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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 analyzed for a total of 128 clinical isolates of staphylococci obtained from a tertiary hospital in Myanmar. The dominant species was \textit{S. aureus} (39%) and \textit{S. haemolyticus} (35%), followed by \textit{S. epidermidis} (6%), and \textit{S. saprophyticus} (5%). Majority of \textit{S. haemolyticus} isolates (71.1%) harbored \textit{mecA}, showing high resistance rates to ampicillin, cephalosporins, erythromycin and levofloxacin, while methicillin-resistant \textit{S. aureus} (MRSA) was only 8% (4 isolates) among \textit{S. aureus} with type-IV SCC\textit{mec}. PVL genes were detected in 20 isolates of \textit{S. aureus} (40%), among which only one isolate was MRSA belonging to ST88/\textit{agr}-III/\textit{coa}-IIIa, and the other 19 methicillin-susceptible \textit{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 \textit{coa} type, XIIIa. ST121 \textit{S. aureus} was the most common in the PVL-positive MSSA (47%, 9/19), harboring genes of bone sialoprotein and variant of elastin binding protein as a distinctive feature. Although PVL-positive MSSA was susceptible to most of antimicrobials 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 antimicrobials. This study indicated higher prevalence of \textit{mecA} associated with multiple drug resistance in \textit{S. haemolyticus} than in \textit{S. aureus}, and dissemination of PVL genes to multiple clones of MSSA, with ST121 being dominant, among hospital isolates in Myanmar.
INTRODUCTION

Staphylococci constitute one of the major normal flora in skin, nasal cavities, and mucosal membrane of humans. However, they are known as common causes of various infections in healthcare settings as well as community. While approximately 30% of healthy individuals are colonized with *Staphylococcus aureus* asymptptomatically [1], this bacterium causes various infections, including skin and soft tissue infections (SSTI), bacteremia, pneumonia, and so forth. Health care-associated methicillin-resistant *Staphylococcus aureus* (HA-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 MRSA (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), ubiquitously distributed to humans, have been also increasing as nosocomial pathogens mainly due to development of prosthetic devices and invasive medical technologies [5]. Representative species causing infections are *S. epidermidis* and *S. haemolyticus*, which often acquire dug 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 (SCCmec) which is inserted in the chromosome of bacterial cell. SCCmec in MRSA has been differentiated into at least eleven genetic types (I-XI) [6,7], among which types I-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 SCCmec types have been brought to healthcare settings [8-10], which makes distinction between HA- and CA-MRSA more difficult in terms of SCCmec 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 harboring PVL genes has been increasing recently in hospitalized patients as well as healthy individuals in the community [14,15].

In Myanmar, *S. aureus* was reported as the major pathogen in bloodstream infections and the third most common bacteria in blood cultures from febrile children [16,17]. However, there is no epidemiological 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, epidemiological features was not determined because of low numbers of isolates analyzed [18]. In the present study, drug resistance and genetic traits including prevalence of *mecA*, ACME, and PVL genes, was analyzed for clinical isolates of staphylococci in a tertiary hospital in Myanmar.

**MATERIALS AND METHODS**

1. **Bacterial isolates and initial genetic analysis**

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

The presence of staphylococcal 16s rRNA, nuc, mecA, 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]. SCCmec type and ACME type were also determined by multiplex PCR using previously published primers and conditions [20,21].

2. Antimicrobial susceptibility testing

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

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

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 were analyzed by using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). For selected isolates, sequence type (ST)
was determined according to the scheme of MLST [26], and agr group classification and protein A gene (spa) typing were performed as described previously [27,28].

Presence of genes encoding enterotoxins and other toxins, adhesins, other proteins related to virulence, and antimicrobial resistance genes were analyzed 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 program ver. 2.1 which is available on the website of DNA Data Bank of Japan (DDBJ) (http://clustalw.ddbj.nig.ac.jp/).

GenBank accession numbers. 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 was 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). Majority of S. haemolyticus (71%, 32/45) and S. epidermidis (75%, 6/8) possessed mecA, while detection rate of MRSA was only 8% (4/50), and all the four MRSA had type IV SCCmec (Table 1). Although SCCmec of some S. haemolyticus and S. epidermidis isolates was assigned to type
IV and V, most of isolates (71%, 27/38) resulted in untypable. ACME-arcA was detected in two and an 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 swab (Table 2).

While the four MRSA isolates showed resistance to oxacillin and ampicillin, they were mostly susceptible to all other antimicrobials, except for gentamicin and erythromycin (Table 3). MSSA isolates were susceptible to most of antimicrobials, while showing low resistance rates to ampicillin, erythromycin, gentamicin, and sulfamethoxazole-trimethoprim. In contrast, mecA-positive S. haemolyticus showed high resistance rates to ampicillin, cephalosporins, erythromycin and levofloxacin. Similar to MRSA, mecA-positive S. epidermidis were susceptible to most of antimicrobials except for oxacillin. None of the staphylococcal isolates were 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 (19), followed by VIIa (8), VIa (6), and IIIa and VIIb (4) (Table 4). Full-length coa was determined for an MSSA strain MMR-v of which 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 twelve coa types were 64.7-70.3% and 69.7-89.2%, respectively (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), staphylocoagulase gene of MMR-v was considered not to be classified into the known twelve 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.
MLST was performed for 27 isolates, i.e., 20 PVL-positive and 7 PVL-negative *S. aureus* isolates, resulting in identification of 11 STs (Table 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 (9 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. 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 similar patterns of toxin/virulence factors and drug resistance to a PVL-positive ST88 MRSA. ST121 PVL-positive MSSA isolates belonged to coa type Va and agr type IV, and harbored 5-6 enterotoxin genes, and bone sialoprotein gene (*bbp*) and a variant of elastin binding protein gene (*ebpS-v*) with internal 180-bp deletion as described previously [18]. 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 antimicrobials.

**DISCUSSION**

In our 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 high prevalence and antimicrobial resistance trend of *S. haemolyticus*, low rate of MRSA, and 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 lower number of blood isolates (11%), frequency of *S. haemolyticus* was comparable to *S. aureus*, and higher than *S. epidermidis*, suggesting significance of this species also in skin infections. In agreement with the view of this species having great capacity to develop resistance to multiple classes of antimicrobials [29,30], high *mecA*-positive rate associated with high resistance rates to various antimicrobials of *S. haemolyticus* was observed in the present study. Although *mecA*-positive rate in *S. aureus* was still low, high rate of methicillin-resistant *S. haemolyticus* as well as *S. epidermidis* may alert potential increase of drug resistant isolates among staphylococcal species, including MRSA.

In the present study, detection rate of PVL genes among *S. aureus* was notably high (40%), which may be related to a high proportion of wound swab 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 the hospital. The 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. 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, neighboring countries to Myanmar, PVL-positive ST88 MSSA and/or MRSA was reported [34,39,40]. Detection of ST88 MRSA in Myanmar suggests potential spread of this
clone in Asia, and concern in healthcare settings due to presence of PVL in this clone.

ST121 MSSA, mostly belonging to agr-IV, are distributed worldwide (mainly Africa, Asia, 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-Val/agr-IV from wound 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 was previously revealed to harbor the genes of bone sialoprotein and variant of elastin binding protein with 180bp-deletion [18], which was also found in PVL-positive ST121 MSSA in the present study. Therefore, it is suggested 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 was of note that a novel staphylocoagulase genotype, coa XIII was assigned to a PVL-positive MSSA strain (MMR-v) belonging to rare ST1153, which was isolated from 55-year old patient with wound infection. D1 and D2 regions of staphylocoagulase which define coa genotype (subtype) is considered to be responsible for antibody recognition as well as contact with prothrombin [43,44]. Accordingly, genetic diversity of D1/D2 regions is suggested to be caused by selection with antibody and/or prothrombin in host. Hence, increased virulence is concerned for the emergence of S. aureus with the new coa type, due to absence of immune response against the novel antigen of the virulence factor. In the present study, ST1930 MSSA were resistant to erythromycin and levofloxacin, which is the distinctive feature of resistance among PVL-positive isolates. Although significance of the
ST1930 MSSA is not evident, *S. aureus* with CC96 to which ST1930 belongs is revealed to secrete variable to high level of alpha toxin [45], suggesting any relevance to increased virulence. Therefore, these novel PVL-positive MSSA clones, ST1153 and ST1930, should be carefully monitored for their prevalence in Myanmar.

Despite low MRSA rate among *S. aureus* in the present study, it was notable that ST59 was identified in two isolates with SCCmec-IV, as well as an MSSA isolate. These isolates were PVL-negative, however, resistant to multiple antimicrobial agents harboring resistance genes such as *erm(B)*. ST59 (CC59) MRSA have been classified into some groups [3,42]; PVL-positive strains with SCCmec-V predominating in Taiwan and other Asian countries (Taiwan clone), SCCmec-IV-harboring PVL-positive strains known as USA1000 clone mostly restricted to the US, and PVL-negative (or positive) MRSA with SCCmec-IV or V in Australia. The ST59 PVL-negative MRSA-IV, the same genetic traits as ST59 isolates in the present study, was detected also at high rate in nasal cavity of children in Taiwan [46]. Thus, it is suggested that ST59 *S. aureus* may be distributed widely in Asia as well as Australia, and occasionally acquire SCCmec 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 hospital in Myanmar. Further studies are needed in this country to survey prevalence of MR-CNS, MRSA, and PVL-positive *S. aureus* and their drug resistance for control of staphylococcal infections in healthcare settings.

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Conflicts of interest

None to declare.

REFERENCES

1. Gorwitz RJ, Kruszon-Moran D, McAllister SK, McQuillan G, McDougal LK, Fosheim GE, et al. Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001-2004. J Infect Dis 2008; 197:1226-34.

2. Chambers HF, Deleo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat Rev Microbiol 2009;7:629-41

3. David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 2010;23:616-87.

4. DeLeo FR, Otto M, Kreiswirth BM, Chambers HF. Community-associated meticillin-resistant *Staphylococcus aureus*. Lancet 2010; 375:1557-68.

5. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. Clin Microbiol Rev 2014;27:870-926

6. International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). Classification of staphylococcal cassette chromosome *mec* (SCCmec): guidelines for reporting novel SCCmec elements. Antimicrob Agents Chemother 2009;53:4961-7.

7. Shore AC, Deasy EC, Slickers P, Brennan G, O'Connell B, Monecke S, et al. Detection of staphylococcal cassette chromosome *mec* type XI carrying highly divergent *mecA*, *mecI*, *mecR1*, *blaZ*, and *ecr* genes in human clinical isolates of clonal complex 130 methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2011;55:3765-73.

8. Davis SL, Rybak MJ, Amjad M, Kaatz GW, McKinnon PS. Characteristics of patients with healthcare-associated infection due to SCCmec type IV methicillin-resistant *Staphylococcus aureus*. Infect Control Hosp Epidemiol 2006;27:1025-31

9. Popovich KJ, Weinstein RA, Hota B. Are community-associated methicillin-resistant...
Staphylococcus aureus (MRSA) strains replacing traditional nosocomial MRSA strains? Clin Infect Dis 2008;46:787-94.

10. Seybold U, Kourbatova EV, Johnson JG, Halvosa SJ, Wang YF, King MD, et al. Emergence of community-associated methicillin-resistant Staphylococcus aureus USA300 genotype as a major cause of health care-associated blood stream infections. Clin Infect Dis 2006;42:647-56.

11. Vanden Esch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, et al. Community-acquired methicillin-resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis 2003;9:978-84.

12. Baba T, Takeuchi F, Kuroda M, Yuzawa H, Aoki K, Oguchi A, et al. Genome and virulence determinants of high virulence community-acquired MRSA. Lancet 2002;359:1819-27.

13. Francis JS, Doherty MC, Lopatin U, Johnston CP, Sinha G, Ross T, et al. Severe community-onset pneumonia in healthy adults caused by methicillin-resistant Staphylococcus aureus carrying the Panton-Valentine leukocidin genes. Clin Infect Dis 2005;40:100-7.

14. Hetem DJ, Westh H, Boye K, Jarlov JO, Bonten MJ, Bootsma MC. Nosocomial transmission of community-associated methicillin-resistant Staphylococcus aureus in Danish Hospitals. J Antimicrob Chemother 2012;67:1775-80.

15. Yao D, Yu FY, Qin ZQ, Chen C, He SS, Chen ZQ, et al. Molecular characterization of Staphylococcus aureus isolates causing skin and soft tissue infections (SSTIs). BMC Infect Dis 2010;10:133.

16. Myat TO, Prasad N, Thinn KK, Win KK, Htike WW, Zin KN, et al. Bloodstream infections at a tertiary referral hospital in Yangon, Myanmar. Trans R Soc Trop Med Hyg 2014;108:692-8.

17. Shwe TN, Nyein MM, Yi W, Mon A. Blood culture isolates from children admitted to Medical Unit III, Yangon Children’s Hospital, 1998. Southeast Asian J Trop Med Public Health. 2002;33:764-71.

18. Aung MS, Urushibara N, Kawaguchiya M, Aung TS, Mya S, San T, Virulence factors and genetic characteristics of methicillin-resistant and -susceptible Staphylococcus aureus isolates in Myanmar. Microb Drug Resist 2011;17:525-35.

19. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for simultaneous identification of community-associated methicillin-resistant Staphylococcus aureus strains USA300 and USA400 and detection of mecA and panton-valentine leukocidin genes, with discrimination of Staphylococcus aureus from coagulase-negative
20. Barbier F, Lebeaux D, Hernandez D, Delannoy AS, Caro V, François P, et al. High prevalence of the arginine catabolic mobile element in carriage isolates of methicillin-resistant *Staphylococcus epidermidis*. J Antimicrob Chemother 2011;66: 29-36.

21. Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J et al. Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: Rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. Antimicrob Agents Chemother 2007; 51: 264-74.

22. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Second Informational Supplement M100-S22. CLSI, Wayne, PA, USA; 2012.

23. Hirose M, Kobayashi N, Ghosh S, Paul SK, Shen T, Urushibara N, et al. Identification of staphylocoagulase genotypes I-X and discrimination of type IV and V subtypes by multiplex PCR assay for clinical isolates of *Staphylococcus aureus*. Jpn J Infect Dis 2010;63: 257-63.

24. Kinoshita M, Kobayashi N, Nagashima S, Ishino M, Otokozawa S, Mise K, et al. Diversity of staphylocoagulase and identification of novel variants of staphylocoagulase gene in *Staphylococcus aureus*. Microbiol Immunol 2008;52:334-48.

25. Watanabe S, Ito T, Sasaki T, Li S, Uchiyama I, Kishii K, et al. Genetic diversity of staphylocoagulase genes (coa): insight into the evolution of variable chromosomal virulence factors in *Staphylococcus aureus*. PLoS One 2009;4:e5714.

26. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 2000;38:1008-15.

27. Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, et al. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J. Clin. Microbiol. 1999;37:3556-63.

28. Strommenger B, Cuny C, Werner G, Witte W. Obvious lack of association between dynamics of epidemic methicillin-resistant *Staphylococcus aureus* in central Europe and *agr* specificity groups. Eur J Clin Microbiol Infect Dis 2004;23:15-9.

29. Tabe Y, Nakamura A, Oguri T, Igari J. Molecular characterization of epidemic multiresistant *Staphylococcus haemolyticus* isolates. Diagn Microbiol Infect Dis 1998;32:177-83.

30. Takeuchi F, Watanabe S, Baba T, Yuzawa H, Ito T, Morimoto Y, et al. Whole-genome sequencing of *Staphylococcus haemolyticus* uncovers the extreme plasticity of its genome
and the evolution of human-colonizing staphylococcal species. J Bacteriol 2005;187:7292-308.

31. Abdulgader SM, Shittu AO, Nicol MP, Kaba M. Molecular epidemiology of Methicillin-resistant *Staphylococcus aureus* in Africa: a systematic review. Front Microbiol 2015;6:348.

32. Breurec S, Zriouil SB, Fall C, Boisier P, Brisse S, Djibo S, et al. Epidemiology of methicillin-resistant *Staphylococcus aureus* lineages in five major African towns: emergence and spread of atypical clones. Clin Microbiol Infect 2011;17:160-5.

33. Moremi N, Mshana SE, Kamugisha E, Kataraihya J, Tappe D, Vogel U, et al. Predominance of methicillin resistant *Staphylococcus aureus* -ST88 and new ST1797 causing wound infection and abscesses. J Infect Dev Ctries 2012;6:620-5.

34. Yu F, Chen Z, Liu C, Zhang X, Lin X, Chi S, et al. Prevalence of *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes among isolates from hospitalised patients in China. Clin Microbiol Infect 2008;14:381-4.

35. Zhang W, Shen X, Zhang H, Wang C, Deng Q, Liu L, et al. Molecular epidemiological analysis of methicillin-resistant *Staphylococcus aureus* isolates from Chinese pediatric patients. Eur J Clin Microbiol Infect Dis 2009;28:861-4.

36. Denis O, Deplano A, De Beenhouwer H, Hallin M, Huysmans G, Garrino MG, et al. Polyclonal emergence and importation of community-acquired methicillin-resistant *Staphylococcus aureus* strains harbouring Panton-Valentine leucocidin genes in Belgium. J Antimicrob Chemother 2005;56:1103-6.

37. Krziwanek K, Metz-Gercek S, Mittermayer H. Trends in the occurrence of MRSA strains in Upper Austria from 2006 to 2009. Clin Microbiol Infect 2011;17:920-3.

38. Vindel A, Trincado P, Cuevas O, Ballesteros C, Bouza E, Cercenado E. Molecular epidemiology of community-associated methicillin-resistant Staphylococcus aureus in Spain: 2004-12. J Antimicrob Chemother 2014;69:2913-9.

39. Afroz S, Kobayashi N, Nagashima S, Alam MM, Hossain AB, Rahman MA, et al. Genetic characterization of *Staphylococcus aureus* isolates carrying Panton-Valentine leukocidin genes in Bangladesh. 2008;Jpn J Infect Dis 2008;61:393-6.

40. Paul SK, Ghosh S, Kawaguchiya M, Urushibara N, Hossain MA, Ahmed S, et al. Detection and genetic characterization of PVL-positive ST8-MRSA-IVa and exfoliative toxin D-positive European CA-MRSA-Like ST1931 (CC80) MRSA-IVa strains in Bangladesh. Microb Drug Resist 2014;20:325-36.

41. Monecke S, Slickers P, Ellington MJ, Kearns AM, Ehrlich R. High diversity of Panton-Valentine leukocidin-positive, methicillin-susceptible isolates of *Staphylococcus aureus* and implications for the evolution of community-associated methicillin-resistant *S.
42. Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M, et al. A field guide to
pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*.
PLoS One 2011;6:e17936.

43. Phonimdaeng P, O'Reilly M, Nowlan P, Bramley AJ, Foster TJ. The coagulase of
*Staphylococcus aureus* 8325-4. Sequence analysis and virulence of site-specific
coagulase-deficient mutants. Mol Microbiol 1990;4:393-404.

44. Watanabe S, Ito T, Takeuchi F, Endo M, Okuno E, Hiramatsu K. Structural comparison of
ten serotypes of staphylocoagulases in *Staphylococcus aureus*. J Bacteriol
2005;187:3698-707.

45. Monecke S, Müller E, Büchler J, Stieber B, Ehricht R. *Staphylococcus aureus* in vitro
secretion of alpha toxin (hla) correlates with the affiliation to clonal complexes. PLoS One
2014;9(6):e100427.

46. Huang YC, Hwang KP, Chen PY, Chen CJ, Lin TY. Prevalence of methicillin-resistant
*Staphylococcus aureus* nasal colonization among Taiwanese children in 2005 and 2006. J
Clin Microbiol 2007;45:3992-5
**Table 1** Frequencies of isolates with PVL genes, ACME, and mecA (SCCmec type) among different staphylococcal species

| Staphylococcal species | mecA | No. of isolates | PVL genes (+) | ACME-arcA (+) (ACME type) | SCCmec type |
|------------------------|------|----------------|---------------|---------------------------|-------------|
|                        |      |                |               |                           | II          |
| S. aureus (n=50)       | +    | 4              | 1             | 0                         | 4           |
|                        | -    | 46             | 19            | 0                         |             |
| S. haemolyticus (n=45) | +    | 32             | 0             | 1(ACMEII)                 | 2           |
|                        | -    | 13             | 0             | 1(ACMEII)                 | 5           |
| S. epidermidis (n=8)   | +    | 6              | 0             | 0                         | 3           |
|                        | -    | 2              | 0             | 0                         | 1           |
| S. saprophyticus (n=7) | +    | 1              | 0             | 0                         | 1           |
|                        | -    | 6              | 0             | 0                         |             |
| others (n=18)          | +    | 3*1           | 0             | 0                         | 2           |
|                        | -    | 15*2          | 0             | 0                         |             |

*1 One isolate each of S. hominis, S. sciuri, and S. vitulus

*2 S. auricularis (1), S. capitis (1), S. cohnii (1), S. hominis (1), S. kloosii (3), S. sciuri (1), S. vitulus (1), S. warneri (3), S. xylosus (3)

*3 S. sciuri

**Table 2** Genotype (ST) of PVL-positive S. aureus isolates

| mecA | coa type | ST   | CC*  | No. of isolates | specimen (nos.) |
|------|----------|------|------|-----------------|-----------------|
| +    | 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), HVS (1) |
| -    | XIIIa    | ST1153| CC1153| 1               | wound (1)       |

* clonal complex of ST
### Table 3  Resistance rates of staphylococcal species against antimicrobial agents

| Antimicrobial agents | **S. aureus** | **S. haemolyticus** | **S. epidermidis** |
|----------------------|--------------|---------------------|-------------------|
|                      | **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)           | 0 (0)             | 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)             |
| L2D                  | 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)            |

*Abbreviations: OXA, Oxacillin; FOX, Cefoxitin; AMP, Ampicillin; CFZ, Cefazolin; CMZ, Cefmetazole; FMX, Flomoxef; IPM, Imipenem; GEN, Gentamicin; ABK, Arbekacin; MIN, Minocycline; ERY, Erythromycin; CLI, Clindamycin; VAN, Vancomycin; TEC, Teicoplanin; L2D, Linezolid; FOI, Fosfomycin; LVX, Levofloxacin; SXT, Sulfamethoxazole-Trimethoprim.*

Resistance to individual antimicrobial agent was judged according to the guidelines of Clinical Laboratory Standards Institute (CLSI). For antimicrobials whose resistance is not defined by CLSI guidelines, EUCAST breakpoints (staphylococcus spp.: FOI, >32µg/ml) and the following definitions (MIC) were employed to determine resistance for *S. aureus*; *S. haemolyticus*: ABK, >4 µg/ml.

### Table 4  Frequencies of PVL and mecA genes among different coa genotype of *S. aureus* isoaltes

| coa type | No. of isolates | PVL(+) | mecA (+) |
|----------|-----------------|--------|----------|
| IIa      | 2               |        | 1        |
| IIIa     | 4               | 2      | 1        |
| Ivb      | 2               |        | 1        |
| Va       | 19              | 9      |          |
| Vb       | 1               |        |          |
| VIa      | 6               | 5      |          |
| VIIa     | 8               | 2      |          |
| VIIb     | 4               | 2      |          |
| X        | 3               |        |          |
| XIII     | 1               | 1      |          |

**Total** | 50 | 20 | 4

*Abbreviations: PVL, Panton-Valentine leukocidin; mecA, meca prophage*
Table 5: Genotypes, virulence factors and drug resistance in the 15 MSSA and MRSA strains. The following genes were undetectable in any strains: tet(L), tet(M), ermA, msrA, aph(3')qIIIa, acc(6')qIi, acc(6')qIm, ant(9)qIa, ant(9)qIb, ant(3'')qIa, aph(2'')qIb, aph(2'')qIc and aph(2'')qId. None of the strains showed resistance to arbekacin, cefazolin, cefmetazole, flomoxef, fosfomycin, teicoplanin, linezolid and vancomycin.

| Genotype | meA/PVL | genes | Strain ID | Age/Sex | Specimen | Leukocidins, haemolysins | Drug resistance gene | Antimicrobial resistance pattern |
|----------|---------|-------|-----------|---------|-----------|------------------------|---------------------|----------------------------------|
| meA/PVL  |         |       | PVL MMRq20A 34/F Blood Va IV ST88 (CC88) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, blaZ | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, blaZ | | | |
| meA/PVL  |         |       | PVL MMRq6A 30/M Blood Va IV ST121 (CC121) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, blaZ | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, blaZ | | | |
| meA/PVL  |         |       | PVL MMRqz0 38/M Wound Va IV ST121 (CC121) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, blaZ | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, blaZ | | | |
| meA/PVL  |         |       | PVL MMRq46A 49/M Wound Va IV ST121 (CC121) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, blaZ | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, blaZ | | | |
| meA/PVL  |         |       | PVL MMRq30B 30/F Wound Va IV ST1155 (CC121) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, cna | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, cna | | | |
| meA/PVL  |         |       | PVL MMRq55B 55/F Wound Va IV ST1153 (CC121) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, blaZ | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, blaZ | | | |
| meA/PVL  |         |       | PVL MMRq44B 53/M Wound Va IV ST1155 (CC121) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, cna, blaq | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, cna, blaq | | | |
| meA/PVL  |         |       | PVL MMRqa 43/M Wound Va IV ST1155 (CC121) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, cna, blaq | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, cna, blaq | | | |
| meA/PVL  |         |       | PVL MMRq14B 20/M Blood Va IV ST1155 (CC121) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, ebpS, cna | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, ebpS, cna | | | |
| meA/PVL  |         |       | PVL MMRq22B 20/F Wound Va IV ST1155 (CC121) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, ebpS, cna | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, ebpS, cna | | | |
| meA/PVL  |         |       | PVL MMRq42A 56/M Wound Va IV ST1155 (CC121) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, cna, blaq, tetK | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, cna, blaq, tetK | | | |
| meA/PVL  |         |       | PVL MMRq57B 66/F Wound Va IV ST1155 (CC121) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, ebpS, cna, blaq, OXA, AMP | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, cna, blaq, OXA, AMP | | | |
| meA/PVL  |         |       | PVL MMRq22B 20/F Wound Va IV ST1155 (CC121) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, ebpS, cna | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, ebpS, cna | | | |
| meA/PVL  |         |       | PVL MMRq42A 56/M Wound Va IV ST1155 (CC121) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, cna, blaq | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, cna, blaq | | | |
| meA/PVL  |         |       | PVL MMRq57B 66/F Wound Va IV ST1155 (CC121) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, ebpS, cna, blaq, OXA, AMP | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, cna, blaq, OXA, AMP | | | |
| meA/PVL  |         |       | PVL MMRq22B 20/F Wound Va IV ST1155 (CC121) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, ebpS, cna | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, ebpS, cna | | | |
| meA/PVL  |         |       | PVL MMRq42A 56/M Wound Va IV ST1155 (CC121) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, cna, blaq | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, cna, blaq | | | |
| meA/PVL  |         |       | PVL MMRq57B 66/F Wound Va IV ST1155 (CC121) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, ebpS, cna, blaq, OXA, AMP | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, clfB, ebpS, cna, blaq, OXA, AMP | | | |
| meA/PVL  |         |       | PVL MMRq22B 20/F Wound Va IV ST1155 (CC121) | lukE, lukD, hla, hlg2, seq, sdrC, sdrD, sdrE, fib, ebpS, cna | AMP | LukD, lukE, seq, sdrC, sdrD, sdrE, fib, ebpS, cna | | | |
Table 1  Frequencies of isolates with PVL genes, ACME, and mecA (SCCmec type) among different staphylococcal species

| Staphylococcal species | mecA | No. of isolates | PVL genes (+) | ACME-arcA (+) (ACME type) | SCCmec 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                         |             |
| others (n=18)          | +    | 3<sup>1</sup>  | 0            | 0                         | 1<sup>3</sup> 2   |
|                        | -    | 15<sup>2</sup> | 0            | 0                         |             |

<sup>1</sup> One isolate each of *S. hominis*, *S. sciuri*, and *S. vitulus*

<sup>2</sup> *S. auricularis* (1), *S. capitis* (1), *S. cohnii* (1), *S. hominis* (1), *S. kloosi* (3), *S. sciuri* (1), *S. vitulus* (1), *S. warneri* (3), *S. xylosus* (3)

<sup>3</sup> *S. sciuri*
Table 2 Genotype (ST) of PVL-positive *S. aureus* isolates

| meca | coa type | ST   | CC*  | No. of isolates | specimen (nos.) |
|------|----------|------|------|-----------------|-----------------|
| +    | 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), HVS (1)  |
| -    | XIIIa    | ST1153| CC1153| 1             | wound (1)       |

* clonal complex of ST
Table 3  Resistance rates of staphylococcal species against antimicrobial agents

| Antimicrobial agents\(^a\) | \(mecA^+\) (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)           |

\(^a\)Abbreviations : OXA, Oxacillin; FOX, Cefoxitin; AMP, Ampicillin; CFZ, Cefazolin; CMZ, Cefmetazole; FMX, Flomoxef; IPM, Imipenem; GEN, Gentamicin; ABK, Arbekacin; MIN, Minocycline; ERY, Erythromycin; CLI, Clindamycin; VAN, Vancomycin; TEC, Teicoplanin; LZD, Linezolid ; FOF, Fosfomycin ; LVX, Levofloxacin; SXT, Sulfamethoxazole-Trimethoprim.

Resistance to individual antimicrobial agent was judged according to the guidelines of Clinical Laboratory Standards Institute (CLSI). For antimicrobials whose resistance is not defined by CLSI guidelines, EUCAST breakpoints (staphylococcus spp.: FOF, >32µg/ml) and the following definitions (MIC) were employed to determine resistance for \(S.aureus\), \(S.haemolyticus\): ABK, >4µg/ml.
Table 4  Frequencies of PVL and *mecA* genes among different *coa* genotype of *S. aureus* islates

| *coa* type | No. of isolates | PVL(+) | *mecA* (+) |
|------------|----------------|--------|------------|
| IIIa       | 2              |        |            |
| IIIa       | 4              | 2      | 1          |
| IVb        | 2              |        | 1          |
| Va         | 19             |        | 9          |
| Vb         | 1              |        |            |
| Vla        | 6              | 5      |            |
| VIIa       | 8              |        | 2          |
| VIIb       | 4              |        | 2          |
| X          | 3              |        |            |
| XIII       | 1              |        | 1          |
| **Total**  | **50**         | **20** | **4**      |
### Table 5: Genotypes, virulence factors and drug resistance in the 15 MSSA and MRSA strains

| mec/PVL genes | Strain ID | Age/Sex Specimen | Leukocidins, haemolysins | Enterotoxins | Adhesins and others | Drug resistance gene | Antimicrobial resistance pattern |
|---------------|-----------|------------------|--------------------------|--------------|-------------------|---------------------|-----------------------------|
| mecA/PVL     | MMR-20A   | 34/M Blood       | IIIa III ST88 (CC38)     | 44E-44I, hla, hlg2 | se1               | adk, adh1, sde, fib, clfB, ebpS | blaZ | AMP |
|               | MMR-24A   | 36/F Wound       | Va IV ST121 (CC211)     | 44E-44D, hla, hlg2 | se2, sei, ser, sei, sero | fib, clfB, cna, sde, ebpS-v | blaZ | AMP |
|               | MMR-6A    | 30/M Pus         | Va IV ST121 (CC211)     | 44E-44A, hla, hlg2 | sei, sei, ser, ser, sero | fib, clfB, cna, sde, ebpS-v | blaZ | AMP |
|               | MMR-20D   | 38/M Wound       | Va III ST1930 (CC96)    | 44E-44D, hla, hlg2 | sei, sei, ser, sero | fib, clfB, cna, sde, ebpS, cna | blaZ, ermC | AMP, ERY, LVX |
|               | MMR-46A   | 49/M Wound       | Va III ST1930 (CC96)    | 44E-44D, hla, hlg2 | sei, sei, ser, sero | fib, clfB, cna, sde, ebpS | blaZ, ermC | AMP, ERY, LVX |
|               | MMR-30B   | 30/M Pus         | Va I ST1155 (CC101)     | 44E-44D, hla, hlg2 | sei, sei, ser, sero | fib, clfB, cna, sde, ebpS, cna | blaZ | AMP |
|               | MMR-55B   | 55/F Wound       | XII II ST1153 (CC1153)  | 44E-44G, hla, hlg2 | sec, sei, sel     | adk, adh1, sde, fib, clfB, ebpS | blaZ | AMP, GEN |
|               | MMR-42A   | 56/M Wound       | X VA ST2349 (CC45)      | 44E-44D, hlg2  | sei, sei, ser     | adk, adh1, sde, fib, clfB, ebpS, cna | blaZ | AMP, GEN |
|               | MMR-22A   | 20/F Blood       | XII ST2390 (Singleton)  | 44E-44D, hlg2  | sei, sei, ser     | adk, adh1, sde, fib, clfB, ebpS, cna, dmB | blaZ | AMP, ERY, CLI |
|               | MMR-42A   | 56/M Wound       | IIIa III ST88 (CC38)    | 44E-44D, hlg2  | sei, sei, ser     | adk, adh1, sde, fib, clfB, ebpS, cna, dmB | blaZ, tetK | OXA, AMP, MIN |
|               | MMR-55B   | 25/F IV SIV       | III ST59 (CC39)         | 44E-44D, hlg2  | sei, sei, ser     | adk, adh1, sde, fib, clfB, ebpS, cna, dmB | blaZ, tetK | OXA, AM, GEN, ERY, CLI, LVX |
|               | MMR-57B   | 66/F Wound       | III ST59 (CC39)         | 44E-44D, hlg2  | sei, sei, ser     | adk, adh1, sde, fib, clfB, ebpS, cna, dmB | blaZ, tetK | OXA, FOX, AM, GEN, ERY, CLI, LVX, SXT |
|               | MMR-22B   | 20/F Blood       | IV ST6 (CC6)            | 44E-44D, hlg2  | sei, sei, ser     | adk, adh1, sde, fib, clfB, ebpS, cna, dmB | blaZ | OXA, AMP |

*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: adk, adh1, sde, sei, ser, sei, sero, sei, ser, sei, sero.

*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 footnotes of Table 3 for abbreviations of antimicrobials and breakpoints for resistance. None of the strains showed resistance to arbekacin, carbenicillin, cetramicillin, flomoxef, fosfomycin, teicoplanin, linezolid and vancomycin.

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].
Table S1. Nucleotide sequence identities of staphylocoagulase gene between *S. aureus* strain MMR-v and strains with established *coa* types

| Strain   | *coa* type | Region of staphylocoagulase* |
|----------|------------|------------------------------|
|          |            | D1  | D2-C | Whole N-terminal region |
| 104      | Ia         | 69.7 | 83.7 | 79.0 |
| 213      | IIa        | 66.5 | 81.3 | 76.3 |
| SH682    | IIIa       | 64.7 | 80.4 | 74.9 |
| Stp-28   | IVa        | 67.3 | 82.4 | 77.3 |
| No.55    | Va         | 65.9 | 76.0 | 72.7 |
| Stp-12   | VIa        | 65.6 | 80.1 | 75.5 |
| JCSC4796 | VIIa       | 67.8 | 89.2 | 82.5 |
| Ku       | VIIIa      | 70.3 | 76.0 | 74.1 |
| 17573    | IXa        | 67.2 | 80.8 | 76.3 |
| 19       | Xa         | 69.0 | 85.3 | 79.9 |
| JCSC6075 | X1a        | 68.7 | 81.4 | 77.1 |
| JCSC1469 | XIIa       | 68.3 | 69.7 | 69.0 |

*D1, D2: divergent regions located at N-terminal side of staphylocoagulase. C: central region.
Whole N-terminal region: D1, D2, and C*