Molecular Identification of *mecA* gene in Methicillin-Resistant *Staphylococcus aureus* Isolated from the Hospitalized Patients in Teaching Hospitals of Ahvaz, Iran

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: *Staphylococcus aureus* is a causative agent of nosocomial infections. Methicillin is one of the most important antibiotics that is used in treatment of *S. aureus* infections; however, resistance to this antibiotic has occured in recent years. The aim of this study was detection of *mecA* gene in methicillin-resistant *S. aureus* (MRSA) strains which were isolated from the hospitalized patients.
Methodology: In this study, 255 *Staphylococci* isolates were collected from the patients with infection in three teaching hospitals. These strains were isolated from different specimens and identified using microscopic and standard biochemical tests. *S. aureus* strains phenotypically resistant to oxacillin, were screened after determination of drug resistance patterns against 9

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antibiotics by disk diffusion method based on CLSI guidelines. Then, DNA was extracted from phenotypic MRSA strains and finally *mecA* gene was amplified by PCR.

Results: Out of 255 *Staphylococci* isolates collected from the patients with infection, 180 isolates were confirmed as *S. aureus.* The results of the antibiogram test revealed the highest and lowest rate of resistance against gentamicin and nitrofurantoin, respectively. These results also showed that out of 180 isolates of *S. aureus*, 59 isolates (32.7%) were identified phenotypically as MRSA and *mecA* gene was confirmed in 58 of them by PCR method.

Conclusion: The results of our study showed high prevalence of HA-MRSA isolates among of the examined patients, as nearly one third of these isolates were resistant to methicillin. So, for prevention of the spread of Health care- associated MRSA infection, it is necessary to augment control measures of hospitals and it is recommended that the patients with staphylococcal infection be treated only after verification of drug sensitivity of isolates by clinical laboratories.

1. INTRODUCTION

Staphylococcus aureus is an important causative agent of nosocomial infections. These infections form a range from relatively mild infections of skin, soft tissue, post-operative wound and bacteremia to life-threatening forms such as septicemia in children and necrotizing pneumonia [1,2]. This opportunistic pathogen mainly infects patients who have had surgery or have invasive devices (such as intravascular catheters). Outbreaks of staphylococcal infections are common in these patients and can be a serious threat to their health [2,3].

After introduction of methicillin as a penicillinase-stable penicillin, methicillin-resistant *S. aureus* (MRSA) strains were reported. It was demonstrated that beta-lactam antibiotics cannot bind to PBP2a which are present in *S. aureus* cell wall, so the peptidoglycan layer synthesis is completed and results in the growth of MRSA [4,5].

MRSA isolates are classified into two major groups. The hospital-associated MRSA (HA-MRSA) isolates that were first detected in hospitals, are responsible for the infections which resulting from implanted devices and are acquired within the healthcare setting, while community-associated MRSA (CA-MRSA) isolates which have emerged in recent years infections, most commonly, cause skin and soft-tissue infections and are classified as being acquired outside of any type of healthcare setting [6,7]. HA-MRSA isolates are typically resistant to multiple non-beta-lactam antibiotics, while in contrast, CA-MRSA strains are commonly susceptible to most of other non-beta-lactam antibiotics [8].

The studies have showed that many of CA-MRSA strains could produce a pore-forming cytotoxin due to the presence of genes encoding Panton-Valentine leucocidin (PVL) in these strains [9,10]. Also, some of reports have showed 70-100% of CA-MRSA isolates which cause of infection, carry PVL genes [10,11].

The PBP2a is encoded by *mecA* gene which is carried on a mobile genetic element named staphylococcal cassette chromosome mec (SCCmec) [12,13]. Although previous reports demonstrated eight SCC mec types for *S. aureus*, but based on the International Working Group (IWG-SCC) studies, it currently has been identified 11 types of SCCmec in *S. aureus* which was numbered from I to XI [14].

In addition to produce of resistance to methicillin, *mecA* gene causes of resistance to all currently available β-lactam antibiotics, with the exception of the newest class of MRSA-active cephalosporins.

Emergence of resistance to methicillin and other antimicrobial agents becomes a major concern, especially in the hospital environments, because of the higher mortality rate due to systemic infections caused by MRSA [15]. At present, hospital acquired MRSA is globally endemic, except in Scandinavian countries and the Netherlands, where it is controlled by extensive measures [13].

The studies indicated that the incidence of MRSA in the past few years has increased worldwide. However, there are considerable differences in the prevalence/incidence among countries. For example there is a high prevalence of MRSA from 20% to 60% in the USA, Japan and Southern European countries, while it is less

than 3% in the Netherlands and Scandinavian countries. Also, some of the reports showed that prevalence of CA-MRSA in Europe is lower than in the United States [16], although in the past 10 years, prevalence of MRSA has increased from 2% to approximately 23% today [17].

The MRSA infections spread easily between humans, either directly or indirectly by contact with healthcare workers or a contaminated environment. Because they are highly resistant to drying, staphylococci can survive for months on fomites [13]. Several studies have revealed that the prevalence of MRSA throughout Iran is increasing [18-20], however, the rates considerably differ in each area. The aim of this study was prevalence determination of *mecA* gene in phenotypic MRSA strains which were isolated from the hospitalized patients in three teaching hospitals in Ahvaz city, Iran.

2. MATERIALS AND METHODS

2.1 Bacterial Isolation

A total of 255 non-duplicate staphylococci isolates were collected from hospitalized patients in three teaching hospitals (Golestan, Imam Khomeini and Razi) associated to Ahvaz Jundishapur University of Medical Sciences in Ahvaz city, Iran. These hospital-acquired bacteria were isolated from clinical specimens such as blood, urine, sputum, wound secretions, abscess and respiratory tract secretions during an 18 months period from Nov. 2009 to May 2010. All specimens were inoculated on to blood agar medium (Merck, Germany) and the isolated colonies were identified as *S. aureus* after Gram staining, using standard biochemical tests such as catalase, coagulase, manitol fermentation and DNase agar (Merck, Germany) test [21].

2.2 Antimicrobial Susceptibility Test

This test was performed for isolated bacteria by Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI, 2014) recommendations using 1 µg oxacillin and Mueller-Hinton (Merck, Germany) agar [22]. The following antibiotic discs were used in antibiogram test on all of *S. aureus*

isolates: nitrofurantoin (300 µg), norfloxacin (10 µg), rifampicin (5 µg), azithromycin (15 µg), oxacillin (1 µg), cotrimoxazole (25 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg) and gentamicin (120 µg) (Mast Company-UK).

2.3 Phenotypic Detection of MRSA

Based on the CLSI guidelines (2014), direct colony suspension method was used for phenotypic detection of MRSA. The inoculated plates were incubated at 35ºC, and then inhibition zones around of the disk were measured after 24 h. Inhibition zones with ≤10 mm for *S. aureus* were considered as resistant [22]. Phenotypical MRSA strains underwent with polymerase chain reaction.

2.4 Polymerase Chain Reaction (PCR)

DNA was extracted from colony of methicillin resistant strains by simple boiling method [23]. The polymerase chain reaction was done to detect *mecA*-gene with the primers mentioned in Table 1.

DNA amplification was performed [24] in an Eppendorf master cycler (Eppendorf Germany) in a final volume of 25 µl containing PCR Buffer (10X) 2.5 µl, MgCl2 (50 mM) 1 µl, dNTP mixed (10 mM) 1.5 µl, forward & reverse primers (10 µM) each one 1 µl, Taq DNA Polymerase (500 U) 0.5 µl, DNA template 2 µl and distilled water 15.5 µl (Sina Gene, Iran). PCR condition for amplification of *mecA* gene was programming based on the Table 2.

PCR products were analyzed by horizontal gel electrophoresis in 1% (wt/vol) agarose gels with trisborate-EDTA (TBE) running buffer at 80 V for 1 hour. DNA molecular size markers were run adjacent to the PCR product (Gene Ruler 50-bp DNA ladder, MBI Fermentas). Then the gels were stained with ethidium bromide and observed in Gel-Documentation system (Vilberlourmat-Germany). *S. aureus* strains ATCC33591 and ATCC29213 were used as positive and negative controls, respectively. Collected data were analyzed using descriptive statistics (SPSS Version 17).

Table 1. Primer sequences used for detection of *mecA* gene

Gene	Sequences size	Product	Reference
*mecA*1	F- 5' GTA GAA ATG ACT GAA CGT CCG ATA A3'	310 bp	15
*mecA*2	R- 5'CCA ATT CCA CAT TGT TTC GGT CTA A3'		

Table 2. PCR condition programming for amplification of *mecA* gene

Steps	Temprature	Time (min)	No. of Cycles
Initial denaturation	95^{oc}	5	1
Denaturation	94^{oc}	1	30
Annealing	61^{oc}	1	
Extension	72^{oc}	1	
Final extension	72^{oc}	5	1
Holding	4^{oc}		Until the sample was analyzed

3. RESULTS

In this study, different clinical specimens were collected from various wards of the hospitals which the most of them were including of wound secretions (Table 3). Out of these surveyed specimens, 51.5% belonged to female and 48.5% to male patients with infection.

3.1 Bacterial Isolates

A total of 255 Staphylococci isolates were examined. Out of these, 180 isolates were confirmed as *S. aureus* by standard biochemical tests. The relative frequency of *S. aureus* isolates was 29.4% and 70.6% among collected samples of Golestan and Imam Khomeini hospitals, respectively. The number examined samples and frequency of confirmed *S. aureus* isolates related to each of three hospitals are shown in Table 4.

Table 3. Frequency of examined clinical specimens which were collected from the patients

Clinical specimens	No	Percentage
Wound	65	36.1
Intravenous catheter	25	13.9
Blood	24	13.3
Discharges & lesions	43	23.9
Abscess	9	5
Trachea	9	5
Urine	5	2.8
Total	180	100

Table 4. Frequency of *S. aureus* strains isolated from three teaching hospitals of the Ahvaz city, Iran

Hospital name	Staphylococci Isolates (No)	*S. aureus*	Percentage
Imam	182	127	70.6
Golestan	61	53	29.4
Razi	12	0	0
Total	255	180	100

3.2 Drug Susceptibility Test

Drug resistance rate of isolated strains from Imam Khomeini hospital to beta-lactam antibiotics was high in comparison with Golestan hospital. This rate among of Imam Khomeini hospital isolates was as follows:

Gentamicin 37.8%, Oxacillin 37%, Azithromycin 36.2%, 28.3%; Cotrimoxazol 34.7%, Norfloxacin 34.6%, Ciprofloxacin 33.9%, Rifampicin 19.7%, Chloramphenicol 2.4%, and Nitrofurantoin 0.7%. These results in two hospitals have been compared in chart 1.

3.3 Polymerase Chain Reaction

Out of 180 confirmed isolates, 59 strains (32.3%), were known phenotypically as MRSA based on resistance to Oxacillin, and *mecA* gene was found in 58 isolates (Fig. 1).

4. DISCUSSION

In 1959 methicillin was used for treatment of Staphylococcal infections and two years later resistant strains to methicillin were identified in England. Up to 1980, there were a few reports on this type of resistance but after that, especially, in the last decade, MRSA has spread and reported from most part of world. Nowadays MRSA is one of the main causes of infection spreading in hospitals all over the world [12].

Prevalence and distribution of MRSA strains vary from country to another country. The major community pathogens in the United States have belonged to CA-MRSA strains, but in Europe, the prevalence of these isolates appears to be less than United States [6,16].

In present study, the frequency of MRSA phenotypically was 32.7% of which 32.2% had *mecA* gene. The findings from the others studies have shown different rates of MRSA in throughout of the world. Some of these reports

showed prevalence MRSA isolates in Pano Aqil 19.8% [25], in Karachi 22.9% [26] and in Sweeden 2.1% [27].

Ekrami et al. [28] have reported the rate of MRSA as 60% among burns patients in Taleghani hospital of Ahvaz city, Iran while Khosravi et al. [20] have shown that 87.3% *S. aureus* isolates were MRSA in burns patients of that hospital, indicating a progressive increase in MRSA prevalence during a two years period. The European Antimicrobial Resistance Surveillance (EARSS) annual report (2005) in Belgium also has shown that frequency of MRSA isolates in

hospitalized patients has increased from 22% in 1999 up to 31.4% in 2005 [29].

Vaez et al. [19] reported high frequency of MRSA (85.9%) in health care setting of Gorgan city in Iran. The results of present study in frequency of MRSA is near or similar to some of other`s studies which have been performed in previous years. For example, prevalence of methicillin-resistant *S. aureus* was been reported as 34.7% by Siddiqi in Lahore, Pakistan [27], 35.5% by Mehdinejad in Ahvaz, Iran [30], 39% by Alp in Turkey [31] and 43% by Saima in Karachi Pakistan [32].
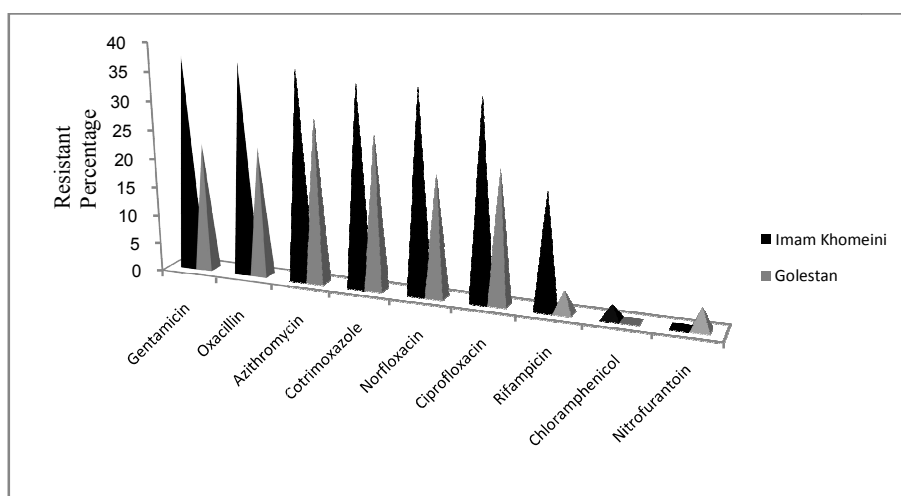

Chart 1. Comparison of resistant *S. aureus* isolates to the antibiotics in two teaching hospitals of the Ahvaz city, Iran
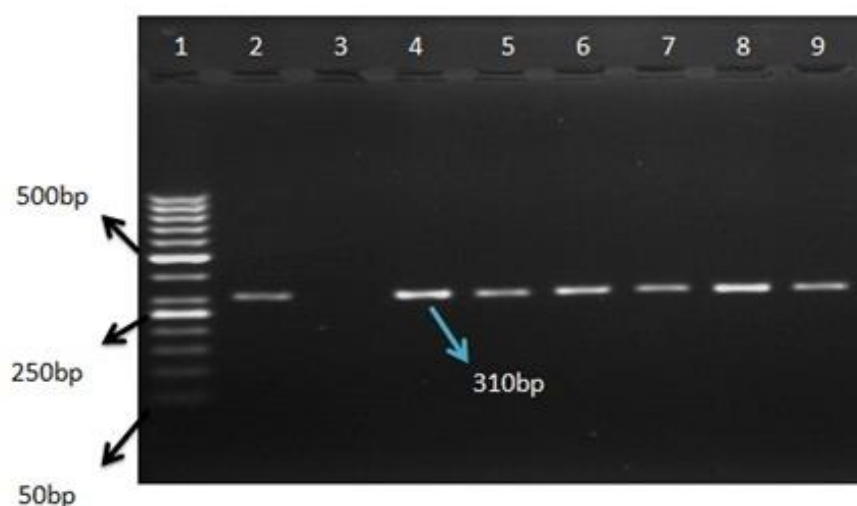

Fig. 1. Detection of *mecA* gene by PCR. Line 1. 50 bp marker, Line 2. *S. aureus mecA* positive control, Line 3. *S. aureus mecA* negative control, Line 4-9. Sample test

The different rates of MRSA in these reports probably are due to presence of risk factors such as recent hospitalization, surgery, dialysis, indwelling percutaneous- medical devices or catheters and residence in a long-term care facility could effect on MRSA infections. Naimi et al. [33] showed that community-associated and health care- associated MRSA isolates are different and distinct demographically, clinically and microbiologically.

In our study, out of 59 strains which were resistant to oxacillin phenotypically, 58 strains had *mecA* gene and only one isolate showed no *mecA* gene by PCR method. Similar to this in a report by Alp`s from Turkey, out of 69 MRSA phenotype isolates, 54 harbored *mecA*-gene and the rest 15 cases did not have this gene [31].

Other mechanisms of resistance to methicillin are probably operating in those siaoltes which were MRSA phenotypes but lacked *mecA* [34].

Although, in present study, resistance rates to the examined antibiotics were generally higher in Imam khomeni hospital patents, but the resistance to gentamycin and oxacillin was more than the other antibiotics.

MRSA has been one of the most important problems in healthcare settings in recent years. Early diagnosis of MRSA is warranted in our hospitals for prevention of mortality. This will also enable use of more appropriate antibiotic other than methicillin and reduce emergence and further spread of MRSA in the community.

5. CONCLUSION

The results of our study showed high prevalence of HA-MRSA isolates among of the examined patients, as nearly one third of these isolates were resistant to methicillin. So, for prevention of the spread of Health care- associated MRSA isolates, it is necessary more control measure of hospitals and it is recommended that the patients with staphylococcal infection be treated only after detection of MRSA isolates by clinical laboratories.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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