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Genotyping of methicillin-resistant *Staphylococcus aureus* in the Sultan Qaboos University Hospital, Oman reveals the dominance of Panton–Valentine leucocidin-negative ST6-IV/t304 clone

E. E. Udo[1], B. A.-H. Al-Lawati[2], Z. Al-Muharmi[2] and S. S. Thukral[2]

1) *Department of Microbiology, Faculty of Medicine, Kuwait University, Safat, Kuwait and* 2) *Department of Microbiology and Immunology, Faculty of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Oman*

Abstract

The objective of this study was to determine the prevalence and distribution of methicillin-resistant *Staphylococcus aureus (*MRSA) genotypes circulating at a tertiary hospital in the Sultanate of Oman. A total of 79 MRSA isolates were obtained from different clinical samples and investigated using antibiogram, pulsed-field gel electrophoresis (PFGE), staphylococcal chromosome cassette mec (SCC*mec*), Spa typing and multilocus sequence typing (MLST). The isolates were susceptible to linezolid, vancomycin, teicoplanin, tigecycline and mupirocin but were resistant to tetracycline (30.4%), erythromycin (26.6%), clindamycin (24.1%), trimethoprim (19.0%), ciprofloxacin (17.7%), fusidic acid (15.2%) and gentamicin (12.7%). Molecular typing revealed 19 PFGE patterns, 26 Spa types and 21 sequence types. SCC*mec*-IV (86.0%) was the dominant SCC*mec* type, followed by SCC*mec*-V (10.1%). SCC*mec*-III (2.5%) and SCC*mec*-II (1.3%) were less common. ST6-IV/t304 (n = 30) and ST1295-IV/t690 (n = 12) were the dominant genotypes followed by ST772-V/t657 (n = 5), ST30-IV/t019/t021 (n = 5), ST22-IV/t852 (n = 4), ST80-IV/t044 (n = 3) and 18 single genotypes that were isolated sporadically. On the basis of SCC*mec* typing and MLST, 91.2% of the isolates were classified as community-associated MRSA and 8.8% of the isolates (consisting of four ST22-IV/t852, one ST239-III/t632, one ST5-III/t311 and one ST5-II/t003) were classified as healthcare-associated MRSA. The study has revealed the dominance of a Panton–Valentine leucocidin-negative ST6-IV/t304 clone and provided insights into the distribution of antibiotic resistance in MRSA at the tertiary hospital in Oman. It also highlights the importance of surveillance in detecting the emergence of new MRSA clones in a healthcare facility.

Keywords: Antibiotic resistance, multilocus sequence typing, molecular typing, methicillin-resistant *Staphylococcus aureus*, Spa typing
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Corresponding author: E. E. Udo, Department of Microbiology, Faculty of Medicine, Kuwait University, PO Box 24923, Safat 13110, Kuwait
E-mail: EDET@hsc.edu.kw

Introduction

The burden of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) is increasing among different patient populations globally [1–3]. Following its initial report in 1961 [4], MRSA has remained an important cause of infections in healthcare facilities and in the community globally [1–3]. Although previously restricted to healthcare facilities, especially large tertiary-care facilities [5], MRSA has been increasingly identified as a major cause of community-associated infections in previously healthy hosts since the late 1990s [6–8]. These new MRSA strains have been described as community-acquired or community-originated MRSA. Community-acquired MRSA can be distinguished from healthcare-associated MRSA isolates on the basis of patient risk factors such as history of previous hospitalization, previous antibiotic treatment, admission to intensive care units, advanced age, location at the time of infection and genetic characteristics [6,7].

Advances in molecular typing techniques, including pulsed-field gel electrophoresis (PFGE) [9], staphylococcal cassette chromosome mec (SCC*mec*) [10], Spa typing [11,12] and multilocus sequence typing (MLST) [13] have facilitated the study of clonal distributions of MRSA strains isolated in

different countries and revealed a diversity in the genetic backgrounds of MRSA isolated in different geographical locations [14]. In addition to the capacity to acquire antibiotic-resistance determinants, some MRSA strains have also acquired the ability to spread rapidly between patients within and between hospitals, thereby causing major problems for infection control. Hence, some epidemic MRSA strains have spread internationally [14]. For example, the epidemic MRSA clones ST239-MRSA-III, ST22-MRSA-IV and ST30-MRSA-IV are widely distributed globally [8] whereas the USA300 MRSA is the dominant MRSA clone in North America and another MRSA clone, the ST80-MRSA-IV clone, is distributed widely in European countries, North Africa, the Middle East and the Gulf Cooperation Council (GCC) countries [15].

Studies on the distribution of MRSA clones in the GCC countries are limited [15–18]. Although MRSA has been reported in the Sultanate of Oman since 1995 [19], there are no data on the MRSA genotypes prevalent in the country. This study was conducted to determine the prevalence and distribution of MRSA clones in a tertiary hospital in the Sultanate of Oman.

Materials and Methods

Setting

The Sultan Qaboos University Hospital (SQUH) is a 550-bed teaching hospital of the Sultan Qaboos University. The hospital has 13 different medical departments, which include Surgery, Oral Health, Ophthalmology, Obstetrics & Gynaecology, Medicine, Human Clinical Anatomy, Haematology, Genetics, Family Medicine and Public Health, Emergency Medicine, Child Health, Behavioural Medicine, Anaesthesia and Intensive Care in addition to technical departments.

MRSA isolates

A total of 79 non-repeat MRSA isolates obtained from clinical samples between March and December 2011 at the SQUH were investigated. Isolation and identification of MRSA from clinical samples were performed in the diagnostic microbiology laboratory of SQUH based on cultural characteristics, Gram stain, positive tube coagulase and DNAse tests. Methicillin resistance was confirmed by the amplification of *mecA* as described previously [20]. The isolates were obtained from samples listed in Table 1. Pure cultures of the isolates were preserved in Cryo-bank vials at −80°C. Molecular typing was performed at the Department of Microbiology, Health Science Centre, Kuwait University, Kuwait.

TABLE 1. Association of Panton–Valentine leucocidin-positive (PVL+) methicillin-resistant *Staphylococcus aureus* isolates with different types of infections

Types of infection	No. of strains	No. (%) of PVL+
Skin and soft tissue infections	29	21 (72.4)
Abscess	19	14 (73.7)
Ulcer[a]	4	2 (50)
Skin lesions/boils/furuncles	3	3 (100)
Folliculitis	1	1 (100)
Cellulitis	1	0 (0.0)
Blisters	1	1 (100)
Wounds	22	9 (40.9)
Postsurgical	11	3 (27.3)
Trauma	11	6 (54.5)
Respiratory tract infections	11	0
Pneumonia	4	0 (0.0)
Others[b]	7	0 (0.0)
Septicaemia/bacteraemia	6	3 (50)
Ear infection	5	1 (20)
Invasive infections (osteomyelitis/arthritis)	2	1 (50)
Colonization[c]	4	0 (0.0)
Total	79	35 (44.3)

[a]Bed Sore, pressure sore, mouth and groin ulcers and diabetic foot ulcer.
[b]Including cystic fibrosis.
[c]Nasal and umbilical.

Antibacterial susceptibility testing

Antibacterial susceptibility testing was performed by the disc diffusion method [21] with the following antimicrobial disks (Oxoid, Basingstoke, UK): benzyl penicillin (2 U), cefoxitin (30 μg), kanamycin (30 μg), mupirocin (200 μg and 5 μg), gentamicin (10 μg), erythromycin (15 μg), clindamycin (2 μg), chloramphenicol (30 μg), tetracycline (10 μg), trimethoprim (2.5 μg), fusidic acid (10 μg), rifampicin (5 μg), ciprofloxacin (5 μg), teicoplanin (30 μg), vancomycin (30 μg) and linezolid (30 μg). Discs containing cadmium acetate (50 μg), propamidine isethionate (100 μg) and mercuric chloride (109 μg) were prepared in the laboratory. Minimum inhibitory concentration (MIC) for cefoxitin, vancomycin and teicoplanin were determined with E-test strips (AB BioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. *Staphylococcus aureus* strain ATCC25923 was used as a quality control strain for susceptibility testing.

SCC*mec* typing

SCC*mec* typing was performed by PCR assays as described previously [22,23].

Detection of genes for Panton–Valentine leucocidin

All isolates were tested for the presence of *lukS-PV-lukF-PV*, which codes for Panton–Valentine leucocidin (PVL), in PCR assays using previously described primers and protocols [24,25]. PCR products were analysed by agarose gel electrophoresis.