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Staphylococcus aureus nasal carriage among outpatients attending primary health care centers: a comparative study of two cities in Saudi Arabia and Egypt

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ABSTRACT

Epidemiological and molecular data on community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) are still scarce in both Egypt and Saudi Arabia. There is almost no data regarding methicillin resistant Staphylococcus aureus (MRSA) prevalence in both countries. This study was conducted to investigate the prevalence and molecular epidemiology of *S. aureus* and MRSA nasal carriage among outpatients attending primary health care centers in two big cities in both countries. A total of 206 nasal swabs were obtained, 103 swabs from each country. *S. aureus* isolates were characterized by antibiotic susceptibility, presence of *mecA* and PVL genes, SCCmec-typing and spa typing, the corresponding Multi locus sequence typing clonal complex was assigned for each spa type based on Ridom StaphType database. MRSA was detected in 32% of the Egyptian outpatients while it was found in 25% of the Saudi Arabian outpatients. All MRSA isolates belonged to SCCmec type V and IVa, where some isolates in Saudi Arabia remained nontypeable. Surprisingly PVL$^+$ isolates were low in frequency: 15% of MRSA Egyptian isolates and 12% of MRSA isolates in Saudi Arabia. Two novel spa types were detected t11839 in Egypt, and t11841 in Saudi Arabia. We found 8 spa types among 20 isolates from Egypt, and 12 spa types out of 15 isolates from Saudi Arabia. Only two spa types t008 and t223 coexisted in both countries. Four clonal complexes (CC5, CC8, CC22, and CC80) were identified in both Egypt and Saudi Arabia. However, the data collected lacked a representation of isolates from different parts of each country as only one health center from each country was included, it still partially illustrates the CA-MRSA situation in both countries. In conclusion a set of control measures is required to prevent further increase in MRSA prevalence.

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Introduction

Staphylococcus aureus is one of the most isolated bacterial pathogens in humans, while it is the causative agent of a wide panel of infections ranging from superficial lesions to life-threatening septicemia. Over the past decades it has remained among the top six clinically important pathogens.[1,2] Methicillin resistant *S. aureus* (MRSA) has been recognized as the principal health-care associated pathogen (HA-MRSA) worldwide. In the last two decades, community acquired methicillin resistant *S. aureus* (CA-MRSA) infections have emerged as well.[3,4] Moreover, CA-MRSA strains have been reported to be more virulent than HA-MRSA strains.[5] Carriage of *S. aureus* by humans is a natural phenomenon associated with health and disease. Although multiple body sites can be colonized in human beings, the anterior nares are the most frequent carriage sites for *S. aureus*. It colonizes the nasal mucosa of approximately 30–50% of individuals. Nasal carriage of *S. aureus* is an important risk factor for a wide range of staphylococcal infections. Accordingly, it has been widely used as an indicator to assess antibiotic resistance of *S. aureus* and MRSA in different populations.[6,7]

Molecular typing of MRSA is an important tool for epidemiological surveillance and for development of infection control measures aimed at preventing the occurrence and dissemination of epidemic clones within hospitals, from the community to hospitals, as well as within community. Several methods have been used for *S. aureus* typing. Spa typing is a reliable, accurate and discriminatory typing method of MRSA; it is based on DNA-sequencing of the repeat region of the *Staphylococcus* protein A gene (spa), where repeats are assigned a numerical code and the spa-type is deduced from the order of specific repeats. The concordance between spa typing and multilocus sequence typing (MLST) has been confirmed, and consequently spa typing could be used to predict multilocus sequence typing clonal complexes (MLST CCs) defined by eBURST software.[8] On the other hand, the feasibility of spa typing as a more expedite and less technically demanding alternative typing method for MRSA has been demonstrated in Canada based on the observed concordance of spa types with pulsed-field electrophoresis (PFGE) for Canadian types of epidemic MRSA.[9] Moreover, Vincent et al. (2013) reported on the cost-effectiveness of spa, *Staphylococcus* chromosomal cassette (SCCmec), and Panton-Valentine leukocidin (PVL) genotyping of MRSA in relation to PFGE and MLST.[10]

Since its first description, infections caused by CA-MRSA strains have been reported worldwide.[11,12] In both Egypt and Saudi Arabia, data on carriage, prevalence and genotyping of CA-MRSA are still scarce.[13,14] The objective of this study was to characterize the MRSA clones dissemination among outpatients attending primary health care centers in both countries. In order to characterize the MRSA strains, different molecular typing methods were used including SCCmec typing, spa-typing and the corresponding multilocus sequence typing (MLST) and clonal complex based on the spa types in the Ridom StaphType database.

Materials and methods

Study area and study population

A cross-sectional descriptive study was conducted from July to October 2011 on a community sample from *Al-kanater Alkhyria* city, Egypt and *Buraydah* city, Saudi Arabia. *Al-Kanater Alkhyria* is one of the major cities in Al-Qalyubia governorate, located about 22 km north of Cairo, the capital city of Egypt. *Buraydah* city is the capital city of AL-Qassim district, located 350 km north of Riyadh, the capital city of Saudi Arabia. We randomly collected nasal swabs from 206 outpatients, 103 from each city. All swabs were taken from outpatients attending primary health care centers which are staffed by a group of general practitioners and nurses, delivering services that include family practice, pediatrics, women's care, family planning and dental care. All outpatients included had no history of MRSA infection, hospital admission or nursing home during the previous year, surgery, dialysis, permanent indwelling catheters, or medical devices inserted through the skin. The objectives of the study were explained for the participants, who have accepted to take nasal swabs. For Egyptian outpatients (EGOs), there were 69% females and 31% males, age ranges from six months to 59 years. For Saudi Arabian outpatients (SAOs), 70% were females and 30% males, age ranges from six months to 60 years.

Specimen collection and screening for nasal carriage

Specimens were collected from both anterior nares using sterile cotton swabs moistened with sterile solution of normal saline. All swabs were kept at 4 °C for 24 h until processing in the laboratory. Each swab was inoculated in tryptic soya broth (Oxoid) and incubated overnight at 37 °C to increase the isolation rate of *S. aureus*. The broth was subcultured on mannitol salt agar (Oxoid) plates and incubated aerobically at 37 °C for 48 h. All presumptive *S. aureus* colonies were identified based on colony morphology, Gram staining, production of catalase, tube coagulase, and DNase test. All *S. aureus* isolates were preserved in glycerol and stored at −80 °C for further experiment.

Antimicrobial susceptibility testing

All *S. aureus* isolates were subjected to antimicrobial susceptibility testing by the standard agar disk diffusion methodology according to Clinical and Laboratory Standards Institute (CLSI). The following panel of antibiotics were used: oxacillin, cefoxitin, vancomycin, erythromycin, clindamycin, gentamycin, trimethoprim-sulphamethoxazole, chloramphenicol, ciprofloxacin, tobramycin, moxifloxacin, and rifampicin. All oxacillin and cefoxitin resistant isolates were further confirmed as methicillin resistant by the ability of the isolates to grow on Muller–Hinton agar supplemented with 4% sodium chloride and 6 μg/mL oxacillin.[15,16]

Molecular characterization

Both methicillin resistant and susceptible *Staphylococcus aureus* isolates were grown in tryptic soya broth at 37 °C