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Drug resistance and genetic characteristics of clinical isolates of staphylococci in Myanmar: high prevalence of PVL among methicillin-susceptible *Staphylococcus aureus* belonging to various sequence types

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Abstract

Prevalence, drug resistance and genetic characteristics were analysed for a total of 128 clinical isolates of staphylococci obtained from a tertiary hospital in Myanmar. The dominant species were *S. aureus* (39%) and *S. haemolyticus* (35%), followed by *S. epidermidis* (6%) and *S. saprophyticus* (5%). The majority of *S. haemolyticus* isolates (71.1%) harboured *mec*A, showing high resistance rates to ampicillin, cephalosporins, erythromycin and levofloxacin, while methicillin-resistant *S. aureus* (MRSA) was only 8% (four isolates) among *S. aureus* with type IV SCC*mec*. Panton-Valentine leukocidin (PVL) genes were detected in 20 isolates of *S. aureus* (40%), among which only one isolate was MRSA belonging to sequence type (ST) 88/*agr*-III/*coa*-IIIa, and the other 19 methicillin-susceptible *S. aureus* (MSSA) isolates were classified into six STs (ST88, ST121, ST1153, ST1155, ST1930, ST3206). An ST1153 MSSA isolate with PVL was revealed to belong to a novel *coa* type, XIIIa. ST121 *S. aureus* was the most common in the PVL-positive MSSA (47%, 9/19), harbouring genes of bone sialoprotein and variant of elastin binding protein as a distinctive feature. Although PVL-positive MSSA was susceptible to most of the antimicrobial agents examined, ST1930 isolates were resistant to erythromycin and levofloxacin. ST59 PVL-negative MRSA and MSSA had more resistance genes than other MRSA and PVL-positive MSSA, showing resistance to more antimicrobial agents. This study indicated higher prevalence of *mec*A associated with multiple drug resistance in *S. haemolyticus* than in *S. aureus,* and dissemination of PVL genes to multiple clones of MSSA, with ST121 being dominant, among hospital isolates in Myanmar.

Keywords: MRSA, Myanmar, Panton-Valentine leukocidin (PVL), ST121, *Staphylococcus*
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Introduction

Staphylococci constitute one of the major normal flora in skin, nasal cavities and mucosal membranes of humans. However, they are known as common causes of various infections in both healthcare and community settings. While approximately 30% of healthy individuals are colonized with *Staphylococcus aureus* asymptomatically [1], this bacterium causes various infections, including skin and soft tissue infections (SSTI), bactaeremia and pneumonia. Healthcare-associated (HA) methicillin-resistant *Staphylococcus aureus* (MRSA) has been recognized as a primary cause of nosocomial infections that acquired multiple drug resistance, associated with its global spread since the 1960s [2]. Thereafter, community-acquired (CA) MRSA have also emerged as cause of infections in individuals who have no healthcare-associated risk [3,4], posing a public health concern worldwide. Coagulase-negative staphylococci (CNS),

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ubiquitously distributed to humans, have been also increasing as nosocomial pathogens mainly as a result of development of prosthetic devices and invasive medical technologies [5]. Representative species causing infections are *S. epidermidis* and *S. haemolyticus,* which often acquire drug resistance, including methicillin resistance *via* same genetic mechanism as that of MRSA.

Methicillin resistance of staphylococcus is characterized by the presence of a transmissible genome element, staphylococcal cassette chromosome *mec* (SCC*mec*), which is inserted in the chromosome of bacterial cell. SCC*mec* in MRSA has been differentiated into at least 11 genetic types (I–XI) [6,7], among which types I to III are commonly found in HA-MRSA, while type IV and V were reported to be frequently in CA-MRSA [3]. However, in the present circumstances, CA-MRSA with the dominant SCC*mec* types have been brought to healthcare settings [8–10], which makes distinction between HA- and CA-MRSA more difficult in terms of SCC*mec* type. The initially identified CA-MRSA strains were characterized by production of Panton-Valentine leukocidin (PVL), a two-component leukolytic toxin [11], which is associated with severe symptoms in a wide spectrum of infections [12,13], including SSTI and necrotizing pneumonia. Prevalence of CA-MRSA harbouring PVL genes has been increasing recently in hospitalized patients as well as healthy individuals in the community [14,15].

In Myanmar, *S. aureus* has been reported to be the major pathogen in bloodstream infections and the third most common bacteria in blood cultures from febrile children [16,17]. However, to our knowledge, there is no epidemiologic study on staphylococci from healthcare settings in Myanmar, and thus information is not available for drug resistance and genetic characteristics on recent clinical isolates of *S. aureus,* including MRSA, and CNS. Although we previously reported genetic traits of MRSA and methicillin-susceptible *S. aureus* (MSSA) isolates from hospital, community and food poisoning cases in Myanmar, the epidemiologic features was not determined because of the low numbers of isolates analysed [18]. In the present study, drug resistance and genetic traits, including prevalence of *mec*A, ACME (arginine catabolic mobile element) and PVL genes, was analysed for clinical isolates of staphylococci in a tertiary-care hospital in Myanmar.

Materials and methods

Bacterial isolates and initial genetic analysis

A total of 128 *Staphylococcus* strains were collected from patients admitted to North Okkalapa General Hospital, Yangon, Myanmar, between January 2012 and August 2013. The main specimen of the isolates was wound swab of surgical site infections (57%), followed by high vaginal swab (12%), blood (11%), pus (10%) and other specimens (sputum, urine, ear exudate) (10%). A single isolate from an individual patient was subjected to this study. Bacterial isolates grown on agar plates were examined by conventional microbiologic methods, and their species were determined by BBL Crystal Gram-Positive ID Kit (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA). Individual bacterial strains were stored in Microbank (Pro-Lab Diagnostics, Richmond Hill, ON, Canada) at −80°C and recovered when they were analysed.

The presence of staphylococcal 16S rRNA, *nuc,* *mec*A, PVL gene (*lukS-PV*/*lukF-PV*) and ACME-*arcA* (arginine deiminase gene) were detected for all isolates by multiplex PCR assay as described by Zhang *et al.* [19]. SCC*mec* type and ACME type were also determined by multiplex PCR using previously published primers and conditions [20,21].

Antimicrobial susceptibility testing

For major staphylococcal species, minimum inhibitory concentrations against 18 antimicrobial agents based on the broth microdilution test were measured by using Dry Plate 'Eiken' DP32 (Eiken Chemical, Tokyo, Japan) for Gram-positive cocci. Breakpoints defined in the Clinical Laboratory Standards Institute (CLSI) guidelines were used to distinguish between resistant and susceptible strains for most of the drugs examined [22].

Genetic typing, detection of virulence factors and drug resistance genes of *S. aureus*

The staphylocoagulase genotype (*coa* type) of *S. aureus* isolates was determined by multiplex PCR using previously published primers and conditions [23]. For the strains for which the *coa* types were not determined for I–X by the multiplex PCR, sequences of D1, D2 and the central region of *coa* were determined as described previously [24,25] to assign the *coa* genotype by sequence homology. Sequence identity to the known *coa* types was analysed by Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi). For selected isolates, sequence type (ST) was determined according to the scheme of multilocus sequence typing (MLST) [26], and *agr* group classification and protein A gene (*spa*) typing were performed as described previously [27,28].

The presence of genes encoding enterotoxins and other toxins, adhesins, other proteins related to virulence and antimicrobial resistance genes were analysed by multiplex or uniplex PCR using primers described previously [18]. Partial sequence of the gene encoding elastin-binding protein (*ebpS*) was determined by PCR and direct sequencing as described previously [18]. Multiple alignment of nucleotide and amino acid sequences determined was performed by the CLUSTAL W 2.1

program (DNA Data Bank of Japan (DDBJ), http://clustalw.ddbj.nig.ac.jp/).

Full-length staphylocoagulase gene (*coa*) sequence of strain MMR-v determined in the present study was deposited in the GenBank database under accession number KT599478.

Results

Among the 128 staphylococcal isolates obtained in the study period, the dominant species identified were *S. aureus* (*n* = 50, 39%) and *S. haemolyticus* (*n* = 45, 35%), followed by *S. epidermidis* (*n* = 8, 6%) and *S. saprophyticus* (*n* = 7, 5%). *S. aureus* and *S. haemolyticus* were isolated from wound swabs at high rates (65% and 62%, respectively), while *S. haemolyticus* was the main species among isolates from blood culture (43%, 6/14). The majority of *S. haemolyticus* (71%, 32/45) and *S. epidermidis* (75%, 6/8) possessed *mec*A, while the detection rate of MRSA was only 8% (4/50), and all the four MRSA had type IV SCC*mec* (Table 1). Although the SCC*mec* of some *S. haemolyticus* and *S. epidermidis* isolates was assigned to types IV and V, most of the isolates (71%, 27/38) were untypable. ACME-*arcA* was detected in two and one isolates of *S. haemolyticus* and *S. epidermidis,* respectively, and their ACME was classified into type II. PVL genes were detected in 20 *S. aureus* isolates (40%), among which only one isolate was MRSA. PVL-positive *S. aureus* were mostly isolated from pus or wound swabs (Table 2).

While the four MRSA isolates showed resistance to oxacillin and ampicillin, they were mostly susceptible to all other antimicrobial agents, except for gentamicin and erythromycin (Table 3). MSSA isolates were susceptible to most antimicrobial agents while showing low resistance rates to ampicillin, erythromycin, gentamicin and sulfamethoxazole/trimethoprim. In contrast, *mec*A-positive *S. haemolyticus* showed high resistance rates to ampicillin, cephalosporins, erythromycin and levofloxacin. Similar to MRSA, *mec*A-positive *S. epidermidis* were susceptible to most of the antimicrobial agents except for oxacillin. None of the staphylococcal isolates was resistant to vancomycin, linezolid and fosfomycin.

Among the 50 *S. aureus* isolates, ten staphylocoagulase (*coa*) genotypes were identified by multiplex PCR or sequencing, and the most common type was Va (*n* = 19), followed by VIIa (*n* = 8), VIa (*n* = 6), and IIIa and VIIb (*n* = 4) (Table 4). Full-length *coa* was determined for an MSSA strain MMR-v of which the *coa* type was untypable by the PCR assay. Sequence identity of *coa*-D1 region and -D2 plus central regions of MMR-v to the known 12 *coa* types were 64.7–70.3% and 69.7–89.2%, respectively (Supplementary Table S1). According to the criteria to determine *coa* type (subtype) proposed by Watanabe *et al.* [25], i.e. >90% identity of the D1 region (*coa* type) and >90% identity of the D1 and central region (*coa* subtype), the staphylocoagulase gene of MMR-v was considered not to be classified into the known 12 *coa* types. Therefore, a new *coa* type, XIIIa, was assigned to this strain. While MRSA belonged to three *coa* types (IIIa, IVb, VIIb), PVL-positive isolates were assigned to five *coa* types (IIIa, Va, VIa, VIIa, XIIIa), with Va being dominant, followed by VIa.

TABLE 2. Genotype (ST) of PVL-positive *Staphylococcus aureus* isolates

*mec*A	*coa* type	ST	CC[a]	No. of isolates	Specimen (*n*)
+	IIIa	ST88	CC88	1	Wound (1)
−	IIIa	ST88	CC88	2	Blood (1), wound (1)
−	Va	ST121	CC121	9	Pus (2), wound (7)
−	VIa	ST1930	CC96	3	Pus (1), wound (2)
−	VIa	ST3206	CC1	2	Pus (1), wound (1)
−	VIIa	ST1155	CC101	2	Pus (1), high vaginal swab (1)
−	XIIIa	ST1153	CC1153	1	Wound (1)

CC, clonal complex; PVL, Panton-Valentine leukocidin; ST, sequence type.
[a]CC of ST.

TABLE 1. Frequencies of isolates with PVL genes, ACME and *mecA* (SCC*mec* type) among different Staphylococcus species

Staphylococcus species	*mec*A	No. of isolates	PVL genes (+)	ACME-*arcA* (+) (ACME type)	SCC*mec* type			
					II	IV	V	NT
S. aureus (*n* = 50)	+	4	1	0		4		
	−	46	19	0				
S. haemolyticus (*n* = 45)	+	32	0	1 (ACMEII)		2	5	25
	−	13	0	1 (ACMEII)				
S. epidermidis (*n* = 8)	+	6	0	0		3	1	2
	−	2	0	1 (ACMEII)				
S. saprophyticus (*n* = 7)	+	1	0	0				1
	−	6	0	0				
Other (*n* = 18)	+	3[a]	0	0	1[b]			2
	−	15[c]	0	0				

PVL, Panton-Valentine leukocidin; NT, nontypeable.
[a]One isolate each of *S. hominis*, *S. sciuri* and *S. vitulinus*.
[b]*S. sciuri*.
[c]*S. auricularis* (1), *S. capitis* (1), *S. cohnii* (1), *S. hominis* (1), *S. kloosii* (3), *S. sciuri* (1), *S. vitulinus* (1), *S. warneri* (3), *S. xylosus* (3).

TABLE 3. Resistance rates of Staphylococcus species against antimicrobial agents

	Resistant isolates, *n* (%)					
	S. aureus		*S. haemolyticus*		*S. epidermidis*	
Antimicrobial agent[a]	*mecA*(+) (*n* = 4)	*mecA*(−) (*n* = 46)	*mecA*(+) (*n* = 32)	*mecA*(−) (*n* = 13)	*mecA*(+) (*n* = 6)	*mecA*(−) (*n* = 2)
OXA	4 (100)	0 (0)	29 (90.6)	4 (30.8)	6 (100)	1 (50)
FOX	1 (25)	0 (0)	28 (87.5)	2 (15.4)	0 (0)	2 (100)
AMP	4 (100)	15 (32.6)	28 (87.5)	1 (7.7)	1 (16.7)	0 (0)
CFZ	0 (0)	0 (0)	23 (71.9)	1 (7.7)	0 (0)	0 (0)
CMZ	0 (0)	0 (0)	17 (53.1)	1 (7.7)	0 (0)	1 (50)
FMX	0 (0)	0 (0)	7 (21.9)	2 (15.4)	0 (0)	1 (50)
IPM	0 (0)	0 (0)	14 (43.8)	0 (0)	0 (0)	0 (0)
GEN	2 (50)	6 (13)	25 (78.1)	0 (0)	1 (16.7)	1 (50)
ABK	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	1 (50)
MIN	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
ERY	2 (50)	7 (15.2)	30 (93.8)	6 (46.2)	0 (0)	2 (100)
CLI	1 (25)	6 (13)	6 (18.8)	3 (23.1)	1 (16.7)	1 (50)
VAN	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
TEC	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
LZD	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
FOF	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
LVX	1 (25)	3 (6.5)	28 (87.5)	1 (7.7)	3 (50)	1 (50)
STX	1 (25)	7 (15.2)	20 (62.5)	1 (7.7)	3 (50)	1 (50)

ABK, arbekacin; AMP, ampicillin; CFZ, cefazolin; CLI, clindamycin; CMZ, cefmetazole; ERY, erythromycin; FMX, flomoxef; FOF, fosfomycin; FOX, cefoxitin; GEN, gentamicin; IPM, imipenem; LVX, levofloxacin; LZD, linezolid; MIN, minocycline; OXA, oxacillin; SXT, sulfamethoxazole/trimethoprim; TEC, teicoplanin; VAN, vancomycin.
[a]Resistance to individual antimicrobial agents was judged according to Clinical Laboratory Standards Institute (CLSI) guidelines. For antimicrobial agents whose resistance is not defined by CLSI guidelines, European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (*Staphylococcus* spp., FOF, >32 μg/mL) and the following definitions (minimum inhibitory concentration) were used to determine resistance for *S. aureus* and *S. haemolyticus:* ABK, >4 μg/mL.

MLST was performed for 27 isolates, i.e. 20 PVL-positive and 7 PVL-negative *S. aureus* isolates, resulting in identification of 11 STs (Tables 2 and 5). ST3206 (CC1) of two PVL-positive MSSA isolates and ST3075 of a PVL-negative MSSA isolate were newly identified in the present study. PVL-positive isolates were differentiated into six STs (ST88, ST121, ST1153, ST1155, ST1930, ST3206), among which ST121 was dominant (nine isolates, 45% of PVL-positive *S. aureus*) and found in only MSSA, and other STs were identified in one to three isolates. A PVL-positive MRSA, strain MMR-42A, belonged to ST88, *coa* type IIIa, *agr* type III, and *spa* type t729. The other three MRSA isolates were classified into ST6 and ST59 (Table 5). ST88 was also identified in PVL-positive MSSA from blood and wound, which exhibited different patterns of toxin/virulence factors and drug resistance from those of a PVL-positive ST88 MRSA. ST121 PVL-positive MSSA isolates belonged to *coa* type Va and *agr* type IV, and harboured five to six enterotoxin genes, the bone sialoprotein gene (*bbp*), and a variant of the elastin binding protein gene (*ebpS-v*) with an internal 180 bp deletion as described previously [18]. The ST59 *S. aureus,* both MRSA (two strains) and MSSA (one strain) without PVL had more resistance genes (*ermB, aac(6′)-Ie-aph(2″)-Ia*) than other MRSA and PVL-positive MSSA, showing resistance to more antimicrobial agents.

TABLE 4. Frequencies of PVL and mecA genes among different coa genotype of Staphylococcus aureus isolates

coa type	No. of isolates	PVL(+)	*mecA*(+)
IIa	2		
IIIa	4	3	1
IVb	2		1
Va	19	9	
Vb	1		
VIa	6	5	
VIIa	8	2	
VIIb	4		2
Xa	3		
XIIIa	1	1	
Total	50	20	4

PVL, Panton-Valentine leukocidin.

Discussion

In the present study, prevalence and drug resistance of staphylococcal species and genetic traits of *S. aureus* were elucidated for clinical isolates in a tertiary hospital in Myanmar. Distinctive features in this study were the high prevalence and antimicrobial resistance trend of *S. haemolyticus,* the low rate of MRSA, and the high rate of PVL among MSSA.

Among CNS species, *S. haemolyticus* has been described as occasionally the second most frequent clinical isolates after *S. epidermidis,* causing primarily bloodstream infections associated with the use of central venous catheters [5]. In the present study, with a lower number of blood isolates (11%), the frequency of *S. haemolyticus* was comparable to *S. aureus* and higher than that of *S. epidermidis,* suggesting the significance of this species in skin infections as well. In agreement with the view of this species having a great capacity to develop resistance to multiple classes of antimicrobial agents [29,30], a high *mecA*-positive rate associated with high resistance rates to various

TABLE 5. Genotypes, virulence factors and drug resistance in 15 MSSA and MRSA strains

				Genotype								
***mecA*/ PVL genes**	**Strain ID**	**Age/ Sex**	**Specimen**	**SCC *mec***	***coa***	***agr***	**ST (CC)**	**Leukocidins, haemolysins[a]**	**Enterotoxins[b]**	**Adhesins and other[a]**	**Drug resistance gene[c]**	**Antimicrobial resistance pattern[d]**
PVL	MMR-20A	34/M	Blood		IIIa	III	ST88 (CC88)	*lukE-lukD, hla, hlg2*	*seq*	*sdrC, sdrD, sdrE, fib, clfB, ebpS*	*blaZ*	AMP
PVL	MMR-k	36/F	Wound		Va	IV	ST121 (CC121)	*lukE-lukD, hla, hlg2*	*seb, sei, sem, sen, seo*	*fib, clfB, cna, bbp, ebpS-v*	*blaZ*	AMP
PVL	MMR-6A	30/M	Pus		Va	IV	ST121 (CC121)	*lukE-lukD, hla, hlg2*	*seb, seg, sei, sem, sen, seo*	*fib, clfB, cna, bbp, ebpS-v*	*blaZ*	AMP
PVL	MMR-z0	38/M	Wound		VIa	III	ST1930 (CC96)	*lukE-lukD, hla, hlg2*	*sea, sec, sei, sel*	*sdrC, sdrD, sdrE, fib, clfB, ebpS, cna*	*blaZ, ermC*	AMP, ERY, LVX
PVL	MMR-46A	49/M	Wound		VIa	III	ST1930 (CC96)	*lukE-lukD, hla, hlg2*	*sea, sec, sei, sel*	*sdrC, sdrD, sdrE, fib, clfB, ebpS*	*blaZ, ermC*	AMP, ERY, LVX
PVL	MMR-30B	30/F	Pus		VIIa	I	ST1155 (CC101)	*lukE-lukD, hla, hlg2*		*sdrC, sdrD, sdrE, fib, clfB, ebpS, cna*		
PVL	MMR-v	55/F	Wound		XIIIa	II	ST1153 (CC1153)	*lukE-lukD, hla, hlg2*	*sec, sei, sel*	*sdrC, sdrD, sdrE, fib, clfB, ebpS*	*blaZ*	
–	MMR-g	46/F	Wound		VIIa	I	ST2549 (CC45)	*hla*	*sea, seg, sei, sem, sen, seo*	*sdrC, sdrD, sdrE, clfB, ebpS, cna*	*blaZ*	AMP, GEN
–	MMR-44B	53/M	Wound		VIIc	I	ST59 (CC59)	*hla, hlg2*	*seb, sek, seq*	*sdrC, sdrD, sdrE, fib, clfB, ebpS*	*blaZ, ermB, aac(6′)-Ie-aph(2″)-Ia, ant(6)-Ia*	AMP, GEN, ERY, CLI, LVX
–	MMR-a	43/M	Wound		VIIa	I	ST3075 (Singleton)	*hla, hlg2*	*sek*	*sdrC, sdrD, sdrE, fib, clfB, ebpS, cna*	*blaZ, ant(4′)-Ia, tet(K)*	AMP, GEN, MIN
–	MMR-14B	20/M	Blood		Xa	II	ST2990 (Singleton)	*lukE-lukD, hlg2*	*sec, sel*	*sdrC, sdrD, sdrE, fib, ebpS, cna, edin-B*	*blaZ*	AMP, ERY, CLI
*mec*A, PVL	MMR-42A	56/M	Wound	IV	IIIa	III	ST88 (CC88)	*lukE-lukD, hlg2*	*sei, sek, seq*	*sdrC, sdrD, sdrE, fib, clfB, ebpS*	*blaZ, tetK*	OXA, AMP, MIN
*mec*A	MMR-55B	25/F	High vaginal swab	IV	VIIb	I	ST59 (CC59)	*hla, hlg2*	*seb, sek, seq*	*sdrC, sdrD, sdrE, fib, clfB, ebpS*	*blaZ, ermB, aac(6′)-Ie-aph(2″)-Ia*	OXA, AMP, GEN, ERY, CLI, LVX
*mec*A	MMR-57B	66/F	Wound	IV	VIIb	I	ST59 (CC59)	*hla, hlg2*	*seb, sek, seq*	*sdrC, sdrD, sdrE, fib, ebpS*	*blaZ, ermB, aac(6′)-Ie-aph(2″)-Ia*	OXA, FOX, AMP, GEN, ERY, CLI, LVX, SXT
*mec*A	MMR-22B	20/F	Wound	IV	IVb	I	ST6 (CC6)	*lukE-lukD, hla, hlg2*	*sea*	*sdrC, sdrD, sdrE, fib, ebpS, cna*	*blaZ*	OXA, AMP

CC, clonal complex; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; PVL, Panton-Valentine leukocidin; ST, sequence type.
[a]The following genes were detected in all strains: *clfA, eno, fnbA, fnbB, hld, hlg* and *hlg2*. *ebpS-v* indicates *ebpS* gene with internal deletion as described previously [18].
[b]The following genes were not detected in any strain: *sed, see, seh, sej, sep, ser, ses, set, seu, eta, etb, etd* and *tst-1*.
[c]The following genes were undetectable in any strains: *tet(L), tet(M), ermA, msrA, aph(3′)-IIIa, acc(6′)-Ii, acc(6′)-Im, ant(9)-Ia, ant(9)-Ib, ant(3″)-Ia, aph(2″)-Ib, aph(2″)-Ic* and *aph(2″)-Id*.
[d]See Table 3 footnotes for abbreviations of antimicrobial agents and breakpoints for resistance. None of the strains showed resistance to arbekacin, cefazolin, cefmetazole, flomoxef, fosfomycin, teicoplanin, linezolid and vancomycin.

antimicrobial agents of *S. haemolyticus* was observed in the present study. Although the *mec*A-positive rate in *S. aureus* was still low, a high rate of methicillin-resistant *S. haemolyticus* as well as *S. epidermidis* may alert us to the potential increase of drug-resistant isolates among staphylococcal species, including MRSA.

In the present study, the detection rate of PVL genes among *S. aureus* was notably high (40%), which may be related to a high proportion of wound swabs and pus (67%) in the specimens examined. Detection of PVL genes in six different STs among 20 *S. aureus* isolates suggests dissemination of PVL phages to multiple clones, while only the dominant clone, ST121, appears to spread within hospitals. Strain MMR-42A is the first PVL-positive MRSA isolated in Myanmar, having SCC*mec*-IV and genetic types ST88/*spa*-t729/*agr*-III/*coa*-III. The ST88 MRSA with SCC*mec* IV or V has been reported in both community and hospital settings in Africa (mostly in East Africa; Tanzania and Madagascar) [31–33] and Asia (mostly in China) [15,34,35], and less frequently in Europe [36–38], exhibiting *agr* type III and various *spa* types, with t186 being dominant. The *spa* type t729 detected in strain MMR-42A is genetically closely related to t186 and was described also for ST88 MRSA isolates in Africa [38], suggesting close relatedness to the previously described ST88 clone. PVL is associated with a part of ST88 MRSA as well as MSSA. In Bangladesh and China, neighbouring countries to Myanmar, PVL-positive ST88 MSSA and/or MRSA was reported [34,39,40]. Detection of ST88 MRSA in Myanmar suggests the potential spread of this clone in Asia, and there should be concern in healthcare settings resulting from the presence of PVL in this clone.

ST121 MSSA, mostly belonging to *agr*-IV, are distributed worldwide (mainly Africa, Asia and Europe) as a common cause of SSTI, often associated with PVL, while MRSA with this ST is rare [41,42]. In our previous study in Myanmar on *S. aureus* isolates from wound/pus, food poisoning and healthy adults [18], PVL genes were detected in only ST121 MSSA strains with *coa*-Va/*agr*-IV from wounds in hospitalized patients. In the present study, ST121 was dominant among PVL-positive isolates and showed genetically identical traits to those of previous MSSA strains in Myanmar. Characteristically, ST121 *S. aureus* in Myanmar has been previously revealed to harbour the genes of bone sialoprotein and a variant of elastin binding protein with 180 bp deletion [18], which was also found in PVL-positive ST121 MSSA in the present study. Therefore, we suggest that a single ST121 PVL-positive *S. aureus* clone has been persisting as a cause of SSTI in Myanmar. Although virulence of ST121 MSSA might be increased with PVL and other toxins, this clone is generally susceptible to most antimicrobial agents. Thus, active promotion of early detection and treatment is recommended for infections with this clone in healthcare settings.

It is of note that a novel staphylocoagulase genotype, *coa* XIII, was assigned to a PVL-positive MSSA strain (MMR-v) belonging to the rare ST1153, which was isolated from 55-year-old patient with wound infection. The D1 and D2 regions of staphylocoagulase which define the *coa* genotype (subtype) is considered to be responsible for antibody recognition as well as contact with prothrombin [43,44]. Accordingly, genetic diversity of the D1/D2 regions is suggested to be caused by selection with antibody and/or prothrombin in the host. Hence, increased virulence is concerned with the emergence of *S. aureus* with the new *coa* type as a result of the absence of immune response against the novel antigen of the virulence factor. In the present study, ST1930 MSSA was resistant to erythromycin and levofloxacin, which is the distinctive feature of resistance among PVL-positive isolates. Although the significance of ST1930 MSSA is not evident, *S. aureus* with CC96, to which ST1930 belongs, is revealed to secrete variable to high levels of alpha toxin [45], suggesting relevance to the increased virulence. Therefore, the prevalence of the novel PVL-positive MSSA clones ST1153 and ST1930 should be carefully monitored in Myanmar.

Despite low MRSA rates among *S. aureus* in the present study, it was notable that ST59 was identified in two isolates with SCC*mec*-IV as well as an MSSA isolate. These isolates were PVL negative, however, resistant to multiple antimicrobial agents harbouring resistance genes such as *erm*(*B*). ST59 (CC59) MRSA has been classified into some groups [3,42], with PVL-positive strains with SCC*mec*-V predominating in Taiwan and other Asian countries (Taiwan clone), SCC*mec*-IV-harbouring PVL-positive strains known as USA1000 clone mostly restricted to the United States and PVL-negative (or positive) MRSA with SCC*mec*-IV or V in Australia. The ST59 PVL-negative MRSA-IV, with the same genetic traits as the ST59 isolates in the present study, was also detected at a high rate in the nasal cavities of children in Taiwan [46]. Thus, we suggest that ST59 *S. aureus* may be distributed widely in Asia as well as Australia and may occasionally acquire SCC*mec* and/or PVL phage, associated with their clonal spread. In Myanmar, caution may be necessary for the ST59 MRSA in hospitals regarding acquisition of PVL genes and more drug resistance.

In summary, the present study elucidated drug resistance and genetic traits of clinical isolates of staphylococci in a tertiary-care hospital in Myanmar. Further studies are needed in this country to survey the prevalence of methicillin-resistant CNS, MRSA and PVL-positive *S. aureus* and their drug resistance for control of staphylococcal infections in healthcare settings.

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Conflict of interest

None declared.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.nmni.2015.12.007.

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