Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated over a 2-year period in a Qatari hospital from multinational patients

T. S. El-Mahdy[1,2], M. El-Ahmady[3,4] and R. V. Goering[5]

1) *Faculty of Pharmacy, Helwan University, Cairo, Egypt,* 2) *College of Clinical Pharmacy, King-Faisal University, Al Ahsa, Saudi Arabia,* 3) *Al Ahli hospital, Doha, Qatar,* 4) *Faculty of Medicine, Zagazig University, Zagazig, Egypt and* 5) *Department of Medical Microbiology and Immunology, School of Medicine, Creighton University, Omaha, NE, USA*

Abstract

Global spread of epidemic methicillin-resistant *Staphylococcus aureus* (MRSA) is an issue of increasing clinical concern especially problematic community-associated (CA) -MRSA. However, data regarding MRSA epidemiology in regions of the Middle East, including Qatar, are still insufficient. A representative subset of 61 MRSA isolates from multinational patients from hospital in Qatar during a 2-year period (2009/2010) was examined. Molecular characterization for MRSA isolates was performed by pulsed-field gel electrophoresis (PFGE), SCC*mec*, *spa* and *dru* typing, and PCR for the presence of the arginine catabolic mobile element and genes for the Panton–Valentine leukocidin (PVL). Prevalence of MRSA among *S. aureus* isolated was 176/840 (21%). Of the 61 MRSA isolates examined, three (5%) represented hospital-acquired infection. By PFGE, 32 isolates (52%) were CA-MRSA USA300 (n = 4), USA400 (n = 3), USA1100/Southwest (SW) Pacific (n = 17) and ST80-MRSA-IV (n = 8) strains. The remaining isolates were well-known healthcare-associated EMRSA-15 (n = 8) and USA800 (n = 13). Three isolates were USA900, one was USA1200 and four were unrelated to any known strains in the international database. Unexpectedly, the USA900 isolates were all positive for PVL and USA400 isolates were PVL negative. Five of the eight EMRSA-15 isolates were PVL positive. ST80-MRSA-IV and USA300 strains exhibited typical *dru* types (dt10a and dt9g, respectively). Eleven different *spa* types were observed in this study. All USA300 isolates were arginine catabolic mobile element positive. The high prevalence of CA-MRSA, especially including USA300, in this setting underscores the importance of global epidemiological monitoring to better understand and hopefully help prevent the emergence and spread of these problem pathogens in patient populations.

Keywords: Arginine catabolic mobile element, *dru*, methicillin-resistant *Staphylococcus aureus*, pulsed-field gel electrophoresis, Panton–Valentine leukocidin, Qatar, SCC*mec*, *Spa*
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Corresponding author: T. S. El-Mahdy, Department of Microbiology and Immunology, Faculty of Pharmacy, Helwan University, Ain Helwan campus, 11795 Cairo, Egypt
E-mail: sata186@hotmail.com

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogen of worldwide concern, a cause of morbidity and mortality in both hospital and community populations. Global spread of epidemic MRSA is an issue of increasing clinical concern especially regarding problematic community-associated MRSA. Several molecular typing techniques have been developed to provide knowledge of both transmission and epidemiology of MRSA strains and to support infection control measures [1]. These techniques include pulsed-field gel electrophoresis (PFGE) as a reference standard for MRSA strain typing [2,3], SCC*mec* typing [4], as well as DNA sequence-based typing such as multilocus sequence typing [5], sequencing of the SCC*mec*-associated direct repeat unit (*dru*) [6] and single-locus

DNA sequencing of repeat regions of the *spa* gene (protein A) [7]. Nucleic acid sequence typing has become more popular than PFGE because of the easy data transfer and excellent comparability of results through online databases [8].

Panton–Valentine leukocidin (PVL) is an *S. aureus*-specific exotoxin pore-forming protein encoded by two co-transcribed genes, *lukF-PV* and *lukS-PV*, and is associated with skin and soft-tissue infections and severe necrotizing pneumonia [9]. Healthcare-associated MRSA strains seldom carry PVL, in contrast community-associated MRSA frequently carries this gene [10].

The arginine catabolic mobile element (ACME) was shown to contribute to bacterial growth, survival, transmission and colonization within the host. Although it is frequently found in coagulase-negative staphylococcal species, a variety of strains such as USA300 (ST8-MRSA-IVa) also carry this element [11]. ACME is integrated into *orfx*, the same site on the *S. aureus* chromosome into which SCC*mec* integrates, and contains the structural gene *arcA* with a distinctive nucleotide sequence, encoding arginine deiminase [12].

Epidemiological data on MRSA in regions of the Middle East, including Qatar, are still insufficient. The present study was undertaken to characterize MRSA strains during a 2-year period (2009/2010) in a private 250-bed tertiary-care general hospital in Doha, Qatar.

Materials and Methods

Bacterial isolates

A representative subset of 61 MRSA were isolated from multinational patients in a tertiary-care hospital in Doha, Qatar over a 2-year period (2009/2010). Only isolates from patients resident in Doha for > 6 months and who had a recent history, 3 months before the study, of antibiotic treatment were selected. Duplicate isolates and isolates with incomplete clinical data were excluded from the study. All the isolates were recovered from different patients and different pathological samples (Table 1). The study was approved by the hospital ethics and research committee. MRSA isolates were detected in the clinical laboratory using VITEK 2 AST-P580 cards (bioMérieux, Marcy l'Etoile, France), cefoxitin disc diffusion, and PBP2 latex agglutination (Denka Seiken Co., Niigata, Japan) according to the manufacturers' instructions. Methicillin sensitive *S. aureus* ATCC 25923 and MRSA ATCC 43300 were used as negative and positive controls, respectively in each run. Further confirmation for suspected MRSA isolates was performed by PCR amplification of the *mecA* gene [13]. All MRSA isolates were also tested for mupirocin and inducible clindamycin susceptibility using VITEK 2 AST-P580 cards according to the manufacturer's instructions.

Clinical and epidemiological information for patients was obtained from clinic documents to determine whether these isolates were recovered from hospital-acquired MRSA or community-acquired MRSA infections based on patient history according to the criteria published by the CDC (Atlanta, GA, USA; http://www.cdc.gov/mrsa/diagnosis/index.html). MRSA strains were considered to be community-acquired MRSA if isolates were recovered from outpatients, within 48 h of hospitalization or if the patient lacked the following hospital-acquired MRSA risk factors: haemodialysis, surgery, residence in a long-term care facility or hospitalization during the previous year, the presence of an indwelling catheter or a percutaneous device at the time of culture, or previous isolation of MRSA from the patient.

Molecular characterization

Genomic DNA was extracted by a 10-min incubation with lysostaphin (0.5 mg/mL) at 37°C followed by boiling for 10 min. After centrifugation, the supernatant was used as a template for amplification. PCR for the presence of the gene encoding PVL was performed as previously described [14]. ACME-*arcA* gene was detected by PCR using ACME-specific primers [12]. PFGE analysis with *Sma*I was carried out according to Goering [15]. Macro-chromosomal restriction fragments were separated for 23 h at 6 V/cm, using a 120° included angle at 14°C using a CHEF-DR II System (Bio-Rad, Hercules, CA, USA), with initial and final pulse times of 1 and 35 s, respectively. *Staphylococcus aureus* 8325 was used as a reference strain. Banding patterns were analysed with BioNumerics V6.5 (Applied Maths, St-Martens-Latem, Belgium) with unweighted pair-group arithmetic averages (UPGMA) and Dice coefficients. Band patterns that were >80% identical were considered related as per the recommendation of Tenover *et al.* [3]. PFGE banding patterns were interpreted to different epidemic-type clones following the published guidelines and database [16].

SCC*mec* typing was performed using six multiplex PCR assays [17,18]. For *dru* typing, the *dru* region was amplified and sequenced as described previously [19]. The BioNumerics tandem repeat sequence typing plug-in tool was used for *dru* sequence analysis and assignment of *dru* types used an alphanumeric nomenclature [19]. For *spa* typing, the primers and thermal cycling conditions of the European Network of Laboratories for Sequenced Based Typing of Microbial Pathogens (SeqNet [http://www.seqnet.org]) were used. Analysis of *spa* sequences and assignment of *spa* types were performed using the Spa typing plug-in tool of the BioNumerics software package. Confirmation of sequenced *dru* and *spa* types was also