethoxazole, tetracycline, and erythromycin (Oxoid) [8].

Data analysis

The prevalence of pneumococcal colonization rate pre-vaccination and post-vaccination was compared with the Chi-square McNemar test. The power analysis has been performed using Stata statistic software.

RESULTS

In this study, we collected the nasopharyngeal swab specimens at two time points (pre-vaccination and 6 months post-vaccination). We identified 27 (52%) and 24 (46%) *S. pneumoniae* isolates out of the 52 paired samples collected during pre- and post-vaccination periods, respectively (Table 1). Among the 52 children, 12 children (23%) tested positive for *S. pneumoniae* colonization at both time points; 15 (29%) tested positive for *S. pneumoniae* pre-vaccination but showed no bacteria detected post-vaccination; 12 (23%) tested negative pre-vaccination but positive for *S. pneumoniae* post-vaccination; and 13 (25%) tested negative for *S. pneumoniae* at both the pre- and post-vaccination time points (Table 1). Power analysis performed using Stata found that the statistical power of the analysis was 82.4% for sample size of 52, take into calculation of the carriage rate of *S. pneumoniae* in HIV-infected children before was 46% based on previouse study [5] with the assumption of 20% decrease in carriage rate after vaccination. The results showed that PCV7 vaccination among HIV-infected children could reduce *Streptococcus pneumoniae* nasopharyngeal carriage from 52–46% that was statistically not significant (Chi-square McNemar test, P=0.701) We observed that those 13 paired samples which were negative for *S. pneumoniae* both pre- and post-vaccination showed either growth of non-pneumococcal bacteria or no bacterial growth at all on the blood agar plate.

We also identified that the pre-vaccination coverage rates for PCV7 (Serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F), PCV10 (Serotypes 4, 6B, 9V, 14, 18C, 19F, 23F, 1, 5, and 7F), and PCV13 (Serotypes 4, 6B, 9V, 14, 18C, 19F, 23F, 1, 5, 7F, 3, 6A,

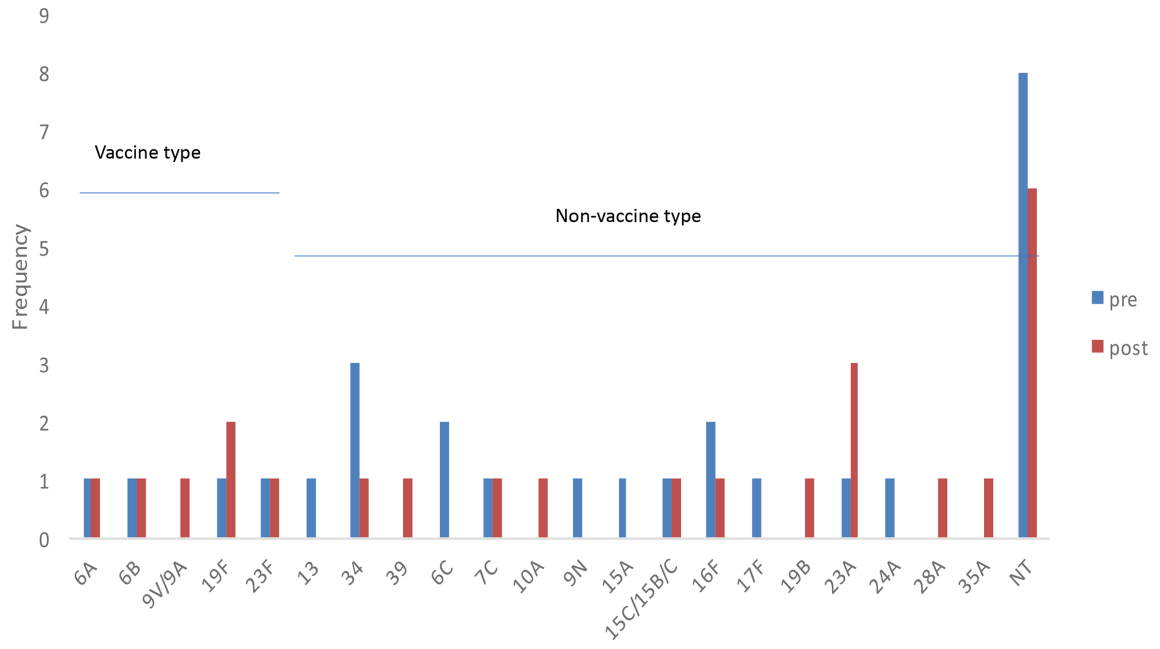

Fig. 1. Frequency of *Streptococcus pneumoniae* serotypes isolated from nasopharynx from children infected with HIV pre- (blue colour) and post- (orange) vaccination (NT=non-typeable isolates). The vaccine type (PCV13) consist of serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

and 19A) were 11, 11 and 15%, respectively. Post-vaccination, the coverage rates for PCV7, PCV10, and PCV13 were 19, 19 and 25%, respectively. In general, non-vaccine type strains were the major serogroup/serotypes detected in this study (85% pre-vaccination and 75% post-vaccination) (Fig. 1).

We found that the *S. pneumoniae* isolates (*n*=51) were most commonly susceptible to chloramphenicol (88%) and clindamycin (88%), followed by erythromycin (84%), trimethoprim-sulphamethoxazole (69%), tetracycline (61%), and penicillin (59%) (Table 2). In comparing the pre- and post-vaccination antibiotic susceptibility profiles, we observed that the pre-vaccination *S. pneumoniae* isolates were most commonly susceptible to chloramphenicol (93%), clindamycin (93%), erythromycin (93%), trimethoprim-sulphamethoxazole (78%), oxacillin (67%), and tetracycline (74%) (Table 2); meanwhile, the post-vaccination strains were most commonly susceptible to chloramphenicol (83%), clindamycin (83%), erythromycin (75%), trimethoprim-sulphamethoxazole (58%), oxacillin (50%), and tetracycline (46%). In this study, we found that nine isolates expressed a lack of susceptibility to at least three antimicrobial agents of different classes; these multi-drug resistant strains included serotype 19F and non-typeable strains (three isolates each) as well as serotype 28A, 35A, and 15B/C (one isolate each).

DISCUSSION

We found that almost half of the children in this study carried pneumococcus in pre- and post-vaccination. This finding was in line with the results of previous studies. The prevalence of *S. pneumoniae* in children infected with HIV aged 4 to 144 months in 2012 in Jakarta, Indonesia was 46%, and healthy

Table 2. Antibiotic susceptibility profile of *Streptococcus pneumoniae* isolated from children infected with HIV

Antimicrobial agents	n (%)	Pre-Vaccination, n (%)	Post-Vaccination, n (%)
Penicillin*	30 (59)	18 (67)	12 (50)
Tetracycline	31 (61)	20 (74)	11 (46)
Sulfamethoxazole/trimethoprim	35 (69)	21 (78)	14 (58)
Erythromycin	43 (84)	25 (93)	18 (75)
Chloramphenicol	45 (88)	25 (93)	20 (83)
Clindamycin	45 (88)	25 (93)	20 (83)

*Susceptibility to penicillin was determined with oxacillin disc.

children aged 0 to 60 months in a different region in Indonesia reported a prevalence that was between 43 and 49.5% [5, 9–12]. A higher prevalence of *S. pneumoniae* colonization was reported among HIV-infected and HIV-uninfected children (80.5%) prior to introduction of the pneumococcal conjugate vaccine in 2013 in Mozambique [13]. Subsequently, in 2015, the prevalence of *S. pneumoniae* colonization among HIV-infected children in Ghana was 27.1% [14]. Our study showed that rates during pre- and post-vaccination in the study population were not significantly different (P=0.701). Another previous study in Fiji also reported that the pneumococcal vaccination in children did not affect the densities of bacteria or the carriage/colonization rates of *S. pneumoniae* and other pathogens [15]. Therefore, this study is also in agreement with results reporting that PCV7 vaccination in HIV-infected children might not reduce pneumococcal colonization significantly.

Previous studies have shown the impact of vaccination on the carriage/colonization of vaccine- and non-vaccine serotypes and antibiotic susceptibility profiles in different countries. A study in the Netherlands showed that vaccine serotypes were largely replaced by non-vaccine serotypes after 3 years of a PCV7 vaccination programme, with serotypes 11A and 19A being the most frequently isolated [16]. Massachusetts, USA, reported the virtual disappearance of vaccine serotypes in *S. pneumoniae* carriage in young children after vaccination; they also reported rapid replacement with non-vaccine serotypes with non-susceptible antibiotics (i.e. penicillin), particularly serotypes 19A and 35B [17]. Another study showed that the pneumococcal conjugate vaccine reduced the risk of vaccine type acquisition and colonization density but increased the risk of non-vaccine type acquisition among vaccines [18]. Meanwhile, in our study, we did not observe any significant change in serotype between the pre- and post-vaccination stages. We found that the majority of *S. pneumoniae* isolates circulating during both of these stages were non-vaccine type strains. Recently, a study in Bengal reported that HIV-infected children had fewer vaccine type colonizations than HIV-uninfected children (23 and 55%, respectively); this study reported no difference in the acquisition of vaccine type nasopharyngeal carriage of pneumococcus in either group after one dose of PCV13 [19]. The findings of that study are in concordance with our present finding that there is no significant difference in vaccine serotype colonization after a single dose of the pneumococcal vaccine.

With regard to comparison of antibiotic susceptibility profiles between pre- and post-vaccination, some studies have reported that after vaccination with the pneumococcal conjugate vaccine, susceptibility to antibiotics showed a decreasing not significantly or change to susceptible. A study in Dallas showed that penicillin-resistant *S. pneumoniae* isolates were reported to increase after pneumococcal conjugate vaccine implementation, but there was no significant change in cefotaxime susceptibility [20]. Bles *et al*. also reported that 69.2% of pneumococcal isolates from the nasopharynges of Tanzanian HIV-exposed infants were intermediately susceptible to benzyl penicillin [21]. These results are in line with those from our study, which found an increase of less susceptible isolates against oxacillin, tetracycline, trimethoprim-sulphamethoxazole, erythromycin, chloramphenicol, and clindamycin. Trimethoprim-sulphamethoxazole and tetracycline are included in commonly used antibiotics to treat patients with respiratory infections in Indonesia [22–24]. This study provided an insight in pneumococcal colonization difference among HIV-infected children vaccinated with PCV7 that might be important for vaccination consideration for HIV children. Besides, presence of vaccine serotypes and antimicrobial susceptibility level might be used as baseline data to determine pneumococcal vaccine implementation and antibiotic treatment should be administered to HIV-infected children when they get infected. However, the antimicrobial susceptibility profiles described in this study were defined according to disc diffusion that we claimed as a limitation of this study. In the previous national recommendation for immunization in Indonesia, pneumococcal vaccine should be given only one time in children above 12 months old. However, in the recent guideline, the Indonesian Paediatric Society recommended giving two doses of pneumococcal vaccine for naïve children 12 months old and above [25]. With this new recommendation, it is possible to increase the immunogenicity and efficacy of the pneumococcal vaccine. In conclusion, serotypes of *S. pneumoniae* isolated from nasopharynges of HIV-infected children varied, and they were more frequently non-vaccine type strains than vaccine type strains.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
This study had been reviewed and approved by the ethical committee of the Faculty of Medicine, Universitas Indonesia (Number: 126/H2.F1/ETIK/2013), Jakarta, Indonesia. An informed parental/guardian (grandfather/grandmother) permission form was completed prior to enrollment.

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