NASOPHARYNGEAL CARRIAGE OF *STREPTOCOCCUS PNEUMONIAE* IN HEALTHY CHILDREN UNDER FIVE YEARS OLD IN CENTRAL LOMBOK REGENCY, INDONESIA

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Abstract. Colonization with *Streptococcus pneumonia*e is mostly symptomless, but can progress to respiratory or even systemic disease. We investigated nasopharyngeal carriage of *Streptococcus pneumoniae* in healthy children under five years of age in Central Lombok Regency, Indonesia. This cross sectional study was carried out in 2012 among 1,200 healthy children aged 2 to 60 months. A multiplex sequential PCR was employed to determine serotype of cultured *S. pneumoniae* and a disk diffusion method to assess susceptibility to antimicrobial drugs. *S. pneumoniae* was cultured from 554 children and the most frequent serotypes found were 6A/B (22% of pneumococcal strains), 19F (11%), 23F (10%), 15B/C (8%), and 19A and 14 (4% each). The majority of strains were still susceptible to clindamycin (97%), erythromycin (87%), chloramphenicol (81%), and penicillin (72%), with only 41% and 38% susceptible to tetracycline and sulfamethoxazole/trimethoprim, respectively. Continuous surveillance of *S. pneumoniae* carriage is important for future pneumococcal vaccination programs in Indonesia.

Keywords: *Streptococcus pneumoniae*, healthy children, nasopharyngeal carriage, Indonesia

INTRODUCTION

Streptococcus pneumoniae is part of the normal flora of the upper respiratory tract and colonizes the nasopharyngeal niche together with other microorganisms (Bogaert *et al*, 2004, 2011). Colonization with *S. pneumonia*e is mostly symptomless, however, it can progress to respiratory or even systemic disease (Bogaert *et al*, 2004). Carriage of *S. pneumoniae* is considered to be an important source of the horizontal spread of this pathogen within the community (Bogaert *et al*, 2004; Oishi *et al*, 2014). Meta-analysis of published data showed that carriage rate for *S. pneu-*

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moniae is generally higher in low-income than in middle- to high-income countries and is higher in young children than in adults (Adegbola *et al,* 2014).

Epidemiological surveillance of *S. pneumoniae* serotype carriage in developing countries is crucial for arriving at informed decisions on implementing appropriate vaccination strategies and for assessing their impacts (Adegbola *et al*, 2014). In general, the prevalence of *S. pneumoniae* carriage in children under five years of age was higher in Africa than other regions of the globe (Le Polain de Waroux *et al*, 2014). Before the introduction of pneumococcal conjugate vaccines (PCVs), serotypes 6A, 6B, 19A, 19F, and 23F are most common serotypes frequently causing pneumococcal disease in low-income countries (Adegbola *et al,* 2014). Current studies have shown that the overall *S. pneumoniae* carriage rate remains unchanged after the pneumococcal vaccination program, but *S. pneumoniae* carriage of vaccine serotypes decreases and is replaced by non-vaccine serotypes (Cho *et al*, 2012; Parra *et al*, 2013; Hammitt *et al*, 2014; Oikawa *et al*, 2014; Daana *et al*, 2015; Gisselsson-Solén *et al*, 2015).

Knowledge of *S. pneumoniae* serotype carriage in Indonesia remains limited. Until now, pneumococcal vaccination is still not a part of the routine basic immunization schedule within the National Immunization Program (Directorate General for Disease Control and Enviromental Health, 2010). Soewignjo *et al* (2001) reported that the prevalence of *S. pneumoniae* carriage is 48% in healthy children aged below 25 months in Lombok Island, Indonesia in 1997 with 66% of isolates belonging to serogroups 6, 23, 15, and 33 or serotype 12. Recently, Farida *et al* (2014) noted that the prevalence rate of *S. pneumoniae* carriage in children aged 6-60 months and adults 45-75 years of age in Semarang, Central Java, Indonesia in 2010 is 43% and 11%, respectively, and that serotypes 6A/B and 15B/C are the most common carried in children. Safari *et al* (2014) reported the prevalence in 2012 of *S. pneumoniae* carriage in HIV-infected children aged 4 to 144 months in Jakarta, Indonesia to be 46%, compared with a hospital-based study conducted in Jakarta among 205 hospitalized children with pneumonia, sepsis or meningitis that identified *S. pneumoniae* as an etiological agent of the disease in only one child (Yuliarti *et al,* 2012).

The present re-investigated nasopharyngeal carriage of *S. pneumoniae* in healthy children under five years old in Central Lombok Regency, Lombok Island, Indonesia 15 years after the first carriage study was conducted (Soewignjo *et al*, 2001). The information obtained will be important for future pneumococcal vaccination programs in Indonesia.

MATERIALS AND METHODS

Study population

We performed a prospective, cross-sectional surveillance study on the nasopharyngeal carriage of *S. pneumoniae* among healthy children aged 2 to 66 months and living in five villages in Central Lombok Regency, Lombok Island, Nusa Tenggara Barat Province, Indonesia: Ubung, Puyung, Praya, Pringgarata, and Mantang (Fig 1). The sample size was calculated based on the previous carriage data in Lombok Island in 1997 (48% of *S. pneumoniae* carriage) (Soewignjo *et al*, 2001) and using the formula:

$$n = Z_{1-\alpha/2} \frac{(1-P)}{\varepsilon^2 P} ,$$

where P is proportion in the population

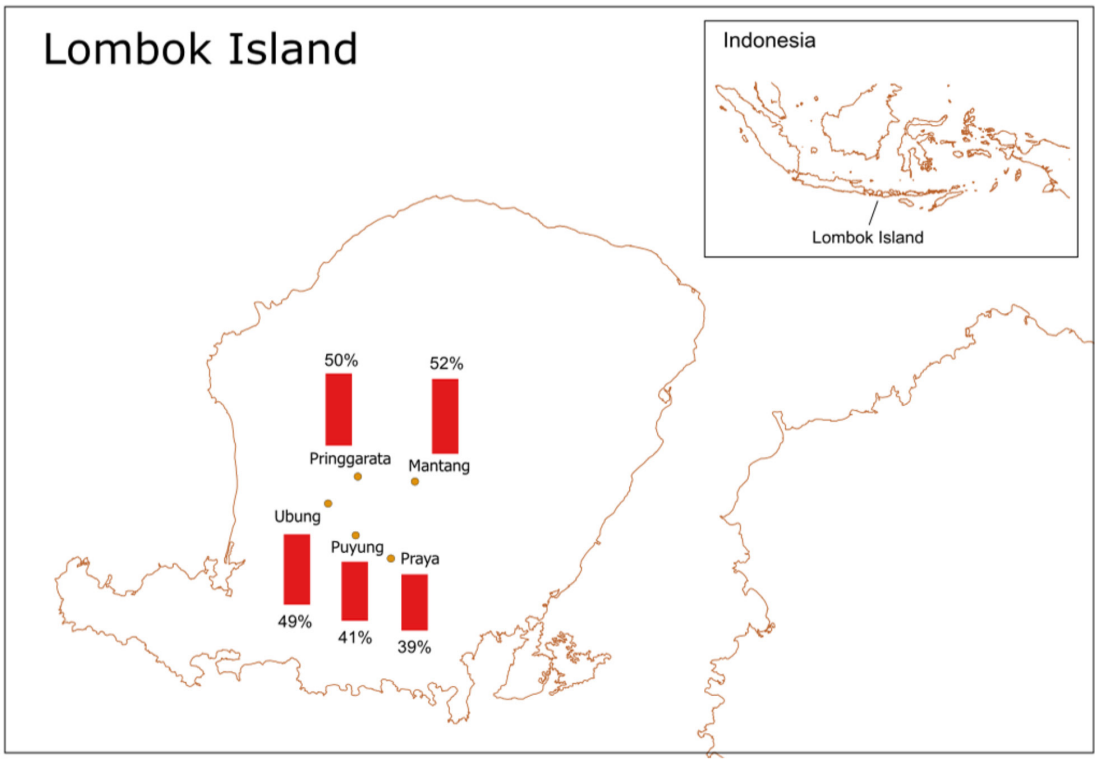

Fig 1–Distribution of nasopharyngeal carriage of *Streptococcus pneumoniae* in healthy children under five years old in five villages, Central Lombok Regency, Indonesia. Red bar indicates percent carriage prevalence. GPS coordinates of the five villages were obtained from OpenStreetMap (http://www.openstreetmap.org) and the distribution map was produced using QGIS program.

(proportion of *S. pneumoniae* carriage in the population from previous study) Z is Z value (1.96 for 95% CI) and ε is the desired precision (Lemeshow *et al*, 1991). The calculated minimum sample size is 417 at 95% confidence interval. In this study, 1,200 children were recruited during March to September 2012, all of whom were registered at 80 "posyandus" located in these five villages. The posyandu (Health and Nutrition Integrated Service Center) provides a range of social and health services for villages, including support in family planning, mother and child health services, nutrition, and immunization (Anwar *et al*, 2010). A posyandu is run by the community and is supported by a medical doctor or midwife from the Primary Health Center (Pusat kesehatan masyarakat/Puskesmas). One posyandu serves on average approximately 50 children under 5 years of age and operates once a month in every village (Anwar *et al*, 2010).

The study was reviewed and approved by the Ethics Committee of the Faculty of Medicine, University of Indonesia, Jakarta (59/PT02.FK/ETIK/2012). The children's parents signed prior informed consent forms and provided clinical and demographic information including the child's address, age, sex, and number of family members in the household. Parents were also asked whether any person living with the child is a smoker (Safari

Table 1
Demographic information of children enrolled in the study of nasopharyngeal *Streptococcus pneumoniae* carriage, Central Lombok Regency, Indonesia.

Characteristics	Number	Number (%) of children carrying *S. pneumoniae*	*p*-value
	1,200	554 (46)	
Age (months)			
1-24	625	332 (53)	0.001
25-48	441	172 (39)	
49-72	134	50 (37)	
Sex			
Male	651	308 (47)	0.386
Female	549	246 (45)	
Village region			
Mantang	240	125 (52)	0.012
Ubung	240	117 (49)	
Puyung	240	98 (41)	
Pringgarata	240	120 (50)	
Praya	240	94 (39)	
Exposure to cigarette			
Yes	874	415 (47)	0.134
Number of family member			
1-3	471	224 (48)	0.127
4-6	646	284 (44)	
> 7	82	45 (55)	
Not available	1	0	

et al, 2014). In this study, children with symptoms of respiratory tract infection were excluded.

Specimen collection

Nasopharyngeal swab specimens were collected using a flexible flocked swab (no. 503SC01, Copan, Brescia, Italy) by trained doctors/nurses and placed in skim milk/tryptone/glucose/glycerol (STGG) medium for transportation on wet ice directly to the Biomedical Laboratory, Provincial General Hospital West Nusa Tenggara, Lombok Island, Indonesia (Satzke *et al*, 2013; Safari *et al*, 2014). Upon arrival, an inoculum was plated onto 5% sheep blood-agar supplemented with 5 mg/l gentamicin (SB-Gent) and incubated at 35°C for 24-48 hours under an atmosphere of 5% CO_2. In the case of growth of multiple alpha-hemolytic colonies on the SB-Gent plate, a single colony was re-cultured and tested for Gram staining and susceptibility to optochin (Safari *et al*, 2014). All gram-positive, optochin-sensitive isolates were stored in STGG medium at -80°C for further analysis.

Serotyping

Serotype determination was performed using a sequential multiplex PCR (smPCR) with an internal positive control of capsule transcriptional regulator gene *wzg* (*cpsA*) universally present in *cps*

operons of almost all serotypes as described previously (Pai *et al*, 2006). Primers used targeted 40 of some 90 known *S. pneumoniae* serotypes.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the disk diffusion method according to CLSI standards for six different antimicrobial disks (Oxoid, Hamshire, UK), namely, chloramphenicol (30 μg), clindamycin (2 μg), erythromycin (15 μg), sulfamethoxazole/trimethoprim (23.75/1.25 μg), and tetracycline (30 μg). Oxacillin disk was used for testing susceptibility to penicillin (CLSI, 2013).

Data analysis

Chi-square test was employed for comparisons among isolates. A p-value <0.05 is considered statistically significant. Stratification analysis also was conducted to further explain factors associated with nasopharyngeal carriage.

RESULTS

We collected nasopharyngeal swabs from 1,200 healthy children from five different villages in Central Lombok Regency, Lombok Island, Nusa Tenggara Barat Province, Indonesia over a six-month period in 2012 (Fig 1). Mean age of children was 25.7 months, with > 50% of the children aged 24 months or younger (n = 625) (Table 1). There were 651 boys (54%) in the study group. The majority of the children (n = 874, 73%) had been exposed to cigarettes and were from households of four family members or larger (54%).

From 554/1,200 (46%) nasopharyngeal samples 557 isolates of *S. pneumoniae* were obtained. In three swab specimens, two pneumococcal strains of different morphotypes were isolated. Pneumococcal carriage rate was higher (53%) in children aged 1-24 months than that of the other age groups (Table 1). Factors associated with nasopharyngeal carriage are young age and living in Mantang (p = 0.041) or Puyung (p = 0.005), but there is no significant different in the age distribution in each village (p = 0.83). There is no significant difference in the carriage rate of *S. pneumoniae* with gender (p = 0.386), exposure to cigarettes (p = 0.134) or with the number of family members (p = 0.127) (Table 1 and Fig 1).

Table 2
Serotype of *Streptococcus pneumoniae* carriage isolates from healthy children under five years old, Central Lombok Regency, Indonesia.

Serotype	Number (%) of isolates
6A/B	120 (22)
19F	64 (11)
23F	58 (10)
15B/C	45 (8)
19A	24 (4)
14	20 (4)
11A	19 (3)
10A	13 (2)
35B	10 (2)
18	9 (2)
Others[a]	60 (11)
Untypable	115 (21)

[a]Serotype (number): 34 (7); 22F(7); 35F(7); 15A(6); 3(5); 20(4); 31(4); 38(4); 4(3); 17F(3); 7F(3); 1(2); 12F(2); 33F(1); 7C(1); 9V(1).

The 10 most frequent serotypes were 6A/B (22%), 19F (11%), 23F (10%), 15B/C (8%), 19A and 14 (4% each), 11A (3%), and 10A, 35B and 18 (2% each) (Table 2). One hundred fifteen strains (21%) were untypable by the smPCR method. Thus, serotype coverage of the 13-valent pneumococcal conjugate vaccine (PCV13) was 56%.

The majority of the strains (73%) were

Table 3
Antimicrobial susceptibility of *Streptococcus pneumoniae* strains carried by healthy children under five years old, Central Lombok Regency, Indonesia.

Antimicrobial agent	Number (%) of susceptible isolates			*p*-value
	Isolates (N = 405)	PCV13 serotype strain[a] (n = 220)	Non-PCV13 serotype strain (n = 185)	
Chloramphenicol	330 (81)	168 (76)	162 (88)	0.0019
Clindamycin	390 (96)	210 (95)	180 (97)	0.1640
Erythromycin	353 (87)	184 (84)	169 (91)	0.0104
Penicillin[b]	292 (72)	162 (74)	130 (70)	0.2259
Sulphamethoxazole/ trimethoprim	155 (38)	74 (34)	81 (44)	0.0182
Tetracycline	165 (41)	81 (37)	84 (45)	0.0399

[a]Strains of serotypes targeted by thirteen-valent conjugated polysaccharide pneumococcal vaccine (PCV13): 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F; [b]Susceptibility to penicillin was determined with oxacillin disk.

still susceptible to clindamycin (96%), erythromycin (87%), chloramphenicol (81%), and penicillin (72%) (Table 3). Only 41% and 38% of the strains were susceptible to tetracycline and sulfamethoxazole/ trimethoprim, respectively. Isolates of PCV13 (3, 6A/B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) serotypes detected are significantly less susceptible to the antimicrobial agents tested compared to isolates of non-PCV13 serotype, except for clindamycin and penicillin (Table 3).

DISCUSSION

In this study, investigation of the carriage of *S. pneumoniae* in healthy children under five years of age in Central Lombok Regency, Lombok Island, Indonesia 15 years after the first carriage study was performed (Soewignjo *et al*, 2001) revealed that *S. pneumoniae* carriage has not changed significantly. This finding was similar with other carriage studies in healthy children in Semarang, West Java in 2010 (43%) and in HIV-infected children in Jakarta (46%) (Farida *et al*, 2014; Safari *et al*, 2014).

In this study, we found that a decrease in sensitivity of *S. pneumoniae* to sulfamethoxazole/trimethoprim (38%) compared to the that (91%) of the previous survey (Soewignjo *et al*, 2001). A study in Semarang, Indonesia in 2010 reported 55% of *S. pneumoniae* strains susceptible to sulfamethoxazole/trimethoprim in children and adults (Farida *et al*, 2014). In HIV-infected children in Jakarta, 41% of strains isolated are still susceptible to this combination of antibiotics (Safari *et al*, 2014). Sulfamethoxazole/trimethoprim is the second most common antimicrobial prescribed for children in public health-care facilities in Indonesia (Hadi *et al*, 2008). High rates of resistance to sulfamethoxazole/trimethoprim were reported among healthy children in different regions of the world, such as 87% in Taiwan (Hsueh *et al*, 1999); 74% in Bangladesh (Saha *et al*, 2003); 49.3% in the Marrakech region, Morocco (Warda *et al*, 2013); and

51.2% in Brazil (Neves *et al*, 2013). We also observed an increase of non-susceptible *S. pneumoniae* strains to penicillin from 0% in 1997 (based on Etest) (Soewignjo *et al*, 2001) to 28% in this study (based on the indirect oxacillin test).

S. pneumoniae isolates of serotypes 6A/B, 19F, 23F, and 15B/C were the most common (51%). *S. pneumoniae* isolates belonging to PCV13 serotype were less susceptible to the majority of antimicrobial agents tested compared to isolates of non-PCV13 serotype in agreement with other studies, for instance, Daana *et al* (2015) reported that the proportion of multi-drug resistant *S. pneumoniae* strains is decreased significantly among Palestinian children (≤ 5 years old) after two years of introduction of PCV7 vaccination. Similarly, Metcalf *et al* (2015) showed that in the United States PCV13 vaccination decreases all PCV13 serotype clones and concurrently decreases strain subsets with resistance and/or adherence features conducive for successful carriage. We suggest that introduction of pneumococcal vaccination program in regions with high rates of antibiotic resistance in *S. pneumoniae* strains potentially lowers (at least temporarily) the rate of resistance to those particular antimicrobial agents, thereby attenuating the incidence of pneumococcal-related diseases. Thus, continuous surveillance of *S. pneumoniae* carriage is an important activity necessary for appropriate implementation of future pneumococcal vaccination programs in Indonesia.

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