Identification and characteristics of drug resistance and genotypes of methicillin-resistant *Staphylococcus aureus* isolated from intensive care units at obstetrics & gynaecology departments: a retrospective analysis

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Abstract

**Background:** The aim of this study was to investigate the prevalence and characteristics of SCC\(^{mec}\) genotypes and drug resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from intensive care units (ICU) at obstetrics & gynaecology departments in a tertiary hospital. **Methods:** MRSA obtained from patients admitted to the ICU were isolated and identified by using the Vitek 2 Compact System with GP21 342 cards. Antimicrobial susceptibility profiles and MRSA screening were determined by using the broth microdilution method according to CLSI guidelines. Determination of resistant genes and SCC\(^{mec}\) genotypes were performed by multiplex PCR. **Results:** Of the 283 patients evaluated, 120 (42.4%) isolates were phenotypically and genotypically confirmed to be MRSA. Among 120 strains, 15 (12.5%) strains were SCC\(^{mec}\) type II, 96 (80%) strains were SCC\(^{mec}\) type III and 9 (7.5%) strains were undifferentiated type. All MRSA strains were recognized as multidrug resistant, exhibiting 100% resistance to cefotaxime and oxacillin, followed by erythromycin and levofloxacin (more than 80% and 90% respectively). Different SCC\(^{mec}\) genotypes in MRSA isolates showed distinct antimicrobial agent patterns. SCC\(^{mec}\) type II was highly resistant to clindamycin (93.3%) with lower resistance to tetracycline (26.7%) with SCC\(^{mec}\) type III being highly resistant to gentamicin (91.7%). Undifferentiated strains were resistant to Cotrimoxazole (77.8%). There was a statistical difference among type II, type III and Undifferentiated strains (\(P<0.05\)). Of interest, a high prevalence of resistance to rifampicin (more than 75%) was also noted in the hospital. With different SCC\(^{mec}\) genotypes, MRSA isolates were sensitive to minocycline, quinupristin, teicoplanin, vancomycin and nitrofurantoin. **Conclusions:** Our data indicate that SCC\(^{mec}\) type II and SCC\(^{mec}\) type III of MRSA are circulating in the ICU and constitute a major source for the infection spread. It is necessary to increase surveillance of MRSA in the ICU and develop adequate infection prevention strategies.

**Keywords:** Methicillin-resistant *Staphylococcus aureus*; MRSA; SCC\(^{mec}\); Nosocomial infection

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common pathogenic bacteria in intensive care units (ICU) with the majority of isolates demonstrating multidrug resistance (MDR) which impacts clinical therapy \([1,2]\). The resistance mechanism of MRSA is mainly secondary to the bacteria acquiring a genetic determinant (methicillin-resistant determinant A and C, abbreviated as meca or mecC), which encode penicillin-binding protein PBP2A or PBP2A respectively \([3]\). These meca and mecC genes exist in the staphylococcal cassette chromosome mec, called SCC\(^{mec}\). SCC\(^{mec}\) can carry other drug resistance genes other than meca and mecC gene resulting in multiple drug resistance \([4]\).

Emerging MRSA and multiple drug resistance are a major public health problem worldwide \([5,6]\). They are the most common cause of healthcare-associated infections (HAI) in patients that are admitted to the ICU \([7]\). HAI occurring in the ICU from MRSA have become particularly problematic since they arise from the treatment received by critically-ill patients \([8]\). Although evidence suggests a significant increase in the proportion of MRSA hospital infections worldwide, ICU at obstetrics & gynaecology departments have reported only a limited number of MRSA isolates in China \([9]\). Effective and safe antimicrobial treatment is essential for treating infections in the ICU. Organizational-wide surveillance of infection-derived bacterial isolates and analysis of their susceptibility to different antimicrobial agents provides crucial information for the most effective antimicrobial therapy \([10]\). Furthermore, a comprehensive analysis of MRSA SCC\(^{mec}\) typing of ICU infections and predicting the development trend of drug-resistant strains is critical in evaluating disease prognosis and essential for reducing infection mortality and morbidity in the ICU.

2. Clinical data and methods

2.1 Study setting and specimen collection

This study was conducted at a university-affiliated hospital in North-East China with approximately 1200
beds. An analysis of retrospective data of MRSA-infected patients in three obstetrics & gynaecology ICUs was conducted during January 2018 to December 2020. A total of 283 obstetric patients were admitted after being transferred from the operating room, general ward and emergency department prior to ICU admission. Patients with concurrent HAI were classified according to infection source such as pneumonia, bloodstream infections, urinary tract infections, surgical site infections or other infections. The pathogenic bacteria associated with a HAI were collected within 48 hours of hospitalization according to the local protocol. Some samples were collected after 48 hours post-hospitalization. The specimens were mainly obtained from sputum or tracheal secretions, pus, blood, ascites, catheters and drainage tubes. Multiple isolates from a single patient were excluded. The isolates from different infected sites of the same patient were also excluded. Approval for collecting clinical samples was granted by the institutional ethics committees of the participating hospital. Informed consent forms were reviewed and signed by all participants before sample collection (Ethical approval number: Protocol Number 2019-01-02).

2.2 Identification and detection of resistance to 14 antibiotics agents

All isolates were identified as *Staphylococcus aureus* by conventional standard procedures and confirmed by VITEK GN1 system with GP21 342 cards (bioMérieux Vitek Inc., Hazelwood, MO, USA). No repetitive isolates from a single patient were included. Susceptibility to 14 antimicrobial agents (Bio-Rad, Hercules, CA, USA) was determined and interpreted by the broth microdilution method akin to that in the Clinical and Laboratory Standards Institute (CLSI) criteria. MRSA screening was determined using oxacillin MIC >6 μg/mL according to CLSI guidelines [11]. Control strains were *Staphylococcus aureus* ATCC29213, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus aureus* ATCC43300, respectively.

2.3 Determination of resistant genes and SCCmec genotypes by multiplex PCR

A series of genes including mecA, femB, mecAa and SCCmec are listed in Table 1. Multiplex PCR amplification and PCR reactions were performed as described elsewhere [12,13]. PCR products of genes were sent to Sangon Biotech Co., Ltd (Shanghai, China) for sequencing and DNAman software (version 6.0) (Lynnon Biosoft, Vaudreuil, QC, Canada) was used to analyse the sequencing results. Reference strains NCTC 85/2082 was used as standard strains with SCCmec type III and Reference strains NCTC N315 was used as SCCmec type II, respectively.

2.4 Statistical analysis

Differences in drug resistance rates of MRSA strains were analysed by Chi-square test. All drug-resistant data were analysed using SPSS version 13.0 (International Business Machines (IBM) Corp., Armonk, NY, USA). Analyses with a value of $P < 0.05$ were considered to be statistically significant.

3. Results

3.1 Epidemiological characteristics of specimens and isolates

From January 2018 to December 2020, a total of 283 obstetric patients from three ICU were enrolled in this study to estimate the quantity and types of infections present in this population. The average age of the patients was 34.88 ± 4.63 years with a range of 17–49 years. Thirty patients (18.8%) were of advanced maternal age (≥35 years). Others (42.9%) patients had pre-existing medical problems. Out of the 283 samples, 120 were classified as SMRA by the VITEK GN1 system and PCR. The remaining samples were classified as *Enterobacteriaceae* and non-*Enterobacteriaceae*, which were not included in this study (data not shown).

3.2 Identification of mecA and femB by multiplex PCR

Both mecA and femB genes were identified by multiplex PCR among all MRSA isolates. The amplicons of mecA and femB genes with a size of 310 bp and 651 bp were illustrated in Fig. 1A, respectively. The findings demonstrated that both mecA and femB genes were found in all MRSA isolates obtained.

3.3 Identification of SCCmec genotypes by multiplex PCR

Out of 120 MRSA isolates, 15 isolates belonged to SCCmec type II (12.5%), 96 isolates were SCCmec type III (80%) and 9 strains belonged to an undefined type. PCR patterns are shown in Fig. 1B.

3.4 Antimicrobial susceptibility analysis

Resistance frequencies of 120 MRSA strains in this study based on CLSI microdilution demonstrated that they were recognized as multidrug resistant, exhibiting 100% resistance to cefoxitin and oxacillin, followed by erythromycin and levofloxacin (more than 80% and 90% respectively). Different SCCmec genotypes in MRAS isolates showed distinct antimicrobial agent patterns. SCCmec type II exhibited a high incidence of resistance to clindamycin (93.3%) but displayed a relatively low prevalence of resistance to tetracycline (26.7%). SCCmec type III exhibited a high incidence of resistance to gentamicin (91.7%) while undefined SCCmec types were resistant to Cotrimoxazole (77.8%). There was a statistical difference among type II, type III and Undifferentiated strains ($P < 0.05$). No vancomycin-resistant isolate was found. Besides, MRSA isolates with different SCCmec genotypes were sensitive to minocycline, quinupristin, teicoplanin and nitrofurantoin (Table 2).
Table 1. Oligonucleotide primers used in this study.

<table>
<thead>
<tr>
<th>Names</th>
<th>Primer sequences (5′ → 3′)</th>
<th>Expected length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mecA</td>
<td>GTAGAAATGACTGAACGTCCGATAA CCAATTCACATTGTCCGTCTAA</td>
<td>310</td>
</tr>
<tr>
<td></td>
<td>TTACAGGTAACTGTGGTACC ATACAAATCCACGCACGCTCT</td>
<td>651</td>
</tr>
<tr>
<td>mecAa</td>
<td>GTGAAGATATACCAAGTGATT ATGCGCTATAGATTGAAAGGAT</td>
<td>147</td>
</tr>
<tr>
<td>SCCmec I</td>
<td>GCTTTAAAGAGTGTCGTTACAGG GTCCTCTCATAGTACGTACC</td>
<td>613</td>
</tr>
<tr>
<td>SCCmec II</td>
<td>CGTTGAAAGATGATGAACGCC GAAATCAATGGTAAATGGACCC</td>
<td>398</td>
</tr>
<tr>
<td>SCCmec III</td>
<td>CCATATTGTACGATGCGG CTCTAGTTGCTAGAACGATCG</td>
<td>280</td>
</tr>
<tr>
<td>SCCmec IVa</td>
<td>GCCTTATTGGAAGAAGACG CTACTCTTCGTAAGCCGTCG</td>
<td>776</td>
</tr>
<tr>
<td>SCCmec IVb</td>
<td>TCTGGAATTCTTACGCTGAC AAAAAATATTGCTCCCCTC</td>
<td>493</td>
</tr>
<tr>
<td>SCCmec IVc</td>
<td>ACAATATTGTTATATCGGCCAGCAC GCATTCTGAATGTGATGCGTGG</td>
<td>200</td>
</tr>
<tr>
<td>SCCmec IVd</td>
<td>CTGGATTGAGATGATGCTGG TGCTCAGGTAATGAAGAAG</td>
<td>881</td>
</tr>
<tr>
<td>SCCmec V</td>
<td>GAAATTGTACTTAAATGAGCG TAAGATGTGACCCCTTGACACC</td>
<td>325</td>
</tr>
</tbody>
</table>

Fig. 1. Representative gel showing banding profiles by multiplex PCR analysis in MRSA isolates. (A) Agar gel electrophoresis of mecA and femB detected by multiplex PCR in MRSA isolates. M: DNA molecular weight; 1–5: MRSA isolates from different samples in ICU; 6: MSSA (ATCC25923); N: Negative control. (B) Agar gel electrophoresis of SCCmec types detected by multiplex PCR in MRSA isolates. M: DNA molecular weight; 1: MRSA isolates SCCmec type III; 2: Reference strains 85/2082 SCCmec type III; 3: Reference strains N315 SCCmec type II; 4–5: MRSA isolates SCCmec type II; 6: MRSA isolates SCCmec undentified type; N: Negative control. MRSA, methicillin resistant Staphylococcus aureus; ICU, intensive care unit.

4. Discussion

Molecular typing of MRSA is an important assay for the epidemiologic investigation and strains of origin in addition to antimicrobial agent selection and therapy. Recent data demonstrate that mecA gene expresses itself in coagulase-negative staphylococcus (CNS) while femB is considered commonly in S. aureus with restricted expression but not present in CNS. Therefore, only when mecA and femB genes are both found to be positive can MRSA be present[14]. This study amplified mecA gene (310 bp) and femB gene (651 bp) from the 120 MRSA strains isolated from clinical samples obtained that were determined to be MRSA.

In the form of a gene complex, mecA gene of MRSA exists in SCCmec that is composed of two gene complexes: mec gene complex and cassette chromosome recombinases (crr). According to the structures of mec and crr, SCCmec can be divided to five types[15]. Evidence exists that a majority of early nosocomial infections were generally considered as SCCmec type I while community-acquired MRSA infections were mostly SCCmec type IV and SCCmec type V [16,17].
Table 2. Comparison of resistant characteristics of SCCmec genotypes in MRSA isolates.

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Type II (n = 15)</th>
<th>Type III (n = 96)</th>
<th>Type UD (n = 9)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R (%)</td>
<td>R (%)</td>
<td>R (%)</td>
<td></td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>15 (100.0)</td>
<td>96 (100.0)</td>
<td>9 (100.0)</td>
<td>—</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>15 (100.0)</td>
<td>96 (100.0)</td>
<td>9 (100.0)</td>
<td>—</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0 (0)</td>
<td>88 (91.7)*</td>
<td>0 (0) #</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>14 (93.3)</td>
<td>17 (17.7)*</td>
<td>3 (33.3)*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Minocycline</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>—</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>—</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>—</td>
</tr>
<tr>
<td>Quinupristin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>—</td>
</tr>
<tr>
<td>Compound sulfamethoxazole</td>
<td>3 (20.0)</td>
<td>6 (6.25)</td>
<td>7 (77.8)*#</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>14 (92.3)</td>
<td>87 (90.6)</td>
<td>8 (88.9)</td>
<td>1.000</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>—</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>12 (80.0)</td>
<td>75 (78.1)</td>
<td>7 (77.8)</td>
<td>1.000</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>14 (93.3)</td>
<td>94 (97.9)</td>
<td>9 (100)</td>
<td>0.4912</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>—</td>
</tr>
</tbody>
</table>

Note: *P < 0.05 compared with the SCC mec type II; #P < 0.05 compared with the SCC mec type III. UD, undifferentiated type.

In the present study, multiplex PCR amplification was conducted to analyze characteristic genes of SCCmec genotypes. The findings showed that the predominant genotype was SCCmec type III (80%) followed by SCCmec type II (12.5%) while no SCCmec type IV or SCCmec type V was found among MRSA strains in the ICU. As SCCmec type IV and SCCmec type V are mainly community-acquired MRSA infections, it can be verified that community-acquired MRSA infections are not present in our ICU. This finding was in agreement with other reports that 250 clinically isolated MRSA strains were mainly SCCmec type III followed by SCCmec type II in 18 hospitals nationwide in China [18]. An explanation may be the fact that the regional difference provided disparate MRSA with a diversity of SCCmec genotypes, thus leading to distinct drug-resistant patterns. In addition, 9 (7.5%) of 120 isolated MRSA strains of this study were undefined SCCmec strains, probably caused by the selected primers and amplification conditions or that they belong to a novel SCCmec gene, which needs further studies.

In the study, all MRSA strains were recognized as multidrug resistant. They showed a high incidence of resistance to β-lactamase, quinolone antibiotics, aminoglycosides, tetracycline and macrolides. One of the benefits from SCCmec genotyping in MRSA isolates is differentiation of antimicrobial agent susceptibility patterns. According to our findings, SCCmec type II demonstrated resistance to clindamycin (93.3%) and tetracycline (26.7%). SCCmec type III was strongly resistant to gentamicin (91.7%) while undefined SCCmec types had high drug-resistance (77.8%) to sulfamethoxazole. Due to frequent immunosuppression, patients in ICU receive more antibacterial agents than ordinary patients. Therefore, the frequencies of drug-resistant strains significantly increase in ICU compared to general wards. All isolates were sensitive to quinupristin, vancomycin and nitrofurantoin. Similar to findings reported by others [12,19].

Interestingly, a high prevalence of resistance to rifampicin was noted in the hospital. In fact, rifampicin was seldom administered for the treatment of MRSA. However, the percentage of rifampicin-resistant MRSA rapidly increased from 15.5% in 2004 to 50.2% in 2008 in China. Several reported that rifampicin resistance in S. aureus isolates including MRSA was associated with mutations of rpoB gene (encoding β subunits of RNA polymerase), which conferred homogeneous methicillin resistance. However, no definitive mechanism has been elucidated to date [19–21].

The treatment of MRSA infection is a very difficult clinical problem. Risk factors for development of an MRSA infection in the ICU include the following: widespread abuse, misuse, or overuse of antibiotics; the frequent renewal of antibiotics; increasing quantity and variety of pathogens present in the ICU; the diagnosis and progression of critical illnesses; postsurgical recovery; the use of invasive medical devices; and prolonged stay in the ICU [22,23]. Patients should be monitored for drug resistance within S. aureus so as to provide a basis for determining appropriate therapy. Meanwhile, hospitals are expected to allocate dedicated ventilators, oxygen, transfusion system, sphygmomanometer and thermometer to each patient and to disinfect the equipment after being used by each person. It is necessary to train the medical staff to infection prevention since researches verify that the hands of the medical staff are an important medium to spread MRSA, which means that hand washing is a vital way to block the spread of MRSA [22,23]. Also, attention should be paid to ventilation and air purification. Moreover, antibiotics should
be used carefully in clinical applications to prevent drug-resistant strains along with disinfection and proper isolation when indicated in order to reduce cross infection of *S. aureus* [24].

This study has a few limitations. First, genotypic or molecular data including Panton-Valentine leukocidin genes among all unidentified strains were not determined. Second, the antibiotic sensitivity of MRSA isolates to Daptomycin was not included. Future research may consider focusing on genetic types and the mechanisms for transmission.

5. Conclusions

The findings of this study indicate that the MRSA isolates are circulating in the ICU at obstetrics & gynaecology departments and constitute a major source of infection at a large hospital in China. This study also found that vancomycin may be a reasonable choice in the treatment of MRSA isolates. There is a strong need for increased hospital-wide surveillance and the development of adequate infection prevention strategies.

Abbreviations

ICU, intensive care unit; CLSI, the Clinical and Laboratory Standards Institute; HAIs, healthcare-associated infections; MRSA, methicillin-resistant *Staphylococcus aureus*.

Author contributions

ZHH conceived, designed the experiments and wrote a draft manuscript. BXL and MCL analyzed, interpreted the results of the experiments and revised the manuscript. PYL performed the experiments. XM collected the clinical data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethical approval for collecting clinical samples was received by the institutional ethics committees of the participating hospital. Informed consent forms were reviewed and signed by all participants before samples collection (Ethical approval number: Protocol Number 2019-01-02).

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Conflict of interest

The authors declare no conflict of interest.

References


