Original Article

The comparison of *Staphylococcus aureus* types 5 and 8 with respect to methicillin resistance in patients admitted to Al-Zahra Hospital by PCR

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Abstract

Background: *Staphylococcus aureus* is a common human pathogen in community- and hospital-acquired infection, and its capsule is involved in pathogenesis. The predominance of 2 capsular polysaccharides types 5 and 8, on the surface of clinical isolates, led to the development of conjugate vaccine (Staph VAX) based on capsular polysacchrides types 5 and 8 conjugated to a carrier protein.

The aim of this study was to determine the prevalence of capsular polysaccharides types 5 and 8 *Staphylococcus aureus* strains among isolates and their comparison with respect to methicillin resistance.

Materials and Methods: We studied the capsular genotypes of 193 isolates that encompassed both hospital- and community-acquired infection in Al-Zahra Hospital of Isfahan city from 2008 to 2009. Cap5 and 8 genes were detected by PCR method. Methicillin resistance was determined by PCR (mecA) and disk diffusion methods as well.

Result: In this population (193 cases), most of the clinical isolates (73%) expressed capsular polysaccharide type 5 (24%) and 8 (49%), whereas 27% were non-typeable. The prevalence of MRSA in type 8 was 67.9%, whereas MRSA isolates in the capsular genotype 5 were 22.2%.

Conclusion: This study *Staphylococcus aureus* confirms that the prevalence of capsular polysaccharide types (5 and 8) are predominant, and *Staphylococcus aureus* type 8 is more resistant to methicillin compared to type 5.

Key Words: Isfahan, methicillin resistance, polymerase chain reaction, *Staphylococcus aureus* types, types 5 and 8

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INTRODUCTION

Staphylococcus aureus is an important hospital and community bacterial pathogen.

The virulence factors of *S. aureus* include adhesin, cytokine, superantigens, exotoxins, enzymes and capsular polysaccharide, which are important in its pathogenicity.[1]

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The capsule is an important virulence factor in this bacteria, which protects it from phagocytosis by scavenger cells and accounts for the higher pathogenicity in capsule-containing strains.[2,3]

11 serotypes of *S. aureus* have been currently identified on the basis of capsular polysaccharide with types 5 and 8 being predominant.

Studies carried out in America and Europe indicates that *S. aureus* types 5 and 8 account for 70-85% of the total infections caused by this bacteria.[1,5-9]

Studies also suggest that 80-90% of the isolates collected from MRSA infections in humans contain type 5 and 8 capsules.[10-12]

Considering the high prevalence of capsules type 5 and 8, it may serve as a suitable candidate for vaccine production.

Extensive research is currently taking place on conjugate vaccines, which are a combination of capsules type 5 and 8 with a protein.[2,13]

The aim of this study include the following: To highlight the importance of *S. aureus* types 5 and 8 in producing infections, to determine the prevalence of these strains in Iran, to clarify their role in causing infections and ultimately to compare their resistance pattern against methicillin antibiotic.

MATERIALS AND METHODS

Sample collection

In the present study, of the total 193 isolates tested, 61 isolates were from skin samples, 34 from lungs, 53 from urine, 12 from synovial and 9 isolates were of other sources.

S. aureus identification

The species were identified on the basis of colony, microscopic and morphologic characteristics and using tests including catalase, coagulase, manitol salt agar and DNase.[14]

DNA extraction

S. aureus isolates were maintained on BHI agar plates for 24 hours, and DNA was extracted using phenol-chlorophorm method.[15]

Capsule identification by PCR

To identify capsules, type 5 and 8 PCR was carried out using the following primers:[1]
cap5 k (5'-GTCAAAGATTATGTGATGCTACTGAG-3')
cap5 k2 (5′-ACTTCGAATATAAAC TTGAATCAATGTTATACAG-3′) for type 5 and
capsul8 k (5′-GCCTTATGTTAGGTGATAAACC-3′)
capsul8 k2 (5′-GGAAAAACACTATCATAGCAGG-3′) for type 8 detection.

The amount used in 30 microliter volume in each sample was as follows:

- Buffer 10 × 3 µLit, MgCl2 1.5 mM, Primer F 0.5 mM, Primer R 0.5 mM, Taq Pol 1.5 unit, dNTP Mix 0.2 mM, DNA Template 5 µLit.

Thereafter, this mixture was placed in the thermocycler for 25 cycles with the following program:

- Pre-denaturation step for 5 min at 94°C, denaturation for 30 sec at 94°C, annealing for 30 sec at 55°C, extension for 1 min at 72°C and finally post-extension for 5 min at 72°C.
- PCR products were analyzed on agarose gel 1.5 and were stained with ethidium bromide.
- The resultant fragments had a length of 361 bps for type 5 and 173 bps for type 8.

MecA detection by PCR

Following verification of *S. aureus* types 5 and 8, the samples were tested for MecA presence.

PCR was carried out using the following primers:[16]
mecA 1 F 5′ TGGCTATCGTGTCACAATCG3′
mecA 2 R 5′ CTGGAACTTGTTGAGCAGAG 3′

The amounts used in 50 µLit volume for each sample was the following:
Buffer 10 × 5 µLit, $MgCl_2$ 1.5 mM, Primer F 0.5 mM, Primer R 0.5 mM, Taq Pol 2 U, dNTP Mix 0.2 mM, DNA Template 10 µLit.

Thereafter, this mixture was placed in the thermocycler for 30 cycles with the following program:
Pre-denaturation for 5 min at 94°C, denaturation for 1 min at 45°C, extension for 1 min at 72°C and finally post-extension for 7 min at 72°C.

The PCR products underwent electrophoresis on agarose gel 1%, and the resultant bands were analyzed after staining with ethidium bromide. The standard MRSA strain ATCC 33591 was used for negative and positive control for PCR test.

Sensitivity detection test by disk diffusion

Following identification of methicillin-resistant types by PCR, the resistance of these strains was tested against methicillin using disk diffusion method.

For anti-biogram tests, the samples were maintained on BHI agar plates for 24 hours, after which a microbial suspension of the same concentration with

0.5 McFarland was prepared and cultured on Muller Hinton agar medium.

Anti-biogram disks were placed on the medium after 40 min.

With respect to oxacillin, a Muller Hinton medium containing 4% Nacl and 6 μ gr oxacillin powder were used.

Thereafter, the plates were incubated for 24 hours at 37°C, and the concentration was measured.[16]

RESULTS

In this study of the total 193 isolates, tested 94 (49%) were type 8, 46 (24%) type 5, and 53 (27%) were of other types.

Furthermore, 112 isolates were found to be susceptible, and 81 were resistant to methicillin. With respect to type 8, 39 isolates (41.5%) were reported susceptible and 55 isolates (58.5%) resistant to methicillin, whereas among type 5, 28 (60.9%) of the isolates were susceptible and 18 (39%) were resistant to methicillin. Among other types, 45 isolates (84.9%) were reported susceptible, and 8 (15.1%) were resistant to methicillin.

Statistically, resistance to methicillin was 67.9% for type 8 and 22.2% for type 5 and 9.9% for other types [Figure 1].

In the present study, methicillin resistance was tested using PCR in 81 samples (42%) and disk diffusion in 100 samples (51.8%).

Strains tested positive by PCR were also methicillin-resistant when tested by disk diffusion [Figure 2].

Although 19 isolates which were found to be methicillin-resistant by disk diffusion were negative for MecA gene [Figure 3].

Table 1: Comparison of *S. aureus* types 5 and 8 with respect to methicillin resistance by PCR and disk diffusion methods

Method	Serotype	Number	Frequency of MRSA (%)	*P* value*
PCR	8	94	55 (58.5)	<0/001
	5	46	18 (29.1)	
	other	53	8 (15.1)	
	total	193	81 (41.9)	
Disk diffusion	8	94	65 (69.1)	<0/0001
	5	46	19 (41.3)	
	other	53	16 (30.2)	

*Chi-square test. Data are respected as percentage

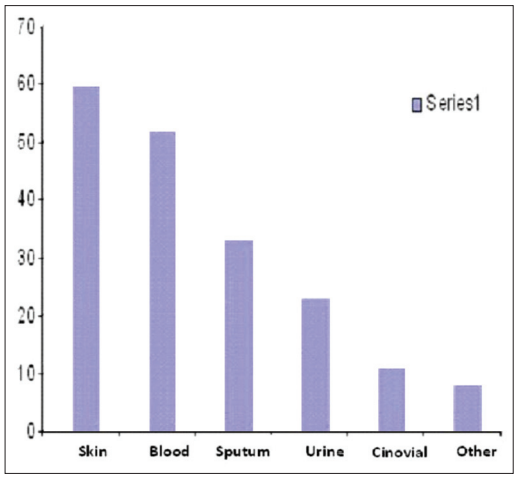

Figure 1: Relative frequency of types (5 and 8) *S. aureus* in various infections

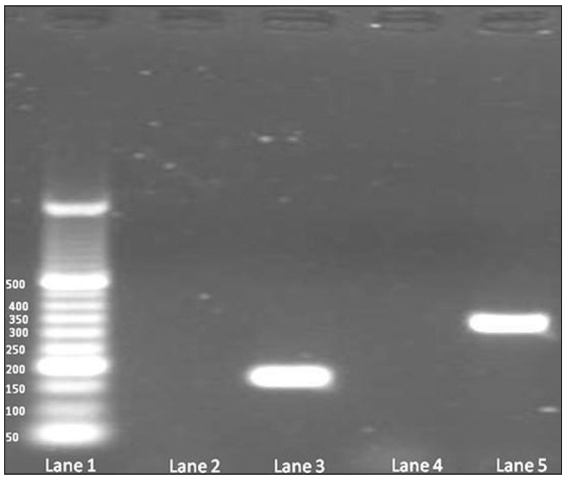

Figure 2: Gel electrophoresis of cap 5 (361 bp) and cap 8 (173 bp) gene along with negative and positive control. Lane 1: DNA ladder 50 bp. Lane 2: Distilled water. Lane 3: Positive control Renold strain (type 8). Lane 4: Distilled water. Lane 5: Positive control Becker (type 5)

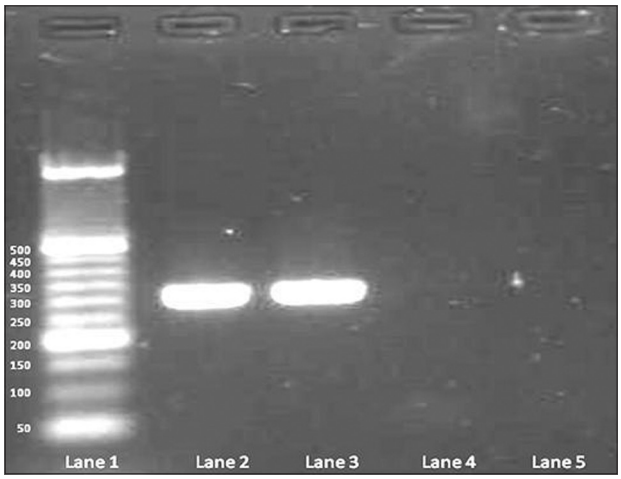

Figure 3: Gel electrophoresis of mecA gene (309 bp) along with negative and positive control. Lane 1: DNA ladder 50 bp. Lane 2: MRSA strain ATCC33591. Lane 3: Positive sample. Lane 4: MSSA strain ATCC2313. Lane 5: Distilled water

With respect to disk diffusion, sensitivity and specifity was estimated 100% and 83%, respectively [Table 1].

DISCUSSION

The aim of this study was the comparison of *S. aureus* types 5 and 8 with respect to methicillin resistance in patients admitted to Al-zahra hospital in Esfahan.

These isolates were collected from different infective sources, mostly skin infections (31.6%).

In this study, 73% of the isolates were either capsular type 8 (49%) or type 5 (24%) with 26% accounting for other types. The prevalence of types 5 and 8 was in accord with other studies conducted in different geographic areas.[1,6-11]

In a study carried out in 2005 by Roghamann *et al.* in America, a total of 259 *S. aureus* isolates were tested, from which capsular types 5 and 8 accounted for 50% and 42%, respectively, with 8% reported for other types.[12]

In another study conducted in 2007 by Verdier *et al.* in France, of a total 195 *S. aureus* isolates tested, 42% were reported to be type 5, 45% type 8 and 13% were reported for other types.[1]

In another study carried out in Argentina in 2009, of the total 118 *S. aureus* isolates collected from patients with osteomyelitis, 76 isolates (64%) were capsular types 5 and 8 where type 5 accounted for 57 isolates and type 8 accounting for 19 isolates.[17]

Our study reports a methicillin resistance of 67.9% in type 8, 22.2% in type 5 and 9.9% in other types. It is evident from the results that methicillin resistance was higher in type 8 compared to type 5.

In a study by Essawi *et al.* in 1998, of the total strains (19%) collected from patients with MRSA, 8 isolates (66.7%) were type 5.[8]

In a study by Na'was *et al.* in 1998, 65 *S. aureus* isolates were tested, of which, 43.1% were reported to be type 5, 44.6% type 8 and 12.3% was reported to be of other types. 9 isolates (13.8%) were found to be methicillin-resistant with 100% of the samples belonging to type 5.[9]

Therefore, our results were in agreement with studies such as Essawi *et al*, and conversely different from studies performed by Verdier, Damain, and Roghamann as they reported methicillin resistance higher in type 5. This difference is probably due to different geographic areas.

In this study of the 193 isolates tested, methicillin resistance was detected in 81 (42%) MRSA samples by PCR and in 100 (51.8%) samples by disk diffusion method.

All strains tested mecA positive using PCR were found to be methicillin-resistant by disk diffusion method.

In a study conducted by Sakoulas in 2001, of the total 203 *S. aureus* strains tested by phenotypy (disk diffusion) and PCR, 2 mecA positive strains were reported to be susceptible to methicillin in the disk diffusion method.[18]

In a study by Cekovska in 2005, a total of 210 *S. aureus* strains were tested for methicillin susceptibility using PCR and disk diffusion.

They reported 3 strains lacking MecA gene to be methicillin-resistant by PCR.[19]

In another study carried out in Turkey in 2008, 416 *S. aureus* strains were assessed with regards to methicillin resistance, of which 210 (51%) strains were shown to be methicillin-resistant using PCR as gold standard. In this study, 26 mecA-negative isolates were reported to be methicillin-resistant using disk diffusion method.[20]

In a research performed in Egypt in 2007, of the 63 clinical isolates tested, 39 isolates were found to be methicillin-resistant by PCR.

Disk diffusion was also performed, which showed of the 39 isolates found to be methicillin-resistant; by PCR, 33 (84.6%) of the isolates were resistant and 6 (15.4%) were susceptible to methicillin.

In addition, of the 24 isolates found to be methicillin susceptible by PCR, 5 isolates (20.8%) were reported methicillin-resistant when tested by disk diffusion method.[16]

Pseudo positive results in disk diffusion could be due to the overproduction of Beta lactamase, production of normal proteins with altered PBP binding abilities, or due to other unknown factors which can produce low levels of resistance to methicillin in *S. aureus* strains lacking the MecA gene.[19]

Furthermore, our results showed the higher prevalence

of *S. aureus* types 5 and 8 which is similar to other countries and that over 70% of the clinical isolates contain capsules type 5 and 8.

Since the vaccine is currently passing the 3rd phase of clinical testing and the prevalent capsules type 5 and 8 are employed in the structure of this vaccine.[2,13] This study indicates a high efficacy of the vaccine in Iran and that proves useful in over 70% of infections in high risk people.

With respect to bacterial resistance, considering the pseudopositive results in disk diffusion, PCR could be used to determine methicillin resistance.

Rapid and precise detection of methicillin-resistant strains can help in rapid onset of antibiotic therapy and the avoidance of extravagant usage of glycopeptid (as the last antibiotic of choice against methicillin-resistant strains) drugs.

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