

Epidemiology of *Staphylococcus aureus* Harboring the *mecA* or Panton-Valentine Leukocidin Genes in Hospitals in Java and Bali, Indonesia

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Abstract. Data of *Staphylococcus aureus* carriage in Indonesian hospitals are scarce. Therefore, the epidemiology of *S. aureus* among surgery patients in three academic hospitals in Indonesia was studied. In total, 366 of 1,502 (24.4%) patients carried *S. aureus*. The methicillin-resistant *S. aureus* (MRSA) carriage rate was 4.3%, whereas 1.5% of the patients carried Panton-Valentine leukocidin (PVL)-positive methicillin-sensitive *S. aureus* (MSSA). Semarang and Malang city (odds ratio [OR] 9.4 and OR 9.0), being male (OR 2.4), hospitalization for more than 5 days (OR 11.708), and antibiotic therapy during hospitalization (OR 2.6) were independent determinants for MRSA carriage, whereas prior hospitalization (OR 2.5) was the only one risk factor for PVL-positive MSSA carriage. Typing of MRSA strains by Raman spectroscopy showed three large clusters assigned type 21, 24, and 38, all corresponding to ST239-MRSA-SCC*mec* type III. In conclusion, MRSA and PVL-positive MSSA are present among patients in surgical wards in Indonesian academic hospitals.

INTRODUCTION

Carriage of *Staphylococcus aureus* is a risk factor for subsequent infection in various settings.¹ Antibiotic treatments of staphylococcal infections has become more challenging over the past decades with the emergence of methicillin-resistant *S. aureus* (MRSA). Nowadays, MRSA is a persistent problem in many healthcare settings around the world.^{2–8} Undetected MRSA-positive patients serve as reservoirs, and roommates of such patients and health care personnel are at significant risk of becoming colonized.^{9–13} In addition, MRSA-positive persons contaminate the hospital's environment turning it into a reservoir for other patients.^{14–17} After discharge from hospital, MRSA-positive patients may transmit their strain to their household members.¹²

Knowledge of the prevalence of MRSA colonization and the frequency of transmission is vital for the implementation of MRSA infection control measures in hospitals.¹⁸ However, little is known about the current epidemiology of MRSA in health care settings in Indonesia. Severin and others reported that among 98 *S. aureus* isolates from 999 patients screened at discharge in 2001–2002 in two cities on the island of Java (Semarang and Surabaya), only two strains were identified as MRSA (carriage rate 0.2%).^{19,20} Among 263 isolates from healthy persons, patients in the community, and patients at the time of admission to the hospital, not a single MRSA was found. However, an unexpectedly high prevalence of Panton-Valentine leukocidin (PVL) genes among methicillin-sensitive *S. aureus* (MSSA) was documented among both patients and healthy individuals. This was of concern, because PVL-positive strains are associated with skin infections and severe necrotizing pneumonia.²¹ In this study, we aimed to gain

more insight in the more recent epidemiology of MRSA and *S. aureus* harboring the *pvl* genes in the Indonesian hospital setting to develop targeted preventive measures. Similar to the study described by Lestari and others,¹⁹ we performed a multicenter study focused on patients at discharge. Molecular characterization of *S. aureus* isolates was carried out and possible risk factors for colonization among these patients were analyzed.

MATERIALS AND METHODS

Setting. Three referral teaching hospitals participated in the study: Sanglah hospital in Denpasar (Bali; 704 beds), Dr. Kariadi hospital in Semarang (Central Java; 779 beds), and Dr. Saiful Anwar hospital in Malang (East Java; 810 beds).

Design. Surgery patients were screened for MRSA carriage at the time of discharge from the hospital. All surgery patients were eligible for inclusion. However, surgery patients discharged within 48 hours after admission were excluded. In case a patient was found MRSA-positive (i.e., the “index case”), additional screening to detect secondary cases was carried out as follows: all patients that had been sharing the room with such an MRSA-positive patient were screened within 1 week and all attending health care workers in that ward. In addition, screening of the innate hospital environment where index cases had been admitted was conducted. In case more than one index case was found within a week, the screening for secondary cases in the hospital was conducted once that referred to those index cases. Four to 6 weeks after discharge of index cases, the household members and household environment of the cases were screened. Index cases living in the rural area or other cities than Denpasar and Semarang were excluded for household members and household environment screening. The additional screening of roommates, health care workers, environment, and household members was not conducted in Malang. The study was performed from July 2007 to December 2008 in

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Denpasar, from February 2008 to October 2009 in Semarang, and from January to March 2011 in Malang. The study was approved by the medical ethics committee of the three academic hospitals.

Screening for *S. aureus* carriage. Samples were obtained using sterile dry cotton swabs (Deltalab, Rubí, Spain) after patients had given informed consent. Cultures of anterior nares, throat, and open skin lesion (if present) were taken from discharge patients and contact patients. Cultures of anterior nares and throat were taken from health care workers and household members. The hospital environment was screened by taking 10 samples minimal, from instruments (stethoscope, blood pressure cuff, and thermometer) and surfaces (bedrails, door handles, telephone handles, dust, sink faucet, and floor). Door handles, kitchen sink and appliances, bed, chairs, table tops, floor, and dust were taken from the household environment of index cases.

Bacterial isolates. Swabs were directly inoculated into 5 mL phenol red mannitol broth (BBL, Le Pont de Claix, France) for overnight incubation at 37°C and then sub-cultured onto *Staphylococcus aureus* and MRSA Chromagar medium (ITK Diagnostics, Uithoorn, The Netherlands) for 24–48 hours incubation at 37°C. Typical colonies of *S. aureus* and MRSA were stored into trypticase soy agar. Confirmation of *S. aureus* was performed by Slidex Staph Plus (bioMérieux, Marcy l'Etoile, France) and the Vitek2 system (bioMérieux).

DNA extraction and detection of *mecA* and *pvl* genes. Bacterial DNA was extracted using a MagNa Pure LC DNA system (DNA isolation kit III; Roche Molecular Biochemicals, Mannheim, Germany).²² The DNA concentration was measured spectrophotometrically and samples were stored at –20°C. Detection of *mecA* and *pvl* genes were performed by polymerase chain reaction (PCR) as previously described.^{21,23}

SCC*mec* typing. The staphylococcal cassette chromosome *mec* (SCC*mec*) of *S. aureus* isolates containing the *mecA* gene was characterized using a multiplex PCR that enables the identification of SCC*mec* types I to VI.²⁴ Positive and negative control strains were included in each PCR run.

Raman spectroscopy. We performed Raman spectroscopy (SpectraCellRA Bacterial Strain Analyzer, RiverD international BV, Rotterdam, The Netherlands) to assess clonal relationship among MRSA and PVL-positive MSSA isolates, as described previously.^{25,26} The American Type Culture Collection (ATCC) strains were included on each measurement day as a control for reproducibility. The analysis of spectra was performed using SpectraCellRA software version 1.9.0.13444:24 (RiverD international). In the SpectraCellRA software, the similarity of two spectra is calculated from the squared Pearson correlation coefficient (R^2) of the sample spectra and the known R^2 - distributions of identical and unrelated isolates.²⁷ For comparing multiple isolates, a similarity or two-dimensional plot is created where the similarity between each combination of isolates is represented as a color-coded square. In this plot, the similarity threshold was set at a 1% false positive rate, which means that for 1% of all indistinguishable isolates a misidentification as unrelated is allowed. Two isolates with a similarity below this value were considered unrelated and designated different Raman types (RTs). The cut-off value was set at a 3% false negative rate, therefore a misidentification as indistinguishable is allowed for 3% of all unrelated strains. Two isolates with a similarity above this value were considered indistinguish-

able and assigned the same RT. In case of a similarity value between both borders the isolates were considered potentially related.

MLST. A random selection of 10 *S. aureus* isolates from the largest clusters generated by Raman spectroscopy were further analyzed by multilocus sequence typing (MLST).²⁸ The MLST sequence type was assigned through the MLST website (<http://www.mlst.net>).

Risk factor analysis. Socio-demographic data, date of admission, date of discharge, ward that discharged the patient, prior hospitalization, intensive care unit admission, surgery procedure, and antibiotic therapy during admission were included in the risk factor analysis. These data were collected from patient records and by interviewing the patients at the moment of discharge using a structured questionnaire. Data obtained from the questionnaires were recorded in a case record form (crf) program. Data were analyzed using statistical software packages SPSS version 16.0 (SPSS Inc., Chicago, IL). A P value < 0.05 was considered as significant.

Definitions. Index cases were defined as patients from whom MRSA was found from any site by screening on discharge; contact patients were patients having shared the room with an index cases; secondary cases were patients, health care workers, hospital environment, household members, and household environment from whom or which MRSA was found with a link in time and location to an index case, and the identical MRSA isolates were determined by Raman spectroscopy.

RESULTS

Carriage rate of MRSA and PVL-positive MSSA among discharge patients. We screened 488, 914, and 100 discharge patients in Sanglah hospital in Denpasar, Dr. Kariadi hospital in Semarang, and Dr. Saiful Anwar hospital in Malang, respectively (Table 1). The carriage rate of *S. aureus* among these was 9.4% (46 of 488) in Denpasar, 32.4% (296 of 914) in Semarang, and 24.0% (24 of 100) in Malang. Overall, the carriage rate of MRSA among discharge patients was 4.3% (64 of 1,502). *Staphylococcus aureus* was less frequently found in patients from Denpasar than in patients from Semarang and Malang, and the carriage rate of MRSA ($P < 0.001$). The MRSA isolates were found in cultures of nares ($N = 50$ isolates; 54.4%), throat ($N = 30$; 32.6%), and open skin lesion ($N = 12$; 13.0%). All MRSA isolates were PVL-negative. The PVL-positive MSSA was found among discharge patients in Semarang and Malang, but not in Denpasar (Table 1).

Carriage rate of MRSA and PVL-positive MSSA among contact patients, health care workers, hospital environment, household members, and household environment. Secondary cases were only found in Semarang: contact patients, 24 of 200 (12.0%) from 54 index cases; hospital environment, 1 of 132 (0.8%) from 3 index cases; and household member, 1 of 10 (10.0%) from 1 index case. The MRSA was not found among health care workers and household environment. The PVL-positive MSSA was only detected in Semarang, which was 2.0% of contact patients and 10.3% among health care workers.

Risk factor analysis for carriage of MRSA and PVL-positive MSSA among discharge patients. Multivariate analysis showed that more than 5 days hospitalization was independently associated with carriage of MRSA (odds ratio [OR]

TABLE 1
Number of subjects and isolates analyzed for carriage rate of *Staphylococcus aureus*, MRSA, and PVL-positive MSSA

City	Group	No. of subjects screened	No. of cultures (environment)	No. of <i>S. aureus</i> isolates	No. of MRSA isolates (%)	No. of PVL-positive MSSA isolates (%)	<i>S. aureus</i> carriage rate (%)	MRSA carriage rate (%)	PVL-positive MSSA carriage rate (%)
Denpasar	Patients at discharge	488		56	4 (7.1)	0	46/488 (9.4)	2/488 (0.4)	0
	Contact patients	21		2	0	0	2/21 (9.5)	0	0
	Health care workers	58		3	0	0	3/58 (5.2)	0	0
	Hospital environment		50	0	0	0	0	0	0
	Household members	12		0	0	0	0	0	0
Semarang	Household environment		20	0	0	0	0	0	0
	Patients at discharge	914		423	76 (18.0)	24 (5.7)	296/914 (32.4)	54/914 (5.9)	21/914 (2.3)
	Contact patients	200		90	33 (26.7)	4 (4.4)	73/200 (36.5)	24/200 (12.0)	4/200 (2.0)
	Health care workers	58		60	0	10 (16.7)	39/58 (67.0)	0	6/58 (10.3)
	Hospital environment		132	13	1 (7.7)	0	13/132 (9.8)	1/132 (0.7)	0
Malang	Household members	10		4	2 (50.0)	0	2/10 (20.0)	1/10 (10.0)	0
	Household environment		15	0	0	0	0	0	0
	Patients at discharge	100		30	12 (40.0)	1 (3.3)	24/100 (24.0)	8/100 (8.0)	1/100 (1.0)
Total	Patients at discharge	1,502		509	92 (18.1)	25 (4.9)	366/1,502 (24.4)	64/1,502 (4.3)	22/1,502 (1.5)
	Contact patients	221		92	33 (35.9)	4 (4.3)	75/221 (33.9)	24/221 (10.9)	4/221 (1.8)
	Health care workers	116		63	0	10 (15.9)	42/116 (36.2)	0	6/116 (5.2)
	Hospital environment		182	13	1 (7.7)	0	13/182 (7.1)	1/182 (0.5)	0
	Household members	22		4	2 (50.0)	0	2/22 (9.1)	1/22 (4.5)	0
	Household environment		35	0	0	0	0	0	

*The denominator is number of *S. aureus* isolates.

MRSA = methicillin-resistant *S. aureus*; PVL = Pantone-Valentine leukocidin; MSSA = methicillin-sensitive *S. aureus*.

11.708; 95% confidence interval [CI] 1.587–86.376) (Table 2). Other identified risk factors were being in the hospital in Semarang (OR 9.404; 95% CI 2.239–39.501) or Malang city (OR 9.003; 95% CI 1.752–46.271), being male (OR 2.374; 95% CI 1.127–5.000), and antibiotic therapy during hospitalization (OR 2.621; 95% CI 1.014–6.773). The only factor that was associated with carriage of PVL-positive MSSA was prior hospitalization (OR 2.466; 95% CI 1.028–5.916).

SCCmec typing. We performed SCCmec typing of 127 MRSA isolates obtained from discharge patients, contact patients,

TABLE 2

Risk factors for carriage of MRSA and PVL-positive MSSA among discharge patients in three Indonesian hospitals: univariate and multivariate analysis

Risk factors	Univariate		Multivariate (backward LR)			Univariate		Multivariate (backward LR)		
	MRSA (+) (n = 55)	MRSA (-) (n = 1,351)	OR	95% CI	p	PVL (+) (n = 21)	PVL (-) (n = 1,385)	OR	95% CI	P
City										NS
Denpasar	2 (3.6)	485 (35.9)	1			0	487 (35.2)			
Semarang	47 (85.5)	786 (58.2)	9.404	2.239–39.501	0.002	20 (95.2)	813 (58.7)			
Malang	6 (10.9)	80 (5.9)	9.003	1.752–46.271	0.009	1 (4.7)	86 (6.2)			
Gender*										
Male	46 (83.6)	875 (64.8)	2.374	1.127–5.000	0.023	12 (57.1)	909 (65.6)			
Female	9 (16.4)	476 (35.2)	1			9 (42.9)	476 (34.4)			
Age*†										
≤ 18 yo	11 (20)	279 (20.7)				3 (14.3)	287 (20.7)			
19–59 yo	36 (65.5)	900 (66.6)				16 (76.2)	920 (66.4)			
≥ 60 yo	8 (14.5)	172 (12.7)				2 (9.5)	178 (12.9)			
Length of stay										NS
≤ 5 days	1 (1.8)	394 (29.2)	1			2 (9.5)	393 (28.4)			
> 5 days	54 (98.2)	957 (70.8)	11.708	1.587–86.376	0.016	19 (90.5)	992 (71.6)			
ICU admission*†										
Yes	3 (5.5)	113 (8.4)				1 (4.8)	115 (8.3)			
No	52 (94.5)	1238 (91.6)				20 (95.2)	1270 (91.7)			
Surgery†										NS
Yes	43 (78.2)	961 (71.1)				9 (42.9)	995 (71.8)			
No	12 (21.8)	390 (28.9)				12 (57.1)	390 (28.2)			
Prior hospitalization†										
Yes	13 (23.6)	336 (24.9)				10 (47.6)	339 (24.5)	2.466	1.028–5.916	0.043
No	42 (76.4)	1015 (75.1)				11 (52.4)	1046 (75.5)	1		
Antibiotic therapy during hospitalization										NS
Yes	50 (90.9)	1116 (82.6)	2.621	1.014–6.773	0.047	13 (61.9)	1153 (83.2)			
No	5 (9.1)	235 (17.4)	1			8 (38.1)	232 (16.8)			

Patients without complete data were not analyzed statistically.

*Univariate analysis of PVL-positive MSSA was not significant ($P > 0.2$).

†Univariate analysis of MRSA carriage was not significant ($P > 0.2$).

MRSA = methicillin-resistant *S. aureus*; PVL = Pantone-Valentine leukocidin; MSSA = methicillin-sensitive *S. aureus*; LR = logistic regression; OR = odds ratio; CI = confidence interval; ICU = intensive care unit.

TABLE 3
Distribution of SCCmec type among MRSA isolates from three Indonesian hospitals (Denpasar, Semarang, and Malang)*

SCCmec type	Number of isolates (%)			
	Denpasar (n = 4)	Semarang (n = 111)	Malang (n = 12)	Total (n = 127)
I	0	2 (1.8)	0	2 (1.6)
I - dcs	2 (50.0)	0	0	2 (1.6)
II	0	0	0	0
II - kdp	0	1 (0.9)	0	1 (0.8)
III	2 (50.0)	107 (96.4)	11 (91.7)	120 (94.4)
III - mecl	0	1 (0.9)	0	1 (0.8)
IV	0	0	0	0
V	0	0	1 (8.3)	1 (0.8)
VI	0	0	0	0

*SCCmec = staphylococcal cassette chromosome mec; MRSA = methicillin-resistant *Staphylococcus aureus*.

hospital environment, and household members. One other MRSA isolate from discharge patient was missing. Type III SCCmec was the main type (94.4%). However, we found few isolates containing type I, type I-dcs, type II-kdp, and type III variant (type III-mecI) in Semarang and Denpasar. Type III-mecI is an isolate that was characterized by SCCmec multiplex PCR by the presence of a pattern very similar to the SCCmec type III but lacking the band corresponding to the amplification of the mecl gene. Interestingly, one SCCmec

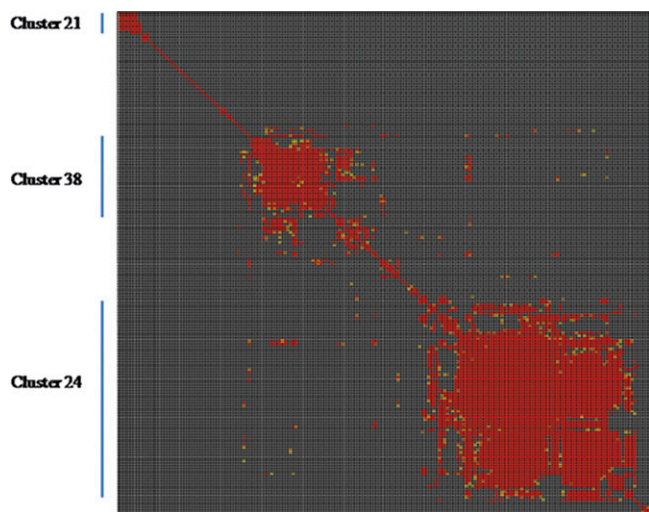


FIGURE 1. Clustering of methicillin-resistant *Staphylococcus aureus* (MRSA) and Panton-Valentine leukocidin (PVL)-positive methicillin-sensitive *S. aureus* (MSSA) isolates from discharge patients in three hospitals in Indonesia as determined by Raman spectroscopy (RT: Raman type). Note: Figure displays 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. Cluster 21 includes MRSA isolates from Semarang: 5 isolates from discharge patients and 2 isolates from contact patients. Cluster 24 contains MRSA isolates from Semarang and Malang: 40 isolates from discharge patients, 18 isolates from contact patients, 1 isolate from hospital environment, 2 isolates from household members. In addition, this cluster contains PVL-positive MSSA Semarang isolates: 7 isolates from discharge patients, 1 isolate from contact patient, and 6 isolates from health care workers. Cluster 38 is consisted of MRSA isolates from Semarang and Malang: 21 isolates from discharge patients and 4 isolates from contact patients. One PVL-positive MSSA isolate belongs to cluster 38.

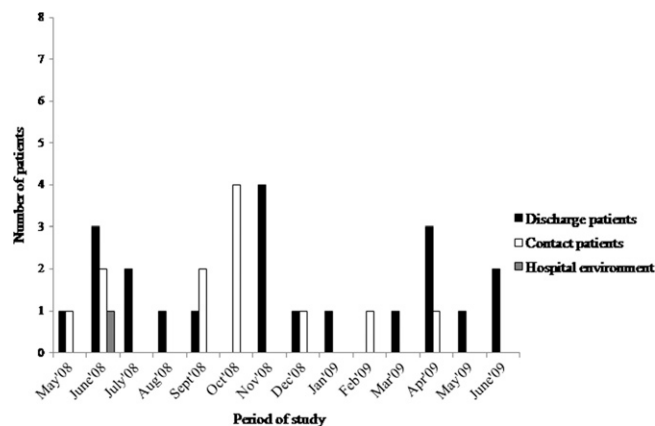


FIGURE 2. Endemicity profile of Raman type 24 MRSA among discharge patients, contact patients, and hospital environment in a surgery ward in Dr. Kariadi hospital, Semarang, Indonesia.

type V was detected from a discharge patient in Malang (Table 3).

Raman spectroscopy. We carried out Raman spectroscopy for 162 *S. aureus* isolates consisting of 127 MRSA and 35 PVL-positive MSSA. The *S. aureus* isolates were obtained from discharge patients (112 isolates), contact patients (37 isolates), health care workers (10 isolates), hospital environment (1 isolate), and household members (2 isolates). One other MRSA isolate and 4 other PVL-positive MSSA isolates from discharge patients were missing.

The Raman spectroscopic analysis showed 61 RTs (Figure 1). The most frequently found type was RT 24 containing 75 *S. aureus* including 61 MRSA and 14 PVL-positive MSSA. The MRSA isolates were obtained from discharge patients (40 isolates), contact patients (18 isolates), hospital environment (1 isolate), and household members (2 isolates), which were mostly (54 isolates) from Dr. Kariadi hospital in Semarang. The RT 24 PVL-positive MSSA isolates were obtained from discharge patients (7 isolates), contact patients (1 isolates), and health care workers (6 isolates) from Semarang. The RT 38 and RT 21 clusters were the second and third most common Raman types including 26 and 7 *S. aureus* isolates, respectively.

Figure 2 shows the endemicity profile of RT 24 MRSA in the surgery ward in Dr. Kariadi hospital. Interestingly, 7 MRSA isolates from Malang were included in the RT 24 together with MRSA isolates from Semarang. No MRSA isolate from Denpasar clustered in one of the three large clusters RT 24, RT 38, or RT 21. Instead, the four MRSA isolates

TABLE 4
Bacterial typing of selected methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from discharge patients in Indonesia*

Isolates number	Raman type	SCCmec type	ST
7192	21	III	239
7237	24	III	239
7300	24	III	239
7337	24	III	239
7047	35	#	ND
7148	38	III	239
7254	38	III	239
7233	38	#	121
7244	40	#	188
7491	43	III	239

*SCCmec = staphylococcal cassette chromosome mec; ST = sequence type; # = PVL-positive MSSA.

from Denpasar could be designated RT 15 (2 isolates), RT 48 (1 isolate), and RT 51 (1 isolate).

MLST analysis. We randomly selected 10 *S. aureus* isolates representing RT 21 (1 isolate), RT 24 (3 isolates), RT 35 (1 isolate), RT 38 (3 isolates), RT 40 (1 isolate), and RT 43 (1 isolate) for MLST. All seven MRSA isolates that were distributed among four different RT clusters belonged to ST239 (Table 4). The PVL-positive MSSA isolates were assigned to ST121 and ST188. One isolate was untypeable by MLST because no PCR product could be generated repeatedly for the *gmk* gene. All *S. aureus* isolates that were analyzed by MLST were from Semarang.

DISCUSSION

In this study, we showed that MRSA were present in the hospital setting in Indonesia, although significant geographical variations exist. The carriage rate among surgery patients screened at discharge was highest in the two hospitals on the island of Java, i.e., 8.0% in Malang and 5.9% in Semarang, versus 0.4% in Denpasar on the island of Bali. Of importance, in our previous 2001–2002 study, no MRSA was found among patients at discharge in Semarang^{19,29}; we now uncovered endemicity of ST239-MRSA-SCC*mec* type III in the same hospital in Semarang several years later. It has been shown by mathematical modeling that the ST239-MRSA-SCC*mec* type III lineage exhibits an enhanced transmissibility compared with other lineages that could explain its successful spread³⁰; this clone is notable for causing prolonged epidemics that are difficult to control in hospitals worldwide, and it is the dominant sequence type in Asia. Raman spectroscopy, a method that attains 95.2% concordance with pulsed-field gel electrophoresis,²⁵ sub-divided isolates belonging to this clone into several sub-clones or RTs, of which RT 24 was the most common. This RT 24 was not only carried by discharge patients but also by contact patients, and contaminated and persisted in a surgical ward of the Semarang hospital over a 14-month period. In such an endemic situation, it is not possible to determine a single (point) source of the MRSA. The RT 24 was also the most frequently found type in Malang hospital, suggesting that this type has also become endemic in that setting. The RT 38, another ST239 sub-clone, was prevalent in another surgical ward in the hospital in Semarang, and was also found in Malang hospital ($N = 4$ isolates).

The MRSA isolates from Sanglah hospital, Denpasar, on the Bali Island clustered in RT 15, RT 48, and RT 51, indicating that the epidemiology of MRSA in Bali differs from that in the two Javanese hospitals.

Interestingly, health care workers were not identified as carriers of MRSA. Although health care workers are generally considered reservoirs for MRSA, this may not be the case in these Indonesian settings. We hypothesize that health care workers may be colonized with other *Staphylococcus* species, such as *S. sciuri*,³¹ or PVL-positive MSSA, which may protect their mucosa from colonization by MRSA. This interference hypothesis, however, needs to be explored further.

In this study, we presented the first risk factors analysis related to MRSA carriage in Indonesian hospitals. According to the multivariate analysis, being male, length of hospitalization, and antibiotic therapy during admission were associated with MRSA carriage among discharge patients, in addition to the determinant city as described previously ($P < 0.05$). Indeed,

these risk factors are in agreement with multiple studies on the nasal carriage of MRSA,^{1,32–35} however no information about these in Indonesian hospital settings has been presented before.

The PVL-positive MSSA were detected in patients at discharge in Semarang and Malang hospitals (PVL-positive MSSA carriage rate ranged between 1.0% and 2.3%) and these data are in agreement with previous reports from Indonesia.^{20,36} However, the prevalence of PVL-positive MSSA among health care workers in Semarang in this study was remarkably high (10.3%). The consequence of this finding is not yet clear. As hypothesized previously, it could provide protection against colonization by other strains of *S. aureus*, including MRSA.

Two isolates of PVL-positive MSSA from patients hospitalized in Semarang were confirmed as ST121 and ST188 by MLST. Both sequence types were also found among PVL-positive MSSA in Surabaya, another city in East Java, Indonesia in 2001–2002.²⁰ A more recent study reported that ST188 PVL-positive MSSA was predominant among discharge patients in Malaysia.³⁷

In concordance with other studies, we did not find PVL-positive MRSA in Indonesia.^{20,36} However, the emergence of such strains is not unlikely because of possible horizontal transfer of the *mecA* gene to PVL-positive MSSA.³⁸ Of note, some PVL-positive MSSA clustered in RT 24, together with ST239-MRSA-SCC*mec* type III isolates.

This study has some limitations. First, we did not ascertain whether the MRSA was acquired in the hospital or before admission, because we did not screen the patients at the time of their admission. Although Raman spectroscopy, MLST, and SCC*mec* typing indicated predominance of typical hospital-acquired MRSA strains, acquisition of such MRSA in the community setting may occur. For example, the single MRSA isolate with a type V SCC*mec* from a patient in Malang could have been acquired in the community. Second, screening of secondary cases in Dr. Saiful Anwar hospital, Malang was not conducted. Consequently, we could not analyze the possible MRSA transmission to the secondary cases at that study site. Third, genetic confirmation with MLST was not performed for all *S. aureus* isolates.

In summary, the prevalence of MRSA among patients in surgery wards in Indonesian hospitals was high in comparison with our earlier analysis, although geographical variations exist. We showed that an endemic situation occurred in Semarang. Therefore, targeted intervention measures including hand hygiene, isolation procedures, cleaning of hospital environment, screening, and decolonization of patients are required to reduce the MRSA acquisition rate in Indonesian hospitals. Recent studies reported that selective MRSA screening to high-risk colonization patients (e.g., patients at intensive care unit admission) and patients detected as MRSA carrier previously will reduce the prevalence of MRSA more efficiently than universal screening at hospital admission.^{6,39} Such a strategy should also be developed for other settings. In addition, it is necessary to study clinical isolates and the burden of disease caused by MRSA in Indonesian hospitals.

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