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Article *in* Tropical Medicine & International Health · March 2016

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VOLUME 21 NO 5 PP 610–618 MAY 2016

Characterisation of clinical *Staphylococcus aureus* isolates harbouring *mecA* or Panton–Valentine leukocidin genes from four tertiary care hospitals in Indonesia

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Abstract

OBJECTIVES To determine the prevalence, antimicrobial susceptibility profiles and clonal distribution of either methicillin-resistant *Staphylococcus aureus* (MRSA) or Panton–Valentine leukocidin (PVL)-positive *S. aureus* obtained from clinical cultures in Indonesian hospitals.

METHODS *S. aureus* isolates from clinical cultures of patients in four tertiary care hospitals in Denpasar, Malang, Padang and Semarang were included. We assessed the antimicrobial susceptibility profiles using the Vitek2® system, determined the presence of the *mecA* gene and genes encoding PVL using PCR and analysed the clonal relatedness with Raman spectroscopy. SCC*mec* typing was performed for all MRSA isolates. Multilocus sequence typing (MLST) was performed for a subset of isolates.

RESULTS In total, 259 *S. aureus* strains were collected. Of these, 17/259 (6.6%) and 48/259 (18.5%) were MRSA and PVL-positive methicillin-susceptible *S. aureus* (MSSA), respectively. The prevalence of MRSA and PVL-positive MSSA ranged between 2.5–8.9% and 9.5–29.1%, respectively and depended on geographic origin. PVL-positive MRSA were not detected. Raman spectroscopy of the strains revealed multiple Raman types with two predominant clusters. We also showed possible transmission of a ST239-MRSA-SCC*mec* type III strain and a ST121 PVL-positive MSSA in one of the hospitals.

CONCLUSIONS We showed that MRSA and PVL-positive MSSA are of clinical importance in Indonesian hospitals. A national surveillance system should be set-up to further monitor this. To reduce the prevalence of MRSA in Indonesian hospitals, a bundle of intervention measures is highly recommended.

keywords Asia, Indonesia, methicillin-resistant *S. aureus*, panton–valentine leukocidin, *Staphylococcus aureus*

Introduction

Staphylococcus aureus is recognised as an important pathogen, both in the hospital and community settings [1]. The emergence and spread of methicillin-resistant *S. aureus* (MRSA), covering both hospital-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA), has been a major problem worldwide [2–6]. Traditionally, HA-MRSA has been associated with multidrug resistance and staphylococcal cassette chromosome *mec* (SCC*mec*) types I, II and III, while CA-MRSA has been associated with SCC*mec* types IV and V and the presence of Panton–Valentine leukocidin (PVL) genes. However, this distinction has blurred in many countries as the CA-MRSA clones have been introduced into the hospital and the HA-MRSA in the community [7].

S. aureus infections, including MRSA infections, have long been underappreciated in resource-limited countries in south and east Asia, where the healthcare agenda prioritises other healthcare issues [8]. With the rapid emergence of antimicrobial resistance worldwide, this topic has, however, increasingly gained interest. Still, only limited data are available on the epidemiology of *S. aureus* infections in Indonesia. We have recently shown that MRSA was carried among patients screened at discharge from hospital in Indonesia, but with significant geographical variation [9]. Among the MRSA strains, the HA-MRSA clone sequence type (ST) 239-SCC*mec* III prevailed. In addition, a high prevalence of PVL was found among carriage and community-onset infectious strains of methicillin-susceptible *S. aureus* (MSSA) from Indonesia [9–13]. However, data on clinical *S. aureus* from Indonesian hospitals are lacking. In this study, we determined the prevalence, antimicrobial susceptibility profiles and clonal distribution of either MRSA or PVL-positive *S. aureus* obtained from clinical cultures in four Indonesian hospitals.

Materials and methods

Setting

Four tertiary care hospitals located on Sumatra island, Java island and Bali island (Figure 1) participated in this study: Sanglah Hospital in Denpasar (Bali; 704 beds), Dr. Saiful Anwar Hospital in Malang (East Java; 810 beds), Dr. M. Djamil Hospital in Padang (West Sumatra; 688 beds) and Dr. Kariadi Hospital in Semarang (Central Java; 779 beds). The study was performed from January 2008 to January 2009 in Padang and Denpasar, from October 2009 to January 2010 in Malang and from April 2008 to October 2009 in Semarang. This study was approved by the medical ethics committee of Dr. Saiful Anwar Hospital (Malang), Sanglah Hospital (Denpasar) and Dr. Kariadi Hospital (Semarang) related to the 'MRSA study' in Indonesia. Isolates were obtained as part of routine diagnostic testing and analysed anonymously for this study.

Bacterial isolates

S. aureus strains were isolated and identified by clinically indicated culture in each hospital involved in this study. *S. aureus* from all departments were included. Isolates were stored in trypticase soy agar until further characterisation could be performed. Confirmation of identification and antibiotic susceptibility testing of the *S. aureus* strains were carried out by the Vitek2® system (bioMérieux, Marcy l'Etoile, France). Antibiotics tested included macrolides (clindamycin, erythromycin), aminoglycosides (gentamicin, tobramycin), fluoroquinolones (ciprofloxacin, levofloxacin and moxifloxacin), glycopeptides (teicoplanin, vancomycin), trimethoprim–sulfamethoxazole, fosfomycin, fusidic acid, linezolid, mupirocin, nitrofurantoin, rifampicin and tetracycline. Only one clinical culture with *S. aureus* per patient was included in this study.

DNA isolation and detection of *mecA* and PVL genes

Bacterial DNA was isolated using the MagNa Pure LC DNA system (DNA isolation kit III; Roche Molecular Biochemicals, Mannheim, Germany) [14]. The DNA

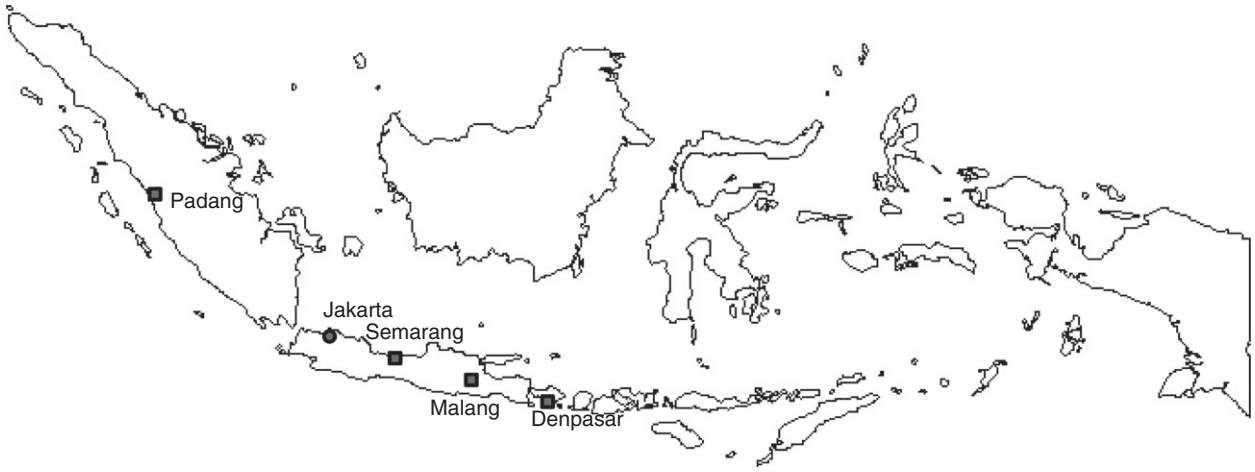

Figure 1 Map of Indonesia depicting the four cities involved in this study (squares). The capital city Jakarta is also indicated (circle).

concentration was measured spectrophotometrically, and the samples were stored at −20 °C. PCRs to detect *mecA* and PVL (*lukF-PV and lukS-PV*) genes were performed as previously described [15, 16].

SCC*mec* typing

Multiplex PCR to characterise the SCC*mec* of *S. aureus* harbouring the *mecA* gene was conducted as previously described [17].

Raman spectroscopy

The clonal relationship among MRSA and PVL-positive MSSA isolates was analysed using Raman spectroscopy (SpectraCell*RA* Bacterial Strain Analyzer, RiverD international BV, Rotterdam, The Netherlands), as previously described [18]; [19]; [9, 20].

Raman spectral analysis was performed using SpectraCell*RA* software version 1.9.0.13444:24 (RiverD international). The squared Pearson correlation coefficient (R^2) determined the similarity of the sample spectra and the known R^2 distribution of the identical and unrelated strains. In five different measurements, we included the ATCC (American Type Collection Culture) 43300 strain as a reproducibility control. A two-dimensional plot was created to compare the similarity of multiple isolates; the similarity of two isolates was presented by a colour scale. The clonal relatedness was determined by setting the similarity threshold and cut-off value as previously described [9].

Multilocus sequence typing (MLST)

Fourteen *S. aureus* isolates were further analysed by MLST to allow international comparison [21]. From each Raman cluster with at least two isolates, one to three isolates (depending on the cluster size) were selected for MLST. For larger clusters, we selected isolates from both the centre and the fringe of the Raman cluster. The MLST sequence type was assigned through the MLST website (http://www.MLST.net to PubMLST.org).

Statistical analysis

Chi-square (χ^2) test was applied to compare the antibiotic resistance rates between MRSA and MSSA isolates as well as PVL-positive MSSA and PVL-negative MSSA isolates using statistical software packages SPSS version 16.0 (SPSS Inc., Chicago, IL). This analysis was only performed in case resistance was present in 10 or more isolates per group. A *P* value less than 0.05 was considered significant.

Results

Prevalence of MRSA and PVL-positive MSSA

A total of 259 *S. aureus* were collected consecutively by clinical culture: Denpasar (Sanglah Hospital), 40 strains; Malang (Dr. Saiful Anwar Hospital), 77 strains; Padang (Dr. M. Djamil Hospital), 79 strains; Semarang (Dr.

Table 1 Origin (ward/outpatient clinic and specimen) of *S. aureus* isolates collected in the study

	No. of isolates (%)			
Ward/outpatient clinic	Blood (*n* = 17)	Pus (*n* = 144)	Sputum (*n* = 37)	Other§ (*n* = 61)
Surgery	0	38 (26.4)[a,i]	1 (2.7)	0
Internal medicine	0	21 (14.6)[b,j]	13 (35.1)[e]	2 (3.3)[q]
Paediatric	0	5 (3.5)[k]	0	5 (8.2)[r]
Emergency	1 (5.9)	6 (4.2)[l]	0	0
ICU	5 (29.4)[g]	2 (1.4)[m]	5 (13.5)	0
Mixed ward*	11 (64.7)[h]	42 (29.2)[c,n]	3 (8.1)	11 (18.0)[f]
Outpatient clinic†	0	30 (20.8)[d,o]	15 (40.5)[p]	28 (45.9)[s]
Unidentified ward‡	0	0	0	15 (24.6)
MRSA (*n* = 17)	0	15/144 (10.4)	1/37 (2.7)	1/61 (1.6)
PVL-positive MSSA (*n* = 48)	4/17 (23.5)	38/144 (26.4)	2/37 (5.4)	4/61 (6.6)

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *S. aureus*; ICU, intensive care unit.
*Occupied by adults only (no children) separated between male and female for mixed medical cases (i.e. internal medicine, neurology, surgery).
†Consisted of outpatient clinics of : surgery, ear nose and throat, dentistry, internal medicine, pulmonology and dermatology.
‡Missing ward identity.
§Sterile sites (amnion fluid, joint fluid, pleural fluid), eye secretion, ear secretion, nose, throat, urine, tissue and vaginal discharge.
MRSA (*n* = 17): [a]5 (29.4%), [b]3 (17.6%), [c]3 (17.6%), [d]3 (17.6%), [e]1 (5.9%), [f]2 (11.8%). PVL-positive MSSA (n=48): [g]1 (2.1%), [h]3 (6.3%), [i]13 (34.2%), [j]4 (8.3%), [k]2 (4.2%), [l]1 (2.1%), [m]1 (2.1%), [n]8 (16.7%), [o]8 (16.7%), [p]2 (4.2%), [q]1 (2.1%), [r]1 (2.1%), [s]3 (6.3%).

Kariadi Hospital), 63 strains. Table 1 shows the origin of *S. aureus* isolates, that is ward or outpatient clinic and type of specimen. The majority of the *S. aureus* strains were found in pus (144/259 [55.6%]), followed by other sites of infection such as sterile sites (amnion fluid, joint fluid, pleural fluid), eye secretion, ear secretion, nose, throat, tissue, urine and vaginal discharge (61/259 [23.6%]), sputum (37/259 [14.3%]) and blood (17/259 [6.6%]). The majority of *S. aureus* from pus were obtained from mixed wards (29.2%) and surgery wards (26.4%). Overall, the prevalence of MRSA among clinical *S. aureus* isolates was 6.6%, whereas the prevalence of PVL-positive MSSA was 18.5%. The highest prevalence of both MRSA (8.9%) and PVL-positive MSSA (29.1%) was found in Padang. None of the MRSA isolates was PVL-positive (Table 2). Fifteen of 17 (88.2%) of the MRSA strains and 38/48 (79.2%) of the PVL-positive MSSA strains were isolated from pus, and of these, five MRSA (5/15, 33.3%) and 13 PVL-positive MSSA (13/38, 34.2%) were from surgery wards. We did not find any MRSA isolates among the blood culture isolates.

Table 2 Prevalence and sources of MRSA and PVL-positive MSSA among clinical specimens in four Indonesian hospitals

City	Clinical specimen	Number of strains (%)		
		S. aureus	MRSA	PVL-pos MSSA
Denpasar	Total	40	1 (2.5)	7 (17.5)
	Blood	5 (12.5)	0	1 (14.3)
	Pus	14 (35.0)	0	5 (71.4)
	Sputum	2 (5.0)	0	0
	Other	19 (47.5)	1* (100)	1* (14.3)
Malang	Total	77	4 (5.2)	12 (15.6)
	Blood	0	0	0
	Pus	33 (42.3)	3 (75.0)	9 (75.0)
	Sputum	24 (30.8)	1 (25.0)	1 (8.3)
	Other	20 (25.9)	0	2† (16.7)
Padang	Total	79	7 (8.9)	23 (29.1)
	Blood	1 (1.3)	0	1 (4.3)
	Pus	69 (87.3)	7 (100.0)	21 (91.4)
	Sputum	5 (6.3)	0	0
	Other	4 (5.1)	0	1‡ (4.3)
Semarang	Total	63	5 (7.9)	6 (9.5)
	Blood	11 (17.5)	0	2 (33.3)
	Pus	28 (44.4)	5 (100.0)	3 (50.0)
	Sputum	6 (9.5)	0	1 (16.7)
	Other	18 (28.6)	0	0
All cities	Total	259	17 (6.6)	48 (18.5)
	Blood	17 (6.6)	0	4 (8.3)
	Pus	144 (55.6)	15 (88.2)	38 (79.2)
	Sputum	37 (14.3)	1 (5.9)	2 (4.2)
	Other	61 (23.6)	1 (5.9)	4 (8.3)

MRSA, methicillin-resistant *Staphylococcus aureus;* MSSA, methicillin-sensitive *Staphylococcus aureus;* PVL, Panton–Valentine leukocidin; Others, body fluid (amnion fluid, joint fluid, pleural fluid), eye secretion, ear secretion, nose, throat, urine, tissue, vaginal discharge.
*Urine.
†Amnion fluid (1 isolate), pleural fluid (1 isolate).
‡Pleural fluid.

Antibiotic susceptibility testing

Table 3 shows the resistance rates of the *S. aureus* isolates. The majority of *S. aureus* isolates were resistant to penicillin (219/259, 84.6%). The MRSA isolates were significantly more resistant to the aminoglycosides (gentamicin and tobramycin), the fluoroquinolones (ciprofloxacin and levofloxacin) and tetracycline. For the other tested antibiotics, this comparison could not be performed because of statistical limitations. The overall resistance rate of MSSA isolates to tetracycline, either PVL-positive or PVL-negative MSSA, was also high (104/242, 43.0%). Of the PVL-negative MSSA, 9.5% were susceptible to all tested antibiotics, 31.4% were resistant to penicillin only, 29.8% were resistant to penicillin and tetracycline and 14.5% were resistant to more than two classes of antibiotics.

SCC*mec* typing

The SCC*mec* typing was performed for all MRSA isolates (n = 17). SCC*mec* type III was predominant in this study, as it was found in isolates from Denpasar (n = 1), Malang (n = 4), Padang (n = 7) and Semarang (n = 4). We found only one isolate, cultured in Semarang, with SCC*mec* type V. The SCC*mec* type V isolate was resistant to aminoglycosides (gentamicin and tobramycin), fluoroquinolones (ciprofloxacin, levofloxacin and moxifloxacin), trimethoprim–sulfamethoxazole and tetracycline.

Raman spectroscopy

We performed Raman spectroscopy of the 17 MRSA and the 48 PVL-positive MSSA isolates. Raman spectroscopic analysis generated 48 Raman types (RTs) (Figure 2). The results of the quality control as performed with the ATCC strain in five different measurements are shown in Figure 2 as well. RT10 was the most frequently found and included 6 *S. aureus* isolates (Malang: MRSA-SCC*mec* type III, 1 isolate; Padang: MRSA-SCC*mec* type III, 1 isolate and PVL-positive MSSA, 1 isolate; Semarang: MRSA-SCC*mec* type III, 1 isolate and MRSA-SCC*mec* type V, 1 isolate; Denpasar: PVL-positive MSSA, 1 isolate). The second most common type was RT18, which consisted of 5 PVL-positive MSSA isolates from Padang.

Table 3 Antibiotic resistances of *S. aureus* isolated from clinical specimens in four Indonesian tertiary care hospitals

	Resistance rate (%)			Resistance rate (%)		
Antibiotics*	MRSA (n = 17)	MSSA (n = 242)	P^1	PVL (+) MSSA (n = 48)	PVL (−) MSSA (n = 194)	P^2
PEN	17 (100)	202 (83.5)	0.083	38 (79.2)	164 (84.5)	0.388
CLI	5 (29.4)	9 (3.7)	ND	2 (4.2)	7 (3.6)	ND
ERY	6 (35.3)	13 (5.4)	ND	3 (6.2)	10 (5.2)	ND
GEN	17 (100)	14 (5.8)	<0.001	0	14 (7.2)	ND
TOB	17 (100)	12 (5.0)	<0.001	0	12 (6.2)	ND
CIP	17 (100)	28 (11.6)	<0.001	3 (6.2)	25 (12.9)	ND
LVX	17 (100)	11 (4.5)	<0.001	1 (2.1)	10 (5.2)	ND
MXF	10 (58.8)	5 (2.1)	ND	0	5 (2.6)	ND
SXT	5 (29.4)	2 (0.8)	ND	0	2 (1.0)	ND
FOF	1 (5.9)	6 (2.5)	ND	0	6 (3.1)	ND
FUA	0	23 (9.5)	ND	7 (14.6)	16 (8.2)	ND
LZD	0	0	–	0	0	–
MUP	0	0	–	0	0	–
RIF	7 (41.2)	15 (6.2)	ND	2 (4.2)	13 (6.7)	ND
TET	17 (100)	104 (43)	<0.001	20 (41.7)	84 (43.3)	0.872
TEC	0	0	–	0	0	–
VAN	0	0	–	0	0	–

All PVL-positive strains were *mecA* negative; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; PVL, Panton–Valentine leukocidin; P^1, significance value between MRSA and MSSA; P^2, significance value between PVL (+) MSSA and PVL (−) MSSA; ND, not determined because of low number of resistance case (less than 10).
*Abbreviations of antibiotics tested: PEN, penicillin; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; LVX, levofloxacin; MXF, moxifloxacin; SXT, trimethoprim–sulfamethoxazole; FOF, fosfomycin; FUA, fucidic acid; LZD, linezolid; MUP, mupirocin; RIF, rifampicin; TET, tetracycline; TEC, teicoplanin; VAN,vancomycin.

MLST

Similar to our previous study [9], we randomly selected 14 strains of *S. aureus* based on Raman spectroscopy analysis for MLST. MLST was performed for 3 MRSA isolates representing RT10 (1 isolate) and RT16 (2 isolates) as well as 11 PVL-positive MSSA isolates representing RT2 (1 isolate), RT4 (2 isolates), RT5 (2 isolate), RT6 (1 isolate), RT18 (3 isolates) and RT20 (2 isolates) (Table 4). The RT10 isolate (MRSA-SCC*mec* type V from Semarang) belonged to ST672, whereas the RT16 MRSA isolates (with SCC*mec* type III) were assigned to ST239. In addition, ST1 was presented by one RT2 and one RT4 PVL-positive MSSA. RT5, RT6 and RT18 PVL-positive MSSA were assigned to ST121. We found different sequence types within the same Raman type of PVL-positive MSSA: (i) RT4 isolates belonged to both ST1 and ST188, (ii) RT18 isolates were assigned to both ST121 (allele profile 6-5-6-2-7-14-5) and a new sequence type, (iii) RT20 isolates belonged to ST2696 (allele profile 6-5-6-6-7-14-5) and a new sequence type. Interestingly, the allele profiles of both new sequence types detected in RT18 and RT20 were the same, 158-5-6-2-7-199-5.

Discussion

This study presents the first multicentre survey of clinical *S. aureus* isolates from Indonesian hospitals. Similar to our previous study [9], we found geographic variation in the prevalence of MRSA among tertiary hospitals in Indonesia. Nevertheless, comparability of the prevalences is limited because of the different study period among hospitals involved. Overall, the prevalence of MRSA detected in this study was 6.6%, which is considered lower than most other countries, but higher than countries in northern Europe [4, 22–24]; [25, 26]. Among *S. aureus* from blood cultures, we did not find any MRSA; however, the number of isolates from blood was small (n = 17). In the European Antimicrobial Resistance Surveillance Network (EARS-Net), the country would be given the colour 'green'. Among *S. aureus* from pus, the prevalence of MRSA was 10.4% (15/144 isolates). The prevalence of PVL-positive MSSA in Indonesian hospitals was high (18.5%), which is consistent with previous studies from Indonesia, but the phenomenon has also been reported recently from other countries [9, 11, 13, 27, 28]. In

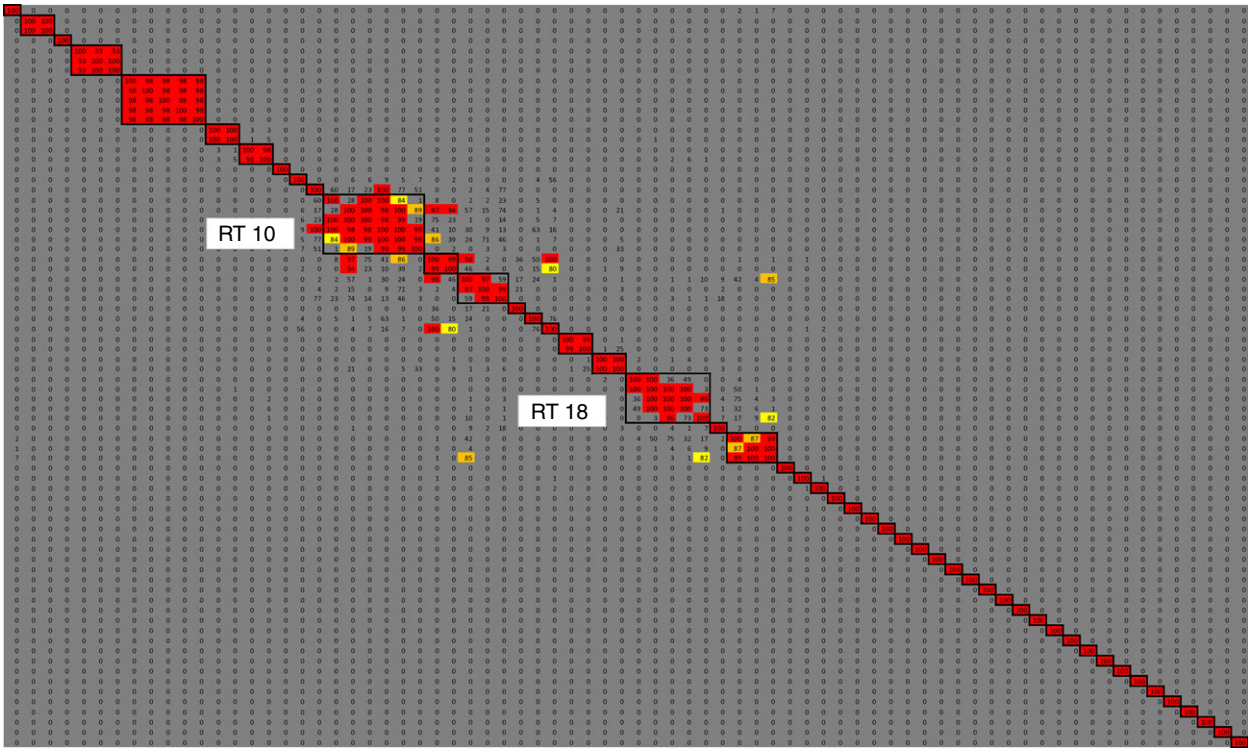

Figure 2 Clustering of MRSA and PVL-positive MSSA isolates from clinical isolates in four hospitals in Indonesia as determined by Raman spectroscopy (RT: Raman type). Note: Figure displays a correlation matrix used to analyze Raman spectral relatedness between isolates. Red clusters indicate isolates that are indistinguishable based on the cut-off value. The grey areas indicate isolates that are non-related based on the similarity threshold. The potentially related isolates are shown by yellow areas to orange areas gradually.

such situations, the emergence of PVL-positive MRSA is a possibility in case the *mecA* gene is transferred to the PVL-positive MSSA strains [9]. Both MRSA and PVL-positive MSSA were most frequently isolated from pus, especially from patients in the surgery ward, suggesting the occurrence of surgical site infections caused by MRSA and PVL-positive MSSA.

Compared to the MSSA strains, the MRSA isolates were resistant to significantly more classes of antibiotics. More than 95% of MRSA strains were resistant to aminoglycosides (gentamicin, tobramycin), fluoroquinolones (ciprofloxacin, levofloxacin) and tetracycline, indicating that these antibiotics were often used as antibiotic therapy. Unfortunately, no clinical data were available to confirm that the patients had been treated with any of these antibiotics before the culture was obtained. Although the PVL-negative MSSA were mostly resistant to penicillin only, they have broadened their resistance profile, now including penicillin and tetracycline as shown before [29]. The addition of tetracycline resistance may be due to the acquisition of the *tet*K and *tet*M genes via plasmid transfer [30].

SCC*mec* typing showed that SCC*mec* type III was predominant among clinical isolates of MRSA, suggesting the presence of HA-MRSA in Indonesian hospitals. In addition, similar to our previous study [9], we found one PVL-negative, SCC*mec* type V MRSA, representing a CA-MRSA, among the isolates from Dr. Kariadi Hospital, Semarang, which was resistant to penicillin, fluoroquinolones, tetracycline and trimethoprim–sulfamethoxazole. However, the strain was also resistant to gentamicin, indicating that the CA-MRSA strains may have penetrated into the hospital setting, and even caused hospital-onset or healthcare-associated infections in Indonesia.

All MRSA and PVL-positive MSSA were analysed using Raman spectroscopy, and a subset of isolates using MLST. Two MRSA isolates from Padang (P63 and P37) were identified as ST239-MRSA-SCC*mec* type III and belonged to RT16. Both MRSA isolates were obtained from wound cultures of patients admitted to the internal medicine ward, which is suggestive of cross-transmission. The ST239-MRSA-SCC*mec* type III clone is a single-locus variant of USA300 [21] and is common in Asian coun-

Table 4 Clonality of MRSA and PVL-positive MSSA clinical isolates in four hospitals in Indonesia

Isolate number		City	Raman type	*SCCmec* type	ST	Specimen	Ward
P47		Padang	2	*	1	Pus	Surgery
P66		Padang	4	*	1	Pus	Surgery
P24		Padang	4	*	188	Pleural fluid	Outpatient clinic
P109	a	Padang	5	*	121	Pus	Surgery
P116	a	Padang	5	*	121	Pus	Surgery
5196		Denpasar	6	*	121	Pus	Unidentified
16		Semarang	10	V	672	Tissue	Mixed
P63	b	Padang	16	III	239	Pus	Internal medicine
P37	b	Padang	16	III	239	Pus	Internal medicine
P101	c	Padang	18	*	121	Pus	Surgery
P119	c	Padang	18	*	121	Pus	Surgery
P111		Padang	18	*	New ST	Blood	Mixed
P107		Padang	20	*	2696	Pus	Ear, nose, throat
P114		Padang	20	*	New ST	Pus	Internal medicine

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*.
*PVL-positive MSSA; ST, sequence type; a, clone 'a' showing the concordance of RT5, PVL-positive MSSA and ST121 on P109 and P116; b, clone 'b' showing the concordance of RT16, SCC*mec* type III and ST239 on P63 and 37; c, clone 'c' showing the concordance of RT18, PVL-positive MSSA and ST121 on P101 and P119.

tries [31]. In our previous study, we also identified the carriage of ST239-MRSA-SCC*mec* type III among discharged patients, particularly in Dr. Kariadi Hospital, Semarang [9]. Interestingly, the MRSA isolate from Semarang that clustered in RT10 was identified as ST672-SCC*mec* type V that was also reported as emerging in India [32]. Two PVL-positive MSSA from Padang (P101 and P119) were assigned to the second largest Raman cluster, RT18, which corresponded to ST121. Both identical PVL-positive MSSA were isolated from wound cultures of surgery patients; hence, nosocomial transmission may have occured. The other two PVL-positive MSSA from Padang (P109 and P116) were assigned to the smaller Raman cluster, RT5, which also corresponded to ST121. Both identical PVL-positive MSSA were isolated from wound cultures of surgery patients indicating nosocomial transmission may also have occurred. Thus, the ST121 PVL-positive MSSA obtained from wound culture of surgery patients in Padang expressed two different phenotypic Raman clusters. New sequence types were identified in both RT18 and RT20 with an allele profile that is similar to ST121 featuring *arcC* and *tpi* variants.

The same Raman type could be assigned to different sequence types that might belong to a single clonal complex. This situation was also encountered in RT4 isolates that were assigned to both ST1 and its double locus variant ST188, both members of clonal complex 1 according to BURST analysis [33]. These discrepancies are not unexpected given the different technical background of the two typing methods. In previous reports, we [20] and others [34] found Raman typing to produce results that were >95% concordant with PFGE, the gold standard for assigning staphylococci to genetic clones. Discrepancies remained, however, albeit at a low rate. In this study, we found two strains with the same RT (4) to have two different STs; however, the two strains were genetically closely related as one (ST188) is a double locus variant of the other (ST1). Apparently, such limited variation in the allelic profile of a few housekeeping genes does not necessarily translate into differences in the strain's Raman spectra. However, for a valid comparison of the two methods, a much larger collection has to be analysed.

Our study has certain limitations. First, it was based on a convenience sample of strains isolated from routine clinical cultures and additional clinical information including some potential risk factors for acquisition of MRSA, and the time of culture related to the admission date was not collected. Therefore, a true distinction between community-acquired and healthcare-associated infection was not possible. Second, as four hospitals from three islands participated, our data should not be considered representative for the whole country, but the data may be used as a point reference [35]. However, a national surveillance system should be set-up in which MRSA and PVL prevalence, epidemic clonal shifts, clone emergence and transmission between community and healthcare settings can be monitored. This would be challenging given the extent of the country and the still limited availability of clinical microbiology services in many

areas. Finally, Raman spectroscopy and MLST were not performed for all *S. aureus* strains; consequently, we could not provide the clonal relatedness of PVL-negative MSSA strains.

In summary, infection with HA-MRSA occurred in Indonesian hospitals, but with geographic variation. The possible penetration of CA-MRSA in Dr. Kariadi Hospital, Semarang, is of concern. In this situation, a bundle of intervention measures to reduce the prevalence of MRSA in Indonesian hospitals is highly recommended.

Acknowledgements

We thank the deans of the Faculty of Medicine, Brawijaya University, Malang; the Faculty of Medicine, Diponegoro University, Semarang; Faculty of Medicine, Andalas University, Padang, and the Faculty of Medicine, Udayana University, Denpasar, the directors of Dr. Saiful Anwar Hospital, Malang; Dr. Kariadi Hospital, Semarang; Dr. M. Djamil Hospital, Padang; and Sanglah Hospital, Denpasar, who facilitated our work in these teaching hospitals. We also thank all staff members who have been involved in the isolation of bacteria. The excellent technical assistance of Michelle de Regt is gratefully acknowledged. This study was financially supported by the Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, The Netherlands.

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