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Antibiotic Resistance, Prevalence of Fibronectin-binding Protein Genes, and Their Role in Biofilm Production Among *Staphylococcus aureus* Strains

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ABSTRACT

Background: Biofilm production increases *Staphylococcus aureus* resistance to antibiotics and also host defense mechanisms. The present study aimed to investigate the antibiotic resistance, fibronectin-binding protein genes frequency, and their contribution with biofilm formation in clinical isolates of *S. aureus*.

Materials and Methods: In this study, 100 clinical isolates of *S. aureus* were collected. The antibiotic susceptibility pattern of the isolates was evaluated by the disk agar diffusion method. The ability of biofilm formation in the studied isolates was also determined by microplate colorimetric assay. Then, all isolates were screened by polymerase chain reaction for the *fnbA* and *fnbB* genes.

Results: Out of 100 clinical isolates of *S. aureus*, the highest and lowest antibiotic resistance rates were against penicillin (94%) and vancomycin (6%), respectively. Thirty-two cases were found to be multi-drug resistant (MDR) among the tested strains. The ability of biofilm production was observed in 89% of the isolates. The PCR results showed that the prevalence of *fnbA* and *fnbB* genes were 91% and 17%, respectively. However, 100 and 21.8% of the MDR isolates had *fnbA* and *fnbB* genes, correspondingly.

Conclusion: The ability to form biofilm in MDR isolates of *S. aureus* is more than non-MDR isolates, especially *fnbA* positive ones. As the bacteria in the biofilm are difficult to kill by antibiotics, attention to the removal or control of the biofilm production seems to be necessary.

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Introduction

Staphylococcus aureus is one of the most important bacterial infectious agents in hospitals and also the most common cause of food poisoning [1]. It causes a wide range of infections ranging from simple skin infections to life-threatening diseases [2]. Moreover, this organism is capable to produce many toxins including enterotoxins, Panton-valentine toxin, and exfoliative toxin [3]. Also, this gram-positive coccus can produce polysaccharides and adherent protein factors, which are involved in biofilm production and adherence of *S. aureus* to the surfaces [4]. Therefore, the presence of biofilm genes in these bacteria is considered as one of their important pathogenic factors and their investigation is very important [5]. Biofilm is a structure composed of a bacterial population that is enclosed by an exopolymeric matrix produced by the bacterium [6]. This property gives the bacterium the ability to bind to different levels as well as increase the intrinsic resistance to different antibiotics [6]. Biofilms comprise a group of microorganisms that interact with a network of internal channels in the extracellular glycoprotein and polysaccharide matrix called extracellular polymeric material [2]. The extracellular polymeric material is composed of polysaccharides, proteins, phospholipids, teichoic acid and other hydrated polymeric substances with 85 to 95% of water and thus can cause binding of various pathogens (especially bacteria) to live tissues and surfaces of medical equipment [7]. This property enhances the bacterial resistance to a variety of antibiotics and host defense mechanisms, as well as facilitates metabolism gene transfer and resistance to antibiotics and disinfectants [7]. Promoting the bacterial survival in harsh environmental conditions, playing a role in pathogenesis and causing chronic diseases, and influencing the development and enhancement of drug resistance through impermeability to antibiotics in the polymer matrix, are the most important features of biofilm [8]. *S. aureus* adhesion genes involved in the cellular accumulation of bacteria in biofilms include *fib*, *fnbA*, *fnbB*, *eno*, *icaADBC*, *sasG* & *C* and *pls* [9]. However, fibronectin-binding proteins A and B (FnBPA and FnBPB) are encoded by the *fnbA* and *fnbB* genes, respectively [10]. These proteins can be covalently attached to the bacterial cell and play an important role in initiating the biofilm production process by binding to fibronectin receptors [9]. On the other hand, the production of fibronectin-binding proteins (FnBP) is essential for the invasion of this organism to eukaryotic cells [11]. The ability to form biofilms is mediated by intercellular adhesin polysaccharides (PIAs) encoded by *IcaA*, *IcaB*, *IcaC*, and *IcaD* genes, which thicken the biofilm layers [2, 9]. In contrast, fibronectin-binding proteins (FnBPA and FnBPB) play important roles in the accumulation, binding and invasion of *S. aureus* to surfaces [12]. Therefore, the present work aimed to investigate the antibiotic resistance, fibronectin-binding protein genes frequency, and their contribution with biofilm formation in clinical isolates of *S. aureus*.

Materials and Methods

Sample collection

In this study, 100 non-duplicate *S. aureus* isolates were collected during 10 months (March to December 2018) from different clinical specimens (blood, urine, ulcer, pus, body fluids, trachea, sputum, etc.) of patients admitted to Zare hospital of Sari city (a burn center) and Imam Khomeini Hospital of Behshahr city (a general center). The isolates were transferred to the Microbiology Laboratory and cultured in Blood Agar (Merck, Germany) and incubated at 37 ˚C for 24 hours. Then, all isolates were identified by routine microscopic and biochemical methods such as gram staining, catalase and coagulase assays, mannitol fermentation and DNase assay [13], and were confirmed by polymerase chain reaction (PCR) using *nuc* gene-specific primers. *S. aureus* ATCC 25923 was used as a control strain for diagnostic tests.

Antimicrobial susceptibility testing (AST) of the isolates

The antibiotic susceptibility pattern of the isolates against 6 antibiotics including penicillin (10 µg), vancomycin (30 µg), erythromycin (15 µg), tetracycline (30 µg), ciprofloxacin (5 µg), and clindamycin (2 µg) (Roscoe, Denmark) was determined by Kirby-Bauer method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [15]. We chose the *S. aureus* ATCC 25923 as a control strain in AST. Vancomycin minimum inhibitory concentrations (MIC)s for *S. aureus* isolates were determined by the broth microdilution method according to CLSI recommendations [15].

Phenotypic evaluation of biofilm production

The ability of biofilm formation in the isolates was investigated by microtiter plate method [16]. Briefly, 180 µL of trypticase soy broth (TSB) containing 1% glucose was poured into 96 well microplate wells. Then, 20 µL of 0. 5 McFarland's equivalent bacterial suspension was added to TSB medium in each well. Next, the microplates were incubated at 37 ˚C for 20 h. After the contents were thoroughly emptied from the wells and washed three times with 0.15M phosphate-buffered saline (PBS), the microplates were completely air-dried. Then, the wells

Table 1. Antibiotic resistance pattern of 100 studied *S. aureus* clinical isolates

	Resistance Pattern		
Antibiotic	**Resistant**	**Intermediate**	**Susceptible**
Ciprofloxacin	37	16	47
Penicillin	94	-	6
Tetracycline	50	40	10
Vancomycin	6	-	94
Clindamycin	35	16	49
Erythromycin	42	24	34

Data are presented as percentages.

were stained with 0.1% crystal violet. After dye evacuation, we washed the wells three times with distilled water and added 200 µL alcohol-acetone (1:4 ethanol to acetone) to the wells in order to release the dye on the wall of bacteria producing biofilm and attached to the well. The amount of dye released at each well was evaluated using the enzyme-linked immunosorbent assay (ELISA) reader (Biotech, USA) at 590 nm. The optimal density (OD) of the samples was then compared with the OD of the control (ODC) and the results were analyzed using the cut-off method. The isolates which showed OD ≤ ODC, were considered as no biofilm producers, while the results as ODC < OD ≤ 2×ODC, 2×ODC < OD ≤ 4×ODC, and 4×ODC < OD were reflected as weak, moderate, and strong biofilm producer isolates, respectively. The TSB medium containing 1% glucose was used as the negative control, while *S. aureus* ATCC 35556 was used as a positive control [16].

PCR test to identify the thermonuclease (nuc), *fnbA* and *fnbB* genes

Genomic DNAs were extracted from clinical isolates of *S. aureus* using a DNA extraction kit (SinaClon, Iran) according to manufacturer's instructions. To confirm the purity of the extracted DNAs, a Nanodrop machine (Thermo Scientific, USA) at 260 nm was used, and the DNAs were electrophoresed on 1.5% agarose gel (Wizbiosolutions, South Korea). PCR was used to identify the *nuc* gene (for the final identification of *S. aureus* isolates) and to detect the presence of *fnbA* and *fnbB* genes in clinical isolates. Primers sequences for the identification of target genes have been described previously [8, 14]. The PCR reaction was performed in a final volume of 25 µL, consisting of 12.5 µL of premix (Denmark, Ampliqon), 10 picomoles of each primer, 1 µL of Taq DNA polymerase, 5 µL of distilled water, and 300 ng of template DNA. The PCR reaction consisted of an initial denaturation step at 95 ˚C for 2 min and 30 cycles of denaturation at 95 ˚C for 25 seconds, followed by 30 s of the annealing stage at 53 ˚C for the nuc gene, 52 ˚C for the *fnbA* gene, and 55 ˚C for the *fnbB* gene, and extension at 72 ˚C for 30 s, along with a final amplification step at 72 ˚C for 5 min. PCR products were electrophoresed on a 1.5% agarose gel (Wizbio, Korea) along with a DNA fragment length marker (GeneDireX, Taiwan) to investigate the presence of the target genes.

Statistical analysis

Data were analyzed by SPSS software, version 22 and mean of quantitative data was analyzed using descriptive software and were presented as Mean±SD. Also, the significance level was evaluated by two-tailed and chi-square tests and P<0.05 was considered statistically significant.

Results

Out of 100 clinical isolates of *S. aureus* in this study, 50 isolates were obtained from Zare Hospital and 50 others were collected from Imam Khomeini Hospital. The mean age of the patients was 42.59±24.94 years. The mean age for men and women was 47.04±24.05 and 38.15±25.25, respectively. There was no significant difference between the two groups in terms of mean age (P=0.07).

The distribution of the isolates, in terms of hospital wards, was as follows: Intensive care units (ICUs) (29%), burn (23%), reconstructive surgery (13%), pediatric (12%), internal (11%), gynecological surgery (5%), male surgery (3%), emergency (2%) and coronary care unit (CCU) (2%).

Table 2. Comparison of antibiotic resistance pattern of MDR and non-MDR *S. aureus* isolates

Antibiotics	Resistance Pattern	No.(%)		P
		MDR	Non-MDR	
		Isolates	Isolates (n=68)	
Ciprofloxacin	Resistant	32(100)	5(7.35)	**0.01**
	Intermediate resistant	-	16(23.52)	
	Susceptible	-	47(69.11)	
Penicillin	Resistant	32(100)	62(91.17)	**0.1**
	Intermediate resistant	-	-	
	Susceptible	-	6(8.82)	
Tetracycline	Resistant	32(100)	18(26.47)	**0.04**
	Intermediate resistant	-	40(58.82)	
	Susceptible	-	10(14.7)	
Vancomycin	Resistant	-	6(8.82)	**0.02**
	Intermediate resistant	-	-	
	Susceptible	32(100)	62(91.17)	
Clindamycin	Resistant	28(87.5)	7(10.29)	**0.02**
	Intermediate resistant	3(9.37)	13(19.11)	
	Susceptible	1(3.12)	48(70.58)	
Erythromycin	Resistant	32(100)	10(14.7)	**0.03**
	Intermediate resistant	-	24(35.29)	
	Susceptible	-	**34(50)**	

Also, there was a significant difference (P=0.000) in the distribution of clinical specimens from different wards between two hospitals, however, the highest frequency of clinical specimens in Imam Hospital included ICU (16 samples, 32%), and internal and pediatrics (11 samples, 22%), while the most frequent isolates in the Zare Hospital belonged to burn ward (23 samples, 46%) and ICU (13 samples, 26%). In general, the frequency of clinical specimens in the present study was as follows:

Wounds (36%), urine (29%), blood (21%), trachea (9%), surgical samples (2%), ascites, pulmonary secretions and sputum each (1%).

Table 3. Frequency of *fnbA* and *fnbB* genes in biofilm -producing and non-biofilm-producing *S. aureus* isolates

Genes	No. (%)		P
	Biofilm-producing Isolates	Non-biofilm-producing Isolates	
fnbA positive(n=91)	83(91.2)	8(8.79)	0.025
fnbA negative(n=9)	6(66.66)	3(33.33)	NS
fnbB positive(n=17)	16(94.11)	1(5.88)	0.01
fnbB negative(n=83)	73(87.95)	10(12.04)	NS

NS: Non-significant.

Table 4. Distribution of biofilm formation ability and its relation to the presence of fibronectin-binding genes

Strains With/Without Genes	No. (%)			
	Biofilm Formation			
	Strong	Moderate	Weak	None
fnbA+ (n=91)	48(52.74)	28(30.76)	7(7.69)	8(8.79)
fnbA- (n=9)	6(66.66)	-	-	3(33.33)
fnbB+ (n=17)	13(76.47)	3(17.64)	-	1(5.88)
fnbB- (n=83)	41(49.39)	25(30.12)	7(8.43)	10(12.04)
f*nbA+* & *B+* (n=15)	13(86.66)	2(13.33)	-	-
FnbA- & *B-* (n=6)	5(83.33)	-	-	1(16.66)

According to the evaluation of antibiotic resistance pattern of the isolates in this study, the highest antibiotic resistance rate was observed against penicillin (94%), tetracycline (50%) and erythromycin (42%), while vancomycin, with 6% resistance rate, was the most effective antibiotic in this research (Table 1). The MIC range of vancomycin against the isolates was 0.25-32 µg/mL, which was consistent with the results of the disk agar diffusion method. In this study, 4%, 9%, 42%, 32%, 7%, and 6% of the isolates showed a MIC range of 0.25, 0.5, 1, 2, 4, and 32 µg/mL, while any of the isolates exhibited a MIC range of 8-16 and ≥64 µg/mL. Statistical analysis of antibiotic resistance results by the chi-square test showed no significant difference between antibiotic resistance pattern of *S. aureus* isolates in two sex groups, different parts of hospitals and different clinical samples (P>0.05). Moreover, 32% of our clinical isolates showed multi-drug resistant (MDR) phenotype. The antibiotic resistance pattern of the MDR and non-MDR clinical isolates of *S. aureus* is compared in Table 2.

According to the results of the PCR, 91% and 17% of *S. aureus* clinical isolates were identified as *fnbA* and *fnbB* positive, respectively. Of the isolates studied, 89 were able to produce biofilms (Table 3). Biofilm production ability was strong in 54 isolates (60.67%), moderate in 28 isolates (31.46%) and weak in 7 isolates (7.86%) (Table 4). The frequencies of *fnbA* and *fnbB* genes in biofilm-producing strains and non-biofilm producer ones are shown in Table 4. Significant differences were observed in the frequency of *fnbA* and *fnbB* genes between biofilm producer and non-biofilm producing isolates in this study (P<0.05). Also, there was a significant difference in the frequency of *fnbA* gene between MDR and non-MDR isolates, while there was no significant difference in *fnbB* gene frequency in these isolates (Table 5). The frequency of biofilm production in MDR and non-MDR strains was 100% and 83.82%, respectively.

Table 5. Frequency of *fnbA* and *fnbB* genes in MDR and non-MDR *S. aureus* isolates

Genes	No. (%)	
	MDR (n=32)	Non-MDR (n=68)
fnbA positive	32(100)	59(86.76)
fnbA negative	-	9(13.23)
P	0.0	NS
fnbB positive	7(21.87)	10(14.7)
fnbB negative	25(78.12)	58(85.29)
P	NS	NS

NS: Non- significant.

Discussion

S. aureus is one of the most important bacteria causing nosocomial infections [17]. The high ability of this bacterium in biofilm production has led to the occurrence of chronic infections and the emergence of MDR *S. aureus* strains. In the present study, the highest antibiotic resistance was reported for penicillin, which is consistent with recent findings. For instance, Jomehzadeh et al. [18] reported 100% penicillin resistance in clinical *S. aureus* isolates. The high resistance of isolates to penicillin in Iran aligns with findings from other Iranian studies, possibly due to factors such as lack of attention to drug dosage, empirical treatment without regard to antibiogram results, incomplete treatment, overuse, and over-the-counter use of penicillin family antibiotics, and high levels of beta-lactamase production by *S. aureus* [19].

The evaluation of biofilm production ability in the present study showed that 89% of the isolates were capable of producing biofilms. This is in line with recent studies, such as the one by Banerjee et al. [20], which reported a similar prevalence of biofilm-producing *S. aureus* isolates (75%). However, the clinical samples in their study were different to those in our research. Vuong et al. in Germany reported, 78% of *S. aureus* clinical isolates were biofilm producers. The percentage of biofilm production varies across different studies but generally indicates the significant role of biofilm production in the pathogenicity of *S. aureus* in clinical settings [21].

In Egypt, Rasmi et al. reported a lower prevalence of the fibronectin-binding gene *fnbA* (13.6%) compared to your study, which found a much higher prevalence of *fnbA* (91%). This difference in *fnbA* prevalence between the two studies likely results from geographical variations, differences in sample sources [22]. Also, in a study conducted by Soltani and colleagues in Iran in 2019 on strains isolated from nasal swabs, 7.2% of the isolates contained *fnbA* gene [23]. The reason for these differences may be related to the type of clinical samples, whereas the skin and nose isolates are normal flora and the virulence factors associated with the pathogenicity are less common in these bacteria. A study performed in the USA on clinical isolates reported the prevalence of 98.7% and 20.1% for *fnbA* and *fnbB* genes, respectively [24], which was much closed to the results of the present study. On the other hand, a study conducted in India also showed a high prevalence of both *fnbA* and *fnbB* genes (77.8% and 81%, respectively), which is consistent with the results of our study regarding *fnbA* gene [25]. Interestingly, Arciola et al. exhibited that 98% of *S. aureus* isolates associated with orthopedic infections contained *fnbA* & *B* genes, indicating the important role of these virulence factors in biofilm production in various infections, especially orthopedic ones [26]. Mater and his colleagues in Iraq, the border with Iran, reported 59% *fnbA* gene frequency [27], while in 2019, Azmi et al. reported the frequency of *fnbA* and *fnbB* genes in Palestine as 78.2% and 29%, respectively [28]. These differences in the prevalence of *fnb* genes indicate the genetic diversity of clinical isolates of *S. aureus* in different regions of the world and the necessity to study this diversity in each region.

Also, 32% of *S. aureus* clinical isolates were MDR in the present study, which showed significantly higher resistance to ciprofloxacin, tetracycline, clindamycin and erythromycin than non-MDR strains. Two other studies conducted in Iran and Palestine also reported 46% and 26.6% prevalence of MDR clinical isolates of *S. aureus*, respectively [28, 29]. However, the MDR rate between strongly positive biofilm-producing isolates in the Palestinian research was reported as 38.5%, while this rate in our study was 66.6%. It seems that strongly biofilm-production had a great impact on the development of antibiotic resistance in our research. On the other hand, we found that the frequency of *fnbA* and *fnbB* genes in MDR strains were 100% and 21.9%, respectively, and all of these isolates were capable of biofilm formation. However, in the study by Azmi et al. 26.6% of the isolates were MDR, while 50.7% and 20% of MDR isolates carried *fnbA* and *fnbB* genes, respectively, and all of which were detected as biofilm producer isolates [28]. Today, with increasing use of antibiotics and increasing prevalence of methicillin and vancomycin resistant *S. aureus* strains and the high ability of this organism in biofilm production, the treatment of infections caused by it has become a major challenge in the world [3].

Conclusion

In conclusion and based on the results of this study and similar results, it can be argued that clinical isolates of *S. aureus* have a high ability to form biofilms and *fnb* genes with high diversity and high prevalence play an important role in biofilm construction. Also, given the high prevalence of the *fnbA* gene, it can be expected that the isolates containing this gene play a more effective role in pathogenesis. Although one of the major causes of infection emergence and drug resistance is attributed to biofilm production, and the presence of related genes, but by identifying and inhibiting the genes, sources, and pathways of infection transmission, Strict adherence to hand hygiene, environmental cleaning, and patient isolation protocols is essential to control the spread of biofilm-forming and MDR *S. aureus* strains. Additionally,

antibiotic stewardship and incorporation of anti-biofilm strategies should be implemented to improve treatment outcomes.

Ethical Considerations

Compliance with ethical guidelines

This study was conducted in accordance with the principles of the Declaration of Helsinki. This study was approved by Research Ethic Committee of Mazandaran University of Medical Sciences, Sari, Iran (Code: IR.MAZUMS.REC.1397.312). Written informed consent was obtained from all participants or their close relatives prior to sample collection. To ensure participant privacy, all identifying information was anonymized, and data were kept strictly confidential throughout the study.

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Authors contribution's

Conceptualization: Hamid Reza Goli and Mohammad Ahanjan; Investigation: Hossein Jafari Soghondicolaei; Writing the original draft: Hossein Jafari Soghondicolaei, Hamid Reza Goli and Mehrdad Gholami ; Review & editing, Hamid Reza Goli and Mehrdad Gholami; Methodology: All Authors.

Conflict of interest

The authors declared no conflict of interest.

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References

[1] Shahmoradi M, Faridifar P, Shapouri R, Mousavi SF, Ezzedin M, Mirzaei B. Determining the biofilm forming gene profile of staphylococcus aureus clinical isolates via multiplex colony PCR Method. Rep Biochem Mol Biol. 2019; 7(2):181-8. [PMID]

[2] Archer NK, Mazaitis MJ, Costerton JW, Leid JG, Powers ME, Shirtliff ME. Staphylococcus aureus biofilms: Properties, regulation, and roles in human disease. Virulence. 2011; 2(5):445-59. [DOI:10.4161/viru.2.5.17724] [PMID]

[3] Kong C, Neoh HM, Nathan S. Targeting Staphylococcus aureus toxins: A potential form of anti-virulence therapy. Toxins (Basel). 2016; 8(3):72. [DOI:10.3390/toxins8030072] [PMID]

[4] Kim MK, Drescher K, Pak OS, Bassler BL, Stone HA. Filaments in curved streamlines: Rapid formation of Staphylococcus aureus biofilm streamers. New J Phys. 2014; 16(6):065024. [DOI:10.1088/1367-2630/16/6/065024] [PMID]

[5] Bridier A, Sanchez-Vizuete Mdel P, Le Coq D, Aymerich S, Meylheuc T, Maillard JY, et al. Biofilms of a Bacillus subtilis hospital isolate protect Staphylococcus aureus from biocide action. PLoS One. 2012; 7(9):e44506. [DOI:10.1371/journal.pone.0044506] [PMID]

[6] Kim SJ, Chang J, Rimal B, Yang H, Schaefer J. Surface proteins and the formation of biofilms by Staphylococcus aureus. Biochim Biophys Acta Biomembr. 2018; 1860(3):749-56. [DOI:10.1016/j.bbamem.2017.12.003] [PMID]

[7] Moormeier DE, Bayles KW. Staphylococcus aureus biofilm: A complex developmental organism. Mol Microbiol. 2017; 104(3):365-76. [DOI:10.1111/mmi.13634] [PMID]

[8] Atshan SS, Nor Shamsudin M, Sekawi Z, Lung LT, Hamat RA, Karunanidhi A, et al. Prevalence of adhesion and regulation of biofilm-related genes in different clones of Staphylococcus aureus. J Biomed Biotechnol. 2012; 2012:976972. [DOI:10.1155/2012/976972] [PMID]

[9] Avila-Novoa MG, Iñíguez-Moreno M, Solís-Velázquez OA, González-Gomez JP, Guerrero-Medina PJ, Gutiérrez-Lomelí M. Biofilm formation by Staphylococcus aureus isolated from food contact surfaces in the dairy industry of Jalisco, Mexico. J Food Qual. 2018; 2018(1):1746139. [DOI:10.1155/2018/1746139]

[10] O'Neill E, Pozzi C, Houston P, Humphreys H, Robinson DA, Loughman A, et al. A novel Staphylococcus aureus biofilm phenotype mediated by the fibronectin-binding proteins, FnBPA and FnBPB. J Bacteriol. 2008; 190(11):3835-50. [DOI:10.1128/JB.00167-08] [PMID]

[11] Charmi far B, Mahdavi S. Frequency of Adherence Genes cna, fnbA and fnbB in Staphylococcus aureus Isolates from Traditional Cheese. Med Lab J. 2017; 11(5):11-5. [DOI:10.29252/mlj.11.5.11]

[12] Speziale P, Pietrocola G, Foster TJ, Geoghegan JA. Protein-based biofilm matrices in Staphylococci. Front Cell Infect Microbiol. 2014; 4:171. [DOI:10.3389/fcimb.2014.00171] [PMID]

[13] Mahon CR, Lehman DC. Textbook of diagnostic microbiology-E-Book: Textbook of diagnostic microbiology-E-Book. Amsterdam: Elsevier Health Sciences; 2022. [Link]

[14] Cremonesi P, Luzzana M, Brasca M, Morandi S, Lodi R, Vimercati C, et al. Development of a multiplex PCR assay for the identification of Staphylococcus aureus enterotoxigenic strains isolated from milk and dairy products. Mol Cell Probes. 2005; 19(5):299-305. [DOI:10.1016/j.mcp.2005.03.002] [PMID]

[15] Clinical and Laboratory Standards Institute. M100 Performance standards for antimicrobial susceptibility testing. Wayne: Clinical and Laboratory Standards Institute; 2018. [Link]

[16] Halebeedu Prakash P, Rajan V, Gopal S. Predominance of SCCmec types IV and V among biofilm producing device-associated Staphylococcus aureus strains isolated from tertiary care hospitals in Mysuru, India. Enferm Infecc Microbiol Clin. 2017; 35(4):229-35. [DOI:10.1016/j.eimce.2017.03.005] [PMID]

[17] Jenkins DR. Nosocomial infections and infection control. Medicine. 2017; 45(10):629-33. [DOI:10.1016/j.mpmed.2017.07.005]

[18] Jomehzadeh N, Emrani SS. Assessment of biofilm formation, antibiotic resistance patterns, and the prevalence of adhesion-related genes in clinical Staphylococcus aureus isolates. Heliyon. 2024; 11(1):e41537. [DOI:10.1016/j.heliyon.2024.e41537] [PMID]

[19] Salehi Rad S, Pourmoshtagh H, Sabour S, Nazari S, Sohrabizadeh S, Azimi T. A 12-year surveillance study on distribution and antimicrobial resistance of gram-positive bacteria in Iran. AMB Express. 2025; 15(1):30. [DOI:10.1186/s13568-025-01833-5] [PMID]

[20] Banerjee B, Gowda P, Ananda KT. Biofilm Formation and antibiotic resistance of S. aureus strains isolated from chronic traumatic wounds. J Pure Appl Microbiol. 2022; 16(1):424-9. [DOI:10.22207/JPAM.16.1.38]

[21] Vuong C, Saenz HL, Götz F, Otto M. Impact of the agr quorum-sensing system on adherence to polystyrene in Staphylococcus aureus. J Infect Dis. 2000; 182(6):1688-93. [DOI:10.1086/317606] [PMID]

[22] Rasmi AH, Ahmed EF, Darwish AMA, Gad GFM. Virulence genes distributed among Staphylococcus aureus causing wound infections and their correlation to antibiotic resistance. BMC Infect Dis. 2022; 22(1):652. [DOI:10.1186/s12879-022-07624-8] [PMID]

[23] Soltani E, Farrokhi E, Zamanzad B, Shahini Shams Abadi M, Deris F, Soltani A, et al. Prevalence and distribution of adhesins and the expression of fibronectin-binding protein (FnbA and FnbB) among Staphylococcus aureus isolates from Shahrekord Hospitals. BMC Res Notes. 2019; 12(1):49. [DOI:10.1186/s13104-019-4055-0] [PMID]

[24] Booth MC, Pence LM, Mahasreshti P, Callegan MC, Gilmore MS. Clonal associations among Staphylococcus aureus isolates from various sites of infection. Infect Immun. 2001; 69(1):345-52. [DOI:10.1128/IAI.69.1.345-352.2001] [PMID]

[25] Gowrishankar S, Kamaladevi A, Balamurugan K, Pandian SK. In Vitro and In Vivo Biofilm Characterization of Methicillin-Resistant Staphylococcus aureus from Patients Associated with Pharyngitis Infection. Biomed Res Int. 2016; 2016:1289157. [DOI:10.1155/2016/1289157] [PMID]

[26] Arciola CR, Campoccia D, Gamberini S, Baldassarri L, Montanaro L. Prevalence of cna fnbA and fnbB adhesin genes among Staphylococcus aureus isolates from orthopedic infections associated to different types of implant. FEMS Microbiol Lett. 2005; 246(1):81-6. [DOI:10.1016/j.femsle.2005.03.035] [PMID]

[27] Mater AD, Degaim ZD, Abdulazeez AS. Detection of Fibronectin Binding Protein (fnb A) gene in Staphylococcus aureus isolates. Univ Thi-Qar J. 2019; 14(1):10-6. [Link]

[28] Azmi K, Qrei W, Abdeen Z. Screening of genes encoding adhesion factors and biofilm production in methicillin resistant strains of Staphylococcus aureus isolated from Palestinian patients. BMC Genomics. 2019; 20(1):578. [DOI:10.1186/s12864-019-5929-1] [PMID]

[29] Rahimi F, Bouzari M, Katouli M, Pourshafie MR. Antibiotic resistance pattern of methicillin resistant and methicillin sensitive Staphylococcus aureus isolates in Tehran, Iran. Jundishapur J Microbiol. 2013; 6(2):144-9. [DOI:10.5812/jjm.4896]