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Original article

Detection of *ica C* gene involved in biofilm formation in *methicillin-resistant Staphylococcus aureus* (*MRSA*) isolates

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Abstract

Background: *Staphylococcus aureus* (*S.aureus*) is one of the gram-positive bacteria that causes a wide range of nosocomial infections. Our present study aimed to investigate genotypic and phenotypic aspects involved in biofilm formation in methicillin-resistant *S.aureus* strains isolated from hospital-acquired infections at Beni-Suef University Hospital **Methodology**: A total of 100 patients, 86 *S. aureus* strains were collected from all departments of Beni Suef University Hospital. The antibiotic resistance pattern, phenotypes of biofilm formation and *ica C* gene were studied using Congo Red Agar (CRA) then molecular conformation was done by PCR. **Results**: We found that 80 out of 86 samples (93%) were mecicillin resistant *S. aureus* (MRSA). The highest frequency of resistance was found for oxacillin (95.3%), cefoxitin (88.4%), and ceftazidime (82.6%). Phenotypic results showed

that 68.8% were high biofilm producers, while 10% and 21.2% were intermediate and low biofilm producers, respectively. From the 63 strong and intermediate biofilm producers isolates, we found that 55 specimens had the *icaC* gene (87.3%). **Conclusion**: Our study concluded that adherence ability and biofilm production are important for enhancing virulence factors among isolates of *S. aureus* strains.

1. Introduction:

S. aureus is one of the most common causes of healthcare and community-acquired infections e.g wound infections, skin ulcers, septicemia, toxic shock and pneumonia. (1)

Multidrug-resistant *S. aureus* (MDRSA) is a severe global concern that is considered a major health problem all over the world. (2)

Biofilm formation contributes to more than 80% of all MRSA infections. In a biofilm, bacterial cells are more resistant to traditional antibiotics and immune factors from the host. Biofilm formation by MRSA isolates is an essential virulence factor influencing its persistence in both the environment and the host (3).

Biofilm formation is divided into three stages at least: initial attachment, maturation, and dispersal. *S. aureus* adheres to different surfaces and takes over the host's tissues as the first step in making biofilm. For this purpose, the bacterium expresses several surface adhesins named "microbial surface components." Moreover, the bacterium recognizes adhesive matrix molecules such as fibronectin-binding proteins A and B (finbA and finbB), clumping factors A and B (clfA and clfB), collagen-binding protein (cna), bone sialoprotein binding protein (bbp), and fibrinogen binding protein (fib) (4).

The next step is the expression of *ica* operon and a surface protein known as biofilm-associated protein (bap). The *Ica* operon produces polysaccharide intercellular adhesin (PIA). Moreover, PIA mediates bacterial cell-to-cell adhesion and biofilm formation in S. aureus biofilms. Among the *ica* genes, *ica C*, *ica A* and *ica D* have shown a significant function in the biofilm formation in *S. aureus* (5).

The final step, dispersal allows the recolonization of other available host sites (6), (7), (8).

Biofilm activity on *S. aureus* increase its resistance pattern to different antibiotics (9), (10), (11) (12).

2. Patients and Methods:

The current study was conducted at different departments of Beni Suef University Hospital including 100 patients during the period from June 2022 to the end of October 2022.

- **Patients**

One hundred patients with wound infections, abscesses, bedsores, respiratory infections, urinary tract infections and burns were enrolled in this study. A full history was taken regarding age, sex, smoking history, sepsis occurrence, antimicrobial use during hospitalization, and history of DM

- **Sample collection and transport**

Samples of blood, sputum and urine, as well as swabs of wound discharge, pus, bed sores, and burned areas, were taken from the cases to test for *S.aureus* colonization. The samples were transported immediately to the Laboratory of Medical Microbiology and Immunology Department, Faculty of Medicine, Beni-Suef University for further processing.

- **Identification of bacterial isolates**

Traditional microbiological standard tests were used to identify the isolates for gram-positive cocci. These tests were colony morphology, gram staining, and catalase and coagulase tests

- **Phenotypic methods for detecting antibiotic susceptibility*):***

Antimicrobial Susceptibility Test by disk diffusion through Kirby-Bauer disk diffusion method

The Clinical Laboratory and Standards Institute (CLSI) guidelines were used for the interpreting of the inhibition halos**.** Inhibition zones should be read by holding the plate up to the light source

- **Phenotypic method for the detection of biofilm formation (CRA method):**

Plates were inoculated by tested MRSA isolates and then were incubated at 37 °C for 24 h. The plates were inspected for the colonies color at 24 and 48 hours. Strains that made biofilm developed black colonies, while strains that did not make biofilm developed red colonies.

- **PCR for detection of *the ica C* gene.**

PCR tested for eighty MRSA isolates to detect the presence of the *ica C* gene.

Ethical considerations

Ethical clearance of the study was obtained from Local Research Ethical Committee) REC) at Beni-Suef University, Faculty of medicine.

The objectives of this study were explained to the patients in Arabic, once the patients had agreed to participate in the study they were requested to sign a consent form. Approval No: FMBSUREC/07062022/Ali

Statistical methodology

• Analysis of data was done by IBM computer using SPSS (statistical program for social science) .

The present study was done at Beni-suef University Hospital from June 2022 to the end of October 2022.

3. Results:

Table (1) and figure (1) demonstrated that the total number of samples included in the present study was 100. The most significant percentage of them were from pus (35.0), followed by wound infection (18.0) and Sputum (15.0).

Table 1: Frequency of the studied samples

Samples	Frequency (n)	Percentage (%)
Urine	5	5.0
Pus	35	35.0
Wound infection	18	18.0
Sputum	15	15.0
Diabetic foot	5	5.0
Blood	12	12.0
Decubitus ulcer	10	10.0
Total	**100**	**100.0**

Figure 1: Distribution of the studied samples according to their sources

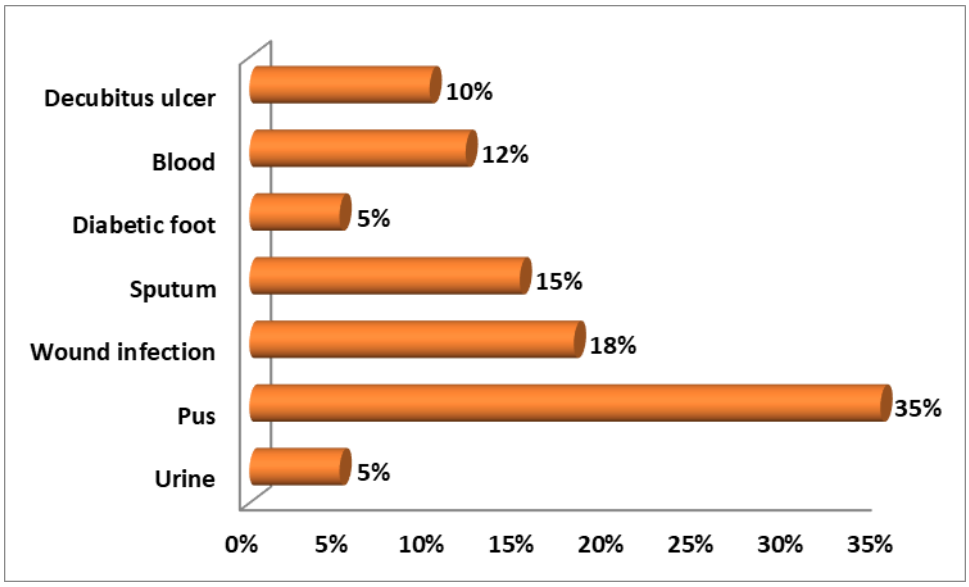

Table (2) and figure (2) demonstrated that the prevalence of staphylococcal bacterial infection among the studied specimens was (86.0%). Among the staphylococcal-infected specimens, 80 out of 86 were Methicillin-resistant (93.0%).

Table 2: Prevalence of *S.aureus* bacterial infection among the studied specimens

Specimens		Frequency	Percentage (%)
***S.Aureus*, n=100**	Positive	86	86.0
	Negative	14	14.0
Methicillin sensitivity, n=86	MRSA	80	93.0
	MSSA	6	7.0

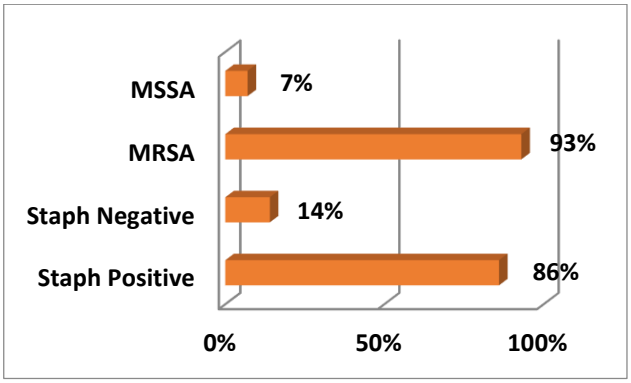

Table (3) demonstrated that the prevalence of *S. aureus* infection was higher among pus (97.1), wound infection (94.4), Sputum (73.3), blood (100.0), decubitus ulcer (70.0), and diabetic foot (60.0) than urine (40.0). There was a statistically significant difference between them (P-value=0.001).

	Staphylococcal infection		Total	P value	X2
	Present	Absent			
Urine	2 (40.0)	3 (60.0)	5 (100.0)	0.001*	22.34
Pus	34 (97.1)	1 (2.9)	35 (100.0)		
Wound infection	17 (94.4)	1 (5.6)	18 (100.0)		
Sputum	11 (73.3)	4 (26.7)	15 (100.0)		
Diabetic foot	3 (60.0)	2 (40.0)	5 (100.0)		
Blood	12 (100.0)	0 (0.0)	12 (100.0)		
Decubitus ulcer	7 (70.0)	3 (30.0)	10 (100.0)		
Total	86 (86.0)	14 (14.0)	100 (100.0)		

*Statistics were done using the Chi-square test /*P-value≤ 0.05 is considered significant.*

Table (4) and figure (3) demonstrated that the resistance rate of the *S.aureus* isolates to the different antibiotics sensitivity discs was as follows: Erythromycin (37.2), Penicillin (62.8), Cefoxitin (88.4), Oxacillin (95.3), clindamycin (23.3), Gentamicin (53.5), Norfloxacin (16.2), Chloramphenicol (29.0), Tetracycline (40.7), Ceftazidime (82.6), Linezolid (0.0), Rifampicin (0.0), Teicoplanin (10.5),

Antibiotics discs	Sensitive	Intermediate	Resistant
Erythromycin	45 (52.3)	9 (10.5)	32 (37.2)
Penicillin	5 (5.8)	27 (31.4)	54 (62.8)
Cefoxitin	3 (3.5)	7 (8.1)	76 (88.4)
Oxacillin	1(1.2)	3 (3.5)	82 (95.3)
Clindamycin	61 (70.9)	5 (5.8)	20 (23.3)
Gentamicin	27 (31.4)	13 (51.1)	46 (53.5)
Norfloxacin	60 (69.8)	12 (14.0)	14 (16.2)
Chloramphenicol	47 (54.7)	14 (16.3)	25 (29.0)
Tetracycline	41 (47.7)	10 (11.6)	35 (40.7)
Ceftazidime	2 (2.3)	13 (51.1)	71 (82.6)
Linezolid	86 (100.0)	0 (0.0)	0 (0.0)
Rifampicin	79 (91.9)	7 (8.1)	0 (0.0)
Teicoplanin	23 (26.7)	54 (62.8)	9 (10.5)
Novobiocin	82 (95.3)	3 (3.5)	1 (1.2)
Meropenem	78 (90.7)	3 (3.5)	5 (5.8)
Co-trimoxazole	78 (90.7)	5 (5.8)	3 (3.5)

Novobiocin (1.2), Meropenem (5.8), and Co-trimoxazole (3.5).

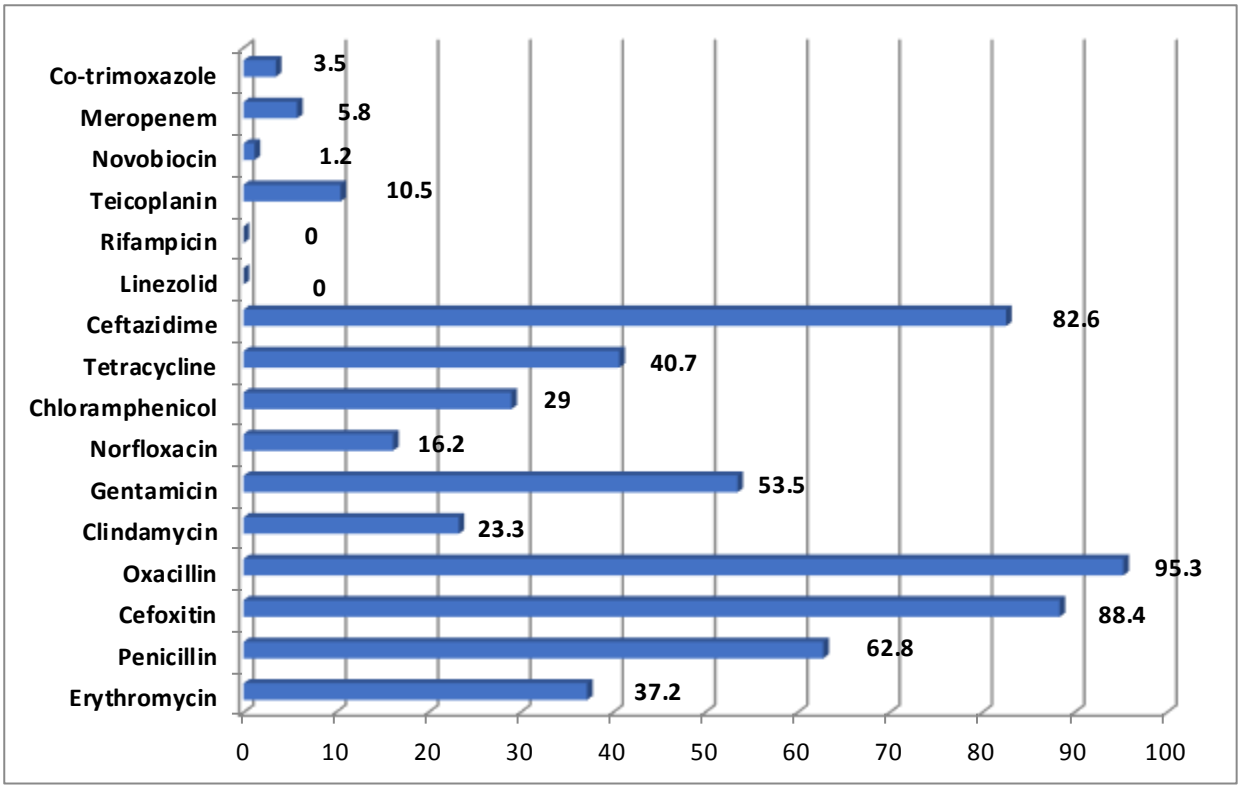

Figure 3: Resistance rate among the *S.aureus* isolates to the different antibiotics discs

Table (5) and figure (4) demonstrated (55 out of 80) of the Methicillin-resistant staphylococcal infected specimens showed strong biofilm-producing (68.8%).

Biofilm producers	Frequency	Percentage (%)
Strong	55	68.8
Intermediate	8	10.0
Weak	17	21.2
Total	80	100.0

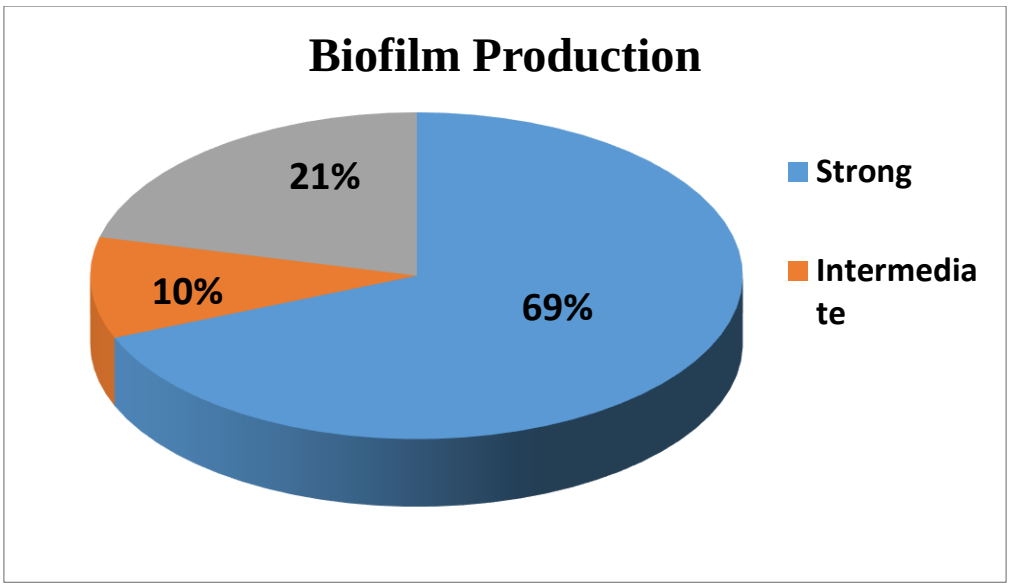

Figure 4: Biofilm-producing pattern among the Methicillin-resistant staphylococcal infected specimens

Table (6) and figure (5) demonstrated that out of 63 specimens with strong and intermediate biofilm production, 55 specimens had the Ica C gene (87.3%).

Ica C gene	Frequency (n=63)	Percentage (%)
Positive	55	87.3
Negative	8	12.7

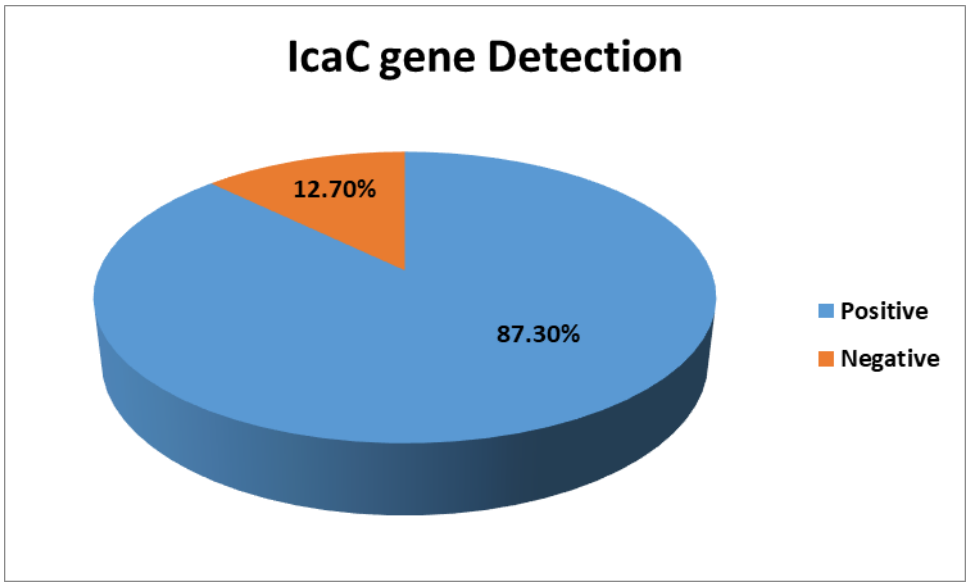

Figure 5: Prevalence of Ica C gene among the strong and intermediate Biofilm producers' specimens

4. Discussion:

Globally, *S. aureus* infections cause a high rate of morbidity and mortality in addition to high economic costs for healthcare institutions (Zhen x., et al., 2020).

Clinicians face serious difficulty treating *S. aureus* infections because of the organism's adaptability, diversity of virulence factors, and rising antibiotic resistance (Chen L. et al., 2020).

The ability of *S. aureus* to form biofilms, which can withstand the effects of the immune system and antibiotics, has been determined to be a crucial element in pathogenesis. It is considered to be responsible for chronic or persistent infections.

Only a small number of research on the expression profiles of genes involved in biofilm creation have been conducted, despite the fact that a more thorough understanding of the process of biofilm formation in *S. aureus* is urgently required.

To better comprehend the molecular mechanism behind biofilm formation, we set out to identify the detection of a single ica C gene that has been efficiently selected in this process. (Mirzaee et al., 2015)

It might be presumed that the *ica C* gene is solely required for biofilm development because it is thought to be a factor in intercellular adhesion. However, this gene has been linked to the development of slime and biofilm (Arciola CR et al., 2001).

The current study was conducted on 100 patients from Beni-Suef University Hospital over six months, from May 2022 to November 2022. Clinical samples were collected including pus, sputum, blood, urine, and swab samples from wound infections, bed sores, and diabetic foot. (86 out of the 100) samples were isolated as *S. aureus*.

The incidence of *S. aureus* among 100 cases investigated was 86 isolates (86%), which was among the high prevalence rates of incidence compared with (Sapkota et al., 2019) and (Koukos et al., 2015), which were 19.96% and 18%, respectively. This could be due to differences in sample size, sampling time, and geographical variation of circulatory clones.

The majority were isolated from pus 34 (34%), followed by wound infection 17 (17%) and sputum 11 (11%), which is consistent with (Sapkota et al., 2019), that have reported a high prevalence of staphylococcal infection among pus samples (49%).

In Europe the percentage of MRSA from all *S. aureus* isolates dropped from 12.9% in 2012 to 10.3% in 2016. However, the occurrence of MRSA isolates shows a clear geographical difference, with the lowest MRSA rates within the range of 1% in the northern countries (Netherlands, Norway, and Denmark) and high MRSA rates in the south ranging from 35% to 50% for countries such as Romania, Portugal, Greece, Malta, and Cyprus (Epidemiologisches Bulletin, Nr. 5/2018) (Musa et al., 2022)

Our study found that 93% of the staphylococcal-infected specimens were MRSA. This result is in line with what (Musa et al., 2022) found ; the prevalence rate of MRSA was 100% and (Jyotshna Sapkota et al.,2019) who found MRSA infections caused by staphylococci were prevalent (70.64%) . These findings agree with our results, which suggest that the type of clinical specimen may influence the methicillin resistance of MRSA isolates. These results may change how MRSA infections are diagnosed and treated, as it may be essential to consider the sample type when determining if MRSA isolates are resistant to methicillin.

However, (Bhat et al., 2016) reported a lower MRSA prevalence among 69 *S. aureus* isolates (17%). The differences between our findings and those of other studies could be due to various factors, such as the geographical location of the study, the period, the type of population, or the type of specimens collected.

The prevalence of methicillin resistance was higher among samples from pus (94.3%), wound infection (83.3%), sputum (73.3%), blood (75.0%), decubitus ulcer (70.0%), and diabetic foot (60.0). However, the prevalence of methicillin resistance in urine was only (40.0%).

Qureshi et al., 2004., reported an 83% MRSA isolation rate from pus in a study conducted in Pakistan. These findings may have implications for diagnosing and managing MRSA infections, as it may be essential to consider the type of specimen when evaluating methicillin resistance.However, (Mehta et al., 1996) and (Rajaduraipandi et al., 2006) made a different observation concerning MRSA prevalence in pus they reported a 33% and 33.6% isolation rate from pus and wound swabs respectively. Other investigations have found MRSA prevalence 29 % in blood isolates, while 76% in urine isolates.

In our study, there was a higher rate of resistance to β-lactam antibiotics: oxacillin (95.3%), cefoxitin (88.4%), Ceftazidime (82.6%), and penicillin (62.8%). These findings coincide with the results of (Das et al., 2016), who found that all MRSA isolates (100%) showed resistance to Penicillin and Oxacillin. Among the MRSA isolates, about 88.2% were resistant to Ceftazidime, and 64.7% were resistant to Ciprofloxacin and Erythromycin.

Our study found a low resistance rate to linezolid 0%, rifampicin 0%, erythromycin 37.2%, and clindamycin 23.3%. These results are different from (Abdel-Maksoud et al., 2016) who reported a higher resistance rate to clindamycin 65%, gentamicin 80.7% and erythromycin 64.4%.

Biofilm prevent penetration of antimicrobial and the concentrations required to eradicate biofilm producing bacteria are higher than those required to eradicate strains that did not produce biofilm due to presence of large amount of exopolysaccharides, slow rate of metabolism and infrequent cell division resulting in decreased sensitivity to antibiotics

targeted at cell wall synthesis (Singh et al., 2021). Furthermore, help in the spread of antibiotic resistant traits in hospital acquired pathogens by increasing mutation rates and by the exchange of genes which are responsible for antibiotic resistance (Jian et al., 2021).

Detection of *ica* genes as a virulence marker of biofilm can be done by some highly accurate methods like PCR analysis. In the present study, PCR for detection of icaC was conducted. It was found that expression of the gene is necessary for phenotypic expression of biofilm in many studies (Kord et al., 2018 and Nasr et al., 2012).

Our study investigated the ability to form biofilm by S. aureus strains isolated from clinical materials from hospitalized patients. Almost all strains (93%) were MRSA but differed in the ability to produce biofilm. However, (Agarwal and Jain et al., 2013), who classified S. aureus into three categories, showed that isolates showing biofilm-producing potential occurred more often among invasive (from blood) and colonizing (from intravenous devices) isolates than in the group of commensals (from skin or nose) isolates.

Our strains also originated from various sources; however, they had a variable ability of forming biofilm. Our results showed that the highest percentage of strong biofilm producers was among the strains isolated from pus (33%), wound infection (15%), sputum (11%), and blood (9%). Because biofilm production is crucial to their persistence in these challenging environmental conditions, this suggested that *S. aureus* isolated from pus and wound infections was a result of environmental selection that led to the prevalence of strong biofilm producers. (Oniciuc et al., 2016) reported that S. aureus strains from different sources produced biofilms with high bio-volumes.

The expression of *ica* genes is strongly linked to biofilm formation. Our findings are consistent with a study that used CLSM analysis to reveal that ica-positive MRSA biofilms have a thicker biofilm and a more compact architecture than ica-negative isolates. The frequency of *ica* genes in clinical MRSA isolates in this study was 68.75% which is considered a high rate similar to that of (Mirzaee et al., 2014) and (Khasawneh et al., 2020) who found that all MRSA isolates harbored icaC gene (100%) and (Parastan et al., 2020) who found (99.04%) of MRSA isolates have *icaC* gene.

In contrast (Ballah et al., 2022) detected low prevalence rate of icaC gene among *S.aureus* isolates(7%)

(Fariña., et al 2017) and (Post., et al 2017) pointed out that although ica genes are responsible for biofilm formation, full phenotypic expression could be conditioned by a few additional genes (atlE, sarA, agrA and mecA) that have a direct or indirect regulatory influence.

The high antibiotic resistance of biofilm producing bacteria, progress has been made on approaches that include anti adhesive strategies

to prevent bacterial surface adhesion, dissolution of already established biofilm, targeting the biofilm matrix for degradation and interference with the biofilm regulation (Mahamuni-Badiger et al., 2020).

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