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Association of intercellular adhesion gene A with biofilm formation in staphylococci isolates from patients with conjunctivitis

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Abstract:

BACKGROUND: There is a great negative impact of biofilm-mediated infection on patient health which necessitates the use of reliable methods for detecting biofilm producers.

AIMS: This study was done to determine biofilm-producing ability and the presence of *intercellular adhesion gene* A in clinical staphylococcal isolates and to assess the reliability of two phenotypic methods used for biofilm detection.

MATERIALS AND METHODS: Fifty staphylococcal strains were isolated from 100 conjunctival swabs from patients attended the Ophthalmology Outpatient Department of the Research Institute of Ophthalmology. Two phenotypic methods were used for detection of biofilm production; qualitative congo red agar (CRA); and quantitative microtiter plate. Polymerase chain reaction was used to determine the presence of *icaA* gene.

RESULTS: In *Staph aureus*, 60% were positive biofilm forming and 40% were negative biofilm forming by both phenotypic methods. All positive biofilm-forming isolates were positive for *icaA* gene production. In coagulase negative staph, 50% were positive biofilm forming and 50% were negative biofilm forming by both phenotypic methods. All positive biofilm-forming strains were positive for *icaA* gene. All negative cases by CRA and microtiter plate methods were negative for *icaA* gene except two isolates. All staphylococcal isolates were subjected to antibiotic susceptibility test to correlate biofilm formation with multidrug resistance in staph.

CONCLUSION: There is high significant correlation between *icaA* gene presence and biofilm forming ability; however, the biofilm-forming ability of some isolates in the absence of *icaA* gene highlights the importance of further genetic investigations of *ica*-independent biofilm formation mechanisms.

Key words:

Biofilm, *icaA* gene, multidrug resistance, staphylococci

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Introduction

Conjunctival infections cause a worldwide problem and affect people of different ages. Proper treatment can reduce symptoms, recovery time, contagious spread, possible reinfection, and risk of complications but prolonged use of antibiotics can cause resistance strains. Infections with multidrug-resistant (MDR) organisms can lead to inadequate or delayed antimicrobial therapy.[1]

Staphylococcus spp. is a clinically relevant pathogen due to its antimicrobial resistance and evasion of the host immune system. Its virulence factors in avoiding host responses and influencing disease make them able to form biofilm. Emergence of resistant staphylococci from the conjunctiva is of

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great concern because its virulence is related to the clinical outcome of ocular infections such as keratitis or endophthalmitis.[2]

Biofilm-forming MDR *Staphylococcus* spp. are major reservoirs for transmission of ophthalmic infections. The ability of bacteria to aggregate and form biofilm is strictly related to the capacity of producing an extracellular mucoid substance: the slime, whose main component is of polysaccharide nature and consists of glycosaminoglycans.[3] Development of biofilm is considered to be a two-step process; first, the bacteria adhere to a surface mediated by a capsular antigen, namely, capsular polysaccharide/adhesin (PS/A), second, the bacteria multiply to form a multilayered biofilm, associated with production of polysaccharide intercellular adhesin (PIA) which mediates cell to cell adhesion.[4] It has been shown that both *Staph aureus* and *S. epidermidis* contain the intercellular adhesion (*ica*) operon responsible for slime production. This operon contains the *intercellular adhesion gene A (icaA) DBC* genes, in addition to the *icaR* gene which exerts a regulatory function. Among *ica* genes, the *icaA and icaD* have been reported to play a significant role in biofilm formation.[3]

The *icaA* gene responsible for PIA poly-*N*-succinyl β-1-6 glucosamine formation plays an independent role in the biofilm composition and intercellular adhesion. *Staphylococcus* spp., especially *S. aureus*, can produce a multilayered biofilm embedded within a glycocalyx or slime layer with heterogeneous protein expression throughout.[5,6]

The aim of this study is to determine association of *icaA* with biofilm formation in staphylococci isolated from patients with conjunctivitis, determine association of *icaA* gene with antibiotic resistance profile in staphylococci causing conjunctivitis, and to assess the reliability of two phenotypic methods used for biofilm detection.

Materials and Methods

Isolates

A total of 50 staphylococcal strains were obtained from 100 conjunctival swabs from patients complaining of inflammatory reactions in the eye (conjunctivitis) without or with other eye diseases or interventions attended the ophthalmology outpatient department of the Research Institute of ophthalmology; the study was conducted in the period from January 2015 to March 2016 who were diagnosed as staphylococcal species bacterial infection. The study protocol was approved by the Faculty of Medicine Ain Shams University Ethical Committee.

Specimen collection

Conjunctival swabbing was taken from the lower fornix of each case and was cultured onto surface of blood agar and mannitol salt agar plates (Oxoid, UK). Inoculated media were incubated aerobically at 37°C for maximum up to 48 h. Identification of *Staphylococcus* isolates was done by conventional methods including hemolytic activity on blood agar, acid production from mannitol, Gram-positive cocci in clusters by light microscopy, and catalase-positive reaction.[7] Final identification was done using Dry spot STAPHYTECT PLUS (Oxoid, UK) which is a latex slide agglutination test card used in the differentiation of *Staphylococcus aureus* by detection of clumping factor, Protein A, and certain polysaccharides.

Antimicrobial susceptibility using disc diffusion method

Antibiotic susceptibility of all isolates was done by disc diffusion method and results' interpretation was done according to.[8] Using a sterile loop, 3–5 well-isolated colonies from the pure culture of the organism were emulsified in sterile saline; the turbidity of the suspension was matched to the turbidity of 0.5 McFarland standards (BioMérieux, France) which was then swabbed on Muller Hinton agar (Oxoid, UK) supplemented with 2% NaCl. Antibiotics tested include penicillin, amoxicillin, ampicillin, cefoxitin, ceftriaxone, ciprofloxacin, vancomycin, amikacin, gentamycin, erythromycin, clindamycin, rifampicin, trimethoprim/sulfamethoxazole, and linezolid. Multidrug resistance was reported as a single isolate resistant (intermediate or complete) to three or more unique antimicrobial classes.[9]

Evaluation of biofilm formation by *Staphylococcus* isolates

Congo red agar (CRA method)

According to Freeman *et al*.,[10] the congo red agar (CRA) medium was prepared with 37 g/l BHI broth, 50 g/l sucrose, 10 g/l agar, and 0.8 g/l congo red. Congo red stain was prepared as a concentrated aqueous solution and autoclaved at 121°C for 15 min separately from other medium constituents and was then added when the agar had cooled to 55°C. Plates were inoculated and incubated at 37°C for 24 h. The plates were inspected for the color of the colonies at 24 and 48 h. A positive result was indicated by black colonies whereas nonproducing strains developed red colonies. The congo red dye directly interacts with certain polysaccharides, forming colored complexes or more likely some metabolic changes of the dye to form a secondary product which could play a more important part in the formation of dark colonies.[11] For colonies color evaluation, a four-color reference scale was used according to Satorres and Alcaráz:[12] black and bordeaux almost black were classified as biofilm producers, while bordeaux and red as nonbiofilm-producing strains. This method was performed in triplicate.

Microtiter plate method

Quantitative determination of biofilm production was carried by MTP according to Stepanović *et al*.[13] Staphylococcal strains were grown overnight at 37°C in BHI supplemented with 2% sucrose. The culture was adjusted to 0.5 McFarland then diluted 1:100 in fresh medium. Individual wells of sterile, polystyrene, 96-well, flat-bottomed tissue culture plates (TPP – Switzerland) were inoculated with 200 µl of the diluted culture then incubated. After 24 h incubation at 37 ˚C, the contents of each well were removed by decantation and each well was washed three times with 300 µl of phosphate-buffered saline (to remove free-floating "planktonic" bacteria). Biofilms formed by adherent ''sessile'' organisms were heat fixed by exposing them to hot air at 60 _C for 60 min, then 150 µl crystal violet (2%) stain was added to each well. After 15 min, the excess stain was rinsed off by decantation and the plate was washed and left to dry. Quantitative analysis of biofilm production was performed by adding 150 ll 95% ethanol to each well, and after 30 min, the optical densities (OD) of stained adherent bacterial films were read using a microtiter-plate reader (Tecan. Infinite F50) at 570 nm. Each assay was performed in triplicate. As a negative control, uninoculated medium was used to determine background OD. The average OD values were calculated for all tested strains and negative controls; the cutoff value (ODc) was established. It is defined as a three standard deviations (SD) above the mean OD of the negative control: ODc = average OD of negative control + (3 SD of negative control). ODc value was calculated for each microtiter plate separately. When a negative value was obtained, it was presented as zero, while any positive value indicated biofilm production. For interpretation of the results, strains were divided into the following categories:

1. Non biofilm producers = OD ≤ ODc
2. Weak biofilm producers = ODc<OD ≤ 2XODc
3. Moderate biofilm producers = 2xODc<OD ≤ 4xODc
4. Strong biofilm producers = 4xODc<OD.

Detection of *intercellular adhesion gene A* gene

DNA extraction

It was done using Qiagen DNeasy (Qiagen, USA), for DNA extraction from bacterial cultures according to manufacturer's instructions[13] (QIAgen DNeasy handbook, July, 2006).

Amplification of intercellular adhesion gene A gene by polymerase chain reaction

DNA from strong biofilm producing isolates was screened for *icaA* gene by polymerase chain reaction (PCR) in thermal cycler (Eppendorf, Hamburg, Germany).[14] Primers used were forward: AAGTCATACACTTGCTGGCG and reverse: CTGTCTGGGCTTCACCATGT. Reaction mixtures (50 µl) contained 25 µl PCR master mixtures, 1 µl of each primer (0.1–0.5 µM final concentration), 18 µl RNase free water, and 5 µl of template DNA. Amplifications were performed with the following thermal cycling profile; an initial denaturation at 94°C for 2 min, followed by 30 cycles of amplification (denaturation at 94°C for 1 min, primer annealing at 60°C for 1 min, and extension at 72°C for 2 min) and a final extension for 4 min. Amplicons for *icaA*-produced fragments of 188 bp were detected by Gel electrophoresis. The amplified product sizes were estimated by comparison with 100 bp DNA ladder (QIAGEN Incorporation) [Figure 1].

Statistical analysis

Qualitative data were presented as frequencies (n) and percentages (%). Friedman's test and Wilcoxon signed-rank test were used to study the associations between the different modalities.

The significant level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistic Version 20 (IBM, USA, Washington).

Results

The study was conducted on 50 staphylococcal isolates; 30 *S. aureus* (60%) and 20 (40%) coagulase-negative staphylococci (CoNS).

Concerning detection of biofilm formation *in S. aureus* isolates by both CRA and MTP methods, 18 (60%) isolates were positive biofilm forming while 12 (40%) were negative. There was a statistically significant association between results of MTP and CRA. All the isolates ($n = 18$) detected as positive biofilm-forming *S. aureus* by CRA were also positive by MTP. Furthermore, all negative isolates ($n = 12$) detected by CRA were also negative by MTP. However, two isolates were different by both methods in the degree of biofilm formation [Table 1].

There was a statistically significant association between biofilm formation as detected by both MTP and CRA

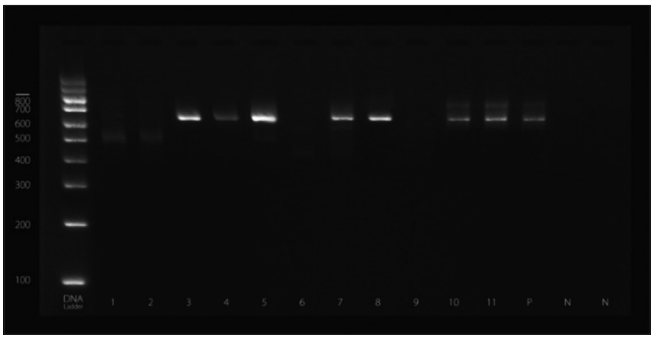

Figure 1: Gel electrophoresis indicating the presence of *intercellular adhesion gene A* gene

methods and the presence of *icaA* gene in *S. aureus* isolates as detected by PCR method. All weak, moderate, and strong cases as diagnosed by MTP and CRA were positive by PCR. All negative cases were negative by PCR [Table 2].

Concerning detection of biofilm formation in CoNS isolates by both CRA and MTP methods, 10 (50%) isolates were positive biofilm forming and 10 (50%) were negative; there was statistically significant association between results of MTP and CRA. All CoNS isolates (n = 10) that were negative for biofilm formation by CRA were also negative by MTP and all isolates (n = 10) that were positive by CRA were positive by MTP methods. However, three isolates were different by both methods in the degree of biofilm formation [Table 3].

There was a statistically significant association between biofilm formation as detected by both MTP and CRA and the presence of *icaA* gene in CoNS isolates as detected by PCR method. All weak, moderate, and strong cases as diagnosed by MTP and CRA were positive for *icaA* gene by PCR. All negative cases by CRA and MTP were negative for *icaA* except two [Table 4].

Concerning the result of antimicrobial susceptibility test, *S. aureus* isolates showed (100%) sensitivity to linezolid followed by cefoxitin (96.7%) then rifampicin (83.3%), ceftriaxone (80.7%), and erythromycin (80%); the least sensitivity was to penicillin (56.7%) and ampicillin (60%) [Table 5].

Concerning the result of antimicrobial susceptibility test, CoNS isolates showed (100%) sensitivity to linezolid followed by vancomycin (80%) then ceftriaxone, ciprofloxacin, and rifampicin (75%); the least sensitivity was to cefoxitin (0%), clindamycin (45%), and sulfamethoprim-trimethoprim (55%) [Table 6].

Concerning antibiotic susceptibility testing, this study shows that out of 30 *S. aureus* isolates, there were 19 (63.3%) isolates MDR to antibiotics, while 11 (55%) out of 20 isolates CoNS were MDR. By making correlation between the existence of *icaA* gene and MDR isolates, there was a statistically significant association between the presence of *icaA* gene and MDR in both *S. aureus* and CoNS. There was 89.5% of the MDR *S. aureus* positive for *icaA* gene and 72.7% of MDR CoNS were positive for *icaA* gene [Table 7].

Table 1: Biofilm formation in *Staphylococcus aureus* isolates by congo red agar and microtiter plate methods

Microtiter	Congo red				
	Weak, *n* (%)	Moderate, *n* (%)	Strong, *n* (%)	Negative, *n* (%)	*P*
Weak	2 (66.7)	1 (12.5)	0	0	0.001*
Moderate	1 (33.3)	6 (75.0)	0	0	
Strong	0	1 (12.5)	7 (100.0)	0	
Negative	0	0	0	12 (100.0)	

*P < 0.05 is significant

Discussion

S. aureus and CoNS are one of the frequent causes of bacterial conjunctivitis which involves biofilm formation.[14] One of the major virulence factors of *S. aureus* and CoNS is the slime production.[15] These bacteria have been known as a main cause of nosocomial infections. It is known that slime formation will significantly enhance the pathogenesis of staphylococcal infections. Furthermore, the slime formation allows staphylococci to escape host defenses and resists the antimicrobial action of antibiotics. Detection of slime production in staphylococcal strains isolated from clinical samples is important. It helps in discovering virulence factors of bacterial pathogenicity and hence the use of suitable ways to overcome them.[16]

It has been shown that both *S. aurues* and CoNS contain the *ica* operon responsible for slime production.[17]

The results of the present study revealed that biofilm production assessed by CRA revealed that 60% isolates of *S. aureus* were biofilm positive and 50% of CoNS were biofilm forming. In the present study, concerning detection of biofilm formation in *S. aureus* by both CRA and MTP methods, there was a highly significant association between the results of the two methods. All the isolates (n = 12) detected as negative biofilm-forming staphylococci by CRA method were also negative by MTP method. Furthermore, all positive biofilm-forming staph (n = 18) detected by CRA were also positive by MTP method. However, three of the biofilm forming *S. aureus* were different by both methods in the degree of formation of biofilm. One was detected as weak biofilm

Table 2: Association between intercellular adhesion gene A presence and biofilm formation as detected by microtiter plate and congo red agar in *Staphylococcus aureus* microorganism

IcaA gene	Congo red					Microtiter				
	Weak, *n* (%)	Moderate, *n* (%)	Strong, *n* (%)	Negative, *n* (%)	*P*	Weak, *n* (%)	Moderate, *n* (%)	Strong, *n* (%)	Negative, *n* (%)	*P*
Positive	3 (100.0)	8 (100.0)	7 (100.0)	0	0.006*	3 (100.0)	7 (100.0)	8 (100.0)	0	<0.001*
Negative	0	0	0	12 (100.0)		0	0	0	12 (100.0)	

*P < 0.05 significant, *IcaA*=Intercellular adhesion gene A

forming by CRA while detected by MTP as moderate biofilm forming. Two were detected as moderate biofilm-forming bacteria by CRA while detected by MTP as one weak and the other strong biofilm-forming *S. aureus*.

While observing the results concerning CoNS, this study revealed statistical association between the results of CRA and MTP in detecting biofilm formation. All CoNS isolates ($n = 10$) that were considered negative for biofilm formation by CRA method were also negative by MTP, and all the positive biofilm forming CoNS isolates ($n = 10$) were considered positive by both methods. However, CRA detected two of the isolates as weak compared to MTP method, which detect them as moderate biofilm-forming bacteria. Another two isolates were considered moderate biofilm forming by CRA while one was detected as weak and the other as strong biofilm-forming bacteria by MTP methods.

The previous results showed the high biofilm-forming ability of *S. aureus* and CoNS in conjunctival infections which was confirmed by both phenotypic methods of biofilm detection. Furthermore, the close results of CRA method and MTP reveal that we can use either method as a screening test for biofilm-forming ability in these organisms. These results agreed with those detected by Gad *et al*.[18] whose study showed high biofilm-forming ability (83.3%) in both *S. aureus* and CoNS isolated from urinary tract catheterized patients. Moreover, the study of Murgan *et al*., showed that 88.6% isolates of staphylococcal isolates from conjunctivitis was biofilm forming. Furthermore,[20,21] showed high percentage high percentage of biofilm formation in CoNS reaching 63% and 71.4%, respectively,[22] suggested that phenotypic methods (the CRA method and the microtiter plate assay) could be used as putative screening methods for the detection of biofilm-positive strains of *S. aureus* and CoNS and may provide a convenient way for early diagnosis of biofilm-related infections.

Table 3: Biofilm formation in coagulase-negative staphylococci isolates by congo red agar and microtiter plate methods

Microtiter	Congo red				
	Weak, *n* (%)	Moderate, *n* (%)	Strong, *n* (%)	Negative, *n* (%)	*P*
Weak	3 (60.0)	1 (20.0)	0	0	0.001
Moderate	2 (40.0)	3 (60.0)	0	0	
Strong	0	1 (20.0)	0	0	
Negative	0	0	0	10 (100.0)	

As regards the result of antimicrobial susceptibility test, *S. aureus* isolates showed (100%) sensitivity to linezolid followed by cefoxitin (96.7%) then rifampicin (83.3%), ceftriaxone (80.7%), and erythromycin (80%); the least sensitivity was to penicillin (56.7%) and ampicillin (60%) comparing to the result of[23] who reported that 31% of *S. aureus* were multidrug resistance (MDR) and 42.7% were oxacillin resistant. Most of these strains were isolated from wound specimens. All isolates were susceptible to vancomycin (100%). They were also susceptible to chloramphenicol, linezolid, nitrofurantoin, rifampicin, and teicoplanin (>80%) but showed resistance to erythromycin and penicillin.

Concerning the result of antimicrobial susceptibility test in CoNS isolates showed (100%) sensitivity to linezolid followed by vancomycin (80%) then ceftriaxone, ciprofloxacin, and rifampicin (75%); the least sensitivity was to cefoxitin (0%), clindamycin (45%), and sulfamethoprim-trimethoprim (55%) compared to the result of Al Tayyar *et al*.[24] who reported that antibiotic susceptibility testing of CoNS species revealed their sensitivity to vancomycin, linezolid, rifampin, and nitrofurantoin, while showing a highly resistant pattern to ampicillin, penicillin, ceftriaxone, cefazolin, amoxicillin-clavulanic acid, and erythromycin. Moreover, the study of Murad *et al*.[25] reported that antimicrobial resistance of CoNS were oxacillin (70.3%), amoxicillin (74.8%), amoxicillin + clavulanate (32.8%), ciprofloxacin (35.2%), ofloxacin (33.6%), ceftriaxone (30.4%), erythromycin (58.3%), clindamycin (16.3%), daptomycin (42.5%), kanamycin (52.2%), fusidic acid (41.7%), doxycycline (24.7%), vancomycin (2.6%), and linezolid (0.8%).

This study shows high association between biofilm formation and *icaA* gene presence in *S. aureus* isolates; *ica A* gene was positive in all the weak, moderate, and strong biofilm-forming *S. aureus* isolates (100%), and all the negative biofilm-forming *S. aureus* (100%) were also observed negative for *icaA gene* presence. In CoNS isolates, it was also observed that all weak, moderate, and strong biofilm-forming isolates as diagnosed by

Table 4: Association between intercellular adhesion gene A presence and biofilm formation as detected by microtiter plate and congo red agar in coagulase-negative staphylococci

IcaA gene	Congo red					Microtitre				
	Weak, *n* (%)	Moderate, *n* (%)	Strong, *n* (%)	Negative, *n* (%)	*P*	Weak, *n* (%)	Moderate, *n* (%)	Strong, *n* (%)	Negative, *n* (%)	*P*
Positive	5 (100.0)	5 (100.0)	0	2 (20)	0.003*	4 (100.0)	5 (100.0)	1 (100.0)	2 (20)	<0.011*
Negative	0	0	0	8 (80)		0	0	0	8 (80)	

*$P < 0.05$ is significant, *IcaA* = Intercellular adhesion gene A

MTP and CRA were positive for *icaA* gene by PCR. All negative cases by CRA and MTP were negative for *icaA* except two. These results agreed with those obtained by Gad *et al*.[18] who found that all biofilm-producing strains of *S. aureus* and CoNS from urinary tract catheterized patients were positive for the *icaA* gene, while the biofilm-negative strains were negative for the gene. Freeman *et al*.[10] and Murugan *et al*.[19] reported that all *S. aureus* and CoNS biofilm-positive strains isolated from intravenous catheters and eye infections were positive for *icaA* gene and that this gene is required for full slime synthesis, which is in agreement with the results of this study. In addition, in the study of Arciola *et al*.,[11] only one out of 65 staphylococci was found to be biofilm negative by CRA while possessing the *icaA* and *icaD* genes.

Table 5: Antimicrobial susceptibility pattern for *Staphylococcus aureus*

Antibiotic	Disc diffusion test result for *Staphylococcus aureus*	
	Sensitive, *n* (%)	Resistant, *n* (%)
Amikacin	23 (76.7)	7 (23.3)
Ampicillin	18 (60.0)	12 (40.0)
Amoxicillin	21 (70.0)	9 (30.0)
Ciprofloxacin	19 (63.3)	11 (36.7)
Gentamycin	20 (66.7)	10 (33.3)
Ceftriaxone	24 (80.0)	6 (20.0)
Clindamycin	23 (76.7)	7 (23.3)
Erythromycin	24 (80.0)	6 (20.0)
Cefoxitin	29 (96.7)	1 (3.3)
Linezolid	30 (100.0)	-
Penicillin	17 (56.7)	13 (43.3)
Rifampicin	25 (83.3)	5 (16.7)
Sulfamethoprim-trimethoprim	19 (63.3)	11 (36.7)
Vancomycin	21 (70.0)	9 (30.0)

Table 6: Antimicrobial susceptibility pattern for coagulase-negative staphylococci

Antibiotic	Disc diffusion test result for *Staphylococcus epidermidis*	
	Sensitive, *n* (%)	Resistant, *n* (%)
Amikacin	14 (70.0)	6 (30.0)
Ampicillin	14 (70.0)	6 (30.0)
Amoxicillin	14 (70.0)	6 (30.0)
Ciprofloxacin	15 (75.0)	5 (25.0)
Gentamycin	13 (65.0)	7 (35.0)
Ceftriaxone	15 (75.0)	5 (25.0)
Clindamycin	9 (45.0)	11 (55.0)
Erythromycin	12 (60.0	8 (40.0)
Cefoxitin	0 (0.0)	20 (100.0)
Linezolid	20 (100.0)	-
Penicillin	12 (60.0)	8 (40.0)
Rifampicin	15 (75.0)	5 (25.0)
Sulfamethoprim-trimethoprim	11 (55.0)	9 (45.0)
Vancomycin	16 (80.0)	4 (20.0)

Some authors attributed the absence of biofilm production in some staphylococcal isolates despite the presence of the *ica* operon to the insertion of a 1332-bp sequence element, known as IS256, in *icaA* causing its inactivation.[26] However, the transposition of IS256 into the *ica* operon has been found to be a reversible process as after repeated passages of the PIA-negative insertional mutants, the biofilm-forming phenotype could be restored. Cafiso *et al*.[27] also proposed that the product of icaR gene (a regulator gene which seems to function as a repressor) could influence transcription of the *ica* operon. Nevertheless, irrespective of *ica* genes expression, *ica* positive isolates should be considered to be potential biofilm producers.[17]

In this study, there was a high significant association between *icaA* gene presence in *Staphylococcus* strains and resistance to various antibiotics. *icaA* gene was positive in 89.5% of the MDR *S. aureus* and 72.7% of MDR CoNS. These results are greatly consistent with those obtained by Murugan *et al*.[19] who reported that all the MDR staphylococcal strains isolated from conjunctival infections carry the *icaA* gene; Abdolmajid *et al*.[28] also reported in their study that 72% of the isolated MDR staph strains carry the ica operon which agreed with this study.

From the previous results, we can conclude the great correlation between biofilm formation and *icaA* gene presence in staph spp. Furthermore, there is a great correlation between MDR Staphylococcal spp. and *icaA* gene carriage. MDR isolates of Staphylococcal spp. usually carry *icaA* gene which is capable of strong biofilm formation that contributes to the higher antibiotic resistance.

Conclusion

The *icaA* gene responsible for slime production among the Staphylococcal isolated from conjunctival infection

Table 7: Association between intercellular adhesion gene A and multidrug resistant in *Staphylococcus aureus* and coagulase-negative staphylococci isolates

IcaA gene	MDR in *Staphylococcus aureus*			MDR in CoNS		
	MDR, *n* (%)	No MDR, *n* (%)	*P*	MDR, *n* (%)	No MDR, *n* (%)	*P*
Positive	17 (89.5)	1 (9)	≤0.001*	8 (72.7)	2 (22.2)	0.009*
Negative	2 (10.5)	10 (91)		3 (27.3)	7 (77.8)	

*$P < 0.05$ is significant, MDR = Multidrug resistant, CoNS = Coagulase negative staphylococci, *IcaA* = Intercellular adhesion gene A

has been found in a significantly high frequency. On the other hand, the biofilm-forming ability of some strains in the absence of *icaA* gene highlights the importance of further genetic investigations of *ica*-independent biofilm formation mechanisms. A suitable and reproducible method is necessary for screening of biofilm producers in any healthcare setup, and CRA test is recommended as it is easier to perform, cheap, and the agreement between the CRA plate test with the molecular detection of *ica* genes indicates that it is a reliable test. Further investigations on inhibiting *icaA* gene are recommended as a gene responsible for biofilm formation may minimize the selection for antimicrobial resistance strains.

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Conflicts of interest
There are no conflicts of interest.

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