Genotypic and phenotypic characterization of methicillin resistance determinants and β-lactamase in Staphylococcus species

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ABSTRACT

Aims: To characterize the genotypic distribution of mec complex, bla complex, methicillin-resistance level (cefoxitin-MIC) and β-lactamase activity in carriage methicillin-resistant Staphylococcus species for a potential correlation.

Methodology and results: Biochemical test, 30 µg cefoxitin diffusion disc test, cefoxitin E-test, mec and bla complexes distributions, Pbp2a and β-lactamase assays were conducted to characterize phenotypic and genotypic of MRSA and MRCoNS in our collection. Phylogenetic tree was constructed using MEGA6 software to trace the diversity of blaZ gene of MRSA and MRCoNS. Sixteen MRSA and nineteen MRCoNS were identified by biochemical tests followed by 30 µg cefoxitin antibiotic disc susceptibility test and mecA gene screening. Twenty nine isolates carry complete mecA genes (2.1 kb), incomplete mec regulator (negative or truncated) and positive Pbp2a assay for both MRSA and MRCoNS. Only MRCoNS SC177 isolate with cefoxitin MIC of 32 µg/mL carries complete mec complex. Thirty-one of thirty-five isolates carry complete bla complex (blaZ, blaRI, blal) with 10 MRSA produce strong β-lactamase and cefoxitin MIC of ≥12 µg/mL. Only 4 MRCoNS with cefoxitin MIC of ≤8 µg/mL produce strong β-lactamase. The diversity of blaZ gene was demonstrated by phylogenetic analysis and unusual amino acid mutation at position 145 for MRSA SA60 isolate may compromise its β-lactamase activity with low cefoxitin MIC level (2 µg/mL).

Conclusions, significance and impact of the study: Isolates that carry complete mecA gene were largely consistent with the expression of Pbp2a. Nevertheless, there is no clear correlation of mec regulator genes in relation to cefoxitin-MIC in both methicillin resistant (MR) isolates that carry Staphylococcus species. On the other hand, various expression level of β-lactamase may correlate with cefoxitin-MIC level in MRSA as compared to MRCoNS.

Keywords: cefoxitin-MIC, mec complex, bla complex, β-lactamase, MR Staphylococcus

INTRODUCTION

Methicillin-resistant Staphylococcus species are resistant toward β-lactam antibiotics and cause a variety of problems such as skin and bloodstream infections, and pneumonia worldwide (Ahlstrand et al., 2011). The complexity of the Staphylococcal Chromosomal Cassette (SCC) mec gene structure that carried with it methicillin-resistance determinant gene named as mecA and its regulatory gene regimen (mecRI and mecI) in Staphylococcus species remains to be elucidated. MecA gene encodes for Pbp2a and serves as a benchmark to diagnose the presence of methicillin-resistant Staphylococcus since it is highly conserved among Staphylococcus species (Al-Abbas, 2012). Regulation of mecA is controlled by mec regulator genes consisting mecRI (transmembrane inducer) and mecI (mec repressor). Both are located immediately upstream of the mecA promoter. mecI encodes a repressor for mecA gene. In the absence of β-lactam antibiotics, it binds mecA promoter and blocks the transcription of mecA gene. MecRI will induce the detachment of MecI by proteolytic cleavage in the presence of β-lactam antibiotics (Arède et al., 2012). However, the regulatory mec structure seems unstable with the varying length of mecI and mecRI

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nucleotide sequences due to truncation and mutation events (Shore and Coleman, 2013).

Previous reports suggested that the expression of methicillin resistance may correlate with over production of β-lactamase, which would partially hydrolyze methicillin and related penicillins (McDougal and Thornsberry 1986; Massiddaa et al., 2006; Olayinka et al., 2009). The enzyme is encoded by blaZ, which is part of bla complex consisting of blaI and blaRI. Bla complex (blaI-blaRI-blaZ) has a similar gene orientation as of mec complex (mecI-mecRI-mecA) (Arède et al., 2013). Interestingly, mecI and blaI have a similar structure and function although they only share 60% nucleotide sequence homology (Garcia-Castellanos, 2003; Safo et al., 2005). Both repressors act interchangeably by binding to the promoter region of either blaZ or mecA operon. On the other hand, the transmembrane inducers (blaRI and mecRI) of the respective operons have a different action: blaRI only reacts with blaI and mecRI only cleaves mecI (McKinney et al., 2001). Although, the respective regulator systems are responsible for their own blaZ or mecA transcription process, a previous study demonstrated that presence of bla locus is associated with a dramatical increase of mecA transcription and the mechanism remains unknown (Katayama et al., 2003).

Some studies also claimed that overexpression of Pbp2a in methicillin-resistant strains doesn’t guarantee that the strains will have a high resistance against methicillin (De Lencastre et al., 1994; Rohrer et al., 2003; Plata et al., 2009). This event usually occurs in methicillin-resistant Staphylococcus species with cefoxitin-MIC level < 8 μg/mL, which is recognized as heterogeneous methicillin-resistant Staphylococcus species (CLSI, 2014). This group of methicillin-resistant (MR) Staphylococcus strains was frequently reported to produce Pbp2a constitutively, and also had an overproduction of β-lactamase. (Boyce and Medeiros, 1987; Malachowa and DeLeo, 2010; Hiramatsu et al., 2013). The role of beta-lactamase in relation to methicillin resistance is not well understood. Nevertheless, it was claimed that mecRI regulation system is much slower compared with the blaRI regulation system, taking hours instead of minutes. Therefore, it is possible that β-lactamase system may immediately interfere with the mec system instead of the mec system itself (Hiramatsu et al., 2013). With such ambiguities, this study was preliminarily undertaken to characterize mec and bla complexes genes distributions, Pbp2a and β-lactamase activity towards methicillin-resistance level (cefotaxin-MIC), and mutation in blaZ gene in relation to β-lactamase activity in a collection of MR Staphylococcus species for any potential explanation on the genotypic and phenotypic associations.

MATERIALS AND METHODS

Bacterial isolate

Sixteen MRSA and nineteen MRCoNS were available in our collection to be further analyzed in this study. The isolates were previously identified from nasal swab of healthy students at the Faculty of Medicine and Health Sciences, UPM in years 2013 and 2014 (Azis et al., 2014). Biochemical test such as Gram stain, coagulase and catalase test (H2O2) and mannitol salt agar (MSA) (Merck, Germany) were performed to differentiate S. aureus and CoNS. Antibiotic susceptibility pattern was determined by disc diffusion method using 30 μg cefotixin diffusion disc based on a guideline from Clinical and Laboratory Standards Institute (CLSI) (2014) (CLSI, 2014). The presence of mecA gene with size 530 bp was screened in all isolates according to Petinaki et al. (2001).

Cefotixin E-test

All mecA positive isolates were subjected for minimum inhibitory concentration (MIC) by standard E-test method (0.016-256 μg/mL) (Oxoid, UK). The concentration of 8 μg/mL was made as a breakpoint for resistance category as recommended by Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2014).

Mec operon screening

For detection of full and partial presence of mecA gene sequence, all mecA positive isolates were analyzed by PCR (Biometra Thermocycler, Germany) using different primer sets targeting mecA gene with expected sizes of 1.0 kb and 2.1 kb according to Petinaki et al., 2001 and Shore et al. (2011). Each PCR reaction was carried out in a 25 μL mixture composed of 12.5 μL of Green GoTaq® PCR mastermix (2X Green GoTaq® Reaction Buffer (pH 8.5), 400 μM dATP, 400 μM dGTP, 400 μM dCTP, 400 μM dTTP and 3 mM MgCl2) (Promega, USA), 7.5 μL nuclease free water (Promega, USA), 2.5 μL of DNA template (25-100 ng) and 1.25 μL of each specific primer pairs (Petinaki et al., 2001; Shore et al., 2011). MecI and mecRI genes were also screened using PCR in all mecA positive isolates using different primer sets targeting different sites described by Petinaki et al. (2001) to trace alteration or deletion part of mec regulator genes. Primer for mecI amplifies 1196 bp targeting the whole mecI including penicillin-binding domain (PB), while primers for the mecRI gene were of two separate sets targeting two part of mecRI, the PB and membrane spanning part (MS) for 747 bp and 310 bp, respectively. PB is located at upstream of mecRI whereas MS is located at the mid-internal region of the mecRI sequence. Each PCR reaction was carried out in a 25 μL mixture composed of 12.5 μL of Green GoTaq® PCR mastermix (2X Green GoTaq® Reaction Buffer (pH 8.5), 400 μM dATP, 400 μM dGTP, 400 μM dCTP, 400 μM dTTP and 3 mM MgCl2) (Promega, USA), 7.5 μL nuclease free water (Promega, USA), 2.5 μL of DNA template (25-100 ng) and 1.25 μL of 0.025 μM of each specific primer pairs (Petinaki et al., 2001).

Bla operon screening and protein analysis

All mecA positive isolates were subjected to blaZ amplification by targeting a gene sequence area of the...
functional part according to Kaase et al. (2008). Expected PCR products with size ~0.5 kb were purified, sequenced and submitted to GenBank (http://www.ncbi.nlm.nih.gov/genbank/). BlaZ nucleotide sequences were translated into an amino acid sequence using Expasy Translate tool (http://web.expasy.org/translate). Multiple sequence alignment using Biology workbench server (http://workbench.sdsc.edu) was conducted for tracing amino acid mutation on blaZ sequences of all blaZ-positive isolates. The phylogenetic tree was constructed using the neighbor-joining method with a bootstrap value of 1000 replication using MEGA 6 based on the amino acid sequence of all blaZ-positive isolates. BlaZ-positive isolates were classified based on β-lactamase signature types and classes according to blaZ protein sequence (Olsen et al., 2006; Bush and Jacoby, 2010). All blaZ sequence were submitted to GenBank and assigned with accession number KX985846.Bla regulator genes (blaRI and blaI) were also screened using primers sets targeting 0.9 kb blaI gene and 1.1 kb blaRI gene sequence as described by Chen et al. (2015). Each PCR reaction was carried out in a 25 µL mixture composed of 12.5 µL of Green GoTaq® PCR mastermix (2X Green GoTag® Reaction Buffer (pH 8.5), 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP and 3 mM MgCl₂) (Promega, USA), 7.5 µL nuclease free water (Promega, USA), 2.5 µL of DNA template (25-100 ng) and 1.25 µL of 0.025 µM of each specific primer pairs (Chen et al., 2015).

**PBP2a and β-lactamase assays**

All mecA positive isolates were tested for the presence of Pbp2a using penicillin-binding protein (PBP2') latex agglutination test (Oxoid,UK) according to manufacturers instruction. Pbp2a assay consisted of 2 major steps which are culture extraction and latex agglutination assay. For protein extraction, several loops of overnight colonies of each isolate (~1.5 ×10⁹ cells) were suspended into 1.5 mL microcentrifuge containing Extraction Reagent 1 solution. The suspension culture was placed in a water bath (Memmert) at 100 °C for 3 min followed by cooling at room temperature (~27 °C). One drop of Extraction Reagent 2 solution was added and the solution was centrifuged at 1500 g for 5 min and the supernatant was used for protein assay. For latex agglutination assay, one circle was labeled on the test card for testing with Test Latex (monoclonal antibody against PBP2') and another for testing with Control Latex (monoclonal antibody with negative binding towards S. aureus proteins). One drop of each Test Latex and Control Latex were added to each labeled circle. After that, 50 µL of extracted supernatant from each isolate were placed on test circle and control circle and mix thoroughly. Finally, the mixture for both circles was incubated for 3 min at room temperature (~27 °C). Any agglutination on latex circle was observed and recorded for each isolate as the presence of Pbp2a protein. For β-lactamase enzyme assay, one drop of 0.5 mM nitrocefin solution (1.0 mg of lyophilized nitrocefin powder + 1.9 mL of rehydrating fluid) was added to the surface of an overnight culture of each isolate. The β-lactamase production of each isolate was determined based on colour changes from yellow to red.

**RESULTS**

**Pre-screening**

All 35 isolates were amplified for 0.5 kb mecA gene to be considered as MRSA or MRCoNS in this study (Table 1) with 98-99% sequence homology upon BLAST analysis databases (data not shown).

**Mec complex and PBP2a assay**

Different part of mec complex (mecA, mecRI, mecI) was amplified to ensure the presence of complete mec complex in methicillin-resistant *Staphylococcus* species. For MRSA (n=16), 88% (n=14) isolates were positive for 1.0 kb while, only 75% (n=12) carried full length of mecA gene (2.1 kb). For MRCoNS (n=19), 95% (n=18) isolates were positive for both mecA gene fragments (1.0 kb and 2.1 kb) except isolate MRCoNS SC164 (positive in 1.0 kb; negative in 2.1 kb) (Table 1). For mecRI, two different locations were amplified named as PB part and MS part. 94% (n=15) MRSA were positive for PB part (but present in truncated form) and 31% (n=5) were positive for MS part. While MRCoNS (n=19) were 21% (n=4) positive for PB part (complete+truncated) and 73% (n=14) positive for MS part (Table 1). For mecI, 18% of MRSA (n=3) and 21% of MRCoNS (n=4) had a positive PCR amplification. Only one MRCoNS SC177 with MIC 32 µg/mL had a complete mec complex (2.1 kb mecA, mecRI, mecI) positive reaction in Pbp2a assay (MRSa=11; MRCoNS=18) except MRSA SA13 isolate with MIC 24 µg/mL; positive for 2.1 kbp mecA gene but negative in Pbp2a assay (Table 1).

**Bla complex and nitrocefin assay**

Among the 35 methicillin-resistant *Staphylococcus*, thirty-one isolates consisting of sixteen MRSA and fifteen MRCoNS had a positive blaZ and blaRI genes amplification with only four of MRCoNS (SC67, SC126, SC144 and SC70) had a negative detection for both genes. For blal gene, 93% of MRSA (n=15) and 63% of MRCoNS (n=12) were positive for blal gene. Isolates that were detected for the blaZ gene were also detected for the blaRI gene but not blal gene in both MRSA and MRCoNS species. Moreover, more MRSA (n=15) carry complete bla complex genes compared to MRCoNS (n=12), respectively. In nitrocefin assay, red, orange and yellow colours indicate a strong, weak and no β-lactamases production, respectively. 69% of MRSA (n=11) and 27% of MRCoNS (n=4) had a red colour, whereas 25% of MRSA (n=4) and 60% of MRCoNS (n=9) showed orange colour in nitrocefin assay. Only 6% of MRSA (n=1) and 33% of MRCoNS (n=5) showed yellow in...
### Table 1: Genotypic distribution of mec and bla complexes and Pbp2a and nitrocefin assays in relation to methicillin-resistance level (cefoxitin-MIC) of MR Staphylococcus species.

<table>
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<th>Isolate</th>
<th>FOX-MIC test (0.016-256 µg/mL)</th>
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<th>mecRI</th>
<th>mecl</th>
<th>pbp2a assay</th>
<th>blaZ</th>
<th>blaRI</th>
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FOX: cefoxitin; SA: MRSA; SC: MRCoNS; *: pre-screening result; +: amplified; -: not amplified; Δ: truncated; AN: Genbank accession number of the partial nucleotide sequence of a blaZ gene; Red: strong β-lactamase production; Orange: weak β-lactamase production; Yellow: no β-lactamase production.
nitrocefin assay (negative β-lactamase) (Table 1). Overall, all MRSA that produced strong β-lactamase (red colour) have MIC ≥12 µg/mL, while for MRCoNS, only isolates with MIC ≤ 8 µg/mL produce strong β-lactamase (red colour).

**Protein signature type of blaZ**

A total of 31 from 35 isolates had a positive blaZ gene amplification. Only four MRCoNS (SC67, SC126, SC144, SC70) showed a negative bla complex (blaZ, blaRI,blaI) and nitrocefin assay, respectively. The phylogenetic tree produced four major groups (I,II,III and IV) consisting of five protein signature types (type 1,2,3,5 and 6) with three β-lactamase family classes based on deduced amino acid sequences of blaZ from the thirty-one isolates (Figure 1).

In the phylogenetic analysis, group I composed of protein signature type 2 and 5 from β-lactamase class D family. Fifteen isolates (MRCoNS=11; MRSA=4) contained protein signature type 5 and became the most prevalent signature type in this study while two isolates (MRSA=2) contained protein signature 2. A majority of isolates (n=13) in group I have cefoxitin resistance level of ≥8 µg/mL except two MRCoNS [SC175 (4 µg/mL), SC119 (6 µg/mL)] for protein signature type 5 and two MRSA [SA7 (6 µg/mL), SA45 (3 µg/mL)] for protein signature 2. Two MRSA (SA7, SA45) for protein signature type 2 had an identical amino acid sequence with S.aureus M25254.

For protein signature type 5, majority isolates (n=12) had two point mutations at amino acid residue 119 from a threonine to alanine and residue 145 from glycine to glutamic acid (Table 2); most isolates (n=11) produced β-lactamase except MRCoNS SC89 although it carried blaZ gene. Other three MRCoNS isolates (SC175=MIC 4, SC119= MIC 6 and SC129= MIC 12) carrying protein signature type 5 had an identical amino acid sequence with E. faecalis U43087 and showed a positive β-lactamase assay (Figure 1).

Group II contained 10 isolates (MRCoNS=2; MRSA=8) of protein signature type I from β-lactamase class A family. Eight isolates had cefoxitin resistance level of ≥8 µg/mL except for MRSA SA42 (6 µg/mL) and MRCoNS SC140 (2 µg/mL) with all of the isolates showed a positive reaction in the β-lactamase assay. Group III composed of one MRSA (SA4=MIC 16 µg/mL) of protein signature type 3 from β-lactamase class C family with a positive β-lactamase assay. Group IV contained three isolates (MRCoNS=2; MRSA=1) with protein signature type 6 from β-lactamase B family. Only MRCoNS SC91 (MIC 1.5 µg/mL) showed a positive reaction in the β-lactamase assay (Figure 1). Besides, MRSA SA60 isolate had a single amino acid mutation at residue 145 as compared to that in S.aureus AF086644 (Table 2).

**Table 2: Protein signature types of blaZ.**

<table>
<thead>
<tr>
<th>Signature type</th>
<th>Isolates</th>
<th>BlaZ variable protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>X52734 (S. aureus), SC156, SA23, SA5, SA3, SA2, SA32, SA34, C13, SA42, SC140</td>
<td>T K V Q R E K L S P Y</td>
</tr>
<tr>
<td>2</td>
<td>M25254 (S. aureus), SA45, SA7</td>
<td>T K V Q R E K L S S C</td>
</tr>
<tr>
<td>3</td>
<td>AP004382 (S. aureus), SA4</td>
<td>T K V Q R E K N F N P Y</td>
</tr>
<tr>
<td>4</td>
<td>U43087 (E. faecalis), SC129, SC119, SC175, SC8, SA55, SC57, SC165, SA6, SA18, SA89, SC6, SC97, SC111, SA65, SC164</td>
<td>T K V Q R G E K L S S C</td>
</tr>
<tr>
<td>5</td>
<td>AF086644 (S. aureus), SC91, SC177, SA60</td>
<td>K N I K R K K N L N P Y</td>
</tr>
</tbody>
</table>

*Signature type based on Olsen et al., 2006, Malik et al. 2007 and Chen et al., 2015.

Strain in bold represents strain from GenBank.

Variable position according to deduced protein sequence of X52734.
**Figure 1:** Phylogenetic analysis of blaZ protein sequences using neighbor-joining method. Bootstrap value constructed using 1000 replication. SA, MRSA; SC, MRCoNS; MIC, Cefoxitin; ++: strong β-lactamase; +: weak β-lactamase; -: no β-lactamase; AY373761, M25254, U43087, X52734, X04121, AP004832, CP002146, AF086644, CP000029: Reference strains retrieved from NCBI.
DISCUSSION

All MR *Staphylococcus* species that carried full length mecA gene (2.1 kb) in this study showed a positive reaction in the Pbp2a assay except isolate MRSA SA13. Isolate MRSA SA13 carried a full-length mecA gene but had a negative Pbp2a assay. This could be due to potential mutation or alteration in mecA nucleotide sequence in the isolate that may lead to failure in Pbp2a production (Lowy, 2003). Regardless of the species, most MR *Staphylococcus* in this study carried partial mecRI with the absence of mecI gene. This situation may be caused by disruption of the mec regulator sequences particularly by the activities of insertion sequences such as IS431 and IS1272 (Malachowa and DeLeo, 2010). The regulator sequences in *Staphylococcus* species have long been recognized as part of mobile genetic elements whereby genetic exchange, deletion and insertion are common to affect the functionality of the genes (Ito et al., 1999; Jansen et al., 2007; Malachowa and DeLeo, 2010). Overall, no obvious correlation was observed between mec regulator gene distributions toward methicillin-resistant level in *Staphylococcus* species. Nevertheless, Oliveira et al. (2011) proposed that full resistance toward β-lactam antibiotics in MR *Staphylococcus* strain usually does not require functional mec regulator (mecRI-mecI) system (Oliveira et al., 2011).

MRSA SA6 isolate had MIC level of 48 µg/mL which is the highest MIC level in this study, although the isolate carried mec repressor gene (mecI). Presumably, mecI may not affect methicillin resistance level in *Staphylococcus* species as a previous observation showed that overexpression of mecI had no effect on the expression of methicillin resistance in many S. aureus strains (Oliveira and de Lencastre, 2011). This suggests the potential involvement of other regulatory factors such as mecR2 (same function as mecRI), a previously unrecognized gene located downstream of mecI by interfering with the binding of mecI into mecA promoter and facilitating proteolysis of mecI (Arède et al., 2012). This study did not attempt to detect the presence of mecR2 in SA6 isolate. Nevertheless, since MRSA SA6 isolate carried non-functional inducer gene (partial mecR), the involvement of mecR2 could be postulated. Furthermore, blaI was also detected in MRSA SA6 isolate and blaI can interfere with the role of mecI as repressor toward mecA by binding with mecI and forming mecI:blaI heterodimers. This may block mecI from binding with mecA gene promoter (Arède et al., 2013).

Only MRCoNS SC177 isolate with cefoxitin MIC 32 µg/mL carried complete mec complex and was positive in Pbp2a and β-lactamase assay. None of MRSA isolates carried complete mec structure in this study. Although this study has no evidence to claim MRCoNS as mec complex reservoir but the presence of the one isolate SC177 among MRCoNS could be consistent with the earlier claim that MRCoNS may serve as a carrier for a complete sequence of mec complex (Shore and Coleman, 2013). Many findings suggested that MRCoNS isolates are the reservoirs for mec complex and have been transferring the genetic determinants into *S. aureus* (Ito et al., 2009; Shore et al., 2011; Tuliniski et al., 2012).

Overall, a majority of MRSA isolates carried a complete bla operon (*blaZ, blaRI, blaI*) with a strong β-lactamase production and cefoxitin-MIC level of ≥ 12 µg/mL although two MRSA isolates (SA13, SA4) were negative in the Pbp2a production (Table 1). Meanwhile, a few MRCoNS which have high cefoxitin-MIC level in this study such as SC177 (32 µg/mL), SC126 (24 µg/mL) and SC144 (16 µg/mL) did not carry bla operon or only contained partial bla operon and showed no β-lactamase activity (Table 1). Overproduction of β-lactamase was claimed as one of the factors of methicillin-resistance determinant especially in heterogeneous MRSA since MRSA with low level cefoxitin-MIC (2 µg/mL-4 µg/mL) but with overproduction of β-lactamase had been observed. On the other hand, this situation was not common in MRCoNS (Petinaki et al., 2002). This observation agrees with previous reports that claimed β-lactamase production in association with MR to be more frequent in *S. aureus* than in CoNS (Saini et al., 2012; Robles et al., 2014).

As for beta-lactamase, its production had been compromised in seven MR isolates and three of them (SA60, SC89, SC177) carried the *blaZ* gene. There is a correlation with the mutation observed on the amino acid sequence of the two (SA60, SC89) from the three isolates. First, *blaZ* amino acid of SA60 isolate has an unusual mutation at position 145 involving lysine (K) to asparagine (N) and separated from others isolates in protein signature type 6 (Figure 1). This mutation may give a major impact on β-lactamase production considering mutation on that position did not occur in protein signature type 6 in *Staphylococcus* species isolated from human that was previously documented by Olsen (Olsen et al., 2006). MRSA SA60 isolate also had the lowest cefoxitin-MIC level (2 µg/mL) among MR isolates in this study and maybe related to negative β-lactamase production or the incomplete mecA in MRSA SA60 isolate. For the latter, although MRSA SA60 isolate showed a negative Pbp2a expression (Table 1) which could be the main factor for its lower MIC level, a few studies claimed that a diverse MIC level was not related to Pbp2a expression alone. Interestingly, a same level of Pbp2a expression was found in isolates with low cefoxitin-MIC level (1.5 µg/mL) as well as that with high cefoxitin-MIC level (1.5 mg/mL) (Berger-Bachi et al., 1992; De Lencastre and Tomasz, 1994; Peacock and Paterson, 2015).

On the other hand, MRCoNS SC89 have an amino acid modification at position 119 involving threonine (T) to alanine (A) (Table 2). Mutation at position 119 may not play an important role in the β-lactamase production and may not affect cefoxitin MIC level (24 µg/mL) since other isolates with the same mutation in signature type 5 positive had a positive β-lactamase production with cefoxitin-MIC level ≥ 8 µg/mL (Figure 1). Besides, mutation was not observed in *blaZ* amino acid of MRCoNS SC177 isolate suggesting that the *blaZ* gene detection cannot be used as an indicator for β-lactamase production since other studies also observed the same situation; no β-lactamase production but presence of *blaZ*.
gene (Pereira et al., 2014; Papanicolas et al., 2014). Another study also highlighted the importance of mutation of bla regulator (blaRI, blaI) rather than blaZ as the primary reason for the different level of β-lactamase production (Milheiro et al., 2011).

Five protein signature types (type 1,2,3,5 and 6) and four β-lactamase classes (A,B,C,D) were demonstrated in phylogenetic tree indicating the diverse protein sequences of blaZ in this study. Surprisingly, class A and class D β-lactamase genes were detected in 25 isolates (15 isolates= class D β-lactamase, 10 isolates= class A β-lactamase) which was previously documented as the largest group of β-lactamas (Bush and Jacoby, 2010). Certain MRSA and MRCoNS shared same protein signature (type 2 and 5) (Figure 1) suggesting possible genetic transfer of blaZ gene that could have taken place within Staphylococcus population although previous finding reported blaZ gene exchange and horizontal spread of blaZ within Staphylococcus species is a rare event (Olson et al., 2006).

CONCLUSION

As a whole, the comparative analysis in this study indicates a potential correlation between β-lactamase production toward methicillin-resistance particularly in MRSA since MRSA with cefoxitin-MIC ≥ 12 µg/mL have a strong β-lactamase production. Furthermore, a majority of MRSA with cefoxitin-MIC ≥12 µg/mL with a strong β-lactamase production carry a complete bla operon. This demonstrates the importance of bla operon and β-lactamase production in rendering a higher methicillin-resistance level among MRSA as compared to MRCoNS. However, these correlations are only based on comparative observation while the exact mechanism on how the production of β-lactamase affecting the methicillin-resistance level is still ambiguous. Perhaps many other factors are involved and remain to be investigated. In the phylogenetic analysis, four groups represent five protein signature types and four classes of β-lactamase indicating a level of diversity of blaZ protein sequence although within a small collection of isolates (n=35) in this study. However, no obvious pattern was observed between β-lactamase classes and cefoxitin-MIC distribution among both MR Staphylococcus species since a majority of MR Staphylococcus was clustered into a group that homogenously carried β-lactamase class D but varied from low to high cefoxitin-MIC level. This study only looks at the distribution in a small sample size of both species which is still subjected to ambiguity and required further validation involving more MR isolates with a better experimental model.

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REFERENCES


