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Clonal dissemination of methicillin-resistant *Staphylococcus aureus* in patients and the hospital environment

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SUMMARY

Background: Methicillin is the drug of choice to treat infections caused by resistant strains of *Staphylococcus aureus*. However, methicillin-resistant *S. aureus* (MRSA) is now becoming endemic in many hospitals worldwide and is the cause of nosocomial outbreaks.
Methods: To assess clonality and dissemination of MRSA strains in the hospitals of Tehran, a total of 60 MRSA strains were isolated from hospitalized patients (n = 44) and hospital equipment and environment (n = 16) of three metropolitan hospitals in Tehran between July 2009 and March 2010. These strains were subjected to antimicrobial susceptibility testing, pulsed-field gel electrophoresis (PFGE), and biochemical fingerprinting using the PhPlate system.
Results: Results showed the presence of between one and three dominant clonal groups within each hospital, with most equipment and environmental strains being identical to the dominant clones of hospitalized patient strains. The rate of resistance of these strains to the 13 antibiotics tested ranging from 2% to 100%, with resistance being highest for penicillin, ciprofloxacin, and tetracycline (>98% of the isolates). Comparison of the strains isolated from the three hospitals using a combination of PFGE and PhP types showed the presence of 11 clonal groups of MRSA among these hospitals; of these, three common clonal groups also had identical antibiotic resistance patterns and were found in more than one hospital.
Conclusions: These data suggest dissemination of a few dominant clonal groups of MRSA strains in hospitals in Tehran, with high level resistance to other commonly used antibiotics.

1. Introduction

Staphylococcus aureus is one of the leading etiologic agents of hospital-acquired (HA) and community-acquired (CA) infections.[1] Staphylococcal diseases range from minor infections of the skin to postoperative wound infections, bacteremia, infections associated with foreign bodies, and necrotizing pneumonia.[2,3] Methicillin-resistant strains of *S. aureus* (MRSA) have been reported to be endemic in many hospitals throughout the world, and particularly affect patients in the intensive care unit (ICU) and those who have undergone major surgery.[4,5]

The uncontrolled use of antibiotics is often seen as part of the cause of the growing resistance of bacteria to antibiotics, and the persistence and spread of resistant bacteria in hospitals is of major concern to physicians treating patients.[6] In the acute care setting, patients spend a substantial amount of time in hospital beds surrounded by a variety of equipment, devices, and environmental surfaces that can potentially harbor bacteria. These bacteria may be transmitted to the patients either through direct contact or via the hands of healthcare workers.[7]

Despite the efforts made to control hospital infections, there is growing evidence that the incidence of nosocomial MRSA transmission is continuing to rise throughout the world. This highlights the need for appropriate interventions to optimize the effectiveness of MRSA infection control strategies.[8] In this respect, many reports have addressed the phenotypic and genotypic diversity of MRSA, which will allow us to monitor the dissemination of these strains, which may in turn facilitate their control.[9]

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Among the genotypic methods, pulsed-field gel electrophoresis (PFGE) has been recorded as the gold standard method for monitoring the epidemiology of MRSA in hospitals and the community.[10] Of the phenotypic methods used to characterize nosocomial strains of MRSA, the PhenePlate (PhP) technique has been suggested as a simple and rapid typing method for testing a high number of MRSA strains for such studies.[11]

In view of the importance of the MRSA strains in clinical settings and their growing resistance to a number of antibiotics, this study was undertaken to investigate the prevalence and spread of MRSA strains isolated from patients and the hospital environment in Tehran, Iran.

2. Patients and methods

2.1. Sampling, isolation, and identification of MRSA strains

Between July 2009 and March 2010, a total of 324 clinical isolates of *S. aureus* were collected from hospitalized patients in three metropolitan hospitals, Feriozgar Hospital (FH, 46 isolates), Shirati Hospital (SH, 217 isolates), and Aliasgar Hospital (AAH, 61 isolates), located in different parts of Tehran, Iran. During the same period, a total of 1181 specimens were also collected from the medical instruments within these hospitals by monthly sampling and were tested for the presence of MRSA. The samples were taken from equipment including oxygen pumps and masks, suction devices, radiology apparatus, ventilators and connection tubes, catheters, monitoring devices, respirators, nasogastric tubes, stethoscopes, cardio-shock devices, and serum stands. Samples were also taken from patient rooms, including televisions and TV control handsets, chairs, desks, curtains, trash cans, computers, weighing machines, baskets for collecting sheets, toilet sinks, chests and drawers, bed sheets, doors and door handles, beds, partitions, patient slippers, refrigerators, drinking water sets, walls, and floors. The hospital equipment and environment samples were collected using sterile swabs and transported to the laboratory in sterile normal saline, where they were cultured in thioglycolate broth and incubated at 37 °C for 24 h, after which they were cultured on Baird–Parker agar plates and incubated at 37 °C for 24–48 h.

All clinical samples were initially searched for the presence of *S. aureus* in the pathology laboratory of each hospital using Baird–Parker agar plates and an incubation at 37 °C for 24 h. These samples were also transported to the laboratory and sub-cultured for purity before further testing.

2.2. PCR

Suspected *S. aureus* isolates were subjected to standard biochemical tests and were confirmed by PCR using species-specific primers to amplify the *nuc*C gene. The primer sets were F 5′AATTAATGTACAAAGGTCAAC3′ and R 5′TGATAAATATGG ACGTGGCT3′. The confirmed *S. aureus* isolates were further tested for the presence of the *mec*A gene: F 5′GTAGAAATGACT GAACGTCCGATAA3′ and R 5′CCAATTCCACATTGTTTCGGTCTAA3′.[12] 12 The reaction mixture contained 10× PCR buffer, Taq DNA polymerase (0.5 U) (HT Biotechnology, Cambridge, UK), each primer (1.6 μM), $MgCl_2$ (1.2 μM), each dNTP (0.64 μM), and 10 μl of purified bacterial DNA. The PCR for amplification of the *nuc*C gene was performed under the following conditions: denaturation for 3 min at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 56 °C, and 30 s at 72 °C; and a final extension step of 5 min at 72 °C. Confirmation of the methicillin-resistant isolates was done after an initial susceptibility test against oxacillin followed by amplification of the *mec*A gene under the following conditions: denaturation for 5 min at 94 °C; 30 cycles of 15 s at 94 °C, 15 s at 61 °C, and 30 s at 72 °C; and a final extension step of 2 min at 72 °C.

2.3. In vitro susceptibility tests

Using the methods of the Clinical and Laboratory Standard Institute (CLSI),[13] all clinical isolates were tested for their resistance to 13 antimicrobial agents using the following antimicrobial impregnated disks (Mast Diagnostics Ltd, Bootle, Merseyside, UK): oxacillin (OX) (1 μg), penicillin (P) (10 U), erythromycin (E) (15 μg), ciprofloxacin (CIP) (5 μg), gentamicin (GM) (120 μg), chloramphenicol (C) (30 μg), rifampin (RA) (5 μg), co-trimoxazole (SXT) (25 μg), tetracycline (T) (30 μg), clindamycin (CD) (2 μg), quinupristin/dalfopristin (SYN) (15 μg), vancomycin (V) (30 μg), and linezolid (LZD) (30 μg). The minimum inhibitory concentration (MIC) of the bacterial isolates resistant to oxacillin was measured using E-test strips (Biodisk AB, Solna, Sweden).

2.4. Typing of MRSA isolates

All isolated MRSA were typed using PFGE and a high-resolution biochemical fingerprinting method, i.e., PhPlate CS plates, which are specifically developed for the typing of Staphylococcus strains (PhPlate AB, Stockholm, Sweden). The PhenePlate system is based on the evaluation of the kinetics of biochemical reactions, performed in liquid medium in 96-well microplates.[13] In brief, a loopful of a fresh bacterial culture was inoculated in 10 ml of PhPlate growth medium containing 0.2% (w/v) proteose peptone, 0.05% (w/v) yeast extract, 0.5% (w/v) NaCl, and 0.011% (w/v) bromothymol blue, and aliquots of 175 μl of each bacterial suspension were inoculated into the 24 wells of each set with the aid of a multichannel pipette. The plates were then incubated at 37 °C and images of the plates were scanned after 16, 24, and 48 h using an HP Scanjet 4890 scanner. After the final scan, the PhPlate software (PhPWin 4.2) was used to create absorbance data (biochemical fingerprint) from the scanned images, in accordance with the manufacturer's instructions. Similarity among the isolates was calculated as the correlation coefficient after a pair-wise comparison of the biochemical fingerprints and clustered according to the unweighted pair group method (UPGMA) with arithmetic averages.[14]

For PFGE, a single and well-isolated colony grown on blood agar was inoculated into 10 ml of tryptic soy broth (TSB) and grown on a reciprocal shaker at 37 °C overnight. A bacterial suspension with 150 μl EC buffer (100 mM EDTA, 1 M NaCl, 6 mM Tris-Hcl, 0.2% deoxycholate, 0.5% sodium lauroyl sarcosine, 0.5% Brij-58) containing 20 μl lysostaphin (20 mg/ml) was added to 150 μl molten agarose gel and placed in plug molds. The plugs were treated overnight at 37 °C with lysing buffer containing lysozyme (1 mg/ml) and 5 mg of RNase per ml to lysis buffer (6 mM Tris (pH 7.5), 1 M NaCl, 100 mM ethylenediaminetetraacetic acid (EDTA; pH 7.5), 0.5% Brij-58, 0.2% sodium deoxycholate, and 1% sodium lauryl sarcosine). After digestion with 20 U *Sma*I (Roche, Manheim, Germany), the plugs were placed in the wells of 1% agarose in 0.5% TBE (Tris/Borate/EDTA) and electrophoresed with switch times ramped from 5 s to 35 s at 6 V with a run time of 24 h at 16 °C in a Bio-Rad CHEF-DRII system. The banding patterns were clustered by the UPGMA method using the software Gelcompare II version 4.0 (Applied Maths, Sint-Martens-Latem, Belgium).

3. Results

3.1. Bacterial isolates

In all, 324 strains of *S. aureus* were isolated from the clinical cases in all three hospitals. Of these, 44 strains (13.6%) were shown