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Molecular characteristics of *Staphylococcus aureus* isolated from a major hospital in Lebanon

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SUMMARY

Objectives: The occurrence and dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA) in healthcare settings and the community and its risk of being introduced into hospitals are matters of great concern. The purpose of this study was to conduct a miniaturized epidemiological analysis of *S. aureus*-associated infections and to characterize the isolates by a variety of molecular typing techniques. Ongoing molecular surveillance is essential to prevent *S. aureus* strains from becoming endemic in the Lebanese healthcare setting.

Methods: A total of 132 *S. aureus* from different clinical specimens were isolated over a 6-month period. Characterization of the isolates was done by detection of the *mec*A gene, Panton–Valentine leukocidin determinant detection, staphylococcal chromosomal cassette (SCC*mec*) typing of MRSA, *S. aureus* protein A (*spa*) typing, multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), and antibiogram analysis.

Results: MRSA represented 30% of the isolates, with PVL being detected in 54% of MRSA and 12% of methicillin-susceptible *S. aureus* (MSSA). A difference between MRSA and MSSA was observed in the *spa* types. Clustering SCC*mec* with MLST identified seven MRSA and 20 MSSA clones, with PVL-positive ST80-MRSA-IV being the dominant clone (7%), while PFGE revealed 32 groups with 80% cutoff similarity.

Conclusions: Although the results of this study are based on samples collected from one hospital, the high diversity observed along with the lack of any equivalence in the genetic backgrounds of the major MSSA and MRSA clones, emphasizes the urgent need for standardized surveillance combined with the application of well-validated typing methods to assess the occurrence of MRSA and subsequently to control its spread.

1. Introduction

Staphylococcus aureus is a major human pathogen that causes a broad range of serious community-acquired and nosocomial diseases in humans, from minor skin infections to severe infections such as septicemia.[1] The increasing prevalence of methicillin-resistant *S. aureus* (MRSA), and its ability to spread in the hospitals and the community, has posed a major challenge for infection control.[2] Today, although community-associated MRSA (CA-MRSA) imposes low biological cost since it carries a small, easily transferable type of staphylococcal cassette chromosome *mec* (SCC*mec*) and exhibits relative resistance to a limited number of antimicrobial agents, it continues to be a major public health crisis. The fast dissemination of CA-MRSA in the community and its ability to invade hospital settings thus replacing the traditional hospital-associated MRSA (HA-MRSA) strains, makes the epidemiological understanding of CA-MRSA even more complex.[1,3]

The Panton–Valentine leukocidin (PVL) toxin, a prophage-encoded bicomponent pore-forming protein, has been strongly linked epidemiologically to prevalent CA-MRSA strains, although its role in pathogenicity remains controversial, with a number of studies showing its association with primary skin infections and necrotizing pneumonia, while others mitigating its significance as a virulence factor.[1,4–7]

Knowledge of epidemic MRSA clones can help in the development of effective strategies to aid in controlling spread, optimizing

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treatment, and revealing the mode of pathogenicity.[8,9] Monitoring the evolutionary process of prevalent MRSA clones through current genotyping techniques is a crucial step to reveal relatedness among isolates, with sequencing of protein A (*spa* typing), SCC*mec* typing of MRSA, multilocus sequence typing (MLST), and pulsed-field gel electrophoresis (PFGE) being the most valuable typing tools.

In Lebanon, there are limited data on the prevalence of *S. aureus* obtained from both inpatient and outpatient populations, there is no information on the methicillin-susceptible *S. aureus* (MSSA)/MRSA population structure, and little attention has been paid to the molecular epidemiology of this pathogen. Previous findings from Lebanon have provided the first snapshot of the genetic population structure of *S. aureus* in the country.[10] Ongoing molecular surveillance is essential to prevent *S. aureus* strains from becoming endemic in the Lebanese healthcare setting. Accordingly, this study was conducted to better understand *S. aureus*-associated infections and to characterize representative isolates by a variety of molecular typing techniques.

2. Methods

2.1. Clinical isolates

Between May and October 2011 (6 months), a total of 132 consecutive non-duplicate *S. aureus* (designated HST 1–132) clinical samples representing all cultured *S. aureus* isolates recovered in the Clinical Microbiology Section of the American University of Beirut Medical Center (AUB-MC) were collected. AUB-MC provides tertiary services for over 300 000 patients annually with a 350-bed inpatient capacity, occupied by an expatriate population from all over Lebanon as well as neighboring countries. As such, the strain diversity in the hospital is likely to reflect the diversity in the region. All isolates were confirmed as *S. aureus* by growth on mannitol salt agar (MSA), Gram staining, and positive catalase reaction, as well as the ability to produce coagulase enzyme using the SLIDEX Staph Plus agglutination kit (bioMérieux, France).

2.2. Statistical analysis

Categorical comparisons were performed using the Chi-square test. A *p*-value of less than 0.05 was considered to be significant. The associations between MRSA and MSSA carriage along with patient demographics and characteristics were evaluated using the R statistical package (v. 3.0.1). Odds ratios (ORs) and their 95% confidence intervals (95% CIs) were also calculated. The functions used in R included "chisq.test()" from the package "stats" and "oddsratio.wald()" from the package "epitools".

2.3. DNA extraction

DNA was extracted using the Nucleospin Tissue genomic DNA kit (Macherey-Nagel, Germany) in accordance with the manufacturer's instructions.

2.4. Molecular approaches

Amplification of the 16S rRNA, PVL, and *mec*A genes was done as described previously.[11] A PVL-negative MRSA reference strain (N315) and a PVL-positive MSSA reference strain (ATCC 49775) were used. SCC*mec* elements of MRSA were typed using previously described PCR primers.[12] The conditions of the PCR were first optimized using the following reference strains: MRSA NCTC 10442 (SCC*mec* I), MRSA N315 (SCC*mec* II), MRSA 85/2082 (SCC*mec* III), MRSA JCSC 4744 (SCC*mec* IVa), MRSA JCSC 2172 (SCC*mec* IVb), MRSA JCSC 47882 (SCC*mec* IVc), and MRSA WIS (SCC*mec* V). Typing of the polymorphic X region of the *S. aureus* protein A (Spa) was carried out by amplifying the *spa* gene as described previously.[13,14] Thirty-six isolates representing all *spa* clonal clusters (*spa*-CCs) were MLST-typed. Amplification of seven housekeeping genes (carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), glycerol kinase (*glpF*), guanylate kinase (*gmk*), phosphate acetyltransferase (*pta*), triose-phosphate isomerase (*tpi*), and acetyl coenzyme A acetyltransferase (*yqiL*)) by MLST was done according to published sequences.[15] Isolates subjected to MLST typing were also typed using PFGE. Genomic DNA was restricted with *Sma*I and the resulting fragments were separated by PFGE.[16]

2.5. Antibiotic susceptibility testing

All isolates were tested for antibiotic resistance by Kirby–Bauer disk diffusion method, in accordance with the standards recommended by the Clinical and Laboratory Standards Institute.[17] Resistance was tested against the following antibiotics: augmentin, cephalothin, ciprofloxacin, clindamycin, erythromycin, gentamicin, oxacillin, rifampin, teicoplanin, tetracycline, and trimethoprim–sulfamethoxazole. All disks were obtained from Oxoid, UK, and *S. aureus* ATCC 25923 was used as a quality control strain. Isolates showing resistance to erythromycin were further tested for inducible resistance, as described previously.[18]

3. Results and discussion

A total of 132 isolates recovered between May and October 2011 were characterized. Skin and soft tissue infections (SSTIs) accounted for 42% of clinical presentations, which is consistent with the high prevalence of SSTIs caused by *S. aureus*[1] (Table 1). Twenty-one MRSA isolates were PVL-positive (21/39; 54%), while 11 MSSA isolates were PVL-positive (11/93; 12%); the percentage of MRSA PVL-positive was significantly higher than that of the MSSA strains ($p = 8.79 \times 10^{-7}$). However, significant differences were not detected between MRSA and MSSA with respect to specimen origin, gender, or patient status (Table 1).

MRSA represented 30% of the isolates collected in this study, which is significantly lower than the percentage reported previously (93/130; 72%) in a study from Lebanon, where a number of randomly collected isolates from the same hospital (AUB-MC) were partially characterized.[10] It is important to note that an infection control and prevention program (ICPP) serves the AUB-MC to limit MRSA infections.[19] Practices in this program include, but are not limited to, standard precautions (hand hygiene, use of gloves and gowns, appropriate handling of patient care equipment and laundry) and contact precautions (patient placed in a single-patient room when available, limiting patient transport outside the room, use of disposable non-critical patient-care equipment when necessary, frequent cleansing and disinfection of the rooms). The occurrence of MRSA among *S. aureus* varies according to the geographical region, with a low frequency (~1%) in some countries in Europe (e.g., the Netherlands, Denmark, and Sweden) and a high frequency (>60%) in countries such as the USA and Japan.[20–22]

SCC*mec* typing revealed the prevalence of the mobile genetic element SCC*mec* type IV (33/39; 85%), commonly known to be associated with CA-MRSA infections.[23–25] All MRSA isolates harboring the SCC*mec* IV cassette were positive for the PVL gene, while five (13%) harboring the SCC*mec* V cassette were PVL-negative. It is noteworthy that one MRSA recovered from the sputum of a 64-year-old female in the intensive care unit, showed resistance to almost all tested antibiotics and harbored SCC*mec* III; SCC*mec* III is known to be associated with HA-MRSA.[25–27] Similar results were obtained in the study conducted by Tokajian et al. in