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ORIGINAL ARTICLE

Fine typing of methicillin-resistant *Staphylococcus aureus* isolates using direct repeat unit and staphylococcal interspersed repeat unit typing methods

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Background/Purpose: Methicillin-resistant *Staphylococcus aureus* (MRSA) typing is an important epidemiologic tool for monitoring trends and preventing outbreaks. However, the efficiency of various MRSA typing methods for each SCC*mec* MRSA isolate is rarely evaluated.
Materials and methods: A total of 157 MRSA isolates from four different regions in Taiwan were typed with five different molecular methods, including SCC*mec* typing, multilocus sequence typing (MLST), *spa* typing, *mec*-associated direct repeat unit (*dru*) copy number determination, and staphylococcal interspersed repeat unit (SIRU) profiling.
Results: There were four SCC*mec* types, eight MLST types, 15 *spa* types, 11 *dru* types, and 31 SIRU profiles. The most common type determined by each molecular typing method was SCC*mec* III (115 isolates, 73.2%), ST239 (99 isolates, 63.1%), t037 (107 isolates, 68.2%), 14

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dru copies (76 isolates, 48.4%), and SIRU profile 3013722 (102 isolates, 65%), respectively. When using the combination of MLST, *spa* typing, and *dru* copy number, ST5-t002-4 ($n = 8$), ST239-t037-14 ($n = 68$), ST59-t437-9 ($n = 9$), and ST59-t437-11 ($n = 6$) were found to be the most common types of SCC*mec* types II ($n = 9$), III ($n = 115$), IV ($n = 21$), and V_T ($n = 11$) isolates, respectively. SCC*mec* type III isolates were further classified into 11 *dru* types. Of the 21 SCC*mec* type IV isolates, 14 SIRU profiles were found. Seven SIRU patterns were observed in the 11 SCC*mec* type V_T isolates.

Conclusion: Different typing methods showed a similar Hunter—Gaston discrimination index among the 157 MRSA isolates. However, *dru* and SIRU typing methods had a better discriminatory power for SCC*mec* type III and SCC*mec* types IV and V_T isolates, respectively, suggesting that *dru* and SIRU can be used to further type these isolates.

Introduction

Staphylococcus aureus is one of the most common pathogens. It can cause diseases such as cellulitis, myositis, food poisoning, septicemia, and toxic shock syndrome.[1] *S. aureus* infections are usually treated with methicillin. Unfortunately, methicillin-resistant *S. aureus* (MRSA) has emerged, and the incidence of infection caused by MRSA is increasing.[2,3] MRSA isolates harbor the *mecA* gene, which encodes the penicillin binding protein 2a (PBP 2a), rendering them resistant to some beta-lactamase antibiotics such as penicillin and methicillin.[4] The mortality rate of MRSA bacteremia has been shown to be as high as 39%.[5] MRSA can be divided into hospital-acquired (HA-MRSA) and community-acquired MRSA (CA-MRSA).[2] HA-MRSA was first isolated in 1961, shortly after the introduction of methicillin,[6] and CA-MRSA was first found in the United States in the 1990s.[2]

Strain typing is an important epidemiologic tool for monitoring trends and preventing outbreaks of microbial infections. Because of their high discriminatory power and good reproducibility, molecular typing methods are increasingly used for epidemiologic studies.[7] For MRSA, several molecular typing methods including staphylococcal chromosomal cassette *mec* (SCC*mec*) typing, multilocus sequence typing (MLST), determination of direct repeat unit (*dru*), and pulse field gel electrophoresis have been developed.[2] MLST requires sequencing technologies that may not be available in every laboratory. Pulse field gel electrophoresis is labor intensive, and results from different laboratories are difficult to compare because of the lack of a universal nomenclature system.[7] The *dru* locus is located in the hypervariable region of the *mec*A gene, between *tnp* and *orf*145 genes.[8] Different MRSA isolates may have different copies of *dru*. Determination of the staphylococcal interspersed repeat unit (SIRU) pattern is another method for MRSA typing. This method accesses the variable number of tandem repeat of the whole genome of MRSA.[9,10] In this study, we compared the efficiency of various MRSA typing methods and determined if the discrimination powers of these methods were different among each SCC*mec* MRSA isolate.

Materials and methods

Bacterial isolates

A total of 157 MRSA isolates from blood cultures were used in this study. These isolates were collected by the SMART (Surveillance of Multicenter Antimicrobial Resistance in Taiwan) program from March to August 2003 from nine medical centers in Taiwan.[11,12] The contributing hospitals of these isolates are listed in Table 1.

DNA extraction

MRSA isolates were grown on BAP agar plates (BBL Microbiology Systems, Becton Dickinson). Three to five colonies of each isolate were suspended in 600 μL of TE buffer (10 mM Tris, 1 mM EDTA, pH8.0). The cells were then pelleted by centrifugation. DNA was extracted from the bacterial pellet using the Genomic DNA Mini Kit (Geneaid, Taiwan) as described previously.[11]

SCC*mec* typing

Identification of various SCC*mec* types were performed by multiplex polymerase chain reaction (PCR) using the genomic DNA from each MRSA isolate as the template as described previously.[13] Types V and V_T were distinguished with the following primers[14]: F: 5′-GAACATTGTTA CTTAAATGAGCG-3′ and R: 5′-TGAAAGTTGTACCCTTGACACC-3′. The amplification was carried out with a 1-minute heating step at 94°C, followed by 30 cycles of 30 seconds at 94°C for denaturation, 60 seconds at 55°C for primer annealing, and 60 seconds at 72°C for extension, and then 5 minutes at 72°C for final extension. The PCR product of SCC*mec* type V was 325 bp, and that of SCC*mec* V_T was 1600 bp.

MLST typing

Seven housekeeping genes (*arc*, *aroE*, *glp*, *gmk*, *pta*, *tpi*, *yqiL*) of *S. aureus* were used for typing. The amplification of a portion of each gene was performed as described