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 PhPPlate 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.

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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.\(^\text{10}\) 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.\(^\text{11}\)

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 (AH, 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, cardiac shock devices, and serum stands. Samples were also taken from patients’ 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 thiglycolate 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 \(nuc\) gene. The primer sets were F 5′-ATTAAATGTCAAAAGGTCACA3′ and R 5′-TGATAAAATGCGACGTGGCT3′. The confirmed \(S.\) aureus isolates were further tested for the presence of the \(mecA\) gene: F 5′-TGATAAAATGCGAACGTGGCGATA3′ and R 5′-CCAAACATGTCAAGGTGGGCATA3′.\(^\text{12}\) 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 μM of purified bacterial DNA. The PCR for amplification of the \(nuc\) 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 \(mecA\) 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),\(^\text{13}\) 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), rifampicin (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 PhPlate system is based on the evaluation of the kinetics of biochemical reactions performed in liquid medium in 96-well microplates.\(^\text{14}\) 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.\(^\text{14}\)

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 lauryl 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 SmaI (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
to be MRSA (Table 1). These isolates were collected from the ICU \((n = 21)\), neonatal ICU \((n = 4)\), kidney \((n = 3)\), blood \((n = 5)\), surgery \((n = 2)\), pulmonary \((n = 2)\), labor \((n = 1)\), and orthopedic \((n = 2)\) wards; for four isolates the location was not defined. The most common sources of MRSA isolates were found to be lung secretion \((n = 17)\), blood \((n = 10)\), and wound infections \((n = 9)\), constituting 71% of the collected MRSA in these hospitals. Other sources included body fluid \((n = 3)\), eye infection \((n = 3)\), urine \((n = 1)\), and pharyngeal infection \((n = 1)\) (Table 1).

Among the 1181 specimens collected from medical instruments and the hospital environment in all three hospitals, 16 MRSA strains (1.3%) were isolated. Of these, the majority of the strains were isolated from oxygen masks \((n = 6)\) and ventilators or ventilator tubes \((n = 3)\), nasogastric tube \((n = 1)\), suction device \((n = 1)\), serum tube \((n = 1)\), patient beds \((n = 3)\), and drinking water fountain \((n = 1)\) (Table 1).

### 3.2. Antibiotic resistance of MRSA strains

All clinical isolates were susceptible to linezolid, vancomycin, and quinupristin/dalfopristin. However, they showed different degrees of resistance to other antibiotics (ranging from 2% to 100%), with the highest resistance shown to penicillin, ciprofloxacin, and tetracycline (>98%). Almost the same pattern of antibiotic resistance was observed among the MRSA isolates from equipment and the hospital environment, with the exception that the isolates from the latter group were susceptible to chloramphenicol (Figure 1). It was also found that the clinical and equipment and environment isolates had a MIC ≥256 μg/ml for oxacillin. Among the isolates, two clinical strains showed MICs of ≥3 μg/ml and ≥12 μg/ml.

### 3.3. Clonality of the MRSA strains

According to the PhP typing method, the MRSA strains belonged to six common types (CT1–CT6), constituting 47 (78%) isolates, and 13 single types (ST). CT1 and CT2 contained MRSA strains from both patients and the hospital environment of all three hospitals (Figure 1). Using the PFGE method, the MRSA strains were divided into 14 pulsotypes made of five common pulsotypes, constituting 51 (85%) isolates, and nine single pulsotypes (Figure 1). Whilst strains belonging to common PhP types or pulsotypes were found in two of the three hospitals, certain pulsotypes (i.e. type A) were found in all three hospitals. Clonal types A and C were found in both clinical and equipment and environment samples. Equipment and environment samples in these clonal types included oxygen mask, ventilator, and bed set. Other isolates obtained from oxygen mask and ventilator showed single PFGE types.

Combination of the PhP types, pulsotypes, and antibiotic resistance patterns of the strains showed the presence of 10 common and 25 single types (i.e., C-types F1–F35) with some F types containing strains from different wards (i.e., F27) or from both patients and the equipment/hospital environment (i.e., F20) (Figure 1).

### 4. Discussion

The three hospitals chosen for this study included a children’s hospital, a mid-size metropolitan hospital, and a major metropolitan hospital with extremely high referral rates; these were chosen in order to obtain a better understanding of the prevalence of MRSA strains in Tehran. Indeed, a high proportion of the clinical strains (67%) were obtained from the major metropolitan hospital (i.e., SH). Despite this, the ratio of MRSA over methicillin-sensitive S. aureus (MSSA) strains in all three hospitals did not differ significantly (data not shown), indicating that the number of patients is not the key determinant of the prevalence of clinical cases of MRSA strains in each hospital. A similar pattern was also observed with the equipment and environment strains of MRSA that were regarded as resident in each hospital. An extensive sampling from each hospital’s environment or instruments over a period of 1 year, provided a high confidence level that the presence of environmental strains of MRSA was not overlooked in these hospitals.

Using two high-resolution phenotypic and genotypic methods, these environmental samples were then compared to those isolated from clinical sources in different wards within each hospital. The results indicated a strong link between these strains. Of the isolates evaluated in this study from the three hospitals, 51 (85%) were found to have pulsotypes associated with inter-hospital dissemination, as they were detected in more than one hospital. Moreover, within each hospital, some strains were isolated from different wards, i.e., pulsotypes A and C, indicating intra-hospital transmission as well. Although phenotypes of MRSA isolated from the environment are not always identical to those affecting patients within a clinical setting, there have been several
outbreaks of MRSA that have been directly linked to an environmental source.15–17 These studies collectively indicate that the environment and equipment found in a clinical setting play a major role in sustaining endemic MRSA.15 Several other studies have also indicated the prevalence of MRSA in the hospital environment. Of these, few have explored specific aspects of the environment such as faucet handles, door handles, computer terminals, and bed handsets.18

In the present study, a wide range of fixed and portable items from the environment of each hospital was sampled and in most cases the MRSA strains were identified on only a few items on several sampling occasions. Interestingly, these items were located either on the wards from which the clinical MRSA strains were isolated or on equipment/instruments that were directly connected to patients.

It has been established that a clonal relationship exists between hospital equipment and patients infected with MRSA.19–21 In our study similar equipment from different hospitals was found to carry MRSA strains. For example, in AAH three MRSA strains were isolated from ventilator tubes, two of which were in ICUs.

**Figure 1.** PFGE analysis of MRSA strains isolated from hospitalized patients and the hospital environment in Tehran, Iran. E, erythromycin; CIP, ciprofloxacin; GM, gentamicin; C, chloramphenicol; RP, rifampin; TS, co-trimoxazole; T, tetracycline; CD, clindamycin. CT1–CT6, common PhP types 1–6; CT1–ST13, PhP single, types 1–13.
results were found in FH hospital, where the MRSA strains were isolated from oxygen pump and oxygen mask in the ICU. The high demand for the use of ICU facilities could be a reason for the high prevalence of MRSA in these units, and/or the nature of the ventilating tubes could enhance the accumulation of MRSA.

In this study we employed a high-resolution biochemical fingerprinting method (PhPlate system) and a high-resolution genotypic method (PFGE) to compare the environmental and clinical strains of MRSA within each hospital and between the hospitals. In addition, we sought to identify clonal dissemination within each hospital and the possible long term residency in these clinical settings. Interestingly strains showing identical or highly similar pulsortypes/PhP types also had identical or highly similar antibiotic resistance patterns. On occasions, some strains with identical PFGE or PhP patterns showed resistance to three or more different antibiotics. Considering clonal dissemination of these strains in different hospitals, such differences in antibiotic resistance patterns could be due to different antibiotic treatment regimes used in each hospital, or to the acquisition of resistance genes by members of these clones after they were cross-transmitted, or to their long residency periods in the hospital environment, or a combination of these. This hypothesis, however, has to be verified by analysis of antibiotic gene cassettes and the order in which the resistance genes have been acquired by these strains.

In conclusion, data obtained from the present study confirmed the presence of at least one clonal group of MRSA strains in each of the three hospitals investigated. Through precise typing, these strains were also shown to be found in the environment of the same hospital and had highly similar antibiotic resistance patterns to those isolated from patients. The fact that the majority of the equipment and environment strains were isolated from instruments on the same wards where the patients were infected, suggests that they were the most probable source of infection. However, there is a possibility that other sources of transmission were also involved in these hospitals.

Findings from this study also showed that these strains could be found in other hospitals, indicating the wide spread and cross-transmission of a few clones of MRSA strains in these hospitals, and probably other hospitals, in Tehran. In addition, effective and regular cleaning and disinfection should be applied in order to prevent environmental contamination and nosocomial infections.

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