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Original Article

Microbiology Section

Assessment of Knack of Clinical *Staphylococcus aureus* Isolates for the Biofilm Formation and Presence of *icaABCD* Family Genes

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ABSTRACT

Introduction: Superbug known as *Staphylococcus aureus* possess a tendency to form biofilm, which has a significant role in causing infection and abating host defense response. Amongst many mechanisms, biofilm formation depends on the *icaABCD* operon involved in the synthesis of a polysaccharide intercellular adhesion.

Aim: To investigate biofilm forming ability of *S. aureus* isolates by phenotypic and genotypic methods.

Materials and Methods: Of the 97 S. aureus clinical isolates collected, the quantitative biofilm formation was determined by microtiter plates. All *S. aureus* isolates were examined for detection of the *icaABCD* genes and mecA gene by using PCR method. Statistical analysis was performed with SPSS program version 17.0.

Results: Among 97 *S. aureus* isolates from blood, wound, skin, surgery, internal, burn and infectious wards, urine and body fluids specimens, five isolates appeared as strong biofilm producer, while 28 displayed moderate biofilm formation, and 55 showed weak biofilm formation. Nine isolates did not reveal biofilm production on microtiter plates. The frequency of icaA, icaB, icaC and icaD genes in *S. aureus* isolates was 81 (83.5%), 71 (73.2%), 51 (52.5%) and 97 (100%), respectively. There was no relation between presence of *icaABCD* genes and biofilm formation (p=0.74).

Conclusion: The presence of biofilm genes may not coincide with the ability to produce biofilm or vice versa. At the results, *S. aureus* clinical isolates possess different capacity to produce biofilm and adhesion. Methicillin resistance and susceptible isolates may not differ in their capacity to form biofilm.

Keywords: Adhesion, Antibiotic susceptibility pattern, Methicillin resistance, Pathogenesis, Polysaccharide

INTRODUCTION

Staphylococcus aureus is a commensal bacterium, notorious for causing diverse clinical infections, including nosocomial infections [1]. Increased attention has been focused on the ability of *S. aureus* to form biofilm and its relation to human diseases. Like other pathogens, the capacity to form biofilm is one of the defence mechanisms of *S. aureus*. Once embedded in biofilms, bacteria prevail over eradication with standard antibiotic regimens and lies inherently resistant to host immune responses [2]. *S. aureus* adheres via cell wall attached adhesions, such as fibronectin and fibrinogen that recognise host proteins coating biomaterial surfaces [3]. Group of staphylococcal surface proteins termed Microbial Surface Components Recognising Adhesive Matrix Molecules (MSCRAMMs) mediates the adherence to the host proteins. The cells then form a multilayered biofilm through intercellular interactions and the production of an extracellular matrix [4]. Research performed on the biofilm of *S. aureus* reveals that the biofilm phenomenon is mediated by the Polysaccharide Intercellular Adhesin (PIA) encoded by the *ica* operon [5]. The Intracellular Adhesion (*ica*) locus including four genes *icaABCD*, synthesis of the PIA and Capsular Polysaccharide/Adhesion (PS/A) proteins in organism, as well as stay as the main biofilm components in this organism [6]. Among the *ica* genes, *icaA* and *icaD* have a chief role in biofilm formation [7]. The *icaA* gene is responsible for the production of enzyme involved in the synthesis of N-acetylglucosamine oligomers from UDP-N-acetylglucosamine encodes N-acetylglucosaminyltransferase. Moreover, *icaD* has been reported to play an important role in the maximal expression of N-acetylglucosaminyltransferase, resulting in the phenotypic expression of the capsular polysaccharide [8]. *icaB* is the deacetylase responsible for the deacetylation of mature PIA and also the transmembrane protein. *icaC* encodes the transmembrane protein that is hypothetically involved in secretion and elongation of the growing polysaccharide [9]. Nevertheless, biofilm formation may occur with strains of *S. aureus* that lack *ica* [10]. We carried out this study to determine relation between the biofilm forming capacity and presence of the *icaABCD* genes and for the first time we compared presence of these genes in Methicillin Resistance *S. aureus* (MRSA) and Methicillin Sensitive *S. aureus* (MSSA) isolates.

MATERIALS AND METHODS

In this cross-sectional study, from February 2016 to March 2017, 97 *S. aureus* isolates obtained from various clinical infections from Sina hospital in Tabriz, Iran. Based on a previous study, and by considering α=0.05, β=0.2, power=80%, P1=0.61 and P2=0.32 and a difference of 10%, the sample size was estimated at 90 [8]. All detected *S. aureus* isolates confirmed by biochemical and genetic tests from patients referred to Sina hospital during study period included to the study. Exclusion criteria were species other than *S. aureus* and or duplicate isolates from the same patients. Of the total 97 *S. aureus* isolates, 87 were obtained from inpatients and 10 had out- patient source. The identity of all *S. aureus* isolates was confirmed by utilising the conventional bacteriological methods including Gram staining, catalase test, coagulase test, DNase test, mannitol salt agar growth, and 6.5% salt tolerance and later conventional Polymerase Chain Reaction (PCR) amplification was performed to verify species identification using the *nuc* gene as described previously [2,11]. The present study was approved by

The Ethic Commission of Tabriz University of Medical Sciences (Number: 1394.930). Patients consent forms were obtained before sampling, forms were in Persian and all patients informed about procedure of sampling and study.

Antimicrobial Susceptibility Testing

Disc diffusion method was performed to determine antimicrobial susceptibility patterns of *S. aureus* in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI) 2016 guidelines [12]. Inoculum peparation was done in normal saline and all inoculums adjusted to 0.5 McFarland standards. Inocula were used in less than 15 minutes to prevent any changes in the number of bacteria. The antimicrobial agents tested were as follows: trimethoprim/sulfamethoxazole (25 µg), erythromycin (15 µg), cefazolin (30 µg), cefoxitin (30 µg), ciprofloxacin (5 µg), penicillin (10 µg), clindamycin (2 µg) and gentamycin (10 µg) (MAST Diagnostics, Merseyside, UK). Vancomycin susceptibility testing of *S. aureus* was performed by using vancomycin screen agar plates containing 6µg/mL vancomycin and vancomycin E-test according to CLSI 2017 guidelines [12,13]. MRSA isolates were detected by using oxacillin screening agar (plates had 4% NaCl and 6 mg/L of oxacillin) and cefoxitin disc diffusion test (30 ug) [14]. *Staphylococcus aureus* ATCC® 33591™, *S. aureus* ATCC® 25923™, *S. aureus* ATCC® 29213™, *Enterococcus faecalis* ATCC® 51299™, and *E. faecalis* ATCC® 29212™ were used as the control strains. For D test examination in isolates, erythromycin and clindamycin discs were placed adjacent to each other during antimicrobial susceptibility test. The growth of the *S. aureus* isolates up to the edges of the disc, flattening of the clindamycin zone near the erythromycin disc (resistant) was considered D test positive.

Biofilm Formation Assay with Microtiter Plate Method

S. aureus biofilm formation was analysed in 96 well flat bottom polystyrene plates (Greiner Bio One, Germany), under static conditions for 48 hours as previously described [15,16]. For biofilm development, inoculum of *S. aureus* equivalent to 10^7 Colony Forming Unit (CFU)/mL was prepared by adjusting culture grown bacterial suspensions in Trypticase Soy Broth (TSB) (Hi-media, India) from overnight cultures to an Optical Density at 600 nm (OD_{600}) of 0.1 and further 100 µL of each adjusted inoculum was added to the wells. After 48 hours incubation at 37°C, plates were tenderly washed only once with 1x Phosphate Buffered Saline (PBS; pH 7.4) and stained with 100 µL of 0.1% Crystal Violet (CV) for 30 minutes at room temperature. Excess CV was expelled by washing, and CV stained biofilm was then solubilised in 200 µL of 95% ethanol and supernatant was transferred to a fresh microtiter plate. Biofilm was evaluated by measuring absorbance of the supernatant at 570 nm. Biofilm assays were performed in triplicate for each clinical strain and the mean biofilm absorbance quality was determined. OD of stained adherent bacteria were determined with a micro ELISA auto reader (model 680, Bio rad), and the wavelength of values was considered as an index of bacteria adhering to surface and forming biofilms. OD readings of wells with ethanol were used as blank and subtracted from all test values. Biofilm production was considered high, moderate, or weak as described previously [9].

DNA Extraction

DNA extraction was done by DNeasy kit (Qiagen Inc.) according to manufacturer's instructions and boiling method [17]. The extracted DNA concentrations were determined by Nanodrop 1000 (NanoDrop, Wilmington, USA). One microliter of each DNA was used as template in the PCR reaction.

Detection of mecA Gene

DNA of *S. aureus* isolates with the concentration of 0.1 ng/µL was used as the templates for PCR analysis. Conventional PCR was carried out using CINNA GEN MASTERMIX (Cinnaclon, Tehran, Iran) and *mecA* primer as described previously [18]. The strain *S. aureus* ATCC® 43300™ (*mecA* positive) was used as positive control in this study. Amplification was carried out in an Eppendorf thermocycler (Eppendorf, Hamburg, Germany) as follows: initial denaturation at 94°C for five minutes, followed by 35 cycles of 30 seconds for denaturation at 94°C, 30 seconds for annealing at 55°C, and one minute for primer extension at 72°C, followed by terminal extension at 72°C for seven minutes [19]. Electrophoresis of PCR products was performed on 1% agarose gel using SYBR™ Safe DNA Gel Stain (Invitrogen) [20]. The stained gels were viewed on a UV transilluminator (Biorad, UK).

Detection of icaABCD Genes

To evaluate the biofilm formation, the presence of *icaABCD* genes was analysed by PCR amplification using specific primers as described previously [21]. PCR amplification was performed with an Eppendorf thermal cycler (Mastercycler® gradient). Amplification program consisted of initial denaturation at 94°C for five minutes, 30 cycles of denaturation at 94°C for 60 seconds, annealing at 55°C for 60 seconds (*icaA),* 52°C for 30 seconds (*icaB),* 55°C for 30 seconds (*icaC),* 55°C for 30 seconds (*icaD)* and extension at 72°C for 60 seconds with a final step of 72°C for 10 minutes [21]. The PCR products were analysed by electrophoresis in a 1.4% agarose gel using SYBR™ Safe DNA Gel Stain (Invitrogen).

STATISTICAL ANALYSIS

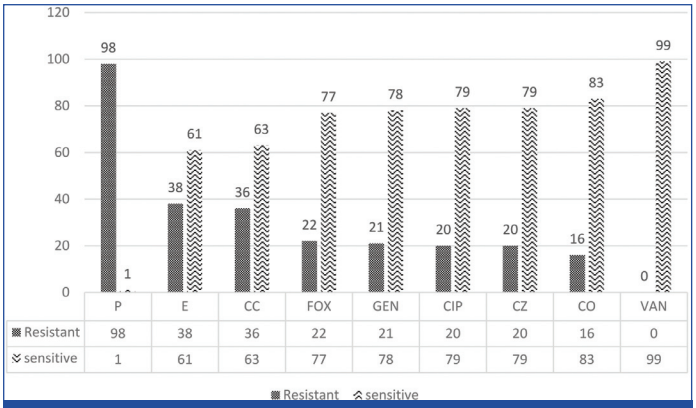
Statistical analysis was performed with SPSS program version 17.0 (SPSS, Chicago, IL, USA). The variables were analysed by univariate analysis using chi square or fisher's exact test, as appropriate. Statistical significance was set at 0.05.

RESULTS

In the present study, 99 isolate of *S. aureus* were collected from various clinical specimens comprising but for biofilm assay and genotyping, 97 *S. aureus* isolates were found suitable according to the study criteria. The mean age of the patients was 40.3±24 years and 55 (55.6%) patients were males. Source of the isolates were:blood (n=39, 40.2%), wound (n=50, 51.54% from skin, surgery, internal, burn and infectious wards), urine (n=5, 5.1%) and body fluids (n=3, 3.09%) from patients admitted to various wards including: infectious diseases (n=20, 20.61%), burn (n=18, 18.55%), intensive care unit (n=17, 17.52%), dermatology (n=14, 14.43%), internal (n=13, 13.4%), surgery (n=7, 7.21%) and out patients (n=8, 8.24%).

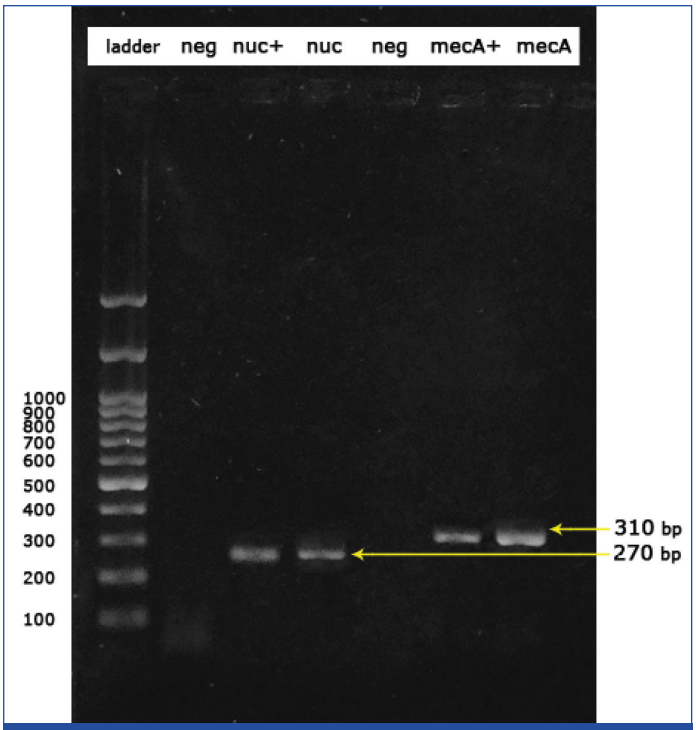
96 (98.9%) of the isolates were penicillin resistance followed by non susceptibility towards erythromycin (38.4%), clindamycin (36.24%), cefoxitin (22.2%), gentamycin (21.2%), ciprofloxacin, cefazolin (each 20.2%), and trimethoprim/sulfamethoxazole (16.2%). Of 36 clindamycin resistant isolates, 9 (25%) were D test positive. All *S. aureus* isolates were susceptible to vancomycin. 22 (22.68%) isolates were recognised phenotypically as Methicillin-Resistant *Staphylococcus aureus* (MRSA) by cefoxitin disk. Of these 22 isolates, 20 were obtained from inpatients and two from outpatients. The result was not significant at $p>0.64$. Of these MRSA isolates 18 (81.8%) of them possessed the *mecA* gene ($p<0.001$). AST patterns of *S. aureus* exhibits in [Table/Fig-1].

Of 97 *S. aureus* isolates, biofilm formation was studied in all isolates. Assessment of biofilm formation in these isolates

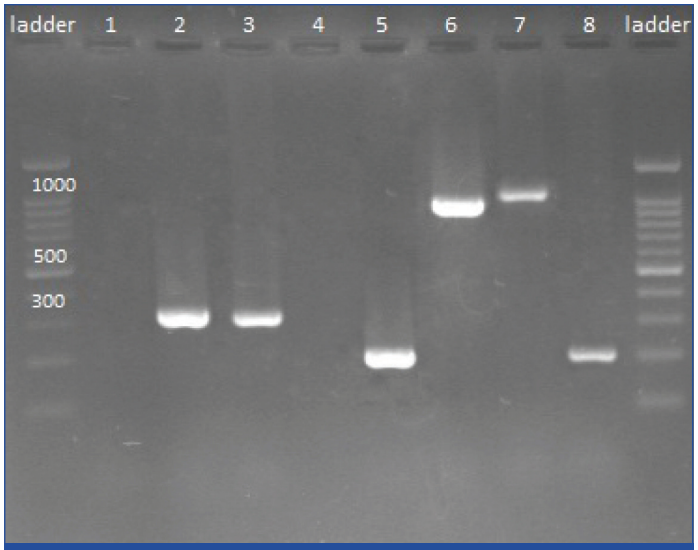


[Table/Fig-1]: Antibiotic susceptibility pattern of *Staphylococcus aureus* isolates*. Y-axis represents percent of isolates, X-axis represents antibiotics tested.
P: Penicillin; E: Erythromycin; CC: Clindamycin; FOX: Cefoxitin; GEN: Gentamycin; CIP: Ciprofloxacin; CZ: Cefazolin; CO: Cotrimoxazole; VAN: Vancomycin

presented five. 5 (15%) of the isolates as strong biofilm producer, while 28 (28.9%) displayed moderate biofilm formation, and 56.7% (n=55) showed weak biofilm formation. By phenotypic method nine isolates did not reveal biofilm production. Among 18 MRSA isolates confirmed by *mecA* gene [Table/Fig-2,3], 1 (9.09%) was strong producer, 4 (22.22%) were moderate producers and 12 (66.66%) of them were found to be weakly adherent while one isolate did not form biofilm. On the other hand, among MSSA isolates, 4 (5.1%) isolates were found to be strong producers,



[Table/Fig-2]: Screening *S. aureus* for the presence of nuc gene for confirmation of species and mecA gene for resistance to methicillin.
Lanes are respectively: ladder:Ladder 100bp, neg:Negative control (Escherichia coli ATCC® 25922 TM), nuc+:nuc positive control (*S. aureus* ATCC® 25923TM), nuc:nuc positive sample (270 base pair), neg:negative sample, mecA+:mecA positive control (*S. aureus* ATCC® 43300TM) (310 bp) and mecA:mecA positive sample (310 bp)



[Table/Fig-3]: Screening of *S. aureus* isolates for the presence of icaABCD and mecA by PCR methods.
Lanes are respectively: ladder: Ladder 100 bp; 1: Negative control (Escherichia coli ATCC® 25922 TM); 2: mecA positive (310 bp (base pair); 3: mecA positive (310 bp); 4: Negative sample; 5: icaA positive (188 bp); 6: icaB positive (900 bp); 7: icaC positive (1100 bp); 8: icaD positive (198 bp)

24 (30.4%) were moderate and 43 (54.4%) were found weakly adherent. Overall, 88 (90.7%) of *S. aureus* isolates were biofilm producers [Table/Fig-4]. [Table/Fig-5] show the relation between *mecA* feature, biofilm capability and type of clinical specimen. Among various clinical sources, except two isolates from blood, all had shown ability to form biofilm. All isolates obtained from urine specimen had shown either weak (60%) or moderate (40%) biofilm producing ability. There was no relation between presence of icaABCD genes and biofilm formation (p=0.74).

S. aureus isolates showing weak or moderate or strong biofilm formation were further analysed to possess biofilm genes and was 81 (83.5%), 71 (73.2%), 51 (52.5%), and 97 (100%) of them revealed *icaA*, *icaB*, *icaC* and *icaD* genes, respectively [Table/Fig-3]. All MRSA isolates were positive for *icaA* and *icaD* genes, while *icaB* gene was detected in 13 (72.2%) isolates and *icaC* being shown by 9 (50%) isolates. On the other hand, all MSSA isolates were positive for *icaD* gene only and 61, 60 and 43 isolates were observed positive for *icaA*, *icaB* and *icaC* genes respectively [Table/Fig-4,5]. However, there was no significant difference between MRSA and MSSA isolates for the presence of icaA*DBC* operon (p=0.789).

Phenotypic and genotypic biofilm forming features of *S. aureus* isolates depicted in [Table/Fig-6] . All isolates irrespective of being MRSA or MSSA were positive for *icaD* gene. 9.09%, 6.84% and 5.77% of isolates were found negative for biofilm activity, nevertheless had *icaA*, *icaB* and *icaC* genes, respectively. On the other hand, 88.8%, 83.3% and 86.6% of *S. aureus* isolates were negative for *icaA*, *icaB* or *icaC* genes respectively, but had shown in vitro weak or moderate or strong biofilm activity.

When source of clinical specimen was compared with ability of isolate to form biofilm and presence of *ica* and *mec*A genes, interestingly

Genes	Biofilm producing ability in MRSA$^{\Psi}$				Total (%)	Biofilm producing ability in MSSA$^{\Psi*}$				Total (%)	p-value*
	Strong	Moderate	Weak	Negative		Strong	Moderate	Weak	Negative		
icaA	1	4	12	1	18 (100)	4	20	37	18	61 (77.2)	0.827
icaB	1	4	8	0	13 (72.2)	4	20	36	19	60 (75.9)	0.561
icaC	1	2	6	9	9 (50)	2	12	29	36	43 (54.4)	0.915
icaD	1	4	13	0	18 (100)	4	24	51	0	79 (100)	0.535

[Table/Fig-4]: Biofilm forming ability and biofilm genes involved in MRSA and MSSA isolates. p-value less than 0.05 was considered significant. Comparison groups were strong to moderate biofilm producers of MRSA and MSSA versus weak or negative ones.
$^{\Psi}$MRSA:Methicillin resistance *S. aureus*; MSSA:Methicillin sensitive *S. aureus*
*Descriptive analysis and chi square tests were applied for analysis

mecA	Clinical specimen	Biofilm ability				Total
		Negative	Weak (+)	Moder-ate (++)	Strong (+++)	
Positive	Blood	0	5	2	1	8
	Wound (other than burns)	0	2	1	0	3
	Burn wound	1	5	1	0	7
	Total	1	12	4	1	18
Negative	Urine	0	3	2	0	5
	Blood	2	15	10	2	29
	Body fluids (other than blood and urine)	0	2	1	0	3
	Wound (other than burns)	6	17	8	2	33
	Burn wound	0	6	3	0	9
	Total	8	43	24	4	79

[Table/Fig-5]: Cross tabulation between mecA characteristic, biofilm ability and type of clinical specimen.

Biofilm genes characteristics		Phenotypic biofilm ability				Total	p-value
		Nega-tive	Weak (+)	Moderate (++)	Strong (+++)		
icaA	positive	8	51	24	5	88	0.0053
	negative	1	5	3	0	9	
Total		9	56	27	5	95	
icaB	positive	5	39	24	5	73	0.038
	negative	4	16	4	0	24	
Total		9	55	28	5	97	
icaC	positive	3	32	14	3	52	0.766
	negative	6	23	14	2	45	
Total		9	55	28	5	97	
icaD	positive	9	55	28	5	97	NS$^{\Psi}$
	negative	0	0	0	0	0	
Total		9	55	28	5	97	

[Table/Fig-6]: Correlation between phenotype and genotype of biofilm production in clinical *S.aureus* isolates. Descriptive analysis and chi square tests were applied for analysis. p-value less than 0.05 was considered significant.

$^{\Psi}$Not significance (because of absence of negative icaD isolate)

no MRSA isolate obtained from urine and body fluids was positive either for *icaB* or *icaC* genes [Table/Fig-7].

Antibiotic susceptible and non susceptible *S. aureus* isolates had no significant difference in biofilm formation [Table/Fig-8].

mecA	ica genes		Clinical specimens					Total
			Urine	Blood	Body flu-ids (other than blood and urine)	Wound	Burn wound	
Positive	icaB	positive	0	6	0	2	5	13
		negative	0	2	0	1	2	5
	Total		0	8	0	3	7	18
Negative	icaB	positive	4	22	3	26	6	61
		negative	1	9	0	7	3	20
	Total		5	31	3	33	9	81
Positive	icaC	positive	0	6	0	1	2	9
		negative	0	2	0	2	5	9
	Total		0	8	0	3	7	18
Negative	icaC	positive	3	20	1	14	6	44
		negative	2	11	2	19	3	37
	Total		5	31	3	33	9	81

[Table/Fig-7]: A comprehensive view of icaB and icaC genes distribution among different clinical specimens and their correlation with mecA gene.

Antibiotics	Non susceptibility		Susceptibility		p-value
	Biofilm former	Nonbiofilm former	Biofilm former	Non biofilm former	
Erythromycin	92.1%	7.9%	90.2%	9.8%	0.636
Clindamycin	91.7%	8.3%	90.5%	9.5%	0.859
Cefazolin	90%	10%	91.1%	8.9%	0.516
Cotrimaxazole	87.5%	12.5%	91.6%	8.4%	0.116
Ciprofloxacin	90%	10%	91.1%	8.9%	0.516
Gentamycin	90.5%	9.5%	91%	9%	0.64
Vancomycin	0%	0%	90.9%	9.1%	NS$^{\Psi}$

[Table/Fig-8]: Antimicrobial resistance pattern of *S. aureus* isolates and its relation with phenotypic biofilm features. Descriptive analysis and chi square tests were applied for analysis. p-value below 0.05 was considered significant.

$^{\Psi}$Not significant (because of absence of vancomycin resistance isolate)

DISCUSSION

S. aureus exploits many virulence factors, ability to adhere and form biofilm on host surfaces to attain the infectious level. The attachment and biofilm formation on abiotic surfaces like catheters and implanted devices are one of the most important virulence factors in *S. aureus* and is responsible for chronic or persistent infections [22]. In this regard, the phenotypic characterisation of adhesion and biofilm formation and related genetic elements involved in diverse clinical isolates of *S. aureus* might permit a better understanding of the complicated process of biofilm formation and infections caused by this microorganism [23]. Several studies have shown that formation of biofilm in *S. aureus* causing catheter associated and nosocomial infections is related to the presence of *icaA* and *icaD* genes [8,24]; however still lacunae exists, particularly in Iran for information regarding the source of bacterium compared with type of biofilm activity and the respective genes being involved. Moreover, research studies available have focused only on the presence of *icaA* and *icaD* genes or had restricted to only one source.

In the present study, all isolates were susceptible to vancomycin; resistance to vancomycin has been sporadically reported from some areas of the world, similar to Iran [25,26].

Microtiter plates were selected for biofilm formation assay and quantify attachment. Yet, presence and expression of biofilm genes ought to be confirmed by genotypic characterisation methods. Present study indicates a high prevalence of the *icaADBC* genes among *S. aureus* isolates. Since, biofilm protects microorganisms from opsonophagocytosis and antimicrobial agent as well as has a direct and indirect impact on healing process, through the production of destructive enzymes and toxin and promoting a chronic inflammatory state, presence of these genes provides vital information on the way of their pathogenesis [7].

In the present investigation study, 88 of the 97 *S. aureus* clinical isolates produced biofilm in vitro, and all the 88 isolates were found to possess the *icaD* gene. On the other hand, few isolates were observed to possess the *ica* genes but were negative on phenotypic test for biofilm formation and the vice versa condition was detected in the many isolates which furnished biofilm activity but were negative for *ica* genes. This can be due to low number of no biofilm producer isolates in present study. However, high rate of biofilm formation and high prevalence of *ica* genes can indicate importance of the presence of these genes in pathogenesis of this bacteria. Another study showed *S. aureus* strains, despite having the *ica* locus may fail to form biofilm in vitro as biofilm formation on inert surfaces is highly sensitive to growth conditions [27]. A previous study reported slime-positive *S. aureus* and *S. epidermidis* strains were deficient in the *icaA* and *icaD* genes as well as the whole *ica* locus. They suggested that the changed

phenotype might be associated with the deletion of the entire *ica* locus [8].

There are several reports concerning prevalence rate of *ica* genes in *S. aureus* from different countries [7]. In the study on 63 MRSA clinical isolates, 29 (46%) of the isolates were shown to have strong ability to produce biofilm, and all the isolates carried *icaD* and *icaC* genes, whereas, the prevalence of *icaA* and icaB was 60.3% and 51%, respectively [22]. Comparatively, *S. aureus* isolates in the present study were not strong biofilm producers. In addition, *S. aureus* isolates from the urine and blood were not strong biofilm producers in comparison to those isolated from wound specimens. In another study by Hou W et al., among 55.56% of *S. aureus* isolates that produced biofilm phenotypically, 11.11% had *icaA* gene, but other genes were not investigated [28]. Compatible to other research findings we found MRSA isolates to harbour higher rate of *icaADBC* genes. However, Smith K et al., and Atshan SS et al., detected no significant correlation between susceptibility to methicillin and biofilm formation [29,30]. In the present study, among 18 MRSA isolates, only one isolate was strong producer, while four were moderate producers and 12 of them were found to be weakly adherent. While, one isolate did not form biofilm. On the other hand, among MSSA isolates, 4 (5.1%) isolates were found to be strong producers, 24 (30.4%) were moderate and 43 (54.4%) were found weakly adherent.

LIMITATION

The main limitation of the present study was presence of small number of non biofilm producing isolates. Future study should have larger number of isolates, includes non-biofilm producing isolates, to have better understanding and confirmation of the these results.

CONCLUSION

In conclusion, though there was a high prevalence of biofilm production among *S. aureus* isolated from inpatients specimens and majority of biofilm producing staphylococci isolates were positive for *ica* genes. Findings of the present study indicate importance and high rate of biofilm formation and the presence of *ica* genes family in pathogenic *S. aureus*. *icaA* and *icaD* were present in all MRSA isolates and all of these isolates were biofilm producer. There was no relation between presence of *ica* genes family and biofilm formation in our isolates. Controlling biofilm formation and use of *ica* genes for defining pathogenesis and control of infection can be an alternative therapies in future treatment.

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