Tropical Medicine and International Health doi:10.1111/tmi.13000

VOLUME 23 NO 1 PP 34–44 JANUARY 2018

Prevalence and characterisation of *Staphylococcus aureus* causing community-acquired skin and soft tissue infections on Java and Bali, Indonesia

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Abstract

OBJECTIVES To define the role of *Staphylococcus aureus* in community settings among patients with skin and soft tissue infections (SSTI) in Indonesia.

METHODS *Staphylococcus aureus* were cultured from anterior nares, throat and wounds of 567 ambulatory patients presenting with SSTI. The *mecA* gene and genes encoding Panton–Valentine leukocidin (PVL; *lukF-PV* and *lukS-PV*) and exfoliative toxin (ET; *eta* and *etb*) were determined by PCR. Clonal relatedness among methicillin-resistant *S. aureus* (MRSA) and PVL-positive *S. aureus* was analysed using multilocus variable-number tandem-repeat analysis (MLVA) typing, and multilocus sequence typing (MLST) for a subset of isolates. Staphylococcal cassette chromosome *mec* (SCC*mec*) was determined for all MRSA isolates. Moreover, determinants for *S. aureus* SSTI, and PVL/ET-positive *vs* PVL/ET-negative *S. aureus* were assessed.

RESULTS *Staphylococcus aureus* were isolated from SSTI wounds of 257 (45.3%) patients, eight (3.1%) of these were MRSA. Genes encoding PVL and ETs were detected in 21.8% and 17.5% of methicillin-susceptible *S. aureus* (MSSA), respectively. PVL-positive MRSA was not detected. Nasopharyngeal *S. aureus* carriage was an independent determinant for *S. aureus* SSTI (odds ratio [OR] 1.8). Primary skin infection (OR 5.4) and previous antibiotic therapy (OR 3.5) were associated with PVL-positive MSSA. Primary skin infection (OR 2.2) was the only factor associated with ET-positive MSSA. MLVA typing revealed two more prevalent MSSA clusters. One ST1-MRSA-SCC*mec* type IV isolate and a cluster of ST239-MRSA-SCC*mec* type III were found.

CONCLUSIONS Community-acquired SSTI in Indonesia was frequently caused by PVL-positive MSSA, and the hospital-associated ST239-MRSA may have spread from the hospital into the community.

keywords exfoliative toxin, Indonesia, methicillin-resistant *Staphylococcus aureus*, Panton–Valentine leukocidin, skin and soft tissue infections, *Staphylococcus aureus*

Introduction

Staphylococcus aureus is known as an important pathogen of skin and soft tissue infections (SSTI) in the community setting [1]. The emergence and transmission of community-associated methicillin-resistant *S. aureus* (CA-MRSA) have become a major problem in several countries [2–7]. In general, CA-MRSA are more susceptible to non-beta-lactam antibiotics compared to hospital-associated MRSA (HA-MRSA). However, CA-MRSA tend to be more virulent than HA-MRSA and may cause highly invasive, rapidly progressive and life-threatening diseases [1]. This phenomenon has been associated with the presence of Panton–Valentine leukocidin (PVL).

CA-MRSA are also associated with staphylococcal cassette chromosome *mec* (SCC*mec*) type IV or V which differentiates them further from HA-MRSA that carry SCC*mec* type I, II or III. Nevertheless, the distinction between CA-MRSA and HA-MRSA has blurred in many countries as CA-MRSA clones have been introduced into the hospital and, vice versa, HA-MRSA have spread from healthcare settings into the community [8].

Only few data are available on the epidemiology of CA-MRSA in Indonesia, the fourth most populous nation in the world. Only a few case reports of the finding of a SCC*mec* type V carrying MRSA strain, one regarding a patient discharged from Dr. Saiful Anwar hospital in Malang and one describing a patient admitted to the Dr. Kariadi hospital in Semarang, have been published previously [9, 10]. Interestingly, a high prevalence of PVL-positive methicillin-susceptible *S. aureus* (MSSA) was observed among carriage and community-onset infection strains in Indonesia [11, 12]. In this study, *S. aureus* isolates from multiple community settings in Indonesia targeting patients suffering from community-acquired SSTI were collected. The presence of *mecA* and genes for PVL and exfoliative toxins was assessed. Further, their clonal relatedness and sequence type (ST) were determined, and we subsequently correlated these data with patients' disease characteristics.

Materials and methods

Setting

Eight primary healthcare centres and two dermatology outpatient clinics of teaching hospitals on Java (Malang and Surabaya city) and Bali (Denpasar city) islands participated in this study (Table S1). Enrolment of patients into the study was from July 2009 to February 2010.

Study population

Patients with SSTI presenting to the participating centres were eligible for enrolment (Table S2). Patients with a history of prior hospitalisation within the past 12 months or other established risk factors, such as surgery, residence in a long-term care facility, dialysis or indwelling percutaneous medical devices and urinary catheters, were excluded (Figure 1). The study was approved by the medical ethics committee of Faculty of Medicine, Brawijaya University, Malang, Indonesia (the ethical clearance was not assigned by a number).

Bacterial isolates

Patients with SSTI who were included in the study and who had given written informed consent were cultured by swabbing their skin lesions. Screening for MSSA and MRSA carriage among patients was performed by culturing their anterior nares and throat. Samples were transported using Amies transport medium (Becton Dickinson). 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 subcultured onto *S. aureus* and MRSA Chromagar medium (ITK Diagnostics, Uithoorn, The Netherlands) and incubated for 24–48 h at 37 °C. Typical colonies of *S. aureus* and MRSA were stored in tubes containing trypticase soy agar until they could be further characterised. Confirmation of *S. aureus* was performed by Slidex Staph Plus (bioMérieux, Marcy l'Etoile, France) and the Vitek2 system (bioMérieux). A subset of *S. aureus* isolates from Denpasar was additionally confirmed by matrix-assisted laser desorption/ionisation (Maldi Biotyper, Bruker Microflex LT, Bruker, London, UK). Antibiotic

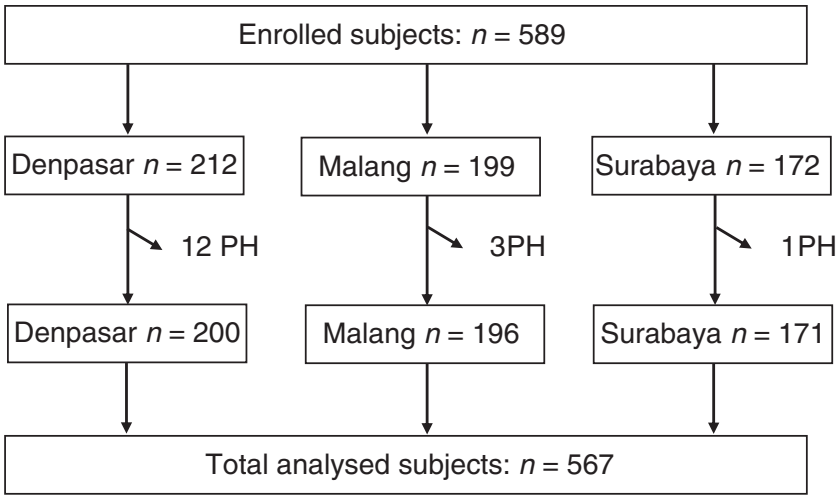

Figure 1 Flow chart with numbers of enrolled and analysed subjects. Reason for exclusion of enrolled subjects from analysis: prior hospitalisation within the past 12 months (PH), other reasons were not found.

susceptibility tests on the MRSA isolates were conducted by Vitek2 system (bioMérieux) (Table S3). Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guideline M100-S20.

DNA extraction and detection of *mecA*, SCC*mec*, and genes for PVL and exfoliative toxins A and B

Bacterial DNA was extracted using the MagNa Pure LC™ DNA system (DNA isolation kit III; Roche Molecular Biochemicals, Mannheim, Germany) [13]. Because of organisational reasons, for a subset of isolates, bacterial DNA was extracted using DNA Mini Kits (QiaAmp DNA Mini Kits; Qiagen Inc, Germany) according to the manufacturer's instruction. The DNA concentration was measured spectrophotometrically and samples were stored at −20 °C. Detection of *mecA*, PVL genes *lukF-PV* and *lukS-PV,* and exfoliative toxin A (*eta*) and B (*etb*) genes was performed by PCR as previously described [14–16]. The SCC*mec* of *S. aureus* isolates containing the *mecA* gene was characterised using a multiplex PCR that enables the identification of SCC*mec* types I to VI [17]. Positive and negative control strains were included in each PCR run.

Multilocus variable-number tandem-repeat analysis (MLVA)

MLVA typing was carried out based on the combination of six loci (SIRU01, SIRU05, SIRU07, SIRU13, SIRU15 and SIRU21) as described previously [18]. Amplification of SIRUs (staphylococcal interspersed repeat units) and the assignment of MLVA type were derived from a previous study by Ikawati *et al.* [19].

Multilocus sequence typing (MLST)

A selection of 12 randomly chosen *S. aureus* isolates was further analysed by MLST [20] as described before. Before the sequence reaction was carried out, the amplicon was purified using a supplement, ExoSAP-IT (Affymetrix product no. 78200; Isogen). The MLST sequence type (ST) was assigned through the MLST website (www.mlst.net).

Risk factor analysis

Socio-demographic data, atopic history, *S. aureus* carriage status, family with similar disease and number of family members were included in the risk factor analysis of *S. aureus* SSTI in the community. As the *S. aureus* carriage status is important in this analysis, only patients that were actually screened for nasopharyngeal carriage were included in this analysis. Duration and type of skin lesion, same complaint before, antibiotic therapy in the previous month and diabetes mellitus were included in the analysis to determine association between the presence of genes encoding PVL and exfoliative toxins and the clinical features and background of the staphylococcal SSTI [21–24]. These data were collected from patient records and by interviewing the patients using a structured questionnaire. Potential risk factors for *S. aureus* SSTI were tested univariately using Pearson chi-square or Fisher analysis. All variables with a P value < 0.2 were included in a multivariate logistic regression model. Backward selection based on the likelihood-ratio test was used to identify significant variables. Likewise, determinants for PVL-positive *vs* PVL-negative *S. aureus* and exfoliative toxin-positive *vs* exfoliative toxin-negative *S. aureus* were analysed. Data were analysed using statistical software packages SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). A P value less than 0.05 was considered significant.

Results

Prevalence of MRSA, PVL- and exfoliative toxin-positive MSSA

A total of 567 patients with SSTI were included in this study, 200 in Denpasar (Bali island), 196 in Malang (Java island) and 171 in Surabaya (Java island). The prevalence of *S. aureus* obtained from wound culture among patients with SSTI was 257/567 (45.3%) with some variation between the three cities (Table 1). In total, we found that eight of 257 (3.1%) *S. aureus*-infected patients had an MRSA infection. In Surabaya, MRSA was significantly more common among the SSTI causing *S. aureus* compared to other cities (7/74 in Surabaya *vs* 1/183 in other cities; $P \leq 0.001$). In Malang, none of the *S. aureus* were MRSA, which was also statistically significant (Table 1). Remarkably, all MRSA isolates were PVL-negative. In contrast, PVL-positive strains were frequently found among the methicillin-susceptible isolates of *S. aureus* (MSSA), 56/249 (22.5%) with significant variation between the three cities. Likewise, exfoliative toxin-coding genes were only detected in MSSA isolates, overall 45/249 (18.1%), only *eta* (29/249, 11.6%), only *etb* (5/249, 2.0%). Exfoliative toxin-positive strains were found in all three cities at approximately similar frequencies (16.0–19.0%) (Table 1). All patients in this study were screened for nasopharyngeal MRSA carriage, except in Surabaya, where 69 of 171 patients included were screened. Table 1 shows the significant variation of *S. aureus* carriage between the three cities either among

Table 1 Prevalence of methicillin-resistant, PVL-, and exfoliative toxin-positive *Staphylococcus aureus* among patients with staphylococcal skin and soft tissue infections in the community setting in Indonesia

	Patients included in				
	Denpasar (n = 200)	Malang (n = 196)	Surabaya (n = 171)	Total (n = 567)	P
S. aureus clinical*	80/200 (40.0)	103/196 (52.6)	74/171 (43.3)	257/567 (45.3)	0.035
S. aureus carriage†	7/80 (8.8)‡	65/103 (63.1)‡	1/18 (5.6)‡	73/201 (36.3)‡	<0.001
	12/120 (10.0)§	33/93 (35.5)§	18/51 (35.3)§	63/264 (23.9)§	<0.001
MRSA*	1/80 (1.2)	0/103 (0)	7/74 (9.5)	8/257 (3.1)	0.441¶
					<0.001**
					0.023††
PVL (+)*'‡‡	28/80 (35.0)	10/103 (9.7)	18/74 (24.3)	56/257 (21.8)	0.001¶
					0.617**
					<0.001††
eta (+)*	11/80 (13.8)	10/103 (9.7)	8/74 (10.8)	29/257 (11.3)	0.819§§
etb (+)*	2/80 (2.5)	2/103 (1.9)	1/74 (1.4)	5/257 (1.9)	
eta and *etb* (+)*	1/80 (1.2)	5/103 (4.9)	5/74 (6.8)	11/257 (4.3)	

Data are absolute numbers of patients (%); MSSA = methicillin-susceptible *S. aureus*; MRSA = methicillin-resistant *S. aureus*; PVL = Panton–Valentine leukocidin; *eta* = exfoliative toxin A gene; *etb* = exfoliative toxin B gene; SSTI = skin and soft tissue infections.
**Staphylococcus aureus* were obtained from wound culture.
†*Staphylococcus aureus* were obtained from either nose only, throat only or both nose and throat.
‡*Staphylococcus aureus* carriage among patients with *S. aureus* SSTI.
§*Staphylococcus aureus* carriage among patients with non-*S. aureus* SSTI.
¶Denpasar *vs* rest (Fisher).
**Surabaya *vs* rest (Fisher).
††Malang *vs* rest (Fisher).
‡‡all PVL-positive strains were MSSA.
§§either in conjunction or independent *eta* and *etb* genes.

patients with *S. aureus* SSTI ($P < 0.001$) or patients with non-*S. aureus* SSTI ($P < 0.001$). We did not find any MRSA among the 136 *S. aureus* carriage patients.

Antibiotic susceptibility testing

Antibiotic susceptibility tests showed that the seven MRSA isolates from Surabaya were all resistant to aminoglycosides, macrolides, ciprofloxacin, levofloxacin, tetracycline and trimethoprim–sulfamethoxazole, whereas four of the seven MRSA isolates were resistant to moxifloxacin as well. All of seven MRSA isolates were sensitive to the remaining antibiotics tested. In contrast, the single MRSA isolate from Denpasar showed susceptibility to all non-beta-lactam antibiotics in the test panel.

SCC*mec* and MLVA typing

SCC*mec* typing was performed for the eight MRSA isolates from wound culture of patients with SSTI. Interestingly, the seven MRSA isolates from Surabaya harboured SCC*mec* type III and the single MRSA isolate from Denpasar harboured SCC*mec* type IV. MLVA typing was carried out for a subset of 56 *S. aureus* isolates consisting of the eight MRSA isolates from wound cultures and 48 PVL-positive MSSA from different sources (wound: 34 isolates; nose screening: six isolates; throat screening: eight isolates). Eight PVL-positive MSSA isolates could not be retrieved from storage for MLVA typing. The MLVA revealed 30 MLVA types (MT) (Table 2), and one isolate was not determinable. The most frequently found type was MT11 consisting of seven PVL-positive MSSA obtained from wound cultures in three cities involved in this study, except one isolate from Malang that was obtained from a nose culture. The second largest cluster was MT19 containing five MRSA isolates from wound cultures, all isolated in Surabaya.

MLST analysis

Twelve *S. aureus* strains were selected for MLST. MLST was performed for two MRSA isolates representing MT2 (one isolate from Denpasar) and MT19 (one isolate from Surabaya); they belonged to ST1-MRSA-SCC*mec* type IV and ST239-MRSA-SCC*mec* type III, respectively. In addition, MLST was conducted for 10 PVL-positive MSSA representing MT5 (one isolate), MT6 (one isolate), MT7 (one isolate), MT10 (one isolate), MT11 (two isolates),

Table 2 MLVA typing results of *Staphylococcus aureus* obtained from three cities in the community setting in Indonesia

Isolate number	City	Source	SIRU profile						MLVA type
			SIRU01	SIRU05	SIRU07	SIRU13	SIRU15	SIRU21	
2241	Malang	T	1	999	2	1	1	10	1
W492	Denpasar	W	1	999	2	2	1	6	2
1121	Malang	N	1	999	3	999	999	999	3
2091	Malang	T	1	999	3	999	4	5	4
W138	Denpasar	W	1	999	3	2	1	5	5
3195	Malang	W	1	999	3	2	1	6	6
10115	Surabaya	W	1	999	3	2	1	6	6
3107	Malang	W	1	999	3	2	2	6	6
3122	Malang	W	1	999	3	3	4	5	7
3222	Malang	W	1	999	3	3	4	5	7
W387	Denpasar	W	1	999	3	3	4	5	7
W546	Denpasar	W	1	999	3	3	4	5	7
TS448	Denpasar	T	1	1	2	1	3	7	8
2089	Malang	T	1	1	3	1	3	7	9
W372	Denpasar	W	1	1	3	1	3	7	9
10124	Surabaya	W	1	1	3	1	3	7	9
W305	Denpasar	W	1	1	3	2	3	1	10
3087	Malang	W	1	1	3	2	3	8	11
1086	Malang	N	1	1	3	2	3	8	11
W501	Denpasar	W	1	1	3	2	3	8	11
W378	Denpasar	W	1	1	3	2	3	8	11
10093	Surabaya	W	1	1	3	2	3	8	11
10022	Surabaya	W	1	1	3	2	3	8	11
3178	Malang	W	1	1	3	2	1	8	11
W396	Denpasar	W	1	1	5	2	3	7	12
1223	Malang	N	2	999	1	2	2	7	13
3011	Malang	W	2	0	3	2	3	9	14
2010	Malang	T	2	0	3	2	3	9	14
3106	Malang	W	2	0	3	2	3	9	14
W357	Denpasar	W	2	1	3	1	3	8	15
10069	Surabaya	W	2	8	1	5	1	8	16
W474	Denpasar	W	2	10	1	4	1	9	17
W31	Denpasar	W	3	999	3	2	4	9	18
12*	Surabaya	W	3	1	2	1	1	6	19
13*	Surabaya	W	3	1	2	1	1	6	19
14*	Surabaya	W	3	1	2	1	1	6	19
18*	Surabaya	W	3	1	2	1	1	6	19
19*	Surabaya	W	3	1	2	1	1	6	19
15	Surabaya	W	3	1	3	1	1	6	20
10066	Surabaya	W	3	1	3	1	1	6	20
W486	Denpasar	W	3	1	3	1	3	8	21
W375	Denpasar	W	3	1	3	1	3	8	21
1128	Malang	N	3	1	3	2	2	8	22
3130	Malang	W	3	1	3	2	2	8	22
10005	Surabaya	W	3	2	3	2	2	8	23
2214	Malang	T	3	4	2	5	4	10	24
2216	Malang	T	3	4	3	3	4	3	25
P24	Surabaya	W	3	4	3	3	4	9	26
3007	Malang	W	3	4	3	3	4	9	26
W320	Denpasar	W	3	4	3	3	4	10	27
W429	Denpasar	W	3	4	3	3	4	10	27
W402	Denpasar	W	4	999	1	3	5	7	28

Table 2 (*Continued*)

Isolate number	City	Source	SIRU profile						MLVA type
			SIRU01	SIRU05	SIRU07	SIRU13	SIRU15	SIRU21	
W335	Denpasar	W	4	999	1	3	4	8	29
1193	Malang	N	4	9	2	3	5	4	30
2194	Malang	T	4	9	2	3	5	4	30
1240	Malang	N	999	999	999	999	999	999	ND

SIRU = Staphylococcal interspersed repeat units; MLVA = multilocus variable-number tandem-repeat analysis; ND = not determinable; W = wound swab; N = nose swab; T = throat swab; *MRSA isolates; 999 = no amplification of SIRU.

MT18 (one isolate), MT22 (two isolates) and MT23 (one isolate). The MLST typing identified ST1301 as the most frequently found sequence type among PVL-positive MSSA isolates corresponding to MT10, MT11, MT22 and MT23, MLVA types with largely concordant SIRU profiles (Table 3).

Risk factor analysis

For the risk factor analysis, all patients from Malang and Denpasar were included, however, from Surabaya, only 69 patients were included, as screening for nose and throat carriage was not performed among the remaining 102 patients. When SSTI due to *S. aureus* was compared univariately to SSTI due to other species, it appeared that *S. aureus*-infected patients were younger than patients suffering from SSTIs by other pathogens ($P = 0.015$). *Staphylococcus aureus*-infected patients were more likely to carry *S. aureus* in their nasopharynx ($P = 0.004$). Applying the Bonferroni correction, we divided $P = 0.05$ by the number of tests (seven) to get the Bonferroni critical value. A test with $P < 0.007$ was then considered as significant. Nasopharyngeal carriage was the only factor associated with *S. aureus* SSTI univariately, with this correction.

By multivariate analysis, nasopharyngeal carriage of *S. aureus* was independently associated with *S. aureus* infection (odds ratio [OR] 1.8; 95% CI 1.172–2.649), whereas age was not. Gender, the number of household members, the ethnicity and the presence of atopy were not associated with *S. aureus* SSTI as compared to SSTI by other pathogens (Table 4).

The associations of PVL-positive or exfoliative toxin-positive strains with premorbid history and clinical features of the SSTI were also investigated (Table 5). We divided $P = 0.05$ by the number of tests (seven) to get the Bonferroni critical value, and a test with $P < 0.007$ was considered as significant. The type of skin infection and antibiotic therapy in the previous month were factors associated with PVL-positive MSSA, univariately. The results of the multivariate analyses showed that PVL-positive MSSA was associated with primary skin infection (OR 5.4; 95% CI 2.1–13.6, $P < 0.001$) and a history of antibiotic therapy in the previous month (OR 3.5; 95% CI 1.7–7.1, $P = 0.001$), whereas exfoliative toxin-positive MSSA was only associated with primary skin infection (OR 2.2; 95% CI 1.0–4.9, $P = 0.047$; significant without correction).

According to the questionnaires obtained from the eight patients with MRSA SSTI, six patients were between 2 and 13 years old and the remaining two were between 20 and 24 years old. Two of these patients had had a previous episode of SSTI and had received antibiotic therapy in the month preceding enrolment in this study.

Table 3 Bacterial typing of 12 selected *Staphylococcus aureus* isolates from community setting in Indonesia

Isolate number	City	SCC*mec* type	SIRU profile	MLVA type (MT)	ST
W492	Denpasar	IV	1.999.2.2.1.6	2	1
W138	Denpasar	#	1.999.3.2.1.5	5	97
3107	Malang	#	1.999.3.2.2.6	6	1
W546	Denpasar	#	1.999.3.3.4.5	7	188
W305	Denpasar	#	1.1.3.2.3.1	10	1301
W501*	Denpasar	#	1.1.3.2.3.8	11	1301
10022*	Surabaya	#	1.1.3.2.3.8	11	1301
W31	Denpasar	#	3.999.3.2.4.9	18	3035
12	Surabaya	III	3.1.2.1.1.6	19	239
CA1128†	Malang	#	3.1.3.2.2.8	22	1301
CA3130†	Malang	#	3.1.3.2.2.8	22	1301
10005	Surabaya	#	3.2.3.2.2.8	23	1301

SCC*mec* = Staphylococcal cassette chromosome *mec*; ST = sequence type; SIRU = Staphylococcal interspersed repeat units; MLVA = multilocus variable-number tandem-repeat analysis; # = methicillin-susceptible *Staphylococcus aureus*; clone a, '*' showing the concordance of PVL-positive MSSA, MLVA type 11 and ST1301; clone b, '†' showing the concordance of PVL-positive MSSA, MLVA type 22 and ST1301; 999 = no amplification of SIRU.

Table 4 Risk factors of *Staphylococcus aureus* (*S. aureus*) skin and soft tissue infection

	Univariate analysis			Multivariate analysis		
	No. of subjects (%)					
Factors	*S. aureus* infection (n = 201)	Not *S. aureus* infection (n = 264)	P	OR	95% CI	P
Age			0.015			NS
≤18 years	148 (73.6)	158 (59.8)				
19–59 years	43 (21.4)	86 (32.6)				
≥60 years	9 (4.5)	15 (5.7)				
Unknown	1 (0.5)	5 (1.9)				
Gender			0.354			
Male	123 (61.2)	147 (55.7)				
Female	78 (38.8)	116 (43.9)				
Unknown	0	1 (0.4)				
Ethnic group			0.615			
Javanese	135 (67.2)	167 (63.3)				
Balinese	54 (26.9)	85 (32.2)				
Maduranese	7 (3.5)	7 (2.7)				
Other*	5 (2.4)	5 (1.8)				
Atopic			0.929			
Yes	64 (31.8)	82 (31.1)				
No	136 (67.7)	180 (68.2)				
Unknown	1 (0.5)	2 (0.7)				
Nasopharyngeal carriage	73 (36.3)	63 (23.9)	0.004	1.762	1.172–2.649	0.006
Family with similar disease	48 (23.9)	49 (18.6)	0.361			
Number of family members						
1–4	104 (51.7)	145 (54.9)	0.89			
5–8	85 (42.3)	106 (40.2)				
≥9	7 (3.5)	7 (2.7)				
Unknown	5 (2.5)	6 (2.3)				

*Bataknese, Chinese, Lomboknese, foreigner.

Discussion

This is the first multicentre survey of *S. aureus* SSTI in the community setting in Indonesia. Similar to our previous studies [9, 10], we found significant geographic variation in the prevalence of MRSA in Indonesia. Overall, the prevalence of MRSA detected in this study was 3.1%, which is within the reported range of 2.5–39% of MRSA among CA-*S. aureus* in Asian countries [2]. Of importance, two previous studies from Indonesia, including one from Surabaya, did not find MRSA in the community setting [11, 12]. In the present study, we uncovered a clone of ST239-MRSA-SCC*mec* type III consisting of six MRSA isolates in Surabaya several years later, indicating infiltration of an HA-MRSA clone into this urban community. Furthermore, one isolate from Denpasar was ST1-MRSA-SCC*mec* type IV, which represents a typical CA-MRSA, although in this case, PVL genes were absent in the isolate. The antibiotic susceptibility patterns among MRSA isolates were in accordance with the HA-MRSA and CA-MRSA susceptibility characteristics reported before [8, 25]. Similar to previous findings in Indonesia, all MRSA isolates were PVL-negative, whereas the prevalence of PVL among MSSA strains causing SSTIs was high, as more than 20% of the isolates carried these cytotoxin-coding genes [9, 10, 12]. Bacterial typing showed that two PVL-positive MSSA isolates from different patients in Malang (CA1128 and CA3130) were assigned to the same clone (ST1301/MT22), which is suggestive of cross-transmission in the community setting. However, we did not investigate a possible epidemiological link between them. Another two PVL-positive MSSA isolates (W501, Denpasar and 10022, Surabaya) were also assigned to the same clone (ST1301/MT11), a finding that is compatible with spreading of this clone between two islands, Bali and Java. The ST1301 clone that was frequently found among PVL-positive MSSA isolates in this study was also detected among clinical *S. aureus* in several hospitals in China [26, 27]. Clonal dissemination of this strain might, thus, have been influenced by travel of persons between Indonesia and China.

Table 5 Association of genes encoding PVL and exfoliative toxins to the clinical feature and background of the staphylococcal skin and soft tissue infections

	Univariate analysis					
	No. of subjects (%)			No of subjects (%)		
Factors	PVL (+) MSSA (n = 56)	PVL (−) MSSA (n = 193)	P	ETs (+) MSSA (n = 45)	ETs (−) MSSA (n = 204)	P
Duration of skin lesion			0.327			0.659
1–3 days	15 (26.8)	35 (18.1)		6 (13.3)	44 (21.6)	
4–7 days	23 (41.1)	85 (44.0)		24 (53.3)	84 (41.2)	
8–14 days	13 (23.2)	36 (18.7)		8 (17.8)	41 (20.1)	
15–30 days	3 (5.4)	23 (11.9)		4 (8.9)	22 (10.8)	
>30 days	1 (1.8)	12 (6.2)		2 (4.4)	11 (5.4)	
Unknown	1 (1.8)	2 (1.0)		1 (2.2)	2 (1.0)	
Type of skin infection			<0.001			0.087
Primary infection*	49 (87.5)	110 (57.0)		34 (75.6)	125 (61.3)	
Secondary infection†	7 (12.5)	83 (43.0)		11 (24.4)	79 (38.7)	
Same complaint before	21 (37.5)	58 (30.1)	0.396	12 (26.7)	67 (32.8)	0.072
Antibiotic therapy previous month	22 (39.3)	27 (14.0)	<0.001	6 (13.3)	43 (21.1)	0.026
Diabetes mellitus	1 (1.8)	6 (3.1)	0.555	2 (4.4)	5 (2.5)	0.066
Atopic history	25 (44.6)	66 (34.2)	0.257	14 (31.1)	77 (37.7)	0.072
Family with similar disease (yes/no question)	17 (30.4)	37 (19.2)	0.142	7 (15.6)	47 (23.0)	0.055
Multivariate analysis						
Factors	**OR**		**95% CI**		***P***	
PVL-positive MSSA						
Type of skin infection						
Primary infection*	5.417		2.137–13.734		<0.001	
Secondary infection†	1					
Antibiotic therapy previous month	3.463		1.693–7.086		0.001	
Family with similar disease					NS	
ETs positive MSSA						
Type of skin infection						
Primary infection*	2.233		1.010–4.936		0.047	
Secondary infection†	1					
Same complaint before					NS	
Antibiotic therapy previous month					NS	
Diabetes mellitus					NS	
Atopic history					NS	
Family with similar disease					NS	

MSSA = methicillin-susceptible *S. aureus*; PVL = Panton–Valentine leukocidin; ETs = exfoliative toxins (either in conjunction or independent exfoliative toxin A gene and exfoliative toxin B gene).
*Impetigo, ecthyma, erysipelas, furuncles, carbuncles, cellulitis, subcutaneous abscesses and paronychia.
†Secondary infections complicating pre-existing skin diseases or traumatic lesions.

Concordant with a previous study [19], multiple MLVA types within one ST were present; ST1301 included MT10, MT11, MT22 and MT23. We also found single locus variants: MT10 and MT11 (SIRU21), MT11 and MT22 (SIRU01), MT22 and MT23 (SIRU05), and two locus variants: MT10 and MT22 (SIRU01 and SIRU21) and MT10 and MT23 (SIRU01 and SIRU05), that indicates that these strains are possibly clonally related [28].

In the community setting, transmission between animals and humans of certain *S. aureus* clones may occur and may be difficult to prevent. In Denpasar, we isolated a *S. aureus* strain belonging to ST97, a clone that has been described in pigs and bovine animals in Europe and Japan [29–31]. However, the MLST profile of *S. aureus* carried by livestock in Indonesia has yet to be determined. To ascertain the level of the transmission of *S. aureus* from animal to human, further investigations on genetic background of livestock-associated *S. aureus* in Indonesia are needed.

This study yielded an MRSA clone represented by ST239-SCC*mec* type III that belonged to MT19,

suggesting penetration of a typical HA-MRSA clone into the community setting. However, all patients infected with this typical hospital clone reported not to have had hospital admission in the preceding 12 months. Therefore, this HA-MRSA might have gained a community foothold some time before this survey and can now be acquired through social contact in the community setting such as a day care centre or at home [32]. However, PVL and SCC*mec* PCR differentiating between CA- and HA-MRSA are not routinely performed.

According to the multivariate analysis, nasopharyngeal *S. aureus* carriage was associated with *S. aureus* SSTI, a finding similar to that in multiple previous studies on *S. aureus* carriage [33, 34]. Decolonisation may therefore be beneficial in such cases. Nevertheless, decolonisation of *S. aureus* nasal carriage to prevent recurrent SSTI using mupirocin nasal ointment remains controversial because of the cross-sectional design of the studies performed so far and because of the high prevalence of *S. aureus* carriage among patients without skin infection. Therefore, a causal relationship between carriage status and SSTI remains to be proven by prospective randomised trials of preventive eradication of *S. aureus* carriage [35].

Notably, an association between primary SSTI and either PVL- or exfoliative toxin-positive MSSA was found in this study. The association with PVL-positive MSSA was stronger (OR 5.4) than the association with exfoliative toxin-positive MSSA (OR 2.2), suggesting a more significant role for PVL in the pathogenesis of primary SSTI (Table 5). Other virulence factors of *S. aureus* might be associated with secondary SSTI, but further investigation is needed. Antibiotic therapy in the previous month was also associated with PVL-positive MSSA; however, we did not find any study reporting the effect of prior antibiotic therapy on the occurrence of PVL-encoding genes in *S. aureus*. Nevertheless, the effect of antibiotics on PVL released by PVL-producing *S. aureus* has been reported [36, 37].

The present study has certain limitations. First, data from three cities in Java and Bali cannot be considered to be representative for the whole country but may serve as point of reference. Therefore, a national surveillance system should be developed to obtain more representative and longitudinal data. Second, we did not ascertain the association of the many virulence factors of *S. aureus* other than PVL and exfoliative toxins to SSTI, for which further investigation is recommended. Third, due to limited resources, MLVA and MLST were not conducted for all *S. aureus* isolates; hence, the clonal relatedness of all strains causing SSTI could not be provided.

In summary, SSTI caused by either CA-MRSA or HA-MRSA was discovered in the community setting in Indonesia. The penetration of a typical HA-MRSA clone in the community setting probably mediated by undetected carriage deserves increased awareness of public health authorities. Promoting household hygiene in general and proper hand hygiene in particular may be a simple and cost-effective method for containing the spread of MRSA in the community; however, further investigation should be performed. Simultaneously reducing the use of antibiotics in ambulatory care would synergise with such efforts.

Acknowledgements

We thank the deans of the Faculty of Medicine, Brawijaya University, Malang, Indonesia, the Faculty of Medicine, Airlangga University, Surabaya, Indonesia, and the Faculty of Medicine, Udayana University, Denpasar, Indonesia, the directors of the Dr. Soetomo Hospital, Surabaya, Indonesia, the Sanglah Hospital, Denpasar, Indonesia and the heads of primary healthcare centres in Lawang, Singosari, Pakisaji, Sumberpucung and Gondanglegi, Malang, Indonesia who facilitated our work. We also thank Suyati Pujiani and Slamet Riyanto for their technical assistance. 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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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Healthcare facilities that participated in this study

Table S2. Types of skin and soft tissue infections presented by patients involved in this study

Table S3. List of antibiotics tested

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