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Nasopharyngeal carriage and serotype distribution of *Streptococcus pneumoniae* among HIV-infected children aged >6 years: before and after vaccination of 13-valent pneumococcal conjugate vaccine

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Purpose: The objective of this study was to determine the prevalence of colonization, serotype distribution, and antimicrobial susceptibility profile of *Streptococcus pneumoniae* (Pneumococcus) isolated from human immunodeficiency virus (HIV)-infected children before and after single-dose of 13-valent pneumococcal conjugate vaccine (PCV13) vaccination.

Materials and Methods: We conducted a prospective cohort study among HIV-infected children above six years of age in Jakarta, Indonesia. Nasopharyngeal swabs were collected from 50 children before vaccination, 12 months, and 18 months after PCV13 vaccination. The swabs were evaluated by bacterial culture, and serotyping were performed using sequential multiplex polymerase chain reactions and Quellung reactions. Antimicrobial susceptibility profiles were determined using the disk diffusion method.

Results: We found *Streptococcus pneumoniae* colonized 46% (23/50) of total children enrolled before vaccination, which decreased to 19% (n=9/47) at 12 months post-vaccination and 29% (14/48) at 18 months post-vaccination. There was no significant difference in the prevalence of pneumococcal colonization between vaccinated and unvaccinated HIV-infected children ($p>0.05$). There was a significant decrease in pneumococcal colonization between the baseline, 12 months, and 18 months after vaccination among vaccinated children ($p<0.05$). Vaccine-type (VT) serotypes (6B, 23F, and 19A) were more prevalent than non-vaccine serotypes before vaccination. Non-vaccine type (NVT) serotypes (6C, 15C) were more prevalent at 12 months post-vaccination. VT serotypes were found

at 18 months post-vaccination in vaccinated children. There was a high prevalence of antimicrobial resistance to *S. pneumoniae* isolates to oxacillin, tetracycline, and sulfamethoxazole-trimethoprim before and after vaccination.
Conclusion: There was a decrease in pneumococcal carriage after PCV vaccination in HIV-infected children, accompanied by changes in serotype distribution from VT serotypes to NVT serotypes.

Keywords: *Streptococcus pneumoniae*; Vaccination; HIV; Children; Indonesia

INTRODUCTION

Streptococcus pneumoniae (Pneumococcus) is a significant contributor to bacterial pneumonia in HIV-infected children, with high mortality and morbidity rates [1]. Invasive pneumococcal infections, including pneumonia and meningitis, are estimated to have caused the global deaths of 294,000 healthy children and 23,300 human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome-infected children aged 1–59 months in 2015 [2]. The case fatality rates (CFR) of invasive pneumococcal infections are approximately 20% in sepsis cases and 50% in meningitis cases in developing countries [3]. Invasive pneumococcal infections also represent a significant cause of morbidity and mortality among individuals infected with HIV in the era of highly active antiretroviral therapy, with disease incidence reaching 100 times that of the healthy population. One in four HIV-infected individuals is at high risk of experiencing a recurrent episode of invasive pneumococcal infection within 12 months [4]. The risk of developing invasive pneumococcal infection in HIV-infected children is 40 times higher than in healthy children [3]. Pneumococcal conjugate vaccine (PCV) vaccination has demonstrated efficacy in reducing invasive pneumococcal infections and colonization in healthy and HIV-infected children due to its immunogenicity and tolerability in immunocompromised patients [5]. The World Health Organization recommends PCV vaccination for HIV-infected children aged 6–18 years who have not previously received the vaccine, specifically PCV13 vaccination in a single dose to reduce the risk of invasive pneumococcal disease [3]. PCV13 is a conjugate vaccine that covers 13 serotypes that cause invasive pneumococcal disease. The incidence of invasive pneumococcal infection has declined following the introduction of the PCV. Yet, studies have identified shifts in pneumococcal serotypes from those in the vaccine type (VT) to non-vaccine type (NVT) in community settings. Indonesia has implemented PCV13 vaccination as a national basic childhood immunization program since late 2022. However, studies on the prevalence of colonization and serotype distribution patterns of both vaccine and non-vaccine PCV types, as well as the antimicrobial susceptibility profile of *S. pneumoniae* bacteria in high-risk child populations such as HIV-infected children in the PCV13 pre-vaccination period, have not been conducted much, especially in Indonesia. The data on the prevalence of colonization and distribution of *S. pneumoniae* serotypes, the presence of serotype switching after PCV13 vaccination, and the antimicrobial susceptibility profiles pattern of *S. pneumoniae* colonizing HIV-infected children to various antimicrobials can be utilized as baseline data before the widespread implementation of the total doses PCV13 vaccination (2+1) program in Indonesia. In this study, we investigated the pneumococcal colonization, the serotype distribution and the antimicrobial susceptibility profile among those above 6 years of age HIV-infected children on pre and post single doses of PCV13 vaccination.

MATERIALS AND METHODS

Study design and site

This prospective cohort study was conducted to determine nasopharyngeal (NP) carriage prevalence, serotype distribution, and antimicrobial susceptibility profile of *S. pneumoniae* isolated from HIV-infected children ≥6 years of age. All participants were randomly allocated into 2 groups through the random sampling method: the vaccinated group, which received one dose of PCV13 vaccination, and the control group, which did not receive PCV13 vaccination. We collected the data through three time points: before PCV13 vaccination, 12 months post-vaccination, and 18 months post-vaccination. We enrolled HIV-positive children ≥6 years of age from the pediatric clinic at Dr. Cipto Mangunkusumo National Central General Hospital (Rumah Sakit Dr. Cipto Mangunkusumo, RSCM) from October 2019 to April 2021. The inclusion criteria requirements were children above 6 years of age with CD4 absolute numbers >350, who had never received any pneumococcal vaccination

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before, who were currently receiving antiretroviral therapy, and consent from a parent/legal guardian to participate in the study. Patients with pneumococcal vaccination history and parents/legal guardians refusing to participate in the study were excluded.

Sample size

The sample size was calculated using the hypothesis test formula for the difference in proportions of 2 dependent populations with a power of 80% and a 5% significance level. According to this calculation, a minimum of 22 children from each group must make up a total sample of 44 children. With an estimated dropout rate of 10%, the study required the enrolment of 50 children.

NP swab specimen collection

NP swab samples were obtained by trained medical staff at 3 distinct time points: before vaccination in October 2019, 12 months post-vaccination in October 2020, and 18 months post-vaccination in April 2021 from both the control and vaccinated groups. The NP swab collection was initially intended for 6 months post-vaccination but was postponed due to coronavirus disease 2019 (COVID-19) pandemic containment measures. The NP swabs were collected using a flexible NP flocked swab (No. 503SC01; Copan, Brescia, Italy). Each swab was placed in 1.0 ml of skim milk tryptone glucose glycerol (STGG) transport medium and shipped to the Bacteriology Laboratory at the Eijkman Institute for Molecular Biology in Jakarta using the ice packs. The NP-STGG specimens were stored at –80°C for 2 to 4 hours after specimen collection. This study design was reviewed and approved by the Faculty of Medicine Ethics Committee, Universitas Indonesia—Dr. Cipto Mangunkusumo Hospital (Number: 1379/UN2.F1/ETIK/2018). Prior to enrolment, an informed parental/guardian consent form was obtained.

S. pneumoniae bacterial identification and isolation

NP swab specimens stored in a –80°C freezer were thawed at room temperature and enriched using broth enrichment method. Swab samples were vortexed and inoculated 200 μL into glass tubes containing 5 mL Todd Hewitt liquid medium supplemented with yeast extract and 1 ml rabbit serum and incubated at 37°C with 5% CO_2 for 5–6 hours. The incubated swab samples were plated on 5% trypticase soya agar with sheep blood and incubated at 37°C with 5% CO_2 for 18–24 hours. The suspected pneumococcal colonies with typical characteristics, such as a greenish zone around the colony with a mucoid or flat depressed center surface, are subcultured onto trypticase soya agar (BBL Taxo; Becton-Dickinson, Franklin Lakes, NJ, USA) with sheep blood and tested for susceptibility to an optochin (ethylhydrocupreine) disc. The colony with a large inhibition zone (>14 mm) are frozen in STGG medium in a –80°C freezer for DNA extraction and antimicrobial susceptibility testing. The colony with small inhibition zone around the optochin disc and atypical pneumococcal colony morphology was confirmed using the bile solubility test using 2% sodium desoxycholate (Sigma Aldrich, Steinheim, Germany) [6].

S. pneumoniae serotype determination

The suspected *S. pneumoniae* isolates are subcultured for DNA extraction, which will be used for sequential multiplex polymerase chain reactions (PCRs) for serotype determination as previously described [6,7]. The pneumococcal isolates with serogroups results from sequential multiplex PCRs will be confirmed using Quellung reactions (Statens Serum Institut, Copenhagen, Denmark).

Antimicrobial susceptibility test

S. pneumoniae isolated from HIV-infected children were tested for common antimicrobials used for pneumococcal infections according to Clinical Laboratory and Standards Institute guidelines using Mueller-Hinton media supplemented with 5% sheep blood. The antimicrobial discs used were oxacillin 1 μg, erithromycin 15 μg, azithromycin 15 μg, tetracycline 30 μg, trimethroprim/sulphametoxazole 1.25/23.75 μg, clindamycin 2 μg, and chloramphenicol 30 μg [8]. Pneumococcal isolates were classified as multidrug-resistant (MDR) isolates if they exhibit resistance to at least three different antibiotic classes.

Data analysis

Statistical analysis was performed to determine the effect of PCV13 vaccination on the prevalence of colonization in HIV-infected children in the pre-vaccination, 12-, and 18-month post-vaccination periods using the McNemar test, and whether there was a difference in the prevalence of *S. pneumoniae* colonization between the control group and the vaccinated group as a whole using the χ^2 test in SPSS version 29 (IBM Corp., Armonk, NY, USA) with a 95% confidence level ($p<0.05$). The power analysis was performed using Stata (StataCorp LLC, College Station, TX, USA).

Pneumococcal serotypes VT were defined as serotypes present in the PCV13 vaccine (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) and NVT were defined as serotypes other than those present in the PCV13 vaccine. *S. pneumoniae* isolates that could not be detected by conventional multiplex PCR assays and the Neufeld test/Quellung reaction were defined as non-typeable (NT) serotypes.

RESULTS

S. pneumoniae NP carriage in pre, 12 months and 18 months post vaccination

The culture-based method results showed that almost half of the total population of HIV-infected children in this study were colonized by *S. pneumoniae* (46%, n=23/50). The vaccinated group had a higher percentage of *S. pneumoniae* colonization (56%, n=14/25) compared to the control group (36%, n=9/25) (**Table 1**). The overall prevalence of *S. pneumoniae* colonization was determined using the total number of subjects who participated in the study as the denominator, while the prevalence of colonization per category used the total number of subjects from each category (control or vaccination) as the denominator. There was a decrease in the prevalence of *S. pneumoniae* colonization in the vaccinated and control groups at 12 months post-vaccination (19%, n=9/47) compared to the pre-vaccination period (46%, n=23/50) (**Table 2**). *S. pneumoniae* colonization in the vaccinated group decreased from 56% (n=14/25) at pre-vaccination to 17% (n=4/23 subjects) at 12 months post-vaccination. The prevalence of *S. pneumoniae* colonization in the vaccinated group was slightly lower (17%, n=4/23) compared to the control group (21%, n=5/24) at 12 months post-vaccination (**Table 2**). Three subjects dropped out of the study at 12 months post-vaccination: 2 from the vaccinated group and one from the control group because they could not participate in the swab collection.

The prevalence of *S. pneumoniae* colonization in the vaccinated group at 18 months post-vaccination was higher (26.1%, n=6/23) compared to the 12-month post-vaccination period (17%, n=4/23), although the increase in prevalence was not statistically significant in the McNemar test results (p>0.05) (**Table 3**). The prevalence *of S. pneumoniae* colonization in children who received the PCV13 vaccine at 18 months (26%) was lower than that observed in the pre-vaccination period (56%). The prevalence of *S. pneumoniae* colonization did not differ significantly between the control and vaccinated groups before and after PCV13 vaccination (p>0.05) (**Table 4**). However, a significant pneumococcal colonization reduction was observed at 12 and 18 months post-vaccination within the vaccinated group, compared to the pre-vaccination period (p<0.05) (**Table 5**).

S. pneumoniae serotype distribution before PCV13 vaccination

Serotype 6B was the VT serotype found in all study subjects and was most prevalent in the control group (33%), but least prevalent in the test group (7%) (**Fig. 1**). Serotypes 23F and 19A were the most prevalent VT serotypes in the vaccinated group (14%). Serotypes 014 (22%) and 003 (11%) were the VT serotypes found in the control group after serotype 6B, but not in the vaccinated group. The distribution of PCV13 NVT

Table 1. *S. pneumoniae* carriage prevalence in HIV-infected children before PCV13 vaccination

S. pneumoniae colonization	*S. pneumoniae* positive	*S. pneumoniae* negative	Total subject numbers
Vaccinated	14 (56%)	11 (44%)	25
Control	9 (36%)	16 (64%)	25
Total	23 (46%)	27 (54%)	50

Values are presented as number of subjects (%).
HIV, human immunodeficiency virus; PCV13, 13 valent pneumococcal conjugate vaccine.

Table 2. *S. pneumoniae* carriage prevalence in HIV-infected children 12 months after PCV13 vaccination

S. pneumoniae colonization	*S. pneumoniae* positive	*S. pneumoniae* negative	Total subject numbers
Vaccinated	4 (17%)	19 (83%)	23
Control	5 (21%)	19 (79%)	24
Total	9 (19%)	38 (81%)	47

Three subjects dropped out of the study at 12 months post-vaccination: 2 from the vaccinated group and 1 from the control group because they could not participate in the swab collection. Values are presented as number of subjects (%).
HIV, human immunodeficiency virus; PCV13, 13 valent pneumococcal conjugate vaccine.

Table 3. *S. pneumoniae* carriage prevalence in HIV-infected children 18 months after PCV13 vaccination

S. pneumoniae colonization	*S. pneumoniae* positive	*S. pneumoniae* negative	Total subject numbers
Vaccinated	6 (26%)	17 (74%)	23
Control	8 (32%)	17 (68%)	25
Total	14 (29%)	34 (71%)	48

Values are presented as number of subjects (%).
HIV, human immunodeficiency virus; PCV13, 13 valent pneumococcal conjugate vaccine.

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Table 4. *S. pneumoniae* carriage prevalence in HIV-infected children before, 12 months, and 18 months after PCV13 vaccination

S. pneumoniae colonization	*S. pneumoniae* positive			p-value
	Before vaccination	12 months post-vaccination	12 months post-vaccination	
Vaccinated	14 (56%)	4 (17%)	6 (26%)	
Control	9 (36%)	5 (21%)	8 (32%)	
Total	23 (46%)	9 (19%)	14 (29%)	>0.05

Values are presented as number of patients (%).
HIV, human immunodeficiency virus; PCV13, 13 valent pneumococcal conjugate vaccine.

Table 5. *S. pneumoniae* pneumococcal carriage comparison among HIV-infected children receiving PCV13 vaccine

Category	Before vaccination	12 months after vaccination	p-value	Before vaccination	18 months after vaccination	p-value
Vaccinated subject	14 (56%)	4 (17%)	<0.05	14 (56%)	6 (26%)	<0.05

Values are presented as number of patients (%).
HIV, human immunodeficiency virus; PCV13, 13 valent pneumococcal conjugate vaccine.

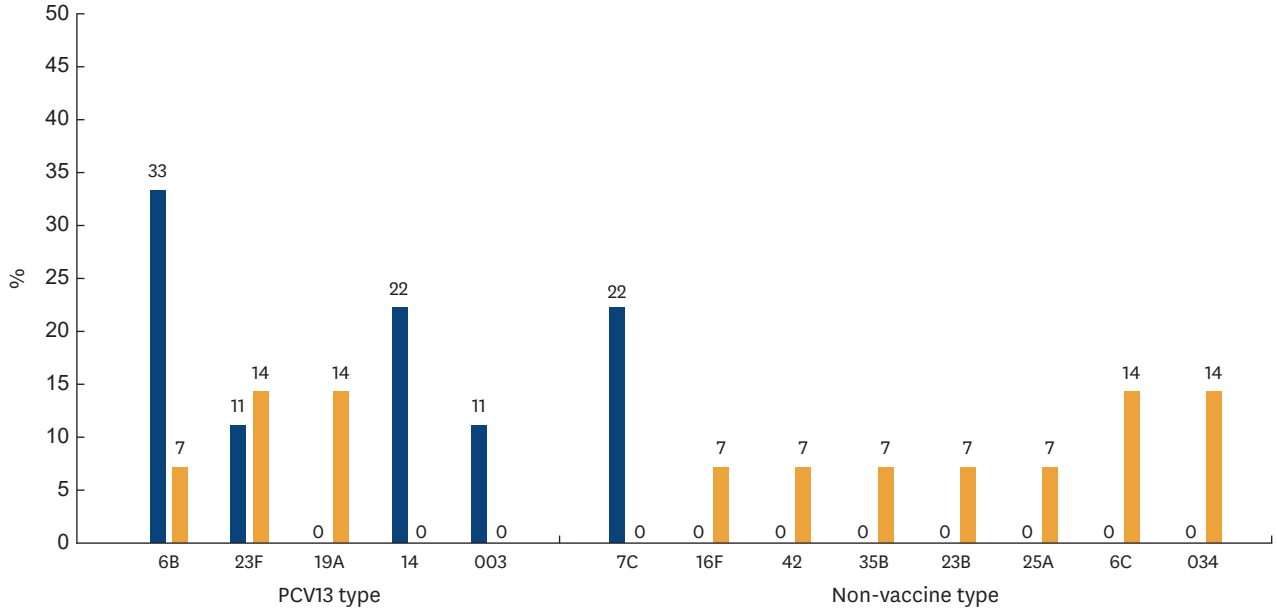

Fig. 1. Pneumococcal serotype distribution before PCV13 vaccination between vaccinated group (Color=Orange, n=14) and control group (Color=Blue, n=9).
PCV13, 13 valent pneumococcal conjugate vaccine.

serotypes in the pre-vaccination period was mostly found in the vaccinated group. Serotype 7C was the only non-vaccine serotype isolated from the control group (22%) (**Fig. 1**). Serotypes 6C and 034 were the most common serotypes found in the nasopharynx of subjects from the vaccinated group (14%). Other non-vaccine serotypes found in the vaccinated group were 16F, 042, 35B, 23B and 25A (7%). The serotype distribution of *S. pneumoniae* before PCV13 vaccination in HIV-infected children showed that non-vaccine serotypes were more prevalent in the vaccinated group than in the vaccinated group, while vaccine serotypes were more prevalent in the control group (**Fig. 1**).

S. pneumoniae serotype distribution 12 months after PCV13 vaccination

VT serotypes 6B, 19F, and 3 were only found in the control group (17%), while serotype 14 was found in one subject from the vaccinated group (25%) (**Fig. 2**). Serotype 6B consistently colonized the control group from before vaccination up to 12 months after vaccination. In the vaccinated group, serotype 6C remained the most prevalent NVT serotype at 12 months post-vaccination (50%), followed by serotype 15C (25%). Serotype 15C was absent in the vaccinated group before PCV13 vaccination (**Fig. 2**). Additionally, serotype 13 and non-typeable serotypes (17%) were found to colonize the control group at 12 months after vaccination (**Fig. 2**).

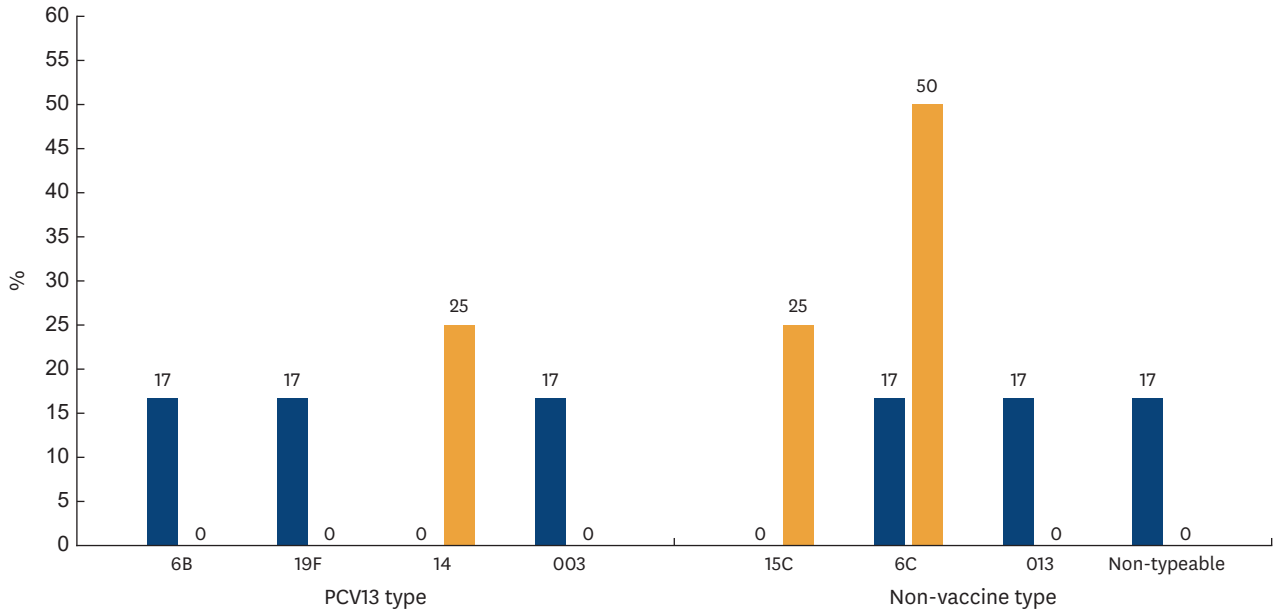

Fig. 2. Pneumococcal serotype distribution 12 months after PCV13 vaccination between vaccinated group (Color=Orange, n=4) and control group (Color=Blue, n=6).
PCV13, 13 valent pneumococcal conjugate vaccine.

S. pneumoniae serotype distribution 18 months after PCV13 vaccination

The control group showed a lower presence of VT serotypes compared to the vaccinated group. Serotypes 6A and 6B (12.5%) were the only 2 serotypes found to colonize the control group. VT serotypes such as serotypes 19F, 14, 23F and 3 (17%) were found in the vaccinated group only at 18 months post-vaccination and were not found in the pre-vaccination or 12-month post-vaccination periods (**Fig. 3**). Serotype 6C was the non-vaccine serotype always found in the control group and vaccinated group, both in the pre-vaccination, 12- and 18-month post-vaccination periods. vaccinated (17%), compared to the control group (12.5%). NT serotypes were also found at 18 months post-vaccination, with a higher prevalence in the control group (25%) compared to the vaccinated group (17%). Other non-vaccine serotypes such as serotypes 11A, 25A, and 7C were more prevalent only in the control group at 18 months post-vaccination. These 3 serotypes were found to colonize the control group with a prevalence of 12.5% each. Serotype 6C was the NVT serotype that persistently colonized few subjects from both the vaccinated and control groups during the pre-vaccination, 12-month, and 18-month post-vaccination periods. Two subjects exhibited persistent colonization by serotype 6C. One subject was from the vaccinated group, with colonization periods spanning from pre-vaccination to 18 months post-vaccination. The other subject was from the control group, with colonization periods occurring at 12 and 18 months post-vaccination (**Fig. 3**).

S. pneumoniae serotype distribution profile before and after PCV13 vaccination

VT serotypes were found more in the control group (78%) than in the vaccinated group (22%), but less in the vaccinated group (36%) than in the control group (64%) before PCV13 vaccine administration. The prevalence of VT serotypes decreased in both the vaccinated (25% to 36%) and control groups (78% to 60%), followed by an increase in the prevalence of NVT serotypes in the vaccinated (64% to 75%) and control groups (22% to 40%) at 12 months after vaccination (**Fig. 4**). VT serotypes were more prevalent than NVT serotypes, with an increase in prevalence from 25% to 67%. NVT serotypes in the vaccinated group decreased in prevalence from 75% to 33%. Non-vaccine serotypes were more prevalent in the control group at 18 months post-vaccination (75%) compared to 12 months post-vaccination (40%). The distribution pattern of S. pneumoniae serotypes in the vaccinated group at 18 months post-vaccination suggests that colonization by VT serotypes may still occur in HIV patients at a certain period of time after single-dose PCV13 vaccination (**Fig. 4**).

Antimicrobial susceptibility profile of *S. pneumoniae* isolated from HIV-infected children before and after PCV13 vaccination

A considerable amount of *S. pneumoniae* isolates from both the control and vaccinated groups showed reduced susceptibility to certain antimicrobial agents, such as oxacillin,

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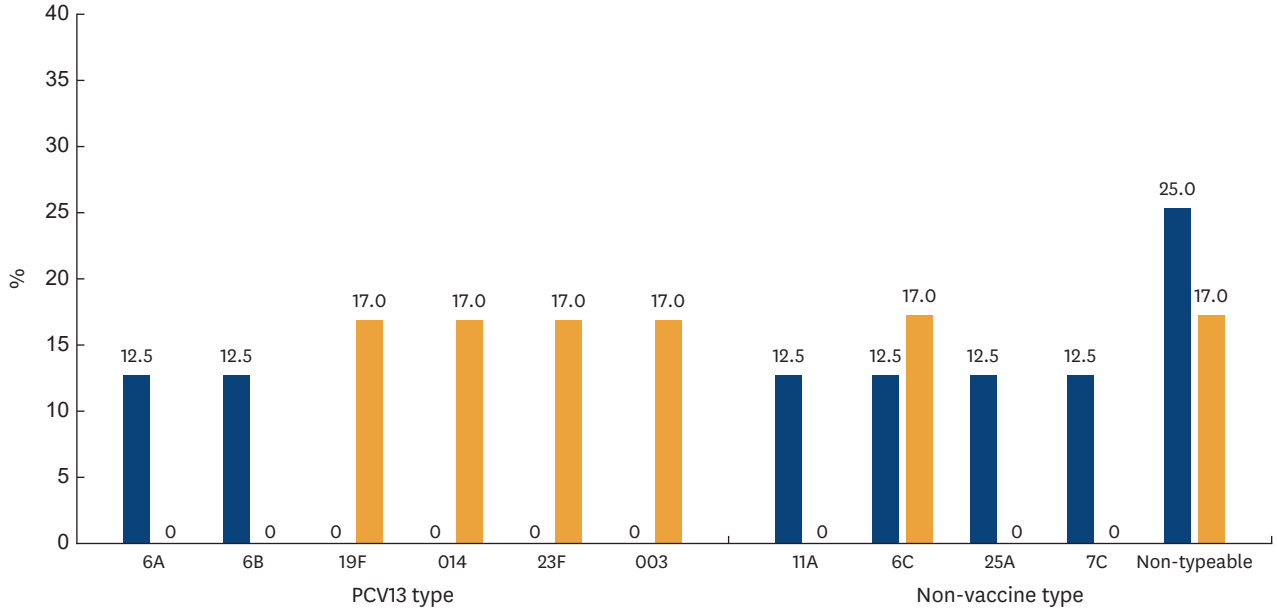

Fig. 3. Pneumococcal serotype distribution 18 months after PCV13 vaccination between vaccinated group (Color=Orange, n=8) and control group (Color=Blue, n=6).
PCV13, 13 valent pneumococcal conjugate vaccine.

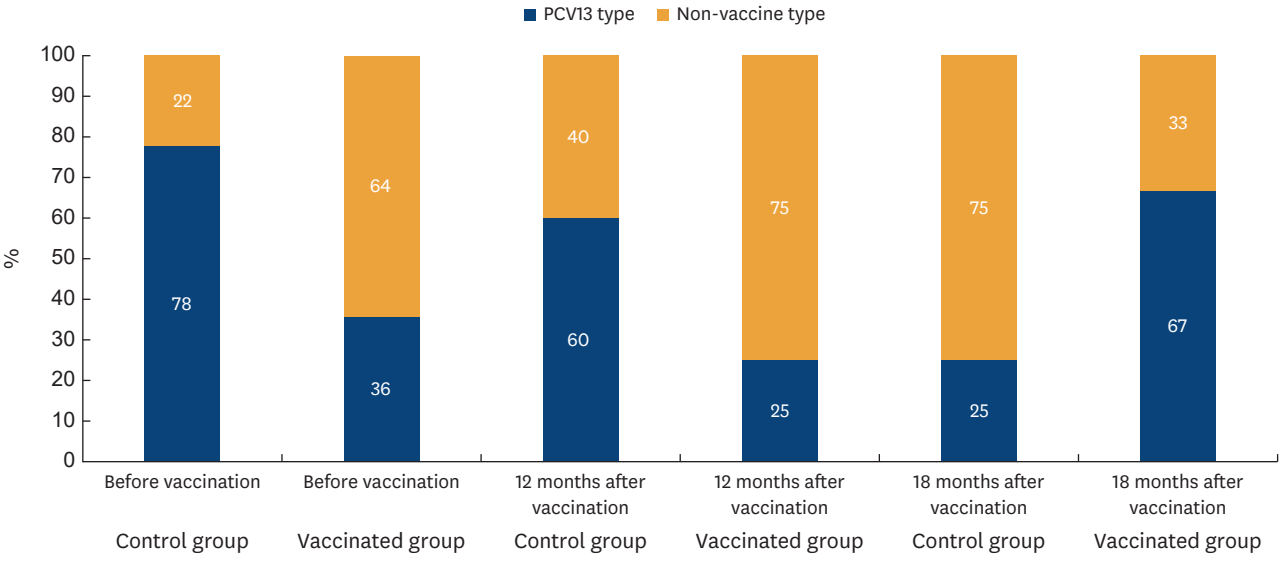

Fig. 4. *S. pneumoniae* serotype distribution before and after PCV13 vaccination.
PCV13, 13 valent pneumococcal conjugate vaccine.

tetracycline, and sulfamethoxazole-trimethoprim (**Table 6**). Most *S. pneumoniae* isolates in both the vaccine and control groups were susceptible to erythromycin, azithromycin, and clindamycin before and after vaccination. All *S. pneumoniae* isolates in both groups remained susceptible to erythromycin and azithromycin (100%) before vaccination and to clindamycin (100%) 18 months after vaccination. There was a decrease in the proportion of isolates susceptible to multiple antimicrobials, particularly in the vaccinated group, at 12 and 18 months post-vaccination. The decrease was found in oxacillin (from 100% to 33%), chloramphenicol (from 75% to 67%), tetracycline (from 75% to 50%), and trimethoprim/sulfamethoxazole (from 100% to 33%). However, in the control group, there was a tendency for the prevalence of oxacillin-susceptibility isolates to increase at 12 and 18 months after vaccination, from 67% to 88% (**Table 7**). Out of 47 isolates, 9 isolates (19%) were MDR (multidrug resistance) *Streptococcus pneumoniae*. The vaccinated group had a higher proportion of MDR isolates

Table 6. Antimicrobial susceptibility profiles of *S. pneumoniae* isolates before vaccination, 12 months, and 18 months after PCV vaccination

Antimicrobials	*S. pneumoniae* isolates					
	Before vaccination		12 months post vaccination		18 months post vaccination	
	Control group (n=9)	Vaccinated group (n=14)	Control group (n=6)	Vaccinated group (n=4)	Control group (n=8)	Vaccinated group (n=6)
Erithromycin	9 (100%)	9 (100%)	5 (83%)	4 (100%)	7 (88%)	5 (83%)
Azithromycin	9 (100%)	9 (100%)	5 (83%)	4 (100%)	7 (88%)	5 (83%)
Chloramphenicol	7 (78%)	11 (79%)	6 (100%)	3 (75%)	7 (88%)	4 (67%)
Clindamycin	9 (100%)	12 (86%)	5 (83%)	4 (100%)	8 (100%)	6 (100%)
Penicillin[a] (oxacillin 1 μg)	6 (67%)	10 (71%)	4 (67%)	4 (100%)	7 (88%)	2 (33%)
Tetracycline	5 (56%)	8 (57%)	5 (83%)	3 (75%)	6 (75%)	3 (50%)
Trimetophrim/ Sulphametoxazole	7 (78%)	8 (57%)	5 (83%)	4 (100%)	5 (63%)	2 (33%)

Values are presented as number of patients (%).
PCV, pneumococcal conjugate vaccine.
[a]*S. pneumoniae* susceptibility to penicillin was tested using a 1 μg oxacillin antibiotic disc according to the 2023 protocol by the Clinical and Laboratory Standards Institute.

Table 7. MDR *S. pneumoniae* isolates before and after PCV13 vaccination among HIV-infected children

No.	Specimen ID	PCV13 vaccination status	Serotype	Serotype category	Antimicrobial resistance pattern						
					Erithromycin	Azithromycin	Chloramphenicol	Clindamycin	Oxacillin	Tetracycline	Trimethroprim/ Sulphamethoxazole
1	1008	Vaccinated	23F	VT				■		■	■
2	1015	Vaccinated	19A	VT	■	■	■	■		■	■
3	1018	Vaccinated	042	NVT			■		■	■	■
4	1029	Vaccinated	23F	VT			■		■		■
5	3018	Vaccinated	NT	NVT			■		■		■
6	3036	Vaccinated	19F	VT	■	■			■		■
7	1028	Control	23F	VT			■		■	■	■
8	2006	Control	19F	VT	■	■		■	■		■
9	3012	Control	6A	VT	■	■	■		■		

MDR, multidrug-resistant; PCV13, 13 valent pneumococcal conjugate vaccine.; HIV, human immunodeficiency virus; NT, non-typeable; VT, vaccine type; NVT, non-vaccine type.

(n=6/9) compared to the control group (n=3/9). Seven isolates were MDR VT serotypes (15%), and 2 were NVT serotypes. The majority of MDR isolates were resistant to chloramphenicol, oxacillin, and sulfamethoxazole-trimethoprim (**Table 7**).

DISCUSSION

We found pneumococcal colonization from HIV-infected children was 46% (n=23/50) in this study, with no significant difference in pneumococcal colonization prevalence between the control group (56%, n=14) and the vaccinated group (36%, n=9) in the period before PCV13 vaccination. These findings align with those of a study conducted by Safari et al. [9] that found 46% (n=41/90) prevalence of *S. pneumoniae* colonization in HIV-infected children who had not received any PCV vaccination between the ages of 4 and 144 months in Jakarta. The study by Muktiarti et al. [10] also found that 52% (n=27/52) of HIV-infected children aged 12-152 months were colonized by *S. pneumoniae* before PCV vaccination. The prevalence of *S. pneumoniae* colonization in this study was slightly greater, as reported by Donkor et al. [11] in 2015, which found a prevalence of *S. pneumoniae* colonization in HIV-infected children aged <15 years in Ghana of 27% (n=32/118). The discrepancy in prevalence may be attributed to several factors, including introducing the PCV13 vaccine in children for routine immunization, which has been implemented in Ghana since 2012 [11]. This contrasts with the circumstances that

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prevailed in Indonesia during the period of this study (2019–2021), where the PCV13 vaccine has not been implemented into a routine immunization program for children aged 2 months. This study concurs that pneumococcal colonization was frequently observed among children infected with HIV prior to the implementation of the PCV13 vaccination, even in comparison to the healthy children population. Before the introduction of PCV13 in Indonesia, Safari et al. identified that 56.3% (n=1,025/1,822) of healthy children in two distinct regions (urban and rural) in Indonesia were colonized by *S. pneumoniae* [12]. The prevalence of *S. pneumoniae* colonization decreased significantly in the vaccinated group from 56% before vaccination to 17% in 12 months after PCV13 vaccination and to 26% in 18 months after vaccination ($p<0.05$). Another previous study from Cambodia also found a significant decrease in the prevalence of *S. pneumoniae* colonization and infection after pneumococcal vaccination in HIV-infected children aged >2 years in Cambodia, from 38.7% in 2007 to 15.8% in 2009, decreasing to 4.3% in 2010 and 2.2% in 2011 [13].

Another study also reported a decrease in the prevalence of *S. pneumoniae* colonization from 52% (n=27/52) in the period before PCV7 vaccination to 46% (n=24/52) at 6 months after PCV7 vaccination in HIV-infected children, although the decrease was not significant ($p=0.70$) [10].

External factors such as COVID-19 mitigation, including restrictions and mask use, may have helped reduce *S. pneumoniae* colonization in HIV-infected children post-vaccination. A study by Nation et al. [14] showed a decrease from 23.6% to 18.3% in June 2020, possibly due to measures like staying at home, physical distancing, and mask use during the COVID-19 period in Vietnam.

Serotype 6B was the most prevalent isolated in HIV-infected children from both the control group and the group vaccinated before PCV13 vaccination in this study. This finding was aligned with a previous study by Arya et al. [15] that serotype 6B was one of the most common VT serotypes found in HIV-infected children before the PCV vaccination. The most common VTs found in this study in the vaccinated group were 23F and 19A, while the VT serotypes most often isolated from the control group were serotypes 6A, 14 and 3. Furthermore, the results of this study are in agreement with reports from Verani et al. [16], indicating that VT serotypes such as 6A, 6B, and 19A (9.2%, 6.2%, and 5.2%) were also frequently found in NP colonization of HIV-infected children before the introduction of the PCV10 vaccine in Mozambique. Another previous study from Indonesia also reported that serotype 19A and serotype 6A/6B were the most frequently isolated serotypes from HIV-infected children aged 4–144 months who were not given the PCV vaccine [9]. Serotypes 6C and 34 (14%) were the most common non-vaccine serotype found in the period before PCV13 vaccination, followed by serotypes 16F, 42, 35B, 23B, and 25A (7%) in this study. These results are in line with report by Muktiarti et al. [10] who found serotype 34 and serotype 16F as the most common non-vaccine serotypes in children infected with HIV before being vaccinated with PCV7. The majority of non-vaccine serotypes were more common in the vaccinated group than vaccine serotypes at 12 months after vaccination. The results align with findings from Mozambique, showing a decrease in VT pneumococcal colonization in HIV-infected children from 35% to 25% over a 3-year period following the introduction of the PCV10 vaccine [17].

The study identified that non-vaccine serotype 6C persisted in one subject from before vaccination until 18 months after. Late antiretroviral therapy use in HIV-infected children could increase the risk of NVT serotype acquisition and delayed *S. pneumoniae* clearance [18]. Additionally, after receiving the PCV13 vaccine, several vaccine serotypes such as 19F, 23F, and 3 were found to colonize the vaccinated group. In another study from Indonesia, serotype 19F was prevalent in HIV-infected children 12 months after PCV7 vaccination [10], and in Ghana, it was the most frequently colonizing serotype in HIV-infected children after PCV13 vaccination [19]. Before PCV13 vaccination, VT serotypes were more common in the control group than in the vaccinated group in this study. In the 12 months following PCV13 vaccination, the prevalence of VT serotypes decreased in control group and the vaccinated group. However, there was an increase in VT serotypes in the vaccinated group, rising from 25% at 12 months post-PCV13 vaccination to 67% at 18 months post-vaccination. This finding was aligned with a previous report from Mozambique that colonization by VT serotypes in healthy children vaccinated with a full dose of PCV10 (3+0 booster) continued to decrease but not in HIV-infected children with the same vaccination status after 2 years of PCV10 introduction. This suggests that herd immunity was not established among HIV-infected children following specific time points after the introduction of the PCV10 vaccine [17].

The results of this study found that several isolates were non-susceptible to several types of antimicrobials such as tetracycline, sulfamethoxazole-trimethoprim (cotrimoxazole) and penicillin (oxacillin). This finding aligns with the report from Ethiopia that pneumococcal isolates in HIV-infected children were resistant to several antimicrobials with resistance to sulfamethoxazole-trimethoprim (70.5%), followed by tetracycline (61%), penicillin (50%), erythromycin (20.5%) and chloramphenicol (8.5%) [20]. Another study

from Indonesia also reported the proportion of isolates sensitive to oxacillin was the lowest at 59% compared to other antimicrobials such as erythromycin (84%), chloramphenicol, and clindamycin (88%) in HIV-infected children who received the PCV7 vaccine [10]. This study also found that erythromycin and azithromycin had the highest proportion of susceptible isolates (92%), followed by clindamycin (88%) in the vaccinated group. Notably, sulfamethoxazole-trimethoprim and tetracycline are the primary antimicrobials used to treat respiratory tract infections in Indonesia [21-23]. The study found that 15% of VT serotype isolates were MDR, whereas only 4% of non-vaccine serotypes showed MDR. Serotypes 23F and 19F were the most prevalent MDR VT serotype isolates in this study. These findings align with other studies from Indonesia that also found high prevalence of MDR isolates from pneumococcal colonization amongst healthy children, particularly serotype 19F [1,2]. However, a limitation of this study is that the antimicrobial susceptibility test for penicillin was carried out using the disc diffusion method with a 1μg oxacillin disc, which may require further confirmation using the broth microdilution method to determine the actual inhibitory value for the minimum concentration of penicillin antibiotics. Further research with a larger number of subjects and several time points after PCV13 vaccination is necessary to understand the prevalence of *S. pneumoniae* colonization and serotype replacement in HIV-infected children who receive the PCV13 vaccine. This will provide clinicians with more comprehensive data to help them make informed decisions about administering the PCV13 vaccine to HIV-infected children. In conclusion, there was a decrease in pneumococcal carriage after PCV13 vaccination in HIV-infected children, accompanied by changes in serotype distribution from VT to NVT. However, there was a slight increase in VTs after 18 months of vaccination. The majority of pneumococcal isolates were less susceptible to oxacillin, tetracycline, and sulfamethoxazole-trimethoprim, reflecting the most commonly used antibiotics in the country.

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