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Original Article

# Nasopharyngeal carriage rate, serotype distribution, and antimicrobial susceptibility profile of *Streptococcus pneumoniae* isolated from children under five years old in Kotabaru, South Kalimantan, Indonesia

**Korrie Salsabila [a], Wisiva Tofriska Paramaiswari [a], Hafsah Amalia [a], Akhmad Ruyani [b], Wisnu Tafroji [a], Yayah Winarti [a], Miftahuddin Majid Khoeri [a], Dodi Safari [a,*]**

[a] *Eijkman Institute for Molecular Biology, Jakarta, Indonesia*
[b] *District Health Office, Kotabaru, South Kalimantan, Indonesia*

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**KEYWORDS**
Antimicrobial resistance;
Carriage;
Children;
Serotype;
*Streptococcus pneumoniae*

**Abstract** *Background: Streptococcus pneumoniae* is a bacterial pathogen that colonizes the human nasopharynx. Colonization is frequently reported to be high in young children. In this study, we investigated the nasopharyngeal (NP) carriage rate, serotype distribution, and antibiotic susceptibility of *S. pneumoniae* in children under five years of age in Kotabaru, South Kalimantan, Indonesia.

*Methods:* NP swab specimens were collected from 399 young children (mean age: 30 months) who participated in the Rampa Village Community Health Center, with 74% of the participants being Bajau children. *S. pneumoniae* was identified using optochin susceptibility and bile solubility tests. Serotyping was performed by sequential multiplex PCR, and antimicrobial susceptibility profiling was performed by disk diffusion and microdilution methods.

*Results:* The NP carriage rate of *S. pneumoniae* was 45% (180/399). The most commonly serotypes were 6A/6B (18%), followed by 15B/15C (17%), 19F (16%), 34 (8%), and 23F (5%); 46% of them were identified as strains of the PCV13 vaccine type. Additionally, almost half of the pneumococcal isolates were non-susceptible to penicillin (40%), whereas non-susceptibility to tetracycline (36.8%), trimethoprim/sulfamethoxazole (29.7%), erythromycin (16.8%), chloramphenicol (9.7%), and clindamycin (8.6%) was also found. We identified 18% (n = 34) of *S. pneumoniae* isolates as multidrug-resistant (MDR) strains, and serotype 19F was the most common (74%) among them.

* Corresponding author. Eijkman Institute for Molecular Biology, Jalan Diponegoro No. 69, Jakarta, 10430, Indonesia.
*E-mail address:* safari@eijkman.go.id (D. Safari).

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*Conclusions:* MDR *S. pneumoniae* vaccine type strains were dominated by serotype 19F. The implementation of a pneumococcal conjugate vaccine program in Indonesia might reduce MDR strains circulating in the community in the future.


## Introduction

*Streptococcus pneumoniae* is a fastidious bacterium responsible for millions of deaths worldwide. This bacterium is a common integrant of the human nasopharynx microbiota, but it can migrate to sterile tissues and organs and cause infections.[1] Globally, *S. pneumoniae* is the major cause of lower respiratory infection morbidity and mortality in children less than five years old.[2] Between 2000 and 2015, there were 294,000 pneumococcal deaths among HIV-uninfected children aged 0—59 months, being pneumonia the most common disease.[3]

The nasopharyngeal (NP) carriage of *S. pneumoniae* plays an important role in the development and transmission of pneumococcal diseases.[4] The collection of pneumococcal isolates with a nasopharyngeal swab from young children allows characterization of pneumococcal strains circulating in the community and describes serotype distribution and antimicrobial susceptibility profiles. Moreover, as a lower-middle-income country, Indonesia has not included 13-pneumococcal conjugate vaccines (PCV13) in routine child immunization schemes. An understanding of the PCV13-type strains circulating, through nasopharyngeal carriage studies, may be useful for recommending the implementation of pneumococcal vaccination in Indonesia. Several NP carriage studies of *S. pneumoniae* have been conducted among Indonesian children before the implementation of a pneumococcal conjugate vaccine (PCV) program. These studies showed that the NP carriage of *S. pneumoniae* ranged between 43% and 55% among young Indonesian children.[5] However, currently, there is no available data on the NP carriage of *S. pneumoniae* from Kalimantan Island, Indonesia, prior to PCV implementation. Therefore, in this study, we investigated the NP carriage rates, serotype distribution, and antimicrobial susceptibility of *S. pneumoniae* in children under 5 years of age in Kotabaru, South Kalimantan, Indonesia, in 2019.

## Methods

### Study population

This study was a cross-sectional survey of NP carriage of *S. pneumoniae* conducted from February to April 2019 among young children aged 0—59 months during monthly community health services at the Community Health Center (Posyandu) at Terapung and Udang Posyandu, Rampa Village, Kotabaru, South Kalimantan, Indonesia. The study was reviewed and approved by the Eijkman Institute Ethical Committee (Project Number: 118). The children's parents signed informed consent forms and provided clinical and demographic information.

### Specimen collection and bacterial identification

NP swab specimens were collected using a flexible nasopharyngeal flocked swab (Copan, Italy; Cat. No. 503CS01) as described previously.[6,7] Swabs were immediately placed into STGG medium (NP-STGG) and vortexed for 10—20 s. The specimens were temporarily stored at −20 °C in Kotabaru, South Kalimantan, shipped on wet ice boxes to the Eijkman Institute for Molecular Biology in Jakarta for 5—6 h, and then stored at −80 °C until use.

Each NP-STGG specimen (200 μL) was transferred into enrichment broth consisting of 5.0 mL Todd Hewitt broth with 0.5% yeast extract supplemented with 1 mL of rabbit serum, followed by incubation at 37 °C. After 5—6 h of incubation, one loop of cultured broth (10 μL) was plated on a blood agar plate and incubated at 37 °C for 18—20 h with 5% $CO_2$. Plates were then examined for the appearance of alpha-hemolytic and flat, depressed in the center, colonies. A single colony was re-cultured and tested by Gram staining and for susceptibility to optochin and bile solubility.[8]

### Sequential multiplex PCR for serotyping

DNA extraction was performed by heating at 100 °C for 5 min. Samples were then placed in a −20 °C freezer for 5 min, and centrifuged at 13,000×*g* for 10 min. Bacterial lysates were stored at −20 °C until further use.[9] Serotypes for the *S. pneumoniae* isolates were deduced using sequential multiplex PCR (smPCR) covering 70 serotypes. The PCR conditions were as follows: pre-denaturation at 94 °C for 4 min, followed by 30 cycles of denaturation at 94 °C for 45 s, annealing at 54 °C for 45 s, and elongation at 65 °C for 2 min 30 s.[8] PCR results were visualized by 2% agarose gel electrophoresis, run at 100 V for 90 min. All isolates defined as non-typeable (NT) by PCR were confirmed by *lytA* gene detection using qPCR.[8]

### Antimicrobial susceptibility test

Antimicrobial susceptibility testing was performed using disk diffusion and microdilution methods according to the Clinical Laboratory Standard Institute, 2019.[10] The antibiotics used in this study were clindamycin (2 μg), chloramphenicol (30 μg), oxacillin (1 μg), tetracycline (30 μg), erythromycin (15 μg), and trimethoprim/sulfamethoxazole (1.25 or 23.75 μg). Briefly, 5 mL of bacterial suspension equivalent to 0.5 McFarland was prepared and spread

**Table 1** Children characteristics related to the nasopharyngeal carriage of *S. pneumoniae*.

| Characteristics | N | Number (%) of children carrying *S. pneumoniae* | p value |
|---|---|---|---|
| **Overall carriage rate** | 399 | 180 (45.1) | |
| **Age (months)** | | | |
| 0—12 | 74 | 38 (51.4) | 0.418 |
| 13—24 | 86 | 40 (46.5) | |
| 25—60 | 239 | 102 (42.7) | |
| **Sex** | | | |
| Male | 184 | 85 (46.2) | 0.762 |
| Female | 215 | 95 (44.2) | |
| **Ethnic group** | | | |
| Bajau | 297 | 143 (48.1) | 0.005 |
| Banjar | 59 | 16 (27.1) | |
| Others (Bugis, Dayak, Jawa, Mandar) | 43 | 21 (48.8) | |
| **No of family member** | | | |
| 0-4 | 230 | 97 (42.2) | |
| 5-8 | 147 | 71 (48.3) | 0.340 |
| >9 | 22 | 12 (54.5) | |
| **Current breastfeeding** | | | |
| Yes | 148 | 71 (48.0) | 0.406 |
| No | 251 | 109 (43.4) | |
| **Exposure to cigarette** | | | |
| Yes | 326 | 156 (47.9) | 0.027 |
| No | 73 | 24 (32.9) | |
| **Household condition** | | | |
| roof with asbes | 347 | 154 (44.4) | 0.832 |
| roof with wood/seng/others | 52 | 26 (50.0) | |
| walls with Bamboo/Brick/Kalsiboard/others | 41 | 14 (34.1) | |
| Walls with wood | 358 | 166 (46.4) | |
| **Fuel Type** | | | |
| Gas | 287 | 124 (43.2) | 0.246 |
| Kerosene | 110 | 55 (50.0) | |
| Wood | 1 | 0 | |
| Solar | 1 | 1 (100) | |
| **Health condition** | | | |
| Fever for the last 3 days | 94 | 41 (43.6) | |
| Cough for the past 24 h | 119 | 60 (50.4) | 0.293 |
| Congestion for the past 24 h | 176 | 85 (48.3) | |
| Breathing difficulties | 119 | 60 (50.4) | |
| **Hospitalization for the last 3 months** | | | |
| No | 387 | 174 (45.0) | 0.756 |
| Yes | 11 | 5 (45.5) | |
| **Visiting health care facilities for the past 3 months** | | | |
| No | 226 | 101 (44.7) | 0.919 |
| Yes | 173 | 79 (45.7) | |
| **Antibiotic consumption for the last 1 month** | | | |
| No | 349 | 162 (46.4) | 0.124 |
| Yes | 24 | 6 (25.0) | |
| Not know | 26 | 12 (46.2) | |

confluently using cotton swabs onto Mueller Hinton agar plates with 5% sheep blood. The plates were incubated at 37 °C for 20—24 h with 5% $CO_2$.

Multidrug-resistant (MDR) isolates are defined as isolates that are resistant to three or more classes of antimicrobials.[11] Microdilution was performed to measure the minimum inhibitory concentration (MIC) of all multidrug-resistant (MDR) strains defined by the disk diffusion method. We used 96-well round bottom plates containing 18 antibiotics at different concentrations (Thermo Fisher Scientific Cat. No. STP6F) for the microdilution assay. Briefly, a colony suspension equivalent to 0.5 McFarland was freshly prepared in 5 mL Mueller Hinton Broth; then, 100 μL was transferred into 11 mL of lysed horse blood medium and 100 μL of the mixture was inoculated into each well, followed by sealing and incubation at 37 °C for 20—24 h.

## Data analysis

Data analysis was performed using SPSS software. The chi-square test was used to determine risk factors contributing to colonization and to determine antimicrobial susceptibility among non-susceptible PCV13 serotypes and non-PCV13 serotypes. Besides, Fisher's exact test was used to assess risk factors with an expected cell count of less than 5. Statistical significance was set at $p < 0.05$.

## Results

In this study, we collected 399 NP swab specimens from children under five years of age in Rampa Village, Kotabaru, South Kalimantan. The majority of children were Bajau (74%), followed by Banjar (15%), Bugis (10%), and other ethnic groups (1%). We found that 180 (45%) children carried *S. pneumoniae* in their nasopharynxes. The characteristics of the children are presented in Table 1. The NP carriage of *S. pneumoniae* was found to be higher among Bajau children (143/297; 48%) than in children from other ethnic groups (36%; 37/102) ($p < 0.05$). Furthermore, we also observed that children exposed to cigarette smoke carried more pneumococcus than non-exposed children (47.9% vs. 32.9%, $p < 0.05$) (Table 1). We observed that there were no differences in pneumococcal carriage rates according to age, sex, number of family members, current breastfeeding status, household condition, use of antibiotics, and fuel type for cooking at the children's house (Table 1).

A total of 185 *S. pneumoniae* strains were isolated, with five children co-colonized by more than one serotype. The most commonly identified serotypes were 6A/6B (34 of 185 cultured *S. pneumoniae* strains; 18%), 15B/15C (31 strains; 17%), and 19F (29 strains; 16%), followed by serotypes 34 (14 strains; 8%), 23F (9 strains; 5%), 35A/35C/42 (8 strains; 4%), 14 (7 strains; 4%), 19A, 13, and 16F (four strains each; 2%), 11A/11D and 7C/7B/40 (3 strains; 2%), and 3, 4, 18C/18F/18B, 39, 17F, 22F/22A, 23B, 33F/33A/37, and 35B, 6C/6D (1 strain each; 1%) (Fig. 1). In this study, we identified five specimens co-colonized by different serotypes as follows: 1) serotypes 19F and 15B/15C; 2) 6A/6B and non-typeable; 3) 19F and 17F; 4) 16F and 6A/6B; and 5) 3 and 6A/6B. Whereas, 14% (25/185) of the isolates were non-typeable by smPCR but *lytA* positive. In this study, we found that 46% percent (86/185) of the strains were vaccine type (PCV13).

The pneumococcal isolates were found to be non-susceptible to penicillin (40%), tetracycline (36.8%), sulfamethoxazole/trimethoprim (29.7%), erythromycin (16.8%), chloramphenicol (9.7%), and clindamycin (8.6%) (Table 2). In general, the vaccine type strains were more susceptible to six different antibiotics than the non-vaccine strains (Table 2). Penicillin and tetracycline resistance levels between the PCV13 and non-PCV13 serotype groups were significantly different ($p = 0.003$ and $p = 0.007$, respectively) (Table 2). Furthermore, we observed that 18% (34/185) of the isolates were MDR strains (Table 3), and that most of them were resistant to tetracycline (91%; 31/34), trimethoprim/sulfamethoxazole (88%; 30/34), and erythromycin, azithromycin, and cefuroxime (85%; 29/34 each) (Table 3). Moreover, 32% (11/34) of these strains were resistant to meropenem (Table 3). We observed a high MIC value for tetracycline at concentrations $>8$ μg/mL. The highest MIC values for trimethoprim/sulfamethoxazole, cefuroxime, azithromycin, and erythromycin, among MDR isolates, were $>4/76$, $>4$, $>2$, and $>2$ μg/mL, respectively; whereas those for penicillin and amoxicillin/clavulanic acid 2:1 were $>4$ and 16/8 μg/mL, respectively. In contrast, the MICs of moxifloxacin, linezolid, vancomycin, and ertapenem were $\leq 1$ μg/mL among MDR strains. All MDR strains were vaccine-type, including serotypes 19F (73.5%), 6A/6B (8.8%), 19A (8.8%), 14 (5.9%), and 23F (2.9%).

## Discussion

Almost half of the healthy children under five years of age in Kotabaru, South Kalimantan, carried *S. pneumoniae* in their nasopharynxes. Most of the specimens were collected from Bajau children (74%). The indigenous Bajau people (Sea Nomads) have lived an entirely marine-dependent existence and spread across Indonesia, and are renowned for their extraordinary breath-holding abilities.[12] The prevalence of NP carriage of *S. pneumoniae* in this study is almost the same as the previous NP carriage study done in Lombok in 2016, at 46%.[13] In general, the prevalence of NP carriage of *S. pneumoniae* ranged between 43% and 55% in Indonesian healthy young children aged less than 5 years.[5] Geographical differences in NP carriage of *S. pneumoniae* in Indonesia may occur due to socio-economic status that varies among Indonesian regions.[5] We also observed that cigarette smoke contributed to the NP carriage of *S. pneumoniae* in children under five years of age in Kotabaru, South Kalimantan. Cigarette smoke increases the number of *S. pneumoniae* colonizations in the upper respiratory tract and increases the possibility of *S. pneumoniae* infecting the lungs.[14]

Understanding the serotypes that circulate in the community through NP carriage studies will be useful for the implementation of future pneumococcal vaccine programs in Indonesia. NP carriage determination is simple, non-invasive, and requires a small sample size. Therefore, it is a suitable method for low-income countries.[15] In this study, we found 16 serotypes of *S. pneumoniae*, and almost half of them were PCV13 serotypes, and the coverage of PCV10 was slightly lower than that of PCV13 (at 44%). Previously, 46% of PCV13 serotypes were observed among total pneumococcus isolates in healthy children in Bandung, Lombok, and Padang.[16] This finding showed that there was a high prevalence of already observed PCV13 serotypes in young Bajau children. PCV13 serotypes are highly associated with invasive pneumococcal disease (IPD).[17] Unfortunately, IPD data in Indonesia remain limited. However, based on the Indonesia Health Profile 2019, pneumonia, as one of the IPDs, is the main cause of death in children aged 0–11 months. Whereas, in children aged 12–59 months, pneumonia is a leading cause of death after diarrhea. In 2019, the prevalence of deaths due to pneumonia in children $<5$ years of age in South Kalimantan was the highest (5.53%) among Indonesian provinces.[18]

In this study, we observed that 18% of the isolates were MDR. A previous study conducted by the SENTRY Antimicrobial Surveillance Program showed that 21% of Latin American isolates were MDR.[19] Moreover, our MDR *S.*

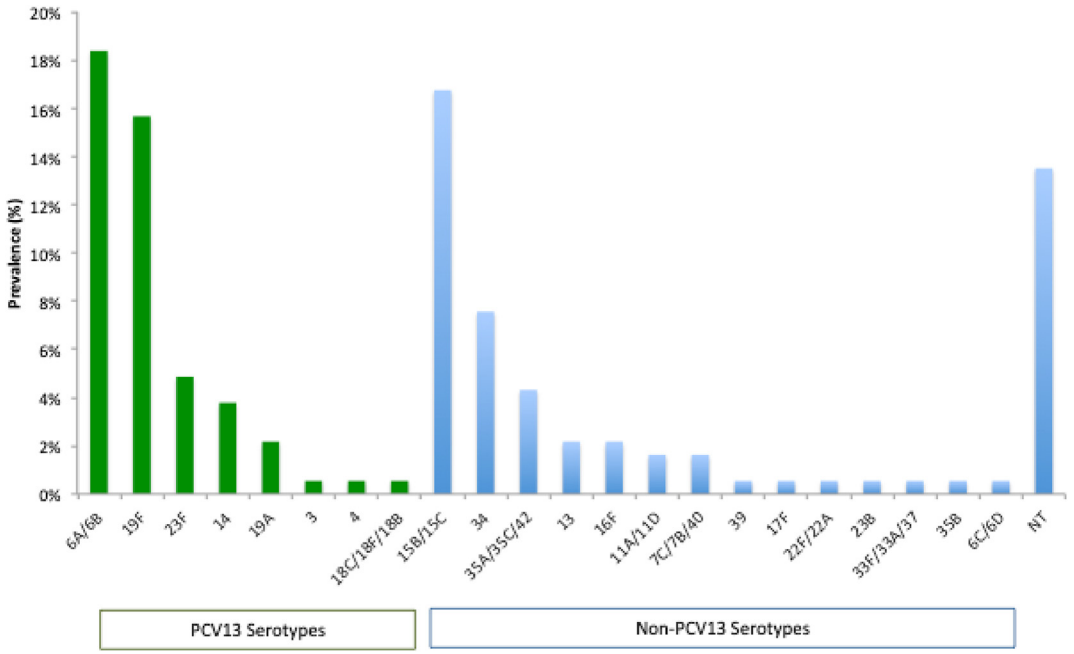


**Figure 1.** Serotype distribution and vaccine coverage among 185 *S. pneumoniae* carriage isolates of children under five years old in Kotabaru, South Kalimantan.

**Table 2** Antimicrobial non-susceptibility of 185 *S. pneumoniae* carriage isolates of children under five years old in Kotabaru, South Kalimantan.

| Antimicrobial Agent | Number (%) of non-susceptible isolates | | | p value |
|---|---|---|---|---|
| | All (n = 185) | PCV-13 serotypes (n = 86)[b] | Non-PCV13 serotypes (n = 99)[c] | |
| Chloramphenicol | 18 (9.7) | 7 (8.1) | 11 (11.1) | 0.498 |
| Clindamycin | 16 (8.6) | 15 (17.4) | 1 (1) | 1.000 |
| Erythromycin | 31 (16.8) | 31 (36) | 0 | N/A |
| Trimethoprim/Sulfamethoxazole | 55 (29.7) | 48 (55.8) | 7 (7.1) | 0.110 |
| Penicillin[a] | 74 (40) | 56 (65.1) | 18 (18.2) | 0.003 |
| Tetracycline | 68 (36.8) | 50 (58.1) | 18 (18.2) | 0.007 |

[a] Susceptibility to penicillin was determined with oxacillin disk. Interpretation followed CLSI 2019 that the clear zone ≥20 mm should be reported as susceptible to penicillin. MIC testing should be performed when the clear zone was ≤19 mm to determine intermediate and resistant to penicillin.
[b] PCV-13 serotypes: 14, 3, 4, Sg18, 19A, 19F, 23F, 6A/6B.
[c] Non-PCV13 serotypes: 13, 34, 39, 11A/11D, 15B/15C, 16F, 17F, 22F/22A, 23B, 33F/33A/37, 35A/35C/42, 35B, 6C/6D, 7C/7B/40, NT.

*pneumoniae* isolates showed a high resistance level to tetracycline as well as to trimethoprim/sulfamethoxazole, cefuroxime, erythromycin, and azithromycin. Noteworthy, tetracycline and trimethoprim/sulfamethoxazole are common antibiotics used for the treatment of patients with an upper respiratory infection.[20] We also discovered a high rate of cefuroxime resistance among MDR isolates. Cefuroxime is used to treat Community-Acquired Pneumonia (CAP) that is caused by intermediately resistant pneumococcus.[21] We found that all of the MDR *S. pneumoniae* strains were vaccine type (PCV13 serotypes), and that serotype 19F was the most common serotype among such MDR strains (74%). Our results are in line with the findings of the Asian Network for Surveillance of Resistant Pathogens (ANSORP), done in 11 Asian countries from 2008 to 2009, where 19F was the most prevalent serotype (59%) among MDR Asian pneumococci.[22]

In addition, we observed that the prevalence of vaccine and non-vaccine serotypes was significantly different among non-susceptible penicillin and tetracycline strains ($p = 0.003$ and $p = 0.007$, respectively). Serotypes 6A/6B and 19F, the dominant serotypes, were also frequently observed among non-susceptible penicillin and tetracycline strains (61/74 [82%] and 61/68 [90%], respectively). Meanwhile, serotypes 15B/15C and non-typeable, which were dominant among non-vaccine serotypes, were scarce among non-susceptible penicillin and tetracycline strains (15/68 [22%] and 8/68 [12%], respectively). Interestingly, serotypes 6A/6B and 19F have been commonly reported as serotypes resistant to penicillin and tetracycline.[7,23–25]

**Table 3** Antibiotic profile for 34 multidrug resistant *S. pneumoniae* isolates from children under five years old in Kotabaru, South Kalimantan, Indonesia.

| Antibiotics | Sussceptible | Intermediate | Resistant | Range MICs |
|---|---|---|---|---|
| | n(%) | n(%) | n(%) | |
| Penicillin[a] | 19 (56) | 9 (26) | 6 (18) | 0.12 - >4 |
| Amoxicilin/Clavulanic Acid 2:1 | 13 (38) | 10 (29) | 11 (32) | ≤2/1—8/4 |
| Cefepime[a] | 18 (53) | 16 (47) | 0 (0) | ≤0.5—2 |
| Cefotaxime[a] | 23 (68) | 11 (32) | 0 (0) | ≤0.12—2 |
| Ceftriaxone[a] | 20 (59) | 13 (38) | 1 (3) | ≤0.12 - >2 |
| Cefuroxime | 5 (15) | 0 (0) | 29 (85) | ≤0.5 - >4 |
| Moxifloxacin | 34 (100) | 0 (0) | 0 (0) | ≤1 |
| Levofloxacin | 28 (82) | 0 (0) | 6 (18) | 1—2 |
| Meropenem | 5 (15) | 18 (53) | 11 (32) | ≤0.25—1 |
| Ertapenem | 33 (97) | 1 (3) | 0 (0) | ≤0.5—2 |
| Vancomycin | 34 (100) | 0 (0) | 0 (0) | ≤0.5—1 |
| Azithromycin | 5 (15) | 0 (0) | 29 (85) | ≤0.25 - >2 |
| Erythromycin | 5 (15) | 0 (0) | 29 (85) | ≤0.25 - >2 |
| Tetracycline | 3 (9) | 0 (0) | 31 (91) | ≤1 - >8 |
| Trimethoprim/Sulfamethoxazole | 0 (0) | 4 (12) | 30 (88) | 1/19 - >4/76 |
| Linezolid | 34 (100) | 0 (0) | 0 (0) | 0.5—1 |
| Clindamycin | 19 (56) | 0 (0) | 15 (44) | ≤0.12 - >1 |
| Chloramphenicol | 33 (97) | 0 (0) | 1 (3) | 2—16 |

[a] Penicillin, ceftriaxone, cefotaxime, and cefepime using CLSI 2019 non-meningitis breakpoints. For penicillin 4 μg/mL was intermediate and ≥8 μg/mL was resistant. While for Cefepime, Cefotaxime, Ceftriaxone, 2 μg/mL was intermediate and ≥4 μg/mL was resistant.

Compared to each other, serotype 19F is more resistant to tetracycline, whereas serotypes 6A/6B is more resistant to penicillin.[26] Therefore, this study found that the prevalence of non-susceptible penicillin and tetracycline strains among vaccine and non-vaccine serotypes was significantly different.

In conclusion, almost half of the serotypes circulating in healthy Bajau children were of the vaccine type. Moreover, all the MDR isolates were of the vaccine type and dominated by the 19F serotype. Among 185 *S. pneumoniae* isolates, the vaccine-type strains showed a higher rate of non-susceptibility to the tested antibiotics. This finding supports the implementation of pneumococcal vaccines as a national vaccine program in Indonesia.

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## Ethical approval

This study was reviewed and approved by The Eijkman Institute Ethical Committee (Project Number: 118).

## Declaration of competing interest

There is no conflict of interest.

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## References

1. Brooks LRK, Mias GI. Streptococcus pneumoniae's virulence and host immunity: aging, diagnostics, and prevention. *Front Immunol* 2018 Jun 22;**9**:1366.
2. Troeger C, Blacker B, Khalil IA, Rao PC, Cao J, Zimsen SRM, et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990—2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis* 2018 Nov; **18**(11):1191—210.
3. Wahl B, O'Brien KL, Greenbaum A, Majumder A, Liu L, Chu Y, et al. Burden of Streptococcus pneumoniae and Haemophilus influenzae type b disease in children in the era of conjugate vaccines: global, regional, and national estimates for 2000—15. *Lancet Glob Health* 2018 Jul 1;**6**(7):e744—57.

4. Gebre T, Tadesse M, Aragaw D, Feye D, Beyene H, Seyoum D, et al. Nasopharyngeal carriage and antimicrobial susceptibility patterns of Streptococcus pneumoniae among children under five in southwest Ethiopia. *Children* 2017 Apr 19;**4**(4):27.
5. Kartasasmita CB, Rezeki Hadinegoro S, Kurniati N, Triasih R, Halim C, Gamil A. Epidemiology, nasopharyngeal carriage, serotype prevalence, and antibiotic resistance of *Streptococcus pneumoniae* in Indonesia [Internet] *Infect Dis Ther* 2020 Aug 30. https://doi.org/10.1007/s40121-020-00330-5 [cited 2020 Oct 12]; Available from:.
6. Satzke C, Turner P, Virolainen-Julkunen A, Adrian PV, Antonio M, Hare KM, 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 Dec;**32**(1):165—79.
7. Safari D, Kurniati N, Waslia L, Khoeri MM, Putri T, Bogaert D, et al. Serotype distribution and antibiotic susceptibility of Streptococcus pneumoniae strains carried by children infected with human immunodeficiency virus. Ratner AJ, editor. *PloS One* 2014 Oct 24;**9**(10):e110526.
8. Carvalho MdG, Pimenta FC, Jackson D, Roundtree A, Ahmad Y, Millar EV, et al. Revisiting pneumococcal carriage by use of broth enrichment and PCR techniques for enhanced detection of carriage and serotypes. *J Clin Microbiol* 2010 May 1;**48**(5):1611—8.
9. Pai R, Gertz RE, Beall B. Sequential multiplex PCR approach for determining capsular serotypes of Streptococcus pneumoniae isolates. *J Clin Microbiol* 2006;**44**:8.
10. Weinstein MP, Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*. 2019.
11. Gladstone RA, Jefferies JM, Faust SN, Clarke SC. Pneumococcal 13-valent conjugate vaccine for the prevention of invasive pneumococcal disease in children and adults. *Expert Rev Vaccines* 2012 Aug;**11**(8):889—902.
12. Ilardo MA, Moltke I, Korneliussen TS, Cheng J, Stern AJ, Racimo F, et al. Physiological and genetic adaptations to diving in Sea Nomads. *Cell* 2018 Apr 19;**173**(3):569—80. e15.
13. Hadinegoro SR, Prayitno A, Khoeri MM, Djelantik IGG, Dewi NE, Indriyani SAK, et al. Nasopharyngeal crriage of *Streptococcus pneumoniae* in healthy children under five years old in Central Lombok Regency, Indonesia. *Southeast Asian J Trop Med Publ Health* 2016;**47**(3):9.
14. Voss M, Wonnenberg B, Honecker A, Kamyschnikow A, Herr C, Bischoff M, et al. Cigarette smoke-promoted acquisition of bacterial pathogens in the upper respiratory tract leads to enhanced inflammation in mice. *Respir Res* 2015 Dec;**16**(1):41.
15. Chan J, Nguyen CD, Dunne EM, Kim Mulholland E, Mungun T, Pomat WS, et al. Using pneumococcal carriage studies to monitor vaccine impact in low- and middle-income countries. *Vaccine* 2019 Oct 8;**37**(43):6299—309.
16. Dunne EM, Murad C, Sudigdoadi S, Fadlyana E, Tarigan R, Indriyani SAK, et al. Carriage of Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, and Staphylococcus aureus in Indonesian children: a cross-sectional study. *PloS One* 2018;**13**(4):e0195098.
17. Gladstone RA, Jefferies JM, Faust SN, Clarke SC. Pneumococcal 13-valent conjugate vaccine for the prevention of invasive pneumococcal disease in children and adults. *Expet Rev Vaccine* 2012 Aug;**11**(8):889—902.
18. Kementerian Kesehatan RI. *Profil Kesehatan Indonesia 2019*. Jakarta: Kementerian Kesehatan Republik Indonesia; 2020.
19. Sader HS, Mendes RE, Le J, Denys G, Flamm RK, Jones RN. Antimicrobial susceptibility of *Streptococcus pneumoniae* from north America, Europe, Latin America, and the Asia-Pacific region: results from 20 Years of the SENTRY antimicrobial surveillance program (1997—2016). *Open Forum Infect Dis* 2019 Mar 15;**6**(Supplement_1):S14—23.
20. Hadi U, Duerink DO, Lestari ES, Nagelkerke NJ, Werter S, Keuter M, et al. Survey of antibiotic use of individuals visiting public healthcare facilities in Indonesia. *Int J Infect Dis* 2008 Nov;**12**(6):622—9.
21. Wiener-Well Y, Raveh D, Schlesinger Y, Yinnon AM, Rudensky B. Cefuroxime for empiric treatment of community-acquired pneumococcal pneumonia: is there a generation gap? *Chemotherapy* 2009;**55**(2):97—104.
22. Kim SH, Song J-H, Chung DR, Thamlikitkul V, Yang Y, Wang H, et al. Changing trends in antimicrobial resistance and serotypes of Streptococcus pneumoniae isolates in asian countries: an asian Network for surveillance of resistant pathogens (ANSORP) study. *Antimicrob Agents Chemother* 2012 Mar;**56**(3):1418—26.
23. Medeiros MIC, Almeida SCG, Bokermann S, Watanabe E, Guerra MLLS, de Andrade D. Antimicrobial susceptibility of Streptococcus pneumoniae isolated from patients in the northeastern macroregion of São Paulo state, Brazil, 1998-2013 [cited 2021 Jun 2] *J Brasileiro de Patologia e Med Laborat [Internet]* 2017;**53**(3). Available from: http://www.gnresearch.org/doi/10.5935/1676-2444.20170029.
24. Said WF, Sukoto E, Khoeri MM, Kumalawati J, Safari D. Serotype distribution and antimicrobial susceptibility of Streptococcus pneumoniae isolates from adult patients in Jakarta, Indonesia. *J Infect Publ Health* 2017 Nov;**10**(6):833—5.
25. Ktari S, Jmal I, Mroua M, Maalej S, Ben Ayed NE, Mnif B, et al. Serotype distribution and antibiotic susceptibility of Streptococcus pneumoniae strains in the south of Tunisia: a five-year study (2012—2016) of pediatric and adult populations. *Int J Infect Dis* 2017 Dec;**65**:110—5.
26. Dzaraly ND, Mohd Desa MN, Muthanna A, Masri SN, Taib NM, Suhaili Z, et al. Antimicrobial susceptibility, serotype distribution, virulence profile and molecular typing of piliated clinical isolates of pneumococci from east coast, Peninsular Malaysia. *Sci Rep* 2021 Dec;**11**(1):8220.