Original Article

Frequency of *fnbA*, *fnbB*, *hla* and *cna* genes in *Staphylococcus aureus* isolates obtained from blood cultures and their antimicrobial susceptibility pattern in Tabriz, Iran

Hamed Emami Kamel Abad [1, 2], Javid Sadeghi [2-3]*, Mohammad Aghazadeh [2-3], Mohammad Ahangarzadeh Rezaee [2-3], Hossein Samadi Kafil [2-3], Mahin Ahangar Oskouee [2-3], Delara Laghousi [4], Fatemeh Yeganeh Sefidan [2], Aidin Lalehzadeh [2], Elham Sheykhsaran [2]

[1]Students' Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran. [2] Department of Bacteriology and Virology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran. [3] Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. [4] Social Determinants of Health Research Center, Health Management and Safety Promotion Research Institute, Tabriz University of Medical Sciences, Tabriz, Iran.

Abstract

Background: The infections caused by *Staphylococcus aureus*, are emerging as a major public health concern. Antibiotic resistance in these isolates makes the treatment procedure harder and costlier. The aim of this study was to determine the frequency of *fnbA*, *fnbB*, *hla* and *cna* genes and antibiotic susceptibility pattern in *Staphylococcus aureus* isolates collected from blood cultures. **Material and Methods:** Totally, 100 *Staphylococcus aureus* isolates were collected from blood cultures at four university hospitals in Tabriz. Susceptibility patterns of isolates to antibiotics including erythromycin, clindamycin, ciprofloxacin, cefoxitin, gentamicin, cotrimoxazole, and linezolid were determined by disk diffusion agar and susceptibility of isolates to vancomycin were identified by microbroth dilution method. The frequency of the *fnbA*, *fnbB*, *hla* and *cna* genes was evaluated through the PCR as well. In addition, the molecular typing of isolates was performed by rep-PCR. **Results:** A high susceptibility rate to vancomycin, linezolid and cotrimoxazole in isolates was observed, while, erythromycin and clindamycin had the lowest efficiency to deal with infections. Totally, 81%, 81%, 73%, and 30% of isolates were containing the *fnbA*, *fnbB*, *cna,* and *hla* genes, respectively. **Conclusion:** In addition to vancomycin, linezolid and cotrimoxazole have the potency to be prescribed in the treatment course of staphylococcal infections.

Keywords: *Staphylococcus aureus*, Blood culture, Susceptibility patterns

Introduction

Staphylococcus aureus (*S. aureus*) is often found in the skin, skin glands, and mucous membranes, particularly in the nostrils of healthy individuals [1]. Approximately, 20-30% of individuals are considered as *S. aureus* carriers in their different tissues. This microorganism is a leading cause of bloodstream infections, especially in hospitals. Staphylococcal bacteremia often indicates a serious medical condition [2, 3]. *S. aureus* has multiple virulence factors and resistance mechanisms. These features may be the reason for the increased disease caused by this bacterium [4]. In the recent decades, staphylococcal infections has increased dramatically in which the mortality rates reach to 15-60% [5, 6]). Among of *S. aureus* virulence factors collagen-binding protein and fibronectin-binding protein A and B are the major factors in the tissue colonization in some clinical conditions such as keratitis and osteomyelitis. Staphylococcal *α-hemolysin* is able to penetrate into the macrophages, epithelial cells, and erythrocytes [7, 8].

The significant mortality rates followed bacteremia and extended hospitalization period forces the extra expenditures to the health care system and patients [9]. A number of antibiotics have been prescribed against staphylococcal infections including beta-lactams and linezolid [10, 11]. Vancomycin, as a glycopeptide also has been prescribed to treatment of invasive methicillin-resistant *S. aureus* (MRSA) infections. Emerging data indicate that vancomycin may be losing its effectiveness against serious infections caused by MRSA with minimum inhibitory

Address for correspondence: Dr. Javid Sadeghi, Department of Bacteriology and Virology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
Email: Sadeghij@tbzmed.ac.ir

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concentration (MIC) values at the higher end of the susceptibility range [12].

Bloodstream infections accountable for approximately 30–40% of all severe sepsis and septic shock cases, and are major causes of fatality rates. Diagnosis of these infections must be performed appropriately by more suitable methods including the molecular techniques [13]. In this study, the prevalence of *fnb*A, *fnb*B, *hla* and *cna* genes in *S. aureus* isolates collected from blood cultures and their antibiotic susceptibility profile, were identified.

Materials and Methods

Bacterial isolation and identification

A total of 100 gram-positive, catalase positive cocci were obtained from septicemic patients during 2018 from Imam Reza, Shahid Madani, Koodakan, and Sina hospitals in Tabriz. Thereafter, collected isolates were subjected to growth on Mannitol salt agar (Selective and differential medium) with the ability to ferment the mannitol and change the medium color to yellow, presence of deoxyribonuclease enzyme (DNase) which cleaves the DNA and release free nucleotides, and phosphate, and tube coagulase test (coagulated plasma) in order to the identification of *S. aureus* isolates [14].

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Antimicrobial susceptibility testing

In order to determine susceptibility patterns in identified isolates, disk diffusion agar method was conducted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [15]. The antimicrobial disks were included ciprofloxacin (5 µg), erythromycin (15 µg), clindamycin (2 µg), cefoxitin (30 µg), gentamicin (10 µg), linezolid (30 µg), cotrimoxazole (25 µg) purchased from (Mast, UK). *S. aureus* ATCC 25923 was used as a control strain.

The minimum inhibitory concentration (MIC) of *S. aureus* isolates against vancomycin was performed by microbroth dilution method. The interpretation of results was done according to the CLSI guidelines. *S. aureus* ATCC 29213 was used as a control strain.

Investigation of the presence of *fnbA*, *fnbB*, *hla* and *cna* genes in the *S. aureus* isolates

In order to this aim, PCR was performed in a value of 20 µL. First, the DNA extraction was done by the boiling method [16]. Thereafter the reagents in water, buffer, dNTPs, Mg Cl_2, template primers, Taq polymerase ingredients was prepared and in the final step, 1 µL of extracted DNA was added to the master mix. The sequences of used primers (Purchased from Sina clone) and PCR conditions are depicted in Table-1. In the last step, PCR amplicons were investigated via electrophoresis for 1 hour on a 1% agarose gel and detected bands were visualized under UV light.

Molecular typing with rep-PCR

S. aureus isolates were typed using rep-PCR with the primer of RW3A (TCGCTCAAAACAACGACACC). PCR amplification was performed under following conditions: 94 °C for 3 min, 30 cycles with 94 °C for 1 min, 54°C for 1 min, 72 °C for 2 min, and final extension 72 °C for 5 min. PCR amplicons were identified by 1% agarose gel electrophoresis.

Statistical analysis

The obtained data were analyzed via SPSS software (Washington, the USA), version 22. The p-value <0.05 was considered as significant.

Results

A total of 100 nonduplicate *S. aureus* isolates were obtained from 54 male and 46 female patients' blood cultures with the mean age of 50+25 years during 2018 from Tabriz hospitals. The isolates were collected from different wards of hospitals (Figure-1). The most isolates were obtained from emergency ward in number of 14 and 12 for male and female patients respectively.

The obtained results from the disk diffusion agar test revealed the most resistance rate to erythromycin in a value of 70% and the lowest resistance rate to linezolid in a value of 3% (Figure-2). All isolates were susceptible to vancomycin.

The percentage of distribution of *fnbA*, *fnbB*, *cna* and *hla* genes in the *S. aureus* isolates were 81, 81, 73 and 30 respectively (Figure 3).

The obtained rep-PCR fragments ranged from 300 to 1500 bp. In patterns, the lowest and highest number of bands was 1 and 9 respectively (Table-2).

Discussion

S. aureus as a well-known bacterium in microbiology laboratories plays a remarkable role in the pathogenesis of human critical infections [17]. The included various virulence factors in this bacterium act through the different mechanisms to penetrate into host tissues and colonization [18]. As a result of heavy economic and health burdens followed the staphylococcal infections, investigating the main virulence factors and their frequency in addition to address the treatment procedure is utmost of importance [19].

Access to the sensitivity and resistance patterns of conventional antibiotics in every region is the main bet to control resistance process and prevent it from occurring [20]. In the present study, these patterns were evaluated through the disk diffusion agar and micro broth dilution methods in blood culture collected *S. aureus* isolates from Tabriz hospitals. Accordingly, the most resistance rate was observed to erythromycin, clindamycin, ciprofloxacin, cefoxitin, gentamicin, cotrimoxazole, and linezolid respectively. The necessary measures must be taken

seriously to fight against the spreading of resistant factors [21]. Based on the obtained results by Hasani et al. the resistance rate to ciprofloxacin, gentamicin, and co-trimoxazole in septicemic patients was in value of 14.3%, 28.6%, and 21.4% respectively [22]. Our findings display a high resistance to ciprofloxacin in comparison to Hasani's results. Notably, majority of investigations express the increase of resistance rate to most of the antibiotics, in particular, gentamicin which had the remarkable resistance report in even other bacterial families [23]. The resistance patterns to antibiotic classes in *S. aureus* isolates had been investigated in earlier studies from different countries as well [24-28]. These findings indicate the growth of resistance to antibiotics even to linezolid, however, in current study, linezolid has the major ability to confront with infections caused by *S. aureus* isolates. This study reveals a total condition about vancomycin and other antibiotic classes, fortunately, vancomycin is still a useful antibiotic in the treatment of staphylococcal infections in our region and the resistance rate has not risen, however, this is not eternal and many factors are able to change this condition. Although vancomycin and other antibiotics, particularly linezolid have effective antibacterial activity, the trivial reported resistance cases is an alarm due to the determinant locations on mobile genetic elements including the plasmids [29]. Because of a high probability to spread of resistance determinants, prevention of these interactions is utmost of importance. The most remarkable solution would be combination therapy [30]. Also, the molecular techniques could be useful to detect the emerging resistance factors.

In this study the frequency of 4 important virulence genes including *fnbA*, *fnbB*, *cna* and *hla* genes in *S. aureus* isolates was evaluated. Goldmann. et al in 2016 reported that responding to the antimicrobial mediators released by mast cells in *S. aureus* isolates is related to the up-regulating the expression of α-hemolysin, fibronectin-binding protein A and several regulatory systems [7]. The α-hemolysin as a pore-forming toxin is present in most strains of *S. aureus* and reported to have a critical role in the pathogenesis of bacterial infections [31]. Menzies et al. indicated the role of fibronectin-binding protein in the pathogenesis of infections as well [32]. It is also reported that lower incidence of endocarditis gives rise to the reduction of fibronectin-binding protein [33].

Conclusion

The results of this study showed that the frequency of *fnb*A, *fnb*B, *cna* and *hla* genes in *S. aureus* strains isolated from blood culture is high and this is a risk factor for endocarditis. Regarding to the susceptibility of these isolates to vaccomycin, this antibiotic can be a threapotic option for treatment of these infections.

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Conflict of interest

None to declare.

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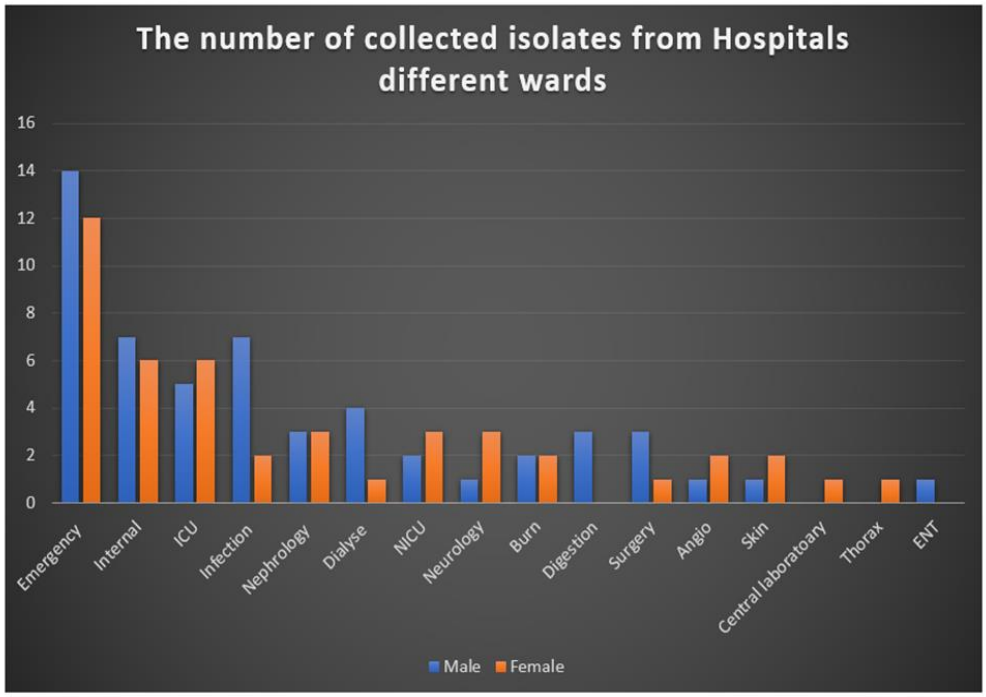
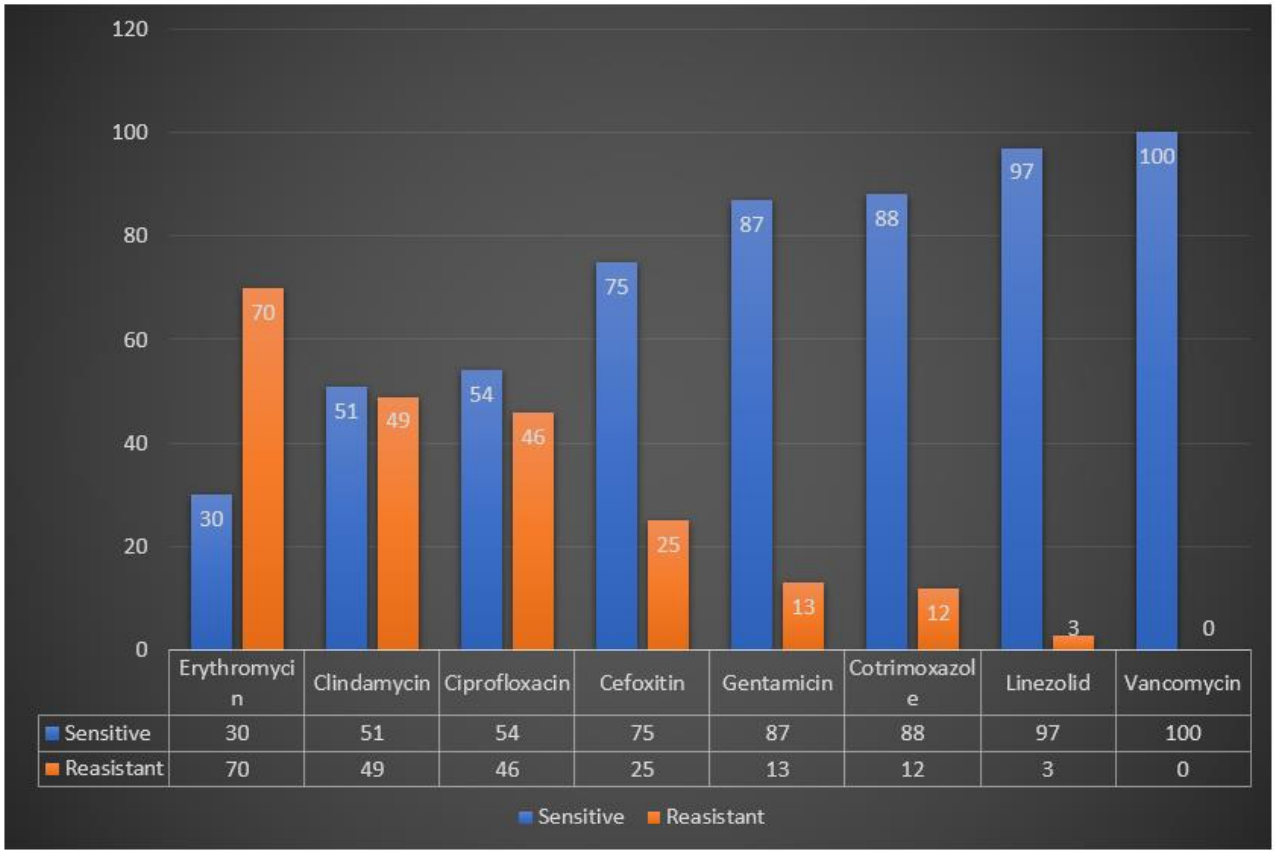


Figure 1. The number of collected isolates from Hospitals different wards



	Erythromycin	Clindamycin	Ciprofloxacin	Cefoxitin	Gentamicin	Cotrimoxazole	Linezolid	Vancomycin
Sensitive	30	51	54	75	87	88	97	100
Reasistant	70	49	46	25	13	12	3	0

Figure 2. Distribution of resistance and susceptibility rates of isolates by the disk diffusion agar method.

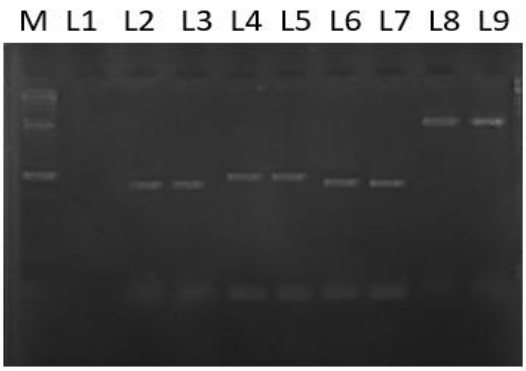


Figure 3. The PCR patterns for *S. aureus* isolates, all PCR products separated in 1% agarose gel. Lane M contained the DNA size marker (50 bp).
fnbA gene: (191 bp) L1: negative isolate, L2: positive isolate, L3: positive control.
fnbB gene: (201 bp) L1: negative isolate, L4: positive isolate, L5: positive control.
cna gene: (192) L1: negative isolate, L6: positive isolate, L7: positive control.
α-hemolysin gene: (603) L1: negative isolate, L8: positive isolate, L9: positive control

Table 1. The PCR program including the sequences of used primers.

Gene		Initial denaturation	Denaturation	Annealing	Extension	Final extension	Amplicon size (bp)
fnbA	Fw: GATACAAACCCAGGTGGTGG	95° C 5 min	95° C 30 sec	57° C 30 sec	72° C 1 min	72° C 5 min	191
	Rv: TGTGCTTGACCATGCTCTTC						
fnb B	Fw: TGTGCTTGACCATGCTCTTC	95° C 5 min	95° C 30 sec	54.5 ° C 30 sec	72° C 1 min	72° C 5 min	201
	Rv: AGTTGATGTCGCGCTGTATG						
cna	Fw: AAAGCGTTGCCTAGTGGAGA	95° C 5 min	95° C 30 sec	55.5 ° C 30 sec	72° C 1 min	72° C 5 min	192
	Rv: AGTGCCTTCCCAAACCTTTT						
hla	Fw: AAA GGT ACC ATT GCT GGTC	95° C 5 min	95° C 30 sec	53 ° C 30 sec	72° C 1 min	72° C 5 min	603
	Rv: CAA TTG GTA ATC ATC ACG AAC						

Table 2. The results of rep-PCR step.

rep-PCR patterns	**Numer of isolates**
300	1
400	3
450	1
500	1
600	3
>1500	1
200-400	1
400-500	1
400-750	1
400-1000	1
400->1500	8
450-850	1
450->1500	1
500-600	1
500-1000	1
550->1500	1
600-700	1
600-800	2

600-1200	1
200-400-700	2
200-400-800	3
200-600-700	1
400-500-800	1
400-600-900	2
400-490-850	6
400-850->1500	1
400-900->1500	1
400-1400->1500	1
400->1500->1500	1
490-600-900	1
490-600->1500	1
500-600-700	2
500-700-800	2
600-1000-1400	1
600-1000->1500	1
200-400-500-800	1
200-400-600-700	1
200-400-700-800	1
240-310-1100-1200	1
300-400-850->1500	1
400-490-600-900	1
400-500-900->1500	3
400-1000-1200-1500	1
490-550-900-1300	1
220-380-400-600-900	1
300-400-580-650-780	1
350-450-850-1300->1500	2
390-490-600-850-1000	2
450-600-800-1000->1500	1
300-400-670-800-950-1200	1
380-490-580-600-900-1000	1
390-490-550-600-850-1000	1
400-490-600-1400->1500->1500	1
480-590-880-1300-1400->1500	1
500-600-1000->1500->1500->1500	1
800-850-1100-1300->1500->1500	1
350-400-600-650-700-800-1000	1
400-490-500-580-600-900-1200	1
410-500-850-900->1500->1500->1500	1
420-510-600-650-900-1300->1500->1500->1500	1
480-500-690-950-1200-1300-1400->1500->1500	1