Characterization of methicillin resistant *Staphylococcus aureus* strains among inpatients and outpatients in a referral hospital in Tehran, Iran

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ABSTRACT

Methicillin resistant *Staphylococcus aureus* is one of the most common causes of a variety of infections ranging from wound infections to urinary tract infections (UTI) in hospital and community. In this study during 3 years we characterized the antibiotic resistance patterns of 491 hospital acquired MRSA and community associated MRSA strains by the guidelines of clinical and laboratory standard institute. A combination of high resolution PhP typing method and SCC*mec* typing were used for clonal dissemination of isolates. Among all 491 MRSA strains, diverse PhP types consisting of 29 common types (CTs) and 4 single types (STs) and also 2 different SCC*mec* types (III and IVa) were detected. In addition, 18 CTs were common among CA- and HA-MRSA strains and the presence of all 4 STs was limited to HA-MRSA strains. All isolates were resistant to penicillin and high level resistance was observed against ciprofloxacin, erythromycin, tobramycin and kanamycin and the rate of resistance to most of the antibiotic tested among HA-MRSA was significantly higher than CA-MRSA isolates. Moreover, all isolates showed susceptibility to linezolid, vancomycin and quinupristin-dalfopristin and very low resistance to fusidic acid, nitrofurantoin and chloramphenicol were detected. Our findings illustrated the increasing rate of clonal dissemination and persistence of highly antibiotic resistant CA-MRSA strains in Tehran hospitals, and also indicated the important role of the hospitals as the reservoir of MRSA strains.

1. Introduction

Staphylococcus aureus is one of the most common causes of a variety of infections ranging from simple wound infections to complicated urinary tract infections (UTI) in hospital and community. During past decades, resistance of *S. aureus* to different classes of antibiotics such as methicillin has increased which making infections by these bacteria difficult to treat, and now methicillin resistant *S. aureus* (MRSA) strains have been prevalent in the hospitals and communities worldwide [1,2]. Methicillin is a semi-synthetic antibiotic which was used in 1960 for treatment of infections caused by penicillin resistant *S. aureus* strains, and the first MRSA strain reported only one year after its introduction in 1961 [3]. One of the most important problems of antibiotic resistance is the emergence of multiple drug resistant (MDR) strains, among hospitalized and outpatients, therefore the options for treatment of

MRSA infections are considerably limited. MRSA infections are associated with a higher mortality rate compared to infections with methicillin-susceptible *S. aureus* (MSSA) [1,4,5].

The *mecA* and novel *mecC* genes are responsible for resistance to methicillin in *S. aureus* strains [6]. The *mecA* gene is a part of the staphylococcal cassette chromosome *mec* (SCC*mec*) which is the only vector for the *mecA* gene and its transfer occurs frequently. According to the diversity in the genetic content and structural organization of SCC*mec* elements, 11 different types have been reported worldwide [7,8]. Detection of the presence of different SCC*mec* types is a useful method for typing of hospital acquired MRSA (HA-MRSA) and community associated MRSA (CA-MRSA) isolates which is used in epidemiological studies of MRSA strains [9]. Different typing methods such as pulsed field gel electrophoresis (PFGE), multilocus sequence typing (MLST), *spa* typing, prophage typing, SCC*mec* and biotyping are used to investigate the epidemiology of MRSA [8,10–13].

The Phene Plate (PhP) system is a simple phenotyping method in which bacterial isolates belonging to the same clonal group show identical metabolic profiles. The kinetics of several biochemical

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reactions (biochemical fingerprinting) in pre-prepared micro plates are measured and analyzed using PhPWIN software [14].

In this study, we characterized the antibiotic resistance patterns and clonal dissemination of CA- and HA-MRSA strains isolated from patients in a referral hospital in Tehran, Iran using SCCmec and high resolution PhP typing methods.

2. Material and methods

2.1. Sample collection and identification of isolates

A total of 2103 *S. aureus* isolates were collected from clinical samples [wound (n = 964, 45.8%), urine (n = 561, 26.7%), blood (n = 337, 16%), sputum (n = 117, 5.6%), CSF (n = 61, 2.9%), ear (n = 35, 1.7%) and eye (n = 28, 1.3%)] in a referral private hospital in Tehran, Iran, during July 2011 and September 2014. Sampling was carried out from hospitalized patients (n = 1229) and outpatients (n = 874) who showed staphylococcal infections. The information about sampling date, hospital wards or clinic, age, and sex of the patients was collected from the laboratory database. All patients who were hospitalized for 72 h and more were excluded as inpatients. All isolates were sub-cultured on HiCrome aureus agar (Himedia, Mumbai, India) and dark brown to black colonies appeared to be pure were collected and identified as *S. aureus* strains using species specific primers for *nucA* gene [15]. *S. aureus* (ATCC 29213) and *Staphylococcus epidermidis* (ATCC 35984) were used as positive and negative references, respectively.

2.2. Antibiotic susceptibility testing

All 2103 *S. aureus* strains were tested for susceptibility to oxacillin (1 μg) using the disk diffusion method according to the guidelines of Clinical and Laboratory Standard Institute (CLSI) for MRSA strains detection [16]. The susceptibility of all oxacillin resistant strains to 17 antibiotics was determined by the guidelines of CLSI [16]. These antibiotics included penicillin (5 μg), ciprofloxacin (5 μg), erythromycin (15 μg), gentamicin (10 μg), chloramphenicol (30 μg), linezolid (30 μg), amikacin (30 μg), kanamycin (30 μg), minocycline (30 μg), sulfamethoxazole-trimethoprim (1.25–23.75 μg), clindamycin (2 μg), tobramycin (10 μg), tetracycline (30 μg), nitrofurantoin (300 μg), quinupristin–dalfopristin (15 μg) and rifampin (2 μg) (Mast Diagnostics, UK). The susceptibility of the strains to fusidic acid was determined as described by McLaws *et al.* [17]. The minimum inhibitory concentrations (MICs) of MRSA strains to oxacillin and vancomycin were determined using broth microdilution assay [18].

2.3. DNA extraction

All isolates were subjected to DNA extraction using the boiling method according to the protocol described previously [2]. Briefly, some pure and distinct colonies of *S. aureus* isolates were suspended in 300 μL of sterile distilled water and vortexed. After 20 min incubation in boiling water, centrifuged at 13000 × g for 15 min and 10 μL of supernatant was used as DNA template in PCR mixture. On the other hand, DNA of MRSA strains was extracted by High Pure PCR Template Preparation Kit (Roche Molecular Biochemicals, Mannheim, Germany).

2.4. SCCmec and ccr typing

The presence of different SCCmec types and subtypes (I–V) among of MRSA isolates was studied using a multiplex-PCR assay with specific primers for SCCmec types and subtypes I, II, III, IVa, IVb, IVc, IVd and V [9]. Also, to identify the type of *ccr*, a multiplex

PCR typing assay consisted of 4 sets of primers specific for each of the *ccr* genes was employed [9].

2.5. Biochemical fingerprinting

The PhP-CS plates (PhPlate AB, Stockholm, Sweden) were employed to type all MRSA isolates according to the guidelines of the manufacturer and the protocol established previously by Rahimi and colleagues [3].

2.6. Detection of pvl gene

Specific primers for *pvl* gene encoding Panton-Valentine leukocidin were used in a PCR reaction as described by McClure *et al.* [19].

2.7. Statistical analysis

Analysis of data was performed using GraphPad Prism 5 statistical software. The Fisher's exact test was used to test for significant associations between categorical variables. P ≤ 0.05 was considered as significant.

3. Results

3.1. Prevalence of MRSA

Of the 2103 *S. aureus* isolates, 23.3% (n = 491) showed resistance to oxacillin and were identified as MRSA, in which 398 (81%) and 93 (19%) MRSA strains were from inpatients and outpatients, respectively. Amongst these, 44% (n = 214), 28% (n = 138) and 15% (n = 72) were isolated from wound, urine and blood, respectively. On the other hand, 288 (59%) MRSA strains were obtained from male and 41% were from female patients. Moreover, the age range of inpatients and outpatients in this study varied between 1 and 90 years. Of these, 20% of isolated strains were from patients in the range of 41–50 years and 19% were found in the age group 51–60 years.

3.2. Antibiotic susceptibility testing

The antibiotic susceptibility testing revealed that all isolates were resistant to penicillin and the highest antimicrobial resistance (more than 90%) was found against ciprofloxacin, erythromycin, tobramycin and kanamycin, respectively (Table 1). Moreover, more than 80% of the isolates were resistant to clindamycin, amikacin and tetracycline. On the other hand, all MRSA strains showed susceptibility to vancomycin, quinupristin–dalfopristin and linezolid, and also low resistance rates were seen to nitrofurantoin, fusidic acid and chlormphenicol in both CA- and HA-MRSA strains. Compare to CA-MRSA, HA-MRSA strains showed significantly higher rates of resistance to different antibiotics except for penicillin, minocycline, nitrofurantoin, fusidic acid and chlormphenicol (Table 1).

Of the 491 MRSA strains, 94.3% (consisted of 65 CA- and 398 HA-MRSA isolates) showed resistance to 2–12 antibiotics tested, and 28 CA-MRSA strains (5.7%) showed susceptibility to all of the antibiotics except for penicillin (data not shown). On the other hand, 96.2% (n = 383) of the HA-MRSA strains showed resistance to more than 8 antibiotics, which was significantly higher than CA-MRSA (60.9%) strains (P < 0.0001).

The MIC values of oxacillin were ranged from 4 to 256 μg/ml, in which 85.7% of MRSA isolates showed high level resistance to oxacillin (MIC = 256 μg/ml) and significant differences were found between HA- and CA-MRSA isolates for resistance to high and low