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Methicillin-resistant *Staphylococcus aureus* isolates with SCC*mec* type V and *spa* types t437 or t1081 associated to discordant susceptibility results between oxacillin and cefoxitin, Central Taiwan

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ABSTRACT

Staphylococcus aureus isolates with discordant susceptibility results between oxacillin and cefoxitin obtained using automated microbiology systems are infrequently observed. From April 2013 to December 2014, 1956 methicillin-resistant *S. aureus* (MRSA) and 1761 methicillin-susceptible *S. aureus* isolates were obtained from different patients. Forty isolates (1.1% and 2% in case of *S. aureus* and MRSA, respectively) with discordant susceptibility results (oxacillin susceptible and cefoxitin resistant) and carrying *mecA* gene were obtained. Except 2 SCC*mec* type IV isolates, 38 MRSA isolates were all SCC*mec* type V (V_T or non-V_T), which were further divided into V_T (n = 22) and non-V_T (n = 16). The most common *spa* type in V_T and non-V_T isolates were t437 (n = 19) and t1081 (n = 13), respectively. Only 55% of patients received effective antimicrobial agents; 2 mortalities were not attributable to MRSA infection. Using standard agar dilution, 17 MRSA isolates (0.46% and 0.87% in case of *S. aureus* and MRSA, respectively) had oxacillin MIC in the susceptible ranges (oxacillin-susceptible MRSA [OS-MRSA]); all carried SCC*mec* type V (V_T, n = 8; non-V_T, n = 9). The most common *spa*-MLST types of OS-MRSA in V_T and non-V_T were t437-ST59 (n = 4) and t1081-ST45 (n = 7), respectively. Concomitant testing by both cefoxitin- and oxacillin-based methods is a practical strategy for OS-MRSA detection in the clinical laboratories. Continuous monitoring of OS-MRSA isolates is necessary to elucidate their impact in clinical infectious diseases.

1. Introduction

Staphylococcus aureus, a versatile microorganism, can cause diverse clinical infectious diseases, ranging from localized superficial skin infections to life-threatening invasive infections, such as endocarditis, sepsis, and toxic shock syndrome (Lowy, 1998). *S. aureus* is also notorious for rapid evolution of antimicrobial resistance, from penicillin (1–2 years) to methicillin (<1 year), and vancomycin (40 years) (Chambers, 2001). Methicillin-resistant *S. aureus* (MRSA) isolates, initially confined to health care facilities since 1960s, have extended into global community after the 1990s (Chambers and Deleo, 2009). In Taiwan, clonal spreading of MRSA was identified, and >50% of the clinical *S. aureus* isolates were found to be MRSA (Ho et al., 2016; Hsueh et al., 2002). Furthermore, >40% of the clinical MRSA isolates harbored biocide-resistant genes (*qacA/B*), and 1–4% showed reduced susceptibility to vancomycin (Ho et al., 2010, 2012b; Lin et al., 2012).

The use of cefoxitin disk to screen for *mecA*-mediated resistance in both *S. aureus* and coagulase-negative staphylococci was first introduced by the Clinical and Laboratory Standards Institute (CLSI) in 2004 (CLSI, 2004). Currently, oxacillin continues to be a surrogate for oxacillin susceptibility testing of *S. aureus* by the MIC method, not by the disk diffusion method, but cefoxitin is the surrogate for oxacilllin susceptibility testing of *S. aureus* by both the disk diffusion and the MIC methods (CLSI, 2013).

In our laboratory, the BD Phoenix™ Automated Microbiology System (Becton Dickinson, Sparks, Maryland, USA) was introduced in 2005; thereafter, almost all MRSA isolates were identified by using BD Phoenix™ Identification/MIC Panels. Initially, the adapted Identification/MIC panel contained oxacillin but no cefoxitin for the detection of MRSA isolates (PMIC/ID-14). In April 2013, another panel (PMIC/ID-62) containing both oxacillin and cefoxitin for antimicrobial susceptibility

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testing replaced the existing one. Subsequently, discordant susceptibility results between oxacillin and cefoxitin were occasionally observed for *S. aureus* isolates. In this study, we intend to undertake molecular typing, study the presence of *mecA* or *mecC*, and understand the clinical characteristics of these interesting, but ambiguous, *S. aureus* isolates.

2. Materials and methods

2.1. MRSA isolates with discordant susceptibility results between oxacillin and cefoxitin by an automated microbiology system

From April 2013 to December 2014, 1956 MRSA and 1761 methicillin-susceptible *S. aureus* (MSSA) isolates were obtained from different patients. Bacterial isolates were identified as *S. aureus*, and the susceptibility to oxacillin and other antimicrobial agents was determined using BD Phoenix™ Automated Microbiology System (Becton Dickinson). The MIC interpretive standards for oxacillin and other antimicrobial agents [except fusidic acid, whose interpretation criteria are given by The European Committee on Antimicrobial Susceptibility Testing (2015)] were illustrated by the CLSI: isolates that test resistant with an oxacillin MIC ≥4 μg/mL or cefoxitin MIC ≥8 μg/mL were reported as MRSA (CLSI, 2013). Those with discordant susceptibility results between oxacillin and cefoxitin by the automated microbiology system were collected.

The oxacillin MIC of each MRSA isolate with discordant susceptibility results between oxacillin and cefoxitin was also determined by the agar dilution (Mueller–Hinton agar) method, as illustrated by the CLSI (2012). The clinical details of each MRSA isolate were obtained by reviewing medical records. Community-acquired MRSA (CA-MRSA) infection was defined as an infection in patients without any history of surgery, hospitalization, long-term care facility residence, dialysis, indwelling device or catheter within the last 1 year, or hospitalization (>48 h) before positive MRSA culture (Buck et al., 2005). Hospital-acquired MRSA infection was defined as those other than CA-MRSA.

2.2. DNA extraction

Approximately 3–5 MRSA colonies isolated on blood agar plate (BBL Microbiology Systems; Becton Dickinson) were suspended in 195 μL of TE buffer (10 mmol/L Tris, 1 mmol/L EDTA, pH 8.0) with 10 μL of lysostaphin (5 mg/mL) for lysis reaction at 37 °C for 30 min. The solution containing lysed bacteria was briefly centrifuged for further DNA extraction by Genomic DNA Mini Kit (Geneaid, New Taipei City, Taiwan).

2.3. SCCmec typing

Identification of *mecA* and various SCC*mec* types was performed by multiplex polymerase chain reaction (PCR) using the genomic DNA from each MRSA isolate according to a previously described method and primers (Kondo et al., 2007; Zhang et al., 2005). The size of PCR products of SCC*mec* type V_T and non-V_T was 1600 bp and 325 bp, respectively, with the following primers (F: 5′-GAACATTGTTACTT AAATGAGCG-3′ and R: 5′-TGAAAGTTGTACCCTTGACACC-3′) and modified PCR conditions: amplification was carried out with a 2-min denaturation step at 94 °C, followed by 30 cycles of 60 s at 94 °C for denaturation, 60 s at 55° for annealing, and 60 s at 72 °C for extension, and then 1 min at 72 °C for the final extension (Ho et al., 2015; Zhang et al., 2005).

2.4. mecC detection

mecC detection was performed according to a previously described method, by using the primers mecAL251_F: 5′-AGATTTAAAGTAG TAGACGGCA-3′ and mecAlga251_R: 5′-TTTCACCGATTCCCAAATCT-3′ (Pichon et al., 2012).

2.5. spa typing

The X-region of *spa* contains 21–27 bp long variable number (3–15) of repeats (Frenay et al., 1996). The X-region of each MRSA isolate was amplified by PCR with the primers 1095F: 5′-AGACGATCCTTCGG TGAGC-3′ and 1517R: 5′-GCTTTTGCAATGTCATTTACTG-3′ as described previously (Harmsen et al., 2003). The amplified products were sequenced, and the results were analyzed using Ridom Staph Type software (version 1.4; Ridom, GmbH, Wurzburg, Germany [http://spa.ridom.de/index.shtml]) to determine the repeat profile and the *spa* type of each isolate (Harmsen et al., 2003).

2.6. Multilocus sequence typing (MLST)

Seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*) of *S. aureus* were used for the MLST typing as described previously (Enright et al., 2000). The amplified products were sequenced, and the sequences thus obtained were analyzed using the software at http://saureus.mlst.net/sql/multiplelocus.asp. MLST analysis was only preformed for the isolates with oxacillin MIC ≤2 μg/mL by standard agar dilution method.

2.7. Pulsed-field gel electrophoresis (PFGE) typing

MRSA isolates were genotyped using PFGE according to the protocol supplied with the CHEF-DR III System (Bio-Rad Laboratories, Hercules, CA, USA). PFGE analysis was carried out by the method as described previously (Prevost et al., 1992). The bacterial genomic DNA was prepared and digested with *SmaI* (New England Bio Labs, Beverly, MA, USA). The PFGE was performed at 6.0 V/cm for 21 h at switch times ramped from 5 to 40 s. The results were analyzed using BioNumerics software (Applied Maths, Kortrijk, Belgium). Pulsotypes were assigned to the same cluster if ≥80% similarity was observed in the dendrogram.

2.8. Panton–Valentine leukocidin (PVL) gene

The PVL toxin gene, *lukS/F*, was detected by PCR using previously reported primers and conditions (Jarraud et al., 2002).

2.9. Statistical analyses

Pearson's chi-square test or Fisher's exact test (when the expected number in any cell was <5) was used to compare various clinical characteristics and antimicrobial susceptibility results between SCC*mec* type V_T and non-V_T MRSA isolates. All statistics were calculated using the SPSS for Windows (version 17.0; Chicago, IL, USA) software. $P \leq 0.05$ was considered statistically significant; all tests of significance were 2 tailed.

3. Results

During the study period of 21 months, there were 40 MRSA isolates (4 isolates in 4 months, 3 isolates in 4 months, 2 isolates in 3 months, 1 isolate in 6 months, and no any isolate in 4 months) with discordant susceptibility results between oxacillin and cefoxitin by the automated microbiology system, all with an oxacillin MIC of ≤2 μg/mL and a cefoxitin MIC of ≥8 μg/mL. The prevalence rate was 2% (40/1956) in case of all MRSA isolates and 1.1% (40/3717) in case of all *S. aureus* isolates. All 40 MRSA isolates harbored *mecA*; however, none carried *mecC*; only 2 isolates showed SCC*mec* type IV (2/40 = 5%), and the other 38 isolates carried SCC*mec* type V (V_T or non-V_T). In these 38 MRSA isolates with SCC*mec* type V (V_T or non-V_T), >50% (n = 22) carried SCC*mec* type V_T. The clinical diagnosis, characteristics, and MIC distribution of various antibiotics obtained using the automated microbiology system for these 40 MRSA isolates are shown in Table 1. No differences were noted in age, gender, source of acquisition (hospital or community), outcome,