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ORIGINAL ARTICLE

Concomitant genotyping revealed diverse spreading between methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *Staphylococcus aureus* in central Taiwan

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KEYWORDS
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Background: *Staphylococcus aureus* is a versatile bacterium, which can lead to various infectious diseases. Various molecular typing methods are applied to the evolution and epidemiology surveys of *S. aureus*, mostly for methicillin-resistant *S. aureus* (MRSA). However, methicillin-susceptible *S. aureus* (MSSA) is still an important pathogen, but their molecular typing is evaluated infrequently.
Methods: Pulsed-field gel electrophoresis (PFGE), *spa* typing, and detection of five virulent genes for 95 MRSA and 56 MSSA isolates (July—December 2008 and July 2008—December 2009, respectively) during an overlapping period were performed.
Results: More diversity was found in MSSA isolates (23 pulsotypes and 25 *spa* types, excluding 4 new-type and 1 nontypable isolates for *spa* typing) than in MRSA isolates (19 pulsotypes and 16

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typing;
pulsed-field gel electrophoresis;
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typing

spa types, excluding 1 new-type and 1 nontypable isolates for *spa* typing). By *spa* typing, t002 ($n = 30$), t037 ($n = 23$), t437 ($n = 21$), t234 ($n = 3$), t1081 ($n = 3$), and t1094 ($n = 3$) were the six major MRSA clones. For MSSA isolates, t189 ($n = 13$), t437 ($n = 4$), t084 ($n = 3$), t213 ($n = 3$), t701 ($n = 3$), and t7200 ($n = 3$) were the six major types. Combining PFGE and spa typing, there were five combinations (pulsotype + spa type) that contained both MRSA and MSSA isolates (pulsotype 9-t437, pulsotype 15-t037, pulsotype 19-t002, pulsotype 21-t002, and pulsotype 28-t1081). For all 151 *S. aureus* or 95 MRSA isolates, the PFGE typing had more discrimination power, but *spa* typing had larger discrimination index for 56 MSSA isolates.

Conclusion: In conclusion, there were different predominant MRSA and MSSA clones clinically. Continuing longitudinal tracking of molecular typing is necessary for elucidating the evolution of this important clinical pathogen.

Introduction

Staphylococcus aureus is a versatile human pathogen, which can cause numerous infectious diseases, ranging from skin, soft tissue, joint and bone infections to food poisoning and pneumonia, even endocarditis, septicemia, and toxic shock syndromes.[1] Besides its high virulence, *S. aureus* is also notorious for its ability to develop resistances to various antibiotics rapidly, including penicillin, methicillin, and even vancomycin.[2] Various molecular typings, including pulsed-field gel electrophoresis (PFGE), *spa* sequencing typing, multilocus sequence typing (MLST), and so on, are applied to the evolution and epidemiology surveys of *S. aureus*, mostly for methicillin-resistant *S. aureus* (MRSA).[3–6] Only a few studies put emphasis on methicillin-susceptible *S. aureus* (MSSA).[7] However, MSSA is still an important pathogen for community or health-care-associated and invasive infections.[8–10] In Taiwan, MRSA-related infections were always a rampant problems, and clonal spreading of specific MRSA strains had been demonstrated.[11,12] However, the molecular epidemiology of MSSA in Taiwan is still limited.[13,14] In this study, we want to elucidate the relationship between clinical MRSA and MSSA isolates from an overlapping period.

Materials and methods

Clinical MRSA and MSSA isolates

As reported in previous studies, 95 MRSA and 56 MSSA isolates were collected from blood culture of different patients.[12,15] The collection periods for MSSA and MRSA were July 2008—December 2009 and July—December 2008, respectively. Identification of clinical isolates was processed initially with a Bactec 9000 system (Becton Dickinson, Sparks, MD, USA). The positive samples were streaked across Trypticase soybean agar with 5% sheep blood (TSA II)/Levine EMB agar (Becton Dickinson) and incubated at 37°C for appropriate periods. Bacterial isolates were identified as *S. aureus*, and the susceptibility to oxacillin was determined using a BD Phoenix automated microbiology system (Becton Dickinson). The minimal inhibitory concentration (MIC) interpretive standards for oxacillin susceptibility were those recommended by the Clinical Laboratory Standards Institute.[16]

DNA extraction

Briefly, isolates were grown on BAP agar plate (BBL Microbiology Systems, Becton Dickinson). Three to five bacterial colonies were suspended in 600 μL of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), and centrifuged briefly. The Genomic DNA Mini Kit (Geneaid, New Taipei City, Taiwan) was used to extract DNA from pelleted cells.

spa typing

The X region of the *spa* gene contains a variable number of repeats of 21—27 bp.[17] The size of the most common repeat is 24 bp. The X region of each MRSA isolate was amplified by polymerase chain reaction (PCR) with primers 1095F: 5′-AGACGATCCTTCGGTGAGC-3′ and 1517R: 5′-GCTTTTGCAATGTCATTTACTG-3′, as described previously.[18] The amplified products were sequenced, and the sequences obtained 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 *spa* type of each isolate.[18]

PFGE typing

All bacterial isolates were genotyped using PFGE according to the manual protocol using a CHEF-DR III system (Bio-Rad Laboratories, Hercules, CA, USA). PFGE analysis was carried out as described previously.[19] The bacterial genomic DNA was prepared and digested with *SmaI* (New England Bio Labs, Beverly, MA, USA). The digested DNA fragments were subjected to PFGE, which was conducted at a voltage of 6.0 V/cm for 21 hours at switch times ramped from 5 seconds to 40 seconds. The gel was stained and analyzed using BioNumerics software (Applied Maths, Kortrijik, Belgium).