

STAPHYLOCOCCUS AUREUS AND STREPTOCOCCUS PNEUMONIAE PREVALENCE AMONG ELDERLY ADULTS IN JAKARTA, INDONESIA

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Abstract. We studied *Staphylococcus aureus* and *Streptococcus pneumoniae* carriage among elderly adults in Jakarta, Indonesia. Nasopharyngeal swabs were collected from 149 adults aged 60-97 years. Both *S. aureus* and *S. pneumoniae* were identified by conventional and molecular methods. Methicillin-resistant *Staphylococcus aureus* (MSRA) was determined by PCR and antibiotic susceptibility using the disk diffusion method. Pneumococcal serotyping was performed with sequential multiplex PCR. We found *S. aureus* and *S. pneumoniae* present in 42 and 4 elderly adults respectively, and MRSA prevalence of 6%. Serotypes 3, 6A/B, 15B/C and 35F were identified among the four pneumococcal isolates. The majority of *S. aureus* isolates were susceptible to chloramphenicol (93%) and sulfamethoxazole/trimethoprim (93%), followed by gentamicin (88%), erythromycin (83%), penicillin (79%) and tetracycline (74%). Thus *S. aureus* prevalence is higher than that of *S. pneumoniae*, and a high frequency of MRSA carried by elderly adults in Jakarta, Indonesia.

Keywords: *Staphylococcus aureus*, *Streptococcus pneumoniae*, carriage, elderly adult, Indonesia

INTRODUCTION

Community-acquired pneumonia (CAP) is the fifth leading cause of death and is the most common cause of death from infectious diseases in people aged 65 years and older (Stupka *et al*, 2009). *Streptococcus pneumoniae* is still the most

common pathogen among the elderly (Simonetti *et al*, 2014). *Haemophilus influenzae*, *Staphylococcus aureus* and *Moraxella catarrhalis* also have been described as pathogens responsible for CAP in elderly adult patients (Stupka *et al*, 2009; Simonetti *et al*, 2014).

S. aureus and *S. pneumoniae* both commonly exist in the nasopharynx of children (McNally *et al*, 2006), affecting children between 3 and 10 years of age (Bogaert *et al*, 2004). In general, *S. pneumoniae* and *S. aureus* are more common in

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children compared to adults (Goldblatt *et al*, 2005; Hill *et al*, 2006; Cardozo *et al*, 2008; Adetifa *et al*, 2012; Palmu *et al*, 2012; Ansaldi *et al*, 2013; Blumental *et al*, 2013; den Heijer *et al*, 2013; Olsen *et al*, 2013). The carriage of *S. aureus* is 26.6% among patients > 75 years old in Paris, France (Lucet *et al*, 2005) and 26.3% in Danish middle-aged and elderly twins (Andersen *et al*, 2012). Meanwhile, *S. pneumoniae* carriage among adults \geq 60 years is 18.7% in the Ligurian population of Italy (Ansaldi *et al*, 2013) and only 1.5% among healthy elderly subjects aged \geq 65 years in Finland (Palmu *et al*, 2012).

Currently, the data available on *S. pneumoniae* and *S. aureus* carriage and disease are still limited among the Indonesian population. It has been reported that *S. aureus* carriage is 9.1% among the community and hospitalized patients in Semarang and Surabaya in 2001-2002 (Lestari *et al*, 2010). Recently, Santosaningsih *et al* (2014) reported that 24.4% of surgery patients in three academic hospitals carried *S. aureus*. Meanwhile, *S. pneumoniae* carriage is 48% in healthy children in Lombok Island in 2001 (Soewignjo *et al*, 2001). Farida *et al* (2014) reported that the carriage of *S. pneumoniae* is 43% and 11% in children aged 6-60 months and adults aged 45-75 years, respectively in Semarang in 2010. Safari *et al* (2014) showed that *S. pneumoniae* carriage is 46% in an HIV-infected group of children in Jakarta. In this present study, we studied nasopharyngeal carriage of *S. pneumoniae* and *S. aureus* in elderly adults in Jakarta.

MATERIALS AND METHODS

Study population

Elderly adults were recruited from those attending routine visits at the Geriatric Clinic, Dr Cipto Mangunkusumo

Hospital, Jakarta, Indonesia during June to September 2011. This study has been reviewed and approved by the ethics committee of the Faculty of Medicine, University of Indonesia, Jakarta, Indonesia. Volunteers signed consent forms and provided demographic information, such as age, sex, and number of family members, and detailed medical information was recorded.

Sample collection

Nasopharyngeal (NP) swabs were collected using a flexible nasopharyngeal flocked swab (Copan, Brescia, Italy) as described previously (O'Brien *et al*, 2003). Swabs were placed into 1.0 ml of skim milk tryptone glucose glycerol (STGG) transport medium, shipped on wet ice directly to the Eijkman Institute, Jakarta. A 20 μ l aliquot of STGG sample solution was plated onto a 5% sheep blood agar plate with and without 5 mg/l gentamicin and incubated at 35°C for 24 hours under 5% CO₂ atmosphere as described previously (Safari *et al*, 2014). In the case of growth of alpha- or beta-hemolytic colonies on the plate, a single colony was re-cultured and tested for Gram-staining, optochin susceptibility for presumptive *S. pneumoniae* isolate (WHO, 2011), and catalase and oxidase for presumptive *S. aureus* (Alesana-Slater *et al*, 2011).

PCR assays

Bacterial DNA was extracted as described previously (Pai *et al*, 2006). PCR targeting staphylococcal nuclease (*nuc*) and methicillin-resistance (*mecA*) genes for detection of presumptive *S. aureus* isolates were performed as described previously (National Food Institute, 2009), and that targeting pneumococcal surface antigen A (*psaA*) and autolysin (*lytA*) genes for detecting presumptive *S. pneumoniae* isolates according to McAvin

Table 1
 Characteristics of elderly adults in Jakarta, Indonesia from whom *S. aureus* and *S. pneumoniae* were isolated.

Characteristics	n	n (%)	
		<i>S. aureus</i>	<i>S. pneumoniae</i>
	149	42 (28)	4 (3)
Age (year)			
60-70	61	15 (25)	2 (3)
71-80	75	24 (32)	2 (3)
>81	13	3 (23)	0 (0)
Sex			
Male	58	19 (33)	1 (2)
Female	91	23 (25)	3 (3)
Number of family members			
1-3	74	20 (27)	2 (3)
4-6	58	20 (34)	1 (2)
>7	17	2 (12)	1 (6)
Respiratory infection			
Yes	41	9 (22)	1 (2)
Co-morbidity			
Diabetes mellitus	50	16 (32)	2 (4)
Heart failure	15	2 (13)	0 (0)
Kidney disease	10	4 (40)	0 (0)
Tuberculosis	8	3 (38)	1 (13)
Others	16	2 (13)	1 (6)
No symptoms	50	15 (30)	0 (0)

et al (2001 and Morrison *et al* (2000). Serotype determination was performed using a sequential multiplex PCR with the presence of the capsular polysaccharide biosynthesis (*cpsA*) gene as an internal control (Pai *et al*, 2006).

Antimicrobial susceptibility test

Antimicrobial susceptibility tests were carried out for *S. aureus* and *S. pneumoniae* isolates using the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI, 2007). All *S. aureus* isolates were tested with chloramphenicol, erythromycin, gentamicin, sulfamethoxazole/trimethoprim, tetracycline, and oxacillin disks (Oxoid,

Hamshire, UK). Four *S. pneumoniae* isolates were tested with chloramphenicol, clindamycin, erythromycin, and sulfamethoxazole/trimethoprim discs (Oxoid, Hamshire, UK).

RESULTS

Forty-two (28%) *S. aureus* and 4 (3%) *S. pneumoniae* isolates were cultured from nasopharyngeal samples collected from 149 elderly adults aged 60-97 years in Jakarta, Indonesia (Table 1). We identified 60 presumptive *S. aureus* isolates, with 42 (28%) PCR positive for *nuc* and 9 (6%) PCR positive for both *nuc* and *mecA*, thus classified as MRSA (Table 2). From 16

Table 2
Identification of *S. aureus* and *S. pneumoniae* isolated from 149 nasopharyngeal swabs of elderly adults in Jakarta, Indonesia.

Bacterial identification	n (%)
<i>S. aureus</i>	
Presumptive isolate	60 (40)
PCR-positive for <i>nuc</i>	42 (28)
PCR-positive for <i>nuc</i> and <i>mecA</i> (MRSA)	9 (6)
<i>S. pneumoniae</i>	
Presumptive isolate	16 (11)
Positive optochin susceptibility test	4 (3)
PCR \-positive for <i>lytA</i> and <i>psaA</i>	4 (3)

Table 3
Antimicrobial susceptibility of *S. aureus* isolated from elderly adults in Jakarta, Indonesia.

Antimicrobial agent	Number (%) of susceptible isolates		
	All (n = 42)	MSSA (n = 33)	MRSA (n = 9)
Oxacillin	33 (79)	33 (100)	0 (0)
Erythromycin	35 (83)	30 (91)	5 (56)
Chloramphenicol	39 (93)	33 (100)	6 (67)
Gentamicin	37 (88)	31 (94)	6 (67)
Sulfamethoxazole/Trimethoprim	39 (93)	33 (100)	6 (67)
Tetracycline	31 (74)	26 (79)	5 (56)

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*.

presumptive *S. pneumoniae* isolates, only 4 isolates were susceptible to the optochin test and PCR-positive for both *psaA* and *lytA* (Table 2). Four different serotypes (3, 6A/B, 15B/C, and 35F) were detected by multiplex sequential PCR.

S. aureus carriage was higher (32%) in the age group of 71-80 years than that of 60-70 years (25%) and above 80 years old (23%) (Table 1). There were 2 *S. pneumoniae* carriages in the age group of 60-70 years (3%) and of 71-80 years (3%). One (2%) *S. pneumoniae* isolate and 9 (22%) *S. aureus* isolates were obtained from elderly adults with respiratory infection (Table 1). No

differences in *S. pneumoniae* and *S. aureus* carriages according to gender, family size and comorbidities.

The majority of *S. aureus* isolates were susceptible to chloramphenicol (93%) and sulfamethoxazole/trimethoprim (93%), followed by gentamicin (88%), erythromycin (83%), oxacillin (79%) and tetracycline (74%) (Table 3). The 9 MRSA strains were not susceptible to oxacillin (Table 3) and were resistant to more antibiotics than methicillin-sensitive *S. aureus* (MSSA) strains (Table 3). All 4 *S. pneumoniae* isolates were susceptible to erythromycin and clindamycin, and 3 to

sulfamethoxazole/trimethoprim and 2 to chloramphenicol (data not shown).

DISCUSSION

In this study, the prevalence of *S. aureus* (28%) was higher than *S. pneumoniae* (3%) among elderly adults (age 60-97 years) in Jakarta, Indonesia, the former being in agreement with that reported in surgery patients from three academic hospitals in Indonesia (24.4%) (Santosaningsih *et al*, 2014), in Paris, France (26.6% in subjects > 75 years) (Lucet *et al*, 2005) and in Danish middle-aged and elderly twins (26.3%; 44-79 years of GE) (Andersen *et al*, 2012). Nguyen *et al* (2014) reported that the *S. aureus* carriage is lower in elderly adults (14.3%; > 60 years of GE) than in children > 5 years of age (30.6%) in urban and rural northern Vietnam. Our findings of *S. aureus* carriage are also in line with that reported from nursing home-acquired pneumonia (29%; \geq 75 years of age) (El-Solh *et al*, 2001), but higher than among community dwellers and hospitalized persons in Semarang and Surabaya, Indonesia (9.1%) (Lestari *et al*, 2010). However, our findings of *S. pneumoniae* carriage in elderly adults are in line with a previously published study on *S. pneumoniae* carriage in elderly adults in Finland (1.5%) (Palmu *et al*, 2012), but lower than that in Ligurian population, Italy (18.7%; \geq 60 years of age) (Ansaldi *et al*, 2013) and in Semarang, Indonesia (11%; 45-75 years of age) (Farida *et al*, 2014).

We found tetracycline susceptibility in 74% of the isolates, in line with the study among community (75%) and hospitalized patients (76%), but susceptibility to oxacillin in our elderly adult population was lower than among community (100%) and hospitalized patients (98%) (Lestari *et al*, 2010). We identified that the frequen-

cy of MRSA among elderly adults (6%) was higher than among surgery patients in three academic hospitals in Indonesia (4.3%) (Santosaningsih *et al*, 2014), among elderly adults (> 60 years of age) in rural and urban northern Vietnam (2.6%) (Nguyen *et al*, 2014), a non-hospitalized population of Braunschweig, northern Germany (1.29%) (Mehraj *et al*, 2014), and among hospitalized patients (2%) and community (0%) in Semarang and Surabaya, Indonesia (Lestari *et al*, 2010). We observed MRSA strains to be more resistant to antimicrobial drugs tested compared to MSSA strains.

In conclusion, among elderly adults in Jakarta, Indonesia *S. aureus* prevalence is higher than that of *S. pneumoniae*, and there is a noticeably high frequency of MRSA strains.

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REFERENCES

- Adetifa IMO, Antonio M, Okoromah CAN, *et al*. Pre-vaccination nasopharyngeal pneumococcal carriage in a Nigerian population: epidemiology and population biology. *PloS One* 2012; 7: e30548.
- Alesana-Slater J, Ritchie SR, Heffernan H, *et al*. Methicillin-resistant *Staphylococcus aureus*, Samoa, 2007-2008. *Emerg Infect Dis* 2011; 17: 1023-9.
- Andersen PS, Pedersen JK, Fode P, *et al*. Influen-

- ence of host genetics and environment on nasal carriage of *Staphylococcus aureus* in Danish middle-aged and elderly twins. *J Infect Dis* 2012; 206: 1178-84.
- Ansaldi F, de Florentiis D, Canepa P, *et al.* Carriage of *Streptococcus pneumoniae* in healthy adults aged 60 years or over in a population with very high and long-lasting pneumococcal conjugate vaccine coverage in children: Rationale and perspectives for PCV13 implementation. *Hum Vaccin Immunother* 2013; 9: 614-20.
- Blumental S, Deplano A, Jourdain S, *et al.* Dynamic pattern and genotypic diversity of *Staphylococcus aureus* nasopharyngeal carriage in healthy pre-school children. *J Antimicrob Chemother* 2013; 68: 1517-23.
- Bogaert D, van Belkum A, Sluijter M, *et al.* Colonisation by *Streptococcus pneumoniae* and *Staphylococcus aureus* in healthy children. *Lancet* 2004; 363: 1871-2.
- Cardozo DM, Nascimento-Carvalho CM, Andrade A-L SS, *et al.* Prevalence and risk factors for nasopharyngeal carriage of *Streptococcus pneumoniae* among adolescents. *J Med Microbiol* 2008; 57: 185-9.
- Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement. Wayne: CLSI, 2007.
- Den Heijer CDJ, van Bijnen EME, Paget WJ, *et al.* Prevalence and resistance of commensal *Staphylococcus aureus*, including methicillin-resistant *S aureus*, in nine European countries: a cross-sectional study. *Lancet Infect Dis* 2013; 13: 409-15.
- El-Solh AA, Sikka P, Ramadan F, Davies J. Etiology of severe pneumonia in the very elderly. *Am J Respir Crit Care Med* 2001; 163: 645-51.
- Farida H, Severin JA, Gasem MH, *et al.* Nasopharyngeal carriage of *Streptococcus pneumoniae* in pneumonia-prone age groups in Semarang, Java Island, Indonesia. *PLoS ONE* 2014; 9: e87431.
- Goldblatt D, Hussain M, Andrews N, *et al.* Antibody responses to nasopharyngeal carriage of *Streptococcus pneumoniae* in adults: a longitudinal household study. *J Infect Dis* 2005; 192: 387-93.
- Hill PC, Akisanya A, Sankareh K, *et al.* Nasopharyngeal carriage of *Streptococcus pneumoniae* in Gambian villagers. *Clin Infect Dis* 2006; 43: 673-9.
- Lestari ES, Duerink DO, Hadi U, *et al.* Determinants of carriage of resistant *Staphylococcus aureus* among *S. aureus* carriers in the Indonesian population inside and outside hospitals. *Trop Med Int Health* 2010; 15: 1235-43.
- Lucet J-C, Grenet K, Armand-Lefevre L, *et al.* High prevalence of carriage of methicillin-resistant *Staphylococcus aureus* at hospital admission in elderly patients: implications for infection control strategies. *Infect Control Hosp Epidemiol* 2005; 26: 121-6.
- McAvin JC, Reilly PA, Roudabush RM, *et al.* Sensitive and specific method for rapid identification of *Streptococcus pneumoniae* using real-time fluorescence PCR. *J Clin Microbiol* 2001; 39: 3446-51.
- McNally LM, Jeena PM, Gajee K, *et al.* Lack of association between the nasopharyngeal carriage of *Streptococcus pneumoniae* and *Staphylococcus aureus* in HIV-1-infected South African children. *J Infect Dis* 2006; 194: 385-90.
- Mehraj J, Akmatov MK, Strömpl J, *et al.* Methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* nasal carriage in a random sample of non-hospitalized adult population in northern Germany. *PLoS ONE* 2014; 9: e107937.
- Morrison KE, Lake D, Crook J, *et al.* Confirmation of *psaA* in all 90 serotypes of *Streptococcus pneumoniae* by PCR and potential of this assay for identification and diagnosis. *J Clin Microbiol* 2000; 38: 434-7.
- National Food Institute (DTU Food). Technical University of Denmark Soborg: DTU Food, 2009. Multiplex PCR for the detection of the *mecA* gene and the identification of *Staphylococcus aureus*. Community Refer-

- ence Laboratory Antimicrobial Resistance 2009.
- Nguyen KV, Zhang T, Vu BNT, *et al.* *Staphylococcus aureus* nasopharyngeal carriage in rural and urban northern Vietnam. *Trans R Soc Trop Med Hyg* 2014; 108: 783-90.
- O'Brien KL, Nohynek H, World Health Organization Pneumococcal Vaccine Trials Carriage Working Group. Report from a WHO Working Group: standard method for detecting upper respiratory carriage of *Streptococcus pneumoniae*. *Pediatr Infect Dis J* 2003; 22: e1-11.
- Olsen K, Sangvik M, Simonsen GS, *et al.* Prevalence and population structure of *Staphylococcus aureus* nasal carriage in healthcare workers in a general population. The Tromsø Staph and Skin Study. *Epidemiol Infect* 2013; 141: 143-52.
- Pai R, Gertz RE, Beall B. Sequential multiplex PCR approach for determining capsular serotypes of *Streptococcus pneumoniae* isolates. *J Clin Microbiol* 2006; 44: 124-31.
- Palmu AA, Kaijalainen T, Saukkoriipi A, Leinonen M, Kilpi TM. Nasopharyngeal carriage of *Streptococcus pneumoniae* and pneumococcal urine antigen test in healthy elderly subjects. *Scand J Infect Dis* 2012; 44: 433-8.
- Safari D, Kurniati N, Waslia L, *et al.* Serotype distribution and antibiotic susceptibility of *Streptococcus pneumoniae* strains carried by children infected with human immunodeficiency virus. *PLoS ONE* 2014; 9: (10) e110526.
- Santosaningsih D, Santoso S, Budayanti NS, *et al.* Epidemiology of *Staphylococcus aureus* harboring the *mecA* or Panton-Valentine leukocidin genes in hospitals in Java and Bali, Indonesia. *Am J Trop Med Hyg* 2014; 90: 728-34.
- Simonetti AF, Viasus D, Garcia-Vidal C, Carratala J. Management of community-acquired pneumonia in older adults. *Ther Adv Infect Dis* 2014; 2: 3-16.
- Soewignjo S, Gessner BD, Sutanto A, *et al.* *Streptococcus pneumoniae* nasopharyngeal carriage prevalence, serotype distribution, and resistance patterns among children on Lombok Island, Indonesia. *Clin Infect Dis* 2001; 32: 1039-43.
- Stupka JE, Mortensen EM, Anzueto A, Restrepo MI. Community-acquired pneumonia in elderly patients. *Aging Health* 2009; 5: 763-74.
- World Health Organization (WHO), Centers for Disease Control and Prevention (CDC). PCR for detection and characterization of bacterial meningitis pathogens: *N. meningitidis*, *H. influenzae*, and *S. pneumoniae*. In: Laboratory methods for diagnosis of meningitis. 2nd ed. Geneva: WHO press, 2011.