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Molecular typing of methicillin-susceptible *Staphylococcus aureus* isolates collected in the Yogyakarta area in Indonesia, 2006

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Abstract

The characterization of 62 community-associated methicillin-sensitive *Staphylococcus aureus* (MSSA) isolates from 440 individuals in the Yogyakarta area of Indonesia in 2006 showed that: (i) almost half of the isolates were associated with methicillin-resistant *S. aureus* lineages [clonal complex (CC)1, CC8 and CC45] and (ii) ten Panton–Valentine leukocidin-positive isolates were associated with CC1 (n = 7), CC30 (n = 1) and CC51 (n = 2). The high Panton–Valentine leukocidin prevalence (16%) among *S. aureus* is of concern because these strains can cause severe infections and the introduction of staphylococcal cassette chromosome *mec* into virulent and epidemic MSSA could pose a serious public health threat.

Keywords: Antibiotic resistance, community, Indonesia, PVL, *S. aureus*, *spa* typing

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Staphylococcus aureus is an important pathogen causing various infectious diseases [1]. *S. aureus* nasal carriers are at increased risk of developing *S. aureus* infection, and *S. aureus* of any genotype can become a life-threatening pathogen [2,3]. Since the 1990s, community-associated methicillin-resistant *S. aureus* (CA-MRSA) has emerged worldwide. CA-MRSA clones are genetically characterized by staphylococcal cassette chromosome *mec* (SCC*mec*) IV or V; some harbour Panton–Valentine leukocidin (PVL) genes and have different geographic distributions, as is the case of clonal complexes (CC)1, CC30, CC59 and CC80 in Asia [4]. The presence of PVL is associated with skin infections and necrotizing pneumonia [4]. However, the role of PVL has recently been questioned, and a possible role of other virulence factors, such as the arginine catabolic mobile element, has been postulated [5]. CA-MRSA could emerge through the transfer of the genes encoding PVL from PVL-positive methicillin-sensitive *S. aureus* (MSSA) into PVL-negative CA-MRSA, because these genes are carried on phages [4,6], or via SCC*mec* transfer into PVL-positive MSSA with a CA-MRSA genetic background. A high PVL prevalence among MSSA supports the hypothesis that SCC*mec* transfer has an important role in the increased prevalence of CA-MRSA [7–9]. For this reason, studies are needed to investigate PVL prevalence among MSSA. The present study aimed to investigate the population structure of *S. aureus* from nasal carriers in the Yogyakarta area of Indonesia and to investigate PVL prevalence.

During September and October 2006, nasal swabs were taken from 220 outpatients and their 220 companions visiting the ear, nose and throat outpatient department at Dr Sardjito Academic Hospital, Yogyakarta, Java, Indonesia. The 220 companions were healthy relatives or household members of patients. Written informed consent was obtained from all participants. *Staphylococcus aureus* isolation and identification was performed as described previously [10]. The susceptibility pattern of the isolates was determined according to CLSI guidelines using the micro-broth dilution method with the antibiotics described previously [10,11]. The isolates were tested for the MRSA-specific *mecA* gene and the *S. aureus*-specific *femA* gene [10].

The genetic background of the isolates was determined using *spa* typing, followed by clustering of the *spa* types into *spa*-clonal complexes (*spa*-CCs) [12]. The associated multilocus sequence typing (MLST) CCs were allocated using the Ridom SpaServer [12–14]. The presence of the genes encoding PVL was determined [15].

Staphylococcus aureus was isolated from 62 individuals (14%), including 39 outpatients (18%) and 23 companions (11%). All isolates harbored the *femA* gene. The outpatients (59% female, 41% male) had a median age of 21 years; their

companions (57% female, 43% male) had a median age of 39 years. The *S. aureus* nasal carriers were not related, nor were they members of the same household. All isolates were susceptible to cefaclor, cefuroxime, ciprofloxacin, gentamicin, linezolid, moxifloxacin, oxacillin, rifampin, teicoplanin, trimethoprim–sulfamethoxazole and vancomycin. Clindamycin and clarithromycin resistance was found among 3%, tetracycline resistance among 24% and penicillin resistance among 92% of the isolates. None of the *S. aureus* isolates harboured the *mecA* gene.

The 37 *spa* types clustered into six *spa*-CCs, 13 singletons and two excluded *spa* types. Five isolates could not be *spa* typed. The six *spa*-CCs were associated with MLST CC1, CC8, CC15, CC45, CC72 and CC121. The group of singletons contained isolates associated with MLST CC1, CC5, CC30 and CC45. Ten of the 62 isolates (16%) harboured PVL genes and were associated with CC1 (n = 7), CC30 (n = 1) and CC121 (n = 2) (Table 1). It is unknown whether the PVL-positive *S. aureus* nasal carriers had a PVL-related infection.

A low *S. aureus* nasal carriage rate (14%) was observed in the present study compared to prevalences described previously (30–50%) [1]. However, a study investigating an Indonesian population inside and outside hospitals in 2001 observed an even lower *S. aureus* nasal carriage rate (9.1%). An MRSA prevalence of 0.6% was observed, whereas no MRSA was observed in the present study [16]. The percentage of clindamycin-resistant isolates (3%) was comparable to that found in a previous study among MSSA isolates from hospitals in the Western Pacific region [17]. Lestari *et al.* [16] showed a higher percentage of erythromycin resistance compared to the present study (3% vs. 12%). Tetracycline resistance was almost equal in both studies (24% vs. 25%). Tetracycline resistance is common among community-associated Indonesian *S. aureus* isolates, and regional differences in antibiotic resistance patterns exist in Indonesia, which suggests regional differences in antibiotic consumption [16].

The MSSA isolates were associated with three lineages commonly observed among MRSA (MLST CC1, CC8 and CC45) and three MLST CCs were found among MSSA (CC15, CC72 and CC121) [8,12]. Eight of the ten PVL-positive MSSA isolates were associated with MLST CC1 and CC30. MLST CC1 is mainly observed in CA-MRSA in Asia, Europe and the USA (USA400 clone), whereas MLST CC30 is mainly observed in Asia and Europe (South West Pacific clone) [4]. Because *mecA* is stable in the MLST CC1 and CC30 lineages [18], PVL-positive MSSA could integrate the highly mobile SCC*mec* IV into their genome, leading to the emergence of CA-MRSA, as has occurred in the South West Pacific [7,19]. Two PVL-positive MSSA isolates associated with MLST CC121 were observed, and similar isolates have been found in the UK and the Cape Verde Islands [9]. However, CC121 is not a good *mecA* recipient [18]. Severin *et al.* [20] described a relatively high PVL prevalence (11%) among MSSA in Surabaya and Semarang, Indonesia. As in the present study, the PVL-positive isolates were associated with MLST CC1 and CC121, whereas the majority of the PVL-negative isolates were associated with MLST CC45 [20].

A limitation of the present study is the relatively small number of isolates tested. It was difficult to estimate the *S. aureus* nasal carriage rate before commencing the study and, consequently, to establish the minimum number of participants to enrol.

TABLE 1. Composition of the *spa*-CCs and the presence of Panton–Valentine leukocidin

spa-CC	No. of strains	No. of *spa* types	*spa* types	Associated MLST CC	No of PVL gene-positive strains
spa-CC 050	11	8	t015, t050, t116, t1823, t2444, t2771, t2773, t2774	45	
spa-CC 189	13	4	t189, t2473, t2765, t2769	1	7[a]
spa-CC 2770	5	4	t521, t2253, t2767, t2770	8	
No founder 4	2	2	t148, t324	72	
No founder 5	2	2	t159, t272	121	2[b]
No founder 6	4	2	t084, t1472	15	
Singletons	17	13	t095, t127, t153, t318, t409, t688, t701, t855, t939, t1458, t2768, t2772, t2775	1/5/30/45[c]	1[d]
Excluded [e]	3	2	t1200, t1544		
Non-typeable	5				
Total	62	37			10

PVL, Panton–Valentine leukocidin; MLST, multilocus sequence typing.
[a]Three strains were *spa* typed as t2769 and four as t189.
[b]One strain was *spa* typed as t159 and one strain as t272.
[c]Including three isolates associated with CC1, two with CC30, one with CC5 and one with CC45.
[d]One isolate associated with CC30 (*spa* type t318).
[e]*spa* types smaller than five *spa* repeats.

In conclusion, PVL-positive MSSA is a 'successful commensal' in Indonesia. The high PVL prevalence among MSSA nasal carriers is of concern because these strains can serve as an endogenous reservoir for infections. Furthermore, the introduction of SCC*mec* into virulent and epidemic MSSA could pose a public health threat.

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Transparancy Declaration

All authors declare that there are no conflicts of interest.

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