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Prevalence and antimicrobial resistance pattern of methicillin resistant *Staphylococcus aureus* (MRSA) strains isolated from clinical specimens in Ardabil, Iran

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ABSTRACT

Background and Objective: Reports on MRSA strains are increasing worldwide. The aim of this study was to find the prevalence of MRSA strains isolated from clinical specimens and to evaluate their resistance profile. Additionally we compared the phenotypic and genotypic methods for detection of methicillin resistance.

Materials and Methods: In this cross-sectional study, a total of 41 isolates of *S. aureus* were collected from clinical specimens at two teaching hospitals in Ardabil, Iran. All isolates were identified at the species level by standard biochemical tests. The methicillin resistance were evaluated using three methods: PCR for *mecA* gene, agar dilution for determination of oxacillin MIC and disk diffusion test to detect methicillin, oxacillin and cefoxitin resistance. Antimicrobial resistance patterns were determined by disk diffusion method.

Results: The results identified 19 (46.3 %) out of 41 isolates as MRSA. Most of the MRSA strains (68.4%) were isolated from patients hospitalized in ICU. All isolates were susceptible to vancomycin, mupirocin and linezolid. Among other antibiotics co-trimoxazole was more active against MRSA isolates. Using PCR as reference method all the phenotypic tests showed 100% specificity. The sensitivity for MIC test and cefoxitin was 100% and for methicillin and oxacillin disks was 77.7% and 89.5%, respectively.

Conclusion: The prevalence of MRSA strains in our hospitals especially in ICU ward was high and disk diffusion testing using cefoxitin or oxacillin MIC test as an alternative to PCR for detection of MRSA is recommended.

Keywords: *Staphylococcus aureus*, Methicillin resistance, Antibiotic resistance

INTRODUCTION

Staphylococcus aureus is one of the most important and frequent cause of nosocomial infections worldwide (1). Emergence of methicillin-resistant *S. aureus* strain (MRSA) in 1961 made staphylococcal infections as a major therapeutic challenge (2). Initially MRSA infections were observed in hospitalized patients and those with chronic illnesses. These types of infections are caused by strains named as hospital-associated MRSA (HA-MRSA) (3). In 1990s another type of MRSA strain was emerged that primarily causes skin and soft tissue infections in healthy people. It is called community-associated MRSA (CA-MRSA) (3). MRSA strains show distinct microbiological, therapeutic and clinical features compared to their methicillin-susceptible (MSSA) counterparts. From microbiological aspect, HA-MRSA strains are resistant to multiple classes

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of antibiotics. This characteristic limits proper therapeutic options against staphylococcal infections (4). Clinically, infections caused by HA-MRSA strains are associated with higher mortality and morbidity (5). Some CA-MRSA strains express additional virulence factors that enable them to causes more serious diseases (6).

Currently, MRSA strains account for many of staphylococcal infections and reports of MRSA strains are increasing worldwide (7). There are also several reports from Iran showing the prevalence of methicillin resistance among clinical isolates of *S. aureus* (8-11). A meta-analysis study recently carried out in Iran by Askari *et al.,* showed that the average prevalence rate of MRSA isolates among clinical specimens were 52.7% (12). Understanding the prevalence, antibiotic resistance patterns and information on accurate and reliable detection methods of MRSA strains are necessary for appropriate antibiotic treatment and effective infection control. Considering these, the current study was performed to find the prevalence and evaluate the antimicrobial resistance profile of MRSA strains isolated from clinical specimens in Ardabil, the northwest region of Iran. Additionally we compared the phenotypic and genotypic methods for detection of methicillin resistance.

MATERIALS AND METHODS

Bacterial Isolates. From July to December 2011, a total of 41 *S. aureus* isolates were collected from patients admitted to two teaching hospitals affiliated to Ardabil University of Medical Sciences. Isolates were examined by conventional methods such as colony morphology on blood agar, Gram stain characteristics and catalase production then identified as *S. aureus* by tube coagulase and DNase tests. Identified strains were stored at -80ºC in Mueller-Hinton broth containing 15% glycerol.

Determination of methicillin resistance. Methicillin resistance was evaluated using three methods: 1) Disk diffusion test using 30 µg cefoxitin disk (≤ 21 mm indicated MRSA), 1 µg oxacillin disk (≤ 10 mm indicated MRSA), and 5 µg methicillin disk (≤ 9 mm indicated MRSA); 2) Oxacillin MIC (Minimum Inhibition Concentration) test (≥ 4 µg/ml indicated MRSA); and 3) Polymerase chain reaction (PCR) for the detection of *mecA* gene (positive indicated MRSA) (13,14). Antibiotic disks were obtained from Himedia (Himedia Laboratories, Pvt. Ltd., Mumbai, India) and oxacilllin powder for MIC determination was purchased from Sigma-Aldrich (St. Louis, MO, USA). All tests were compared for sensitivity and specificity with PCR for *mecA* gene as reference method. Sensitivity was calculated by dividing the number of *mecA*-positive isolates detected as resistant using phenotypic methods by the total number of *mecA*-positive strains (ether susceptible or resistant). Specificity was calculated through dividing the number of *mecA*-negative isolates classified as sensitive based on phenotypic criteria by the total number of *mecA*-negative isolates (15).

Antimicrobial susceptibility testing. The isolates were tested for antibiotic sensitivity using the Kirby Bauer disk diffusion method by employing the following disks (disk); penicillin (10) co-amoxiclav (30), chloramphenicol (30), tetracycline (30), ciprofloxacin (10), ceftriaxone (100), cefazolin (30), clindamycin (2), imipenem (10), co-trimoxazole (25), rifampicin (30), gentamicin (10) pristinamycin (15), linezolid (30) and mupirocin (5). MICs of oxacillin and vancomycin to the both MRSA and MSSA isolates were determined by agar dilution method. All procedures were carried out and interpreted according to CLSI guideline (13). *S. aureus* ATCC 25923, ATCC 29213 and ATCC 33591 were used as control strains in disk diffusion and agar dilution methods.

PCR amplification of *mecA* gene. Total bacterial DNA was extracted from *S. aureus* using DNP™ Genomic DNA Extraction Kit (Cinagen, Tehran, Iran). Oligonucleotide primers (14): 5'-AAAATCGATGGTAAAGGTTGGC-3'(forward) and 5'- AGTTCTGCAGTACCGGATTTGC-3' (revers) were synthesized by Bioneer company (Daejon, South Korea). PCR was performed in a 20 × µL AccuPower™ PCR PreMix (Bioneer) with 10 pmol of each primers under the following conditions: initial denaturation at 95°C for 5 min, followed by 34 cycles of 95°C for 1 min, 55°C for 1 min and 72°C for 1 min, and a final incubation at 72°C for 5 min. The amplified DNA fragments (PCR product: 533 bp) were separated on 1% (w/v) agarose gel, stained with ethidium bromide and visualized under ultraviolet light. *S. aureus* ATCC 29213 and ATCC 33591were used as *mecA* negative and positive controls respectively.

Statistical analysis. Chi-square test was used to

Table 1. Prevalence of *S. aureus* among clinical specimens in relationship with specimen type.

Specimen type	MSSA n (%)	MRSA n (%)	Total
Sputum	6 (27.3)	11 (57.8)	17
Blood	6 (27.3)	3 (15.7)	9
Urine	9 (40.9)	1(5.2)	10
Wound	1 (4.5)	3 (15.7)	4
Cerebral spinal fluid	-	1(5.2)	1
Total	22 (100)	19 (100)	41

Table 2. Prevalence of S. *aureus* among clinical specimens in relationship with hospital wards.

Ward	MSSA n (%)	MRSA n (%)
Emergency	2 (13.3)	2 (11.1)
Surgery	1 (6.6)	3 (16.6)
Infectious	4 (26.6)	-
Intensive care unit	3 (20)	13 (68.4)
Outpatient (Clinic)	4 (26.6)	-
Total	22 (100)	19 (100)

compare the prevalence of MRSA and MSSA strains between specimen type and hospital wards.

RESULTS

A total of 41 non duplicate *S. aureus* isolates including 22 (53.6%) MSSA and 19 (46.3%) MRSA were isolated from different clinical specimens that have been sent to the Microbiology Laboratory. The prevalence of MRSA was significantly higher (P = 0.0001) in sputum (n = 11, 57.8%) than other specimens respectively (Table 1). The majority of isolates with MSSA phenotype were cultured from urine specimens (n = 9, 40.9%).

Table 2 shows the distribution of isolate in relationship with hospital wards. The prevalence of MRSA isolates (68.4%) were significantly higher (P = 0.0001) in patients from intensive care unit (ICU). MRSA accounted for about 81.25% of *S. aureus* strains from patients at ICU.

The MICs for oxacillin and vancomycin are listed in Table 3. The MICs for oxacillin were between 64 to ≥ 512 µg/mL and ≤ 0.25 to 1 µg/mL for MRSA and MSSA strains respectively. The MICs for vancomycin against both MRSA and MSSA strains were 1 µg/mL. Only, 1 MRSA strain showed MIC equal to 2 µg/mL. These strains did not fall into vancomycin resistant category according to CLSI (13).

Table 4 represents the resistance pattern of *S. aureus* isolates (MRSA and MSSA) to the tested antibiotics. In this study the entire *S. aureus* isolates were susceptible to vancomycin, mupirocin and linezolid. Among other antibiotics imipenem and co-trimoxazole showed to be the most effective antibiotics against MRSA isolates. PCR testing revealed the presence of *mecA* gene in all isolates (Fig 1) which were determined as methicillin resistant by the phenotypic methods. The sensitivities of oxacillin MIC test and cefoxitin disk were 100%, whereas the sensitivities of methicillin and oxacillin disks were 77.7% and 89.5% respectively.

Table 3. Frequency and range of oxacillin and vancomycin MICs of S. *aureus* (MRSA and MSSA) isolated from clinical specimens by agar dilution method.

MIC	Oxacillin		MIC	Vancomycin	
µg/ml	MRSA, n (%)	MSSA, n (%)	µg/ml	MRSA, n (%)	MSSA, n (%)
≤ 0.25	-	14 (63.6)	1	18 (94.7)	22 (100)
0.5	-	2 (9)	2	1 (5.3)	-
1	-	6 (27.2)			
64	2 (25)				
128	1(12.5)				
≥ 512	16 (62.5)				

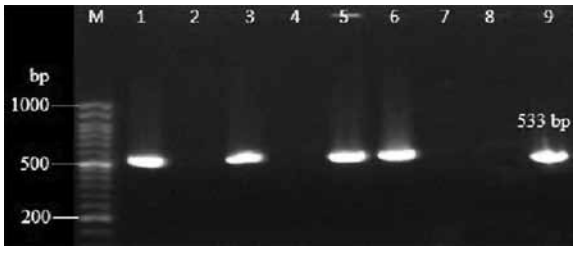

Fig1. PCR detection of *mecA* gene among S. *aureus* isolates. Lane M: 50 pb DNA size marker, Lane 1: positive control strain ATCC 33591. Lane 2: negative control strain ATCC 29213. Lanes 3, 5, 6 and 9 *mecA* positive isolates. Lanes: 4, 7 and 8 *mecA* negative isolates.

DISCUSSION

Since the emergence of MRSA in 1961, there has been a steady increase in the prevalence of this type of *S. aureus* strains worldwide (7). Currently, more than 50% of *S. aureus* infections are caused by MRSA strains in the US (3). The reports from Iran also indicate the increasing incidence of MRSA in clinical specimens over the time (8-12). In this study out of 41, 19 (46.3%) of isolates were MRSA strains. Worldwide, HA-MRSA prevalence varies considerably, from <1 percent in Scandinavia to >50 percent in other countries (7). The estimated prevalence in our study locates in upper limits of the reported ranges. However there is not a uniform prevalence reported for MRSA infection in the different studies. The heterogeneity is probably due to applying different infection control measures, antibiotic administration, human population, study design and laboratory testing for determining methicillin resistance (12). In this study methicillin and oxacillin disks could not detect all MRSA isolates but cefoxitin disk and oxacillin MIC test showed the sensitivity equal to PCR. These results are similar to the previous reports (16). However, the emergence of *mecA* positive oxacillin susceptible and *mecA* negative oxacillin resistant–MRSA strains reduces the sensitivities of both the phenotypic and genotypic methods (17-19). Thus, combination of genotypic and phenotypic tests is necessary to detect the methicillin resistance in *S. aureus* accurately.

MRSA infections pose a significant concern for ICU patients (19). In this study, the incidence rate of MRSA infection in ICU patients was significantly higher than other wards, with an estimated prevalence as high as 68.4% and within ICU MRSA strains were responsible for about 81% of *S. aureus* infections. Previously it has been documented that MRSA accounted for 57% of all ICU acquired *S. aureus* infections (19). However, recent reports indicate declining ICU acquired MRSA infections with applying appropriate infection control measures, rapid and reliable detection of methicillin resistance

Table 4. Antibiotic susceptibility profiles of S. *aureus* strains isolated from clinical specimens by disk diffusion method.

Antibiotic	MSSA (N = 22), n (%)			MRSA (N = 19), n (%)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Vancomycin[a]	22 (100)	-	-	19 (100)	-	-
Penicillin	6 (27.2)	-	16 (72.7)	-	-	19 (100)
Co-amoxiclav	10 (45.4)	-	12 (54.5)	2 (10.5)	-	17 (89.4)
Chloramphenicol	22 (100)	-	-	-	-	19 (100)
Tetracycline	20 (90.9)	-	2 (9)	3 (15.7)		16 (84.2)
Ciprofloxacin	22 (100)	-	-	1 (5.2)	5 (26.3)	13 (68.4)
Ceftriaxone	16 (72.7)	6 (27.2)	-	2 (10.5)	-	17 (89.4)
Cefazolin	21 (95.4)	-	1 (4.5)	3 (15.7)	1 (5.2)	15 (78.9)
Clindamycin	20 (90.9)	-	2 (9)	1 (5.2)	-	18 (94.7)
Imipenem	22 (100)	-	-	14 (73.6)	3 (15.7)	2 (10.5)
Co-trimoxazole	18 (81.8)	1 (4.5)	3 (13.6)	17 (89.4)	-	2 (10.5)
Erythromycin	20 (90.9)	-	2 (9)	3 (15.7)	-	16 (84.2)
Gentamicin	22 (100)	-	-	4 (21)	-	15 (78.9)
Rifampicin	22 (100)	-	-	3 (15.7)	-	16 (84.2)
Pristinamycin	18 (81.8)	1 (4.5)	3 (13.6)	5 (26.3)	-	14 (73.6)
Linezolid	22 (100)	-	-	19 (100)	-	-
Mupirocin	22 (100)	-	-	19 (100)	-	-

a. Vancomycin susceptibility profile was determined using agar dilution method.

and effective antibiotic therapy (2, 20).

In this study, all isolates were susceptible to vancomycin, mupirocin, linezolid. The absence of resistance to mupirocin may be related to the low usage of this antibiotic in the study setting. However others have recently reported the incidence of high-level mupirocin resistant *S. aureus* strains isolated from patients in Iran (11). Mupirocin is topical agent often used to eradicate nasal carriage and control outbreaks of methicillin-resistant *S. aureus* (11).

The vancomycin is the drug of choice for the treatment of infections due to MRSA (21). Several studies reported emergence of vancomycin resistant clinical MRSA isolates around the world (22-24). In our study all of the isolates displayed MICs of ≤ 2 μg/ml to vancomycin and were susceptible to vancomycin.

Multiple-drug resistant characteristics of MRSA and emergence of glycopeptide resistant strains have been frequently caused treatment failure of MRSA infections (25). These findings have promoted researchers to seek new antibiotics for the treatment of MRSA infections (26). Linezolid and pristinamycin showed good activity in vitro and in vivo and are promising therapeutic options against staphylococcal infections (27-28). In this study all isolates were susceptible to linezolid. Similar to our previous study on *S. aureus* strains isolated from health care workers in the same setting (15), 3 (13.6%) MSSA and 14 (73.6%) MRSA strains were found to be resistant to pristinamycin. This antibiotic is not generally used in Iran for treatment of bacterial infectious. Therefore, emergence of pristinamycin resistant *S. aureus* strains in our hospitals could be surprising. There is also a similar finding that has been reported from India (29). Some previous studies demonstrated that antimicrobial selective pressure and microbial cross-transmission are involved in pristinamycin resistance acquisition in *S. aureus* (30).

The high resistance rate for most commonly used antibiotics was observed among MRSA isolates in comparison to MSSA. Most of the MRSA strains were resistant against multiple classes of commonly used antibiotics. Except for above mentioned antibiotics, co-trimoxazol and imipenem showed the lowest resistance rate for MRSA isolates. However, MRSA strains should be considered as resistant to all β-lactam agents other than the cephalosporins with anti-MRSA activity as stated by CLSI (13). Resistance of MRSA to co-trimoxazol in general is low. Several studies reported a decrease in resistance of MRSA to co-trimoxazol over the time (31-32).

In conclusion, the frequency of MRSA infection in our hospitals was found to be high and this finding highlights the need for applying appropriate infection control measures and effective antibiotic therapy. Moreover results emphasize the use of cefoxitin disk diffusion or oxacillin MIC tests as accurate phenotypic methods in clinical laboratories if PCR for *mec*A gene detection is not feasible.

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