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Antimicrobial susceptibility, virulence determinant carriage and molecular characteristics of *Staphylococcus aureus* isolates associated with skin and soft tissue infections

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ABSTRACT

A better understanding of the antimicrobial susceptibility, carriage of virulence determinants and molecular characteristics of *Staphylococcus aureus* isolates associated with skin and soft tissue infections (SSTIs) may provide further insights related to clinical outcomes with these infections. From January 2012 to September 2013, a total of 128 non-duplicate *S. aureus* isolates were recovered from patients with SSTIs. All 128 *S. aureus* SSTI isolates carried at least five virulence genes tested. Virulence genes detected among at least 70% of all tested isolates included *hld* (100%), *hla* (95.3%), *icaA* (96.9%), *clf* (99.2%), *sdrC* (79.7%), *sdrD* (70.3%), and *sdrE* (72.7%). The prevalence of MRSA isolates with 10 virulence genes tested (54.4%, 31/56) was significantly higher than that among MSSA isolates (35.2%, 25/71) ($p < 0.05$). The positive rates of *seb*, *sen*, *sem*, *sdrE* and *pvl* among MRSA isolates were significantly higher than among MSSA isolates ($p < 0.05$). ST7 and ST630 accounting for 10.9% were found to be the predominant STs. The most prevalent *spa* type was t091 (8.6%). MRSA-ST59-SCC*mec* IV was the most common clone (12.3%) among MRSA isolates whereas among MSSA isolates the dominant clone was MSSA-ST7 (15.5%). Six main clonal complexes (CCs) were found, including CC5 (52.3%), CC7 (11.7%), CC59 (8.6%), CC88 (6.3%), CC398 (4.7%), and CC121 (3.1%). A higher carriage of *seb* and *sec* was found among CC59 isolates. In comparison to CC5 and CC7 isolates, those with the highest carriage rates (>80.0%) of *sdrC* and *sdrD*, CC59 isolates had lower prevalence of these two virulence genes. All CC59 isolates were susceptible to gentamicin and trimethoprim/sulfamethoxazole, while CC5 and CC7 isolates had resistance rates to these two antimicrobials of 25.4% and 20.9%, and 40.0% and 40.0%, respectively. The resistance rates for tetracycline, clindamycin, and erythromycin among CC5 isolates were lower than among CC7 and CC59 isolates. In conclusion, the molecular

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typing of *S. aureus* SSTI isolates in the present study showed considerable heterogeneity. ST7 and ST630 became prevailing clones. Different *S. aureus* clones causing SSTIs were associated with specific antimicrobial resistance and virulence gene profiles.

Introduction

Staphylococcus aureus, particularly methicillin-resistant *S. aureus* (MRSA), is an important human pathogen responsible for many infectious diseases including skin and soft tissue infections (SSTIs), foreign-body infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, sepsis, and bloodstream infections in both hospital and community settings.[1] The ability of this clinically important pathogen to successfully persist within the hosts is largely due to the carriage of a battery of virulence factors which promote adhesion, acquisition of nutrients, and evasion of host immunologic responses.[2,3] Some *S. aureus* isolates also produce one or more additional exoproteins, such as toxic shock syndrome toxin-1 (TSST-1), staphylococcal enterotoxins (SEs), exfoliative toxins (ETs), and leukocidins.[2–4] Recently, Panton-Valentine leukocidin (*pvl*) encoded by two contiguous and cotranscribed genes (*lukS-PV* and *lukF-PV*) is an important virulence factor for community-acquired MRSA (CA-MRSA) affecting individuals without apparent risk factors for hospital acquisition.[5,6] *S. aureus* is the most common bacterial pathogen identified from SSTIs.[7] SSTIs caused by MRSA is associated with a high incidence of treatment failure and recurrence.[8] A better understanding of the antimicrobial susceptibility, carriage of virulence determinants, and molecular characteristics of *S. aureus* isolates associated with SSTIs may provide further insights related to clinical outcomes of these infections. Molecular typing has proved to be an important tool to investigate MRSA epidemiology. Pulsed-field gel electrophoresis (PFGE) patterns, SCC*mec* typing, *spa* typing, and multi-locus sequence typing (MLST) have been proven useful for monitoring the evolutionary process of pandemic MRSA clones.[1] In China, ST239-MRSA-III is a predominant MRSA clone among adults, while ST59-MRSA-IV is the most prevalent clone among children.[9,10] In a previous study we investigated the molecular typing of *S. aureus* isolated from patients with SSTIs at our hospital from December 2002 to June 2008 and found that ST239, ST1018, ST59, ST7, and ST88 were the most prevalent sequence types.[11] A shift of important clones has been observed in several studies.[12–14] A report from China found a rapid change of MRSA over a 15-year period at a tertiary care hospital, when the ST239-MRSA-III-t037 clone was replaced by the emerging ST239-MRSA-III-t030 clone.[15] Understanding the shift of important clones at the local and international levels is of great significance. To understand the shift of *S. aureus* clones associated with SSTIs, the present study aimed to investigate the antimicrobial susceptibility, carriage of virulence determinants, and molecular characteristics of *S. aureus* isolates associated with SSTIs at the hospital in 2012–2013.

Materials and methods

Collection of clinical isolates and S. aureus confirmation

From January 2012 to September 2013, a total of 128 non-duplicate *S. aureus* isolates (single isolate per patient) were collected at The First Affiliated Hospital of Wenzhou Medical University, China from pus samples of hospitalized patients with SSTIs. Lesions requiring incision and drainage or with spontaneously draining purulent fluid, carbuncles, furuncles, boils, cellulitis with purulent drainage, chronic ulcer, and deep wounds were included. *S. aureus* isolates from patients with SSTIs with clinical signs and symptoms of infection such as increased white blood cell counts, fever, local redness, swelling, and exudate were considered invasive isolates and included for investigation. Isolates were identified as *S. aureus* using Gram stain, positive catalase and coagulase test results, and Vitek microbiology analyzer (bioMérieu, Marcy l'Etoile, France). *S. aureus* ATCC25923 was used as a control strain.

Ethics statement

This study was approved by the Institutional Ethics Review Board of The First Affiliated Hospital of Wenzhou Medical University. All patients provided written informed consent for this study. The written informed consents were also obtained from the next of kin, caretakers, or guardians on behalf of the minors/children enrolled.

Antimicrobial susceptibility testing

S. aureus susceptibility to penicillin (10 units), erythromycin (15 μg), clindamycin (2 μg), rifampicin (5 μg), tetracycline(30 μg), linezolid (30 μg), mupirocin (5 μg), quinupristin/dalfopristin (15 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), gentamicin (10 μg), ciprofloxacin (5 μg), Chloramphenicol (30 μg), and nitrofurantoin (300 μg) were determined using disc diffusion test recommended by the Clinical and Laboratory Standards Institute (CLSI).[16] All discs were obtained from Oxoid Ltd. Vancomycin MICs for *S. aureus* isolates were determined by agar dilution method. Interpretive standards for the antimicrobial susceptibility test and D-test for tested *S. aureus* isolates were in accordance with the guidelines provided by CLSI.[16] Susceptibility of *S. aureus* to mupirocin was determined by disc diffusion, with a zone diameter ≥14 mm on a 5 μg disc indicating susceptibility as described previously.[17,18] *S. aureus* ATCC 25923 and *Escherichia coli* ATCC25922 were used as reference strains for antimicrobial susceptibility testing.