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Original article

Phenotypic and genotypic detection of biofilm formation and methicillin resistance among *Staphylococcus aureus* isolates

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ABSTRACT

Background: *Staphylococcus aureus (S. aureus)* especially methicillin and multi drug resistant *S. aureus* (MRSA and MDRSA) has the tendency to form biofilm. Our aim was to study the *in vitro* biofilm-forming ability of *S. aureus* isolates and to examine the relationship between biofilm formation and antibiotic resistance pattern. **Methods**: One hundred and forty one clinical isolates of *S. aureus* were isolated from wound pus and tracheal aspirate samples. Biofilm formation of these isolates was detected by Congo red (CRA) method and micro-titer plate (MTP) methods and confirmed by polymerase chain reaction (PCR) through detection of biofilm genes. **Results**: The percentage of biofilm-producing isolates was found to be 81.6% and 58.2% for MTP and CRA methods respectively. The majority of MRSA isolates were positive for biofilm genes. **Conclusions**: Biofilm-producing isolates exhibit high tendency to develop multidrug resistance and methicillin resistance.

Introduction

The concept of biofilm was previously defined by Fletcher and Floodgate (1973), "Biofilm is the unique pattern of growth in the life cycle of microbes that provides specific properties, advantages and higher level of organization of free living bacterial cells during colonization" [1]. Flemming and Wuertz (2019) further clarify the description of biofilms as aggregates of microorganisms with distinct sessile cells followed by cell division to form small clusters, microcolonies, and larger sums [2].

The formation and development of biofilms is a complicated procedure including, different stages. Stages of biofilm development include: attachment of bacterial cells to a suitable surface, development of biofilm structure, maturation of biofilm, and dispersion [3]. Persistent infections caused by biofilm producing bacteria are difficult to treat due to resident multidrug-resistant (MDR) strains.

Staphylococcus aureus (*S. aureus*) is a strong biofilm producer. The biofilm makes antimicrobial cannot reach the organism, and allows it to escape killing by the host immune system and elevating antibiotic resistance [4]. One important element in the process of biofilm formation is the intercellular adhesion (ica) operon , a gene cluster (*icaADBC*) encoding the production of polysaccharide intercellular adhesion (PIA), clumping factor A and B (clfA and clfB) and fibronectin binding proteins A and B (fnbA and fnbB), which mediates adherence of bacteria and the accumulation of multilayer biofilm [5].

Infections with *S. aureus* are difficult to treat because of evolved resistance to antimicrobial drugs [6]. Our aim was to study the *in vitro* biofilm-

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forming ability of *S. aureus* isolates and to examine the relationship between biofilm formation and antibiotic resistance pattern.

Material and Methods

This study was carried out at the Microbiology and Immunology Department, Faculty of Medicine, Minia University in a period from January 2020 to February 2021.

In this study, a total numbers of 290 samples were collected as follows: Two hundred pus samples were obtained from surgical or burn wounds with clinical symptoms and signs of wound infection (suppurative discharge and signs of active infection like fever, redness, and edema) attending Outpatient clinics of the Plastic Surgery Department, Minia University Hospital. Ninety endotracheal secretion samples were obtained from inpatients showing signs and symptoms of lower respiratory tract infection in intensive care unit (ICU) of Neurological Department, Minia University Hospital.

Collection and transport of samples:

Samples were collected by sterile cotton swabs and transferred immediately within 1 hour in a transport medium to Microbiology and Immunology Department for processing as soon as possible.

Isolation and identification of *S. aureus*:

Phenotypic identification

All samples were inoculated into nutrient agar, blood agar and mannitol salt agar plates. The plates were incubated at 37°C for 24–48 h.

Identification of the isolated *S. aureus* colonies were done according to the standard microbiological techniques [7].

Staphylococcus aureus isolates appeared as golden yellow colonies on nutrient agar; on blood agar produce β-hemolysis, on mannitol salt agar *S. aureus* produce yellow colonies. Microscopic examination applied to all isolates after staining by Gram stain and the cells appeared as Gram positive cocci arranged in grape-like clusters. Biochemical tests were done for the identified isolates and *S. aureus* isolates were catalase, coagulase and DNase test positive.

Genotypic identification

For genetic confirmation of all *S. aureus* isolates, Conventional PCR was performed to detect the presence of species specific *16s-rRNA* gene.

DNA extraction: All strains were grown overnight in nutrient broth. 1-ml of each overnight culture was pelleted by centrifugation at 8,000 × *g* for 3 min, suspended in 200 µlof TE buffer containing 2µl lysozyme (10 mg/ml; Sigma), and incubated for 30 minutes at 37°C. The DNA was then extracted using the Thermo Scientific GeneJET GenomicDNA Purification Kit (#K0721) according to the manufacturer's instructions.

Polymerase chain reaction amplification: PCR amplification was performed with PCR thermal cycler (Master cycler® gradient). 25µl was prepared for each reaction using 2x TOP simple Dye Mix-nTaq PCR kit as follows: 12.5µlof PCR master mix, 2µl of primers (1µl forward and 1µl reverse), 6.5µl of PCR water and 4µl of genomic DNA. The sequence of the primers used was listed in **table (1)**.

The amplification program for species specific *16s-rRNA* gene was: initial denaturation at 94 °C for 5min, 30 cycles of denaturation at 94 °C for 60sec, annealing at 56 °C for 30 sec and extension at 72 °C for 90 sec with a final extension step of 72 °C for10 min [8].

Preservation of isolates

After full identification, *S. aureus* isolates were preserved on glycerol broth and then frozen at -20° C**.**

Detection of biofilm formation among *S. aureus* isolates

Phenotypic detection of biofilm formation

Congo red agar (CRA) method: *S. aureus* isolates were cultivated on Brain heart infusion agar with 0.08% Congo red supplemented with 30% sucrose. The strains were inoculated in streaks and incubated at 37 °C under aerobic conditions for 24 and 48 h. The staphylococci biofilm producers appeared as black colonies, while the non-biofilm producer strains formed red or pale colonies [9].

Micro-titer plate (MTP) method: Biofilm formation was measured by the micro-titer plate method as previously described by **TRYP**, [10]. Briefly, all *S. aureus* isolates were inoculated in trypticase soy broth (TSB) containing 1% glucose. The bacterial cultures were adjusted to match the turbidity to that of the 0.5 McFarland standards and added into each well of a sterile 96-well flat-bottom micro-titer plateand then incubated at 37◦C for 48 h under aerobic conditions. After incubation the planktonic cells were washed with deionized water and the adherent cells in each well were air dried at 60°C for 60 min. Then, the adherent cells were stained with 100µl of 0.1% crystal violet solution for 15 min. Excess stain was rinsed off by washing

under running tap water then150 µ of 95% ethanol was gently added and left at room temperature for 30 min. The optical density (OD) of each well was measured at 490nm using an ELISA plate reader (Biotech ELX800, Winooski, USA). The experiment was performed in triplicates, and the absorbance of wells containing sterile TSB was used as a negative control and was taken as the cutoff point to quantities the biofilm formation abilities (optical density cutoff (ODc) = average OD of negative control + 3× standard deviation of negative control). Formation of biofilm was analyzed and categorized based on the absorbance of the stained wells. The strains were classified into the following categories: no biofilm production (OD ≤ ODc), weak biofilm producer (ODc< OD ≤ 2ODc), moderate biofilm producer (2ODc < OD ≤ 4ODc) and strong biofilm producer (4ODc < OD).

Genotypic detection of biofilm formation

The presence of biofilm related genes: *icaA, icaB* and *icaD* genes as well as *fnbA* were analyzed by conventional PCR on the previously extracted DNA of *S. aureus* isolates using specific primers for each and the previously mentioned PCR kit. The sequence of primers for the tested genes was listed in **table (1)**:

Amplification program for *icaA, and icaD* consisted of initial denaturation at 94 °C for 5min, 30 cycles of denaturation at 94 °C for 60sec, annealing at 55 °C for 60 sec and extensionat 72 °C for 60 sec with a final extension step at 72 °C for10 min [13]. Amplification program for *icaB* and *fnbA* consisted of initial denaturation at 94 °C for 5 min, 30cycles of denaturation at 94 °C for 60 sec, annealing at 52 °C for 30 sec and extension at 72°C for 90 sec with a final step at 72 °C for 10 min [13].

The PCR products were analyzed by electrophoresis in a 1.5% agarose grl. The 100-base pair DNA ladder was loaded into the first lane of each gel to evaluate band sizes. The PCR products were visualized under a UV light transilluminator.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done by disc diffusion method according to CLSI guidelines 2019 [15]. The following antimicrobial discs were used: vancomycin (30µg), linezolid (30µg), ampicillin (10µg), amoxicillin clavulanic (30µg), ceftriaxone (30µg) erythromycin (15µg) and teicoplanin (30µg) (Oxoid, UK). Test inoculum (0.5 McFarland standards) was inoculated onto Muller Hinton agar by lawn culture. Antibiotic discs were placed on the agar plate and incubated overnight at 37 °C for 24h. The isolates showing resistance to at least one agent in three or more antimicrobial categories were identified as multi drug resistant (MDR).

Phenotypic detection of methicillin resistant *S. aureus* (MRSA):

All the isolates identified as *S. aureus* were further screened for methicillin resistance by disc diffusion method using the oxacillin (1µg). The zones of inhibition≤ 10 were considered to be MRSA according to CLSI guidelines 2019 [15].

Genotypic detection of MRSA

The presence of *mecA* gene was examined by conventional PCR on the previously extracted DNA of *S. aureus* isolates using specific primers (table 1) and the previously mentioned PCR kit. The amplification program for *mecA* consisted of initial denaturation at 94°C for 4 min, followed by 30 cycles of 45 sec at 94°C, 45 sec at 50°C, and 60 sec at 72°C, with a final extension step at 72°C for 2 min. The *mecA* gene was detected at 310bp [16].

Table 1. The sequence of primers used for amplification of the tested genes.

Gene	Primer	Reference
16S- rRNA	5'GTA GGT GGC AAG CGT TAT CC 3' 3'CGCACATCAGCGTCAG 5'	[8]
icaA	5'ACACTTGCTGGCGCAGTCAA 3' 3'TCTGGAACCAACATCCAACA 5'	[11]
icaB	5'AGAATCGTGAAGTATAGAAAATT 3' 3'TCTAATCTTTTTCATGGAATCCGT 5'	[12]
icaD	5'ATGGTCAAGCCCAGACAGAG 3' 3'AGTATTTTCAATGTTTAAAGCAA 5'	[13]
fnbA	5' CATAAATTGGGAGCAGCATCA 3' 3' ATCAGCAGCTGAATTCCCATT 5'	[14]
mecA	5'GTAGAAATGACTGAACGTCCGATAA 3' 3'CCAATTCCACATTGTTTCGGTCTAA 5'	[15]

Statistical analysis

SPSS software (version 20; SPSS, Chicago, IL, USA) was used for categorical and numerical data analysis. P value of <0.05 was considered statistically significant.

Results

Bacterial isolates

Out of 290 bacterial isolates, 141/290 (48.6%) isolates were phenotypically identified as *S. aureus*, 94(66.7%) isolates were from burn or surgical wound samples of the plastic surgery department and 47(33.3%) isolates were from tracheal aspirate samples of ICU Neurological Department. Amplification of species specific *16s-rRNA* confirmed all the initially identified 141 isolates *as S. aureus* by detection of 228bp band of species specific 16s-rRNA gene.

The percentage of isolates recovered from males and females were 63.1% (89/141) and 36.9% (52/141), respectively. The highest proportions of isolates 37.6 % (53/141) were from patients aged 60 years and older, followed by patients 20 years and younger 29.8% (42/141), the remaining 46 (32.6%) were from patients 20 to <60 years old.

Detection of biofilm formation among *S. aureus* isolates

Phenotypic detection of biofilm formation

All isolates of *S. aureus* (141) were tested for their ability to form biofilm using CRA and MTP methods are shown in **supplementary figure (1& 2)**.

The percentage of biofilm-producing *S.aureus* by MTP method was 81.6% (115/141). Isolates were divided into four categories according to the results of MTP method: non biofilm producers 26 /141 (18.4%), weak biofilm producers 67/141(47.5.7%), moderate biofilm producers 40/141(28.4%) and strong biofilm producers 8/141(5.6%).

Using the CRA method, 58.2% (82/141) of isolates had a biofilm phenotype black colony and 41.8% (59/141) isolates produced a non-biofilm phenotype red colony. In this study, there were 33 *S.aureus* isolates reported as biofilm producing by MTP-method not detected by CRA-method. These data are shown in **table (2)**.

Regression analysis shows that the source of the isolates could be a predictor of biofilm formation (Odds ratio =1.8 and *p* value= 0.04).

Genotypic detection of biofilm formation

All 141 *S. aureus* isolates were tested for the presence of biofilm related genes; *icaA, icaB, icaD* and *fnbA* by conventional PCR. All the primers used in the experiment showed specificity with a single band as shown in **supplementary figure (3)**.

Generally, the majority of *S. aureus* isolates were positive for *icaA* (91.4%; 129/141), *icaB* (92.9%; 131/141), *icaD* (90%; 127/141) and *fnbA* (95.7%; 135/141) genes.

Figure 1 shows the distribution of different biofilm genes in biofilm and non-biofilm producing *S. aureus* isolates reported by MTP-method (*p* value=0.001).

As regards to the correlation of phenotypic biofilm formation with the presence of biofilm genes; 15, 18, 16 and 20 *S. aureus* isolates were non-biofilm forming when assessed by MTP but were positive for *icaA, icaB, icaD or fnbA* genes respectively. On the other hand, all biofilm producing isolates were positive for at least two or more genes.

The sensitivity and specificity of MTP method were estimated to be89.1% and 92.3%, respectively which were higher and significant than the Sensitivity and specificity of CRA method; 57% and 30.8% respectively (*p* value = 0.000).

Phenotypic and genotypic detection of MRSA

Detection of MRSA was done using oxacillin disc.

The percentage of MRSA using oxacillin disc was 79.4% (112⁄141). The *mecA* gene **(Supplementary figure 4)** was detected in 93.6% (132⁄141) of the studied *S. aureus* isolates. The *mecA* gene was detected in all, 100% (112⁄112) MRSA isolates and in 68% (20⁄29) of methicillin sensitive *S. aureus* (MSSA) isolates, the difference was significant (*p* value=0.002).

The distributions of MRSA and methicillin sensitive *S. aureus* (MSSA) (according to oxacillin disc results) as a regard source of isolates, sex, age and history of antibiotic administration was presented in **table (3)**. MRSA was significantly higher in tracheal aspirate samples of the ICU patients in comparison to wound samples of plastic surgery department patients (93.6% versus 72.3%, *p* value=0.002). MRSA was significantly higher in older age group (≥ 60 years old) than other age groups (*p* value=0.02).

The results of the regression analysis show that the source of isolates (Odds ratio =5.6 and *p* value= 0.007) and age (Odds ratio =0.5 and *p* value= 0.008) could be significant predictors of MRSA.

Comparison of the antimicrobial resistance patternsin biofilm-producing and non-biofilm producing *S. aureus* isolates

As shown in **table (4)**, the biofilm-producing *S. aureus* were associated with higher incidence of antimicrobial resistance when compared to the non-producers. Furthermore, 95 (82.6%) of the biofilm-producing isolates were MDR, in contrast to 12(46.2%) of the biofilm non-producers were MDR (*p*=0.005).

Comparison between wound samples of plastic surgery department (outpatients)and tracheal aspirate samples from ICU (inpatients)

Antibiotic resistance and biofilm formation patterns among isolates from the plastic surgery department and ICU of neurological department are presented in **table (5)**. The table shows that the percentages of resistance to all antibiotics in ICU were higher than the plastic surgery department, while the percentage of biofilm formation was higher in the plastic surgery department.

Furthermore, MDR pattern was significantly higher in tracheal aspirate samples of the ICU patients in comparison to wound samples of plastic surgery department patients (93.6% versus 73.4%, *p* value=0.004).

Table 2. The results of biofilm formation in *S. aureus* isolates.

Total N of isolates (141)	**Frequency (%) of Biofilm formation by:**			
	MTP-method			**CRA-method**
	Weak	**Moderate**	**Strong**	
Source of isolates:				
Wound[1]: (N= 94)	48(51.1)	24(25.5)	8(8.5)	62(65.9)
Aspirate[2]: (N= 47)	19(43.1)	16(34.2)	0(0)	20(42.5)
p value	0.04*			0.01*
Sex:				
Male:(N=89)	49(55.1)	14(15.7)	8(8.9)	55(67.1)
Female:(N=52)	18(34.6)	26(50)	0(0)	27(32.9)
p value	0.4			0.09
Age in years:				
2-20:(N= 42)	9(21.4)	21(50)	2(4.7)	17(40.4)
20-40: (N= 13)	5(38.4)	0(0)	3(23.1)	10(76.9)
40-60: (N= 33)	26(78.7)	2(6)	0(0)	25(75.7)
≥60: (N= 53)	27(50.9)	17(32)	3(5.7)	30(56.6)
p value	0.2			0.01*
History of antibiotic administration:	56(45.1)	34(27.4)	8(6.4)	65(52.4)
Yes: (N=124)	11(64.7)	6(35.2)	0(0)	17(100)
No: (N=17)				0.00*
P value	0.03			

1: Wound samples of the plastic surgery department (outpatients), 2: Tracheal aspirate samples of the ICU (inpatients). N: number. *: means significant *p* value.

Table 3. Distributions of MRSA and MSSA**.**

Total N of isolates (141)	**MRSA Frequency (%)**	**MSSA Frequency (%)**	***p* value**
Source of isolates:			
Wounds: (N=94)	68(72.3%)	26 (27.6%)	0.002*
Tracheal aspirates: (N=47)	44(93.6%)	3(6.4%)	
Sex:			
Male: (N=89)	72(80.8%)	17(19.2%)	0.5
Female: (N=52)	40(76.9%)	12(23.1%)	
Age in years:			
2-20: (N=42)	30(71.4%)	6(14.2)	
20-40: (N=13)	13(100%)	0(0%)	0.02*
40-60: (N=33)	21(63.6%)	12(36.3)	
≥60: (N=53)	48(90.5)	11(20.8)	
History of antibiotic administration:			
Yes: (N=124)	98(79.1)	26(20.9)	0.7
No: (N=17)	14(82.3)	13(76.4)	

N: number, Chi square test used to determine *p* value. *: means significant *p* value.

Table 4. Comparison of the antimicrobial resistance patterns in biofilm producing and non-biofilm producing *S. aureus* isolates.

Antibiotic resistance	**Biofilm producers N=115**	**Non-biofilm producers N=26**	*p* value
	Frequency (%)	Frequency(%)	
Vancomycin	64(55.6%)	5 (19.2%)	0.001*
Linezolide	38 (33.3%)	6 (23%)	0.05
Ampicillin	92 (80%)	23 (88.4%)	0.5
Amoxicillin clavulanic	66 (57.3%)	8 (30.7%)	0.002*
Ceftriaxone	105(91.3%)	23 (88.4%)	0.1
Erythromycin	46(40%)	5(19.2%)	0.04*
Teicoplanin	16 (13.9%)	0 (0%)	0.05
Cefoxitin	75(65.2%)	14(53.8%)	0.1
MRSA	96(83.4)	16(61.5%)	0.02*
MDR	95 (82.6%)	12 (46.2%)	0.005*

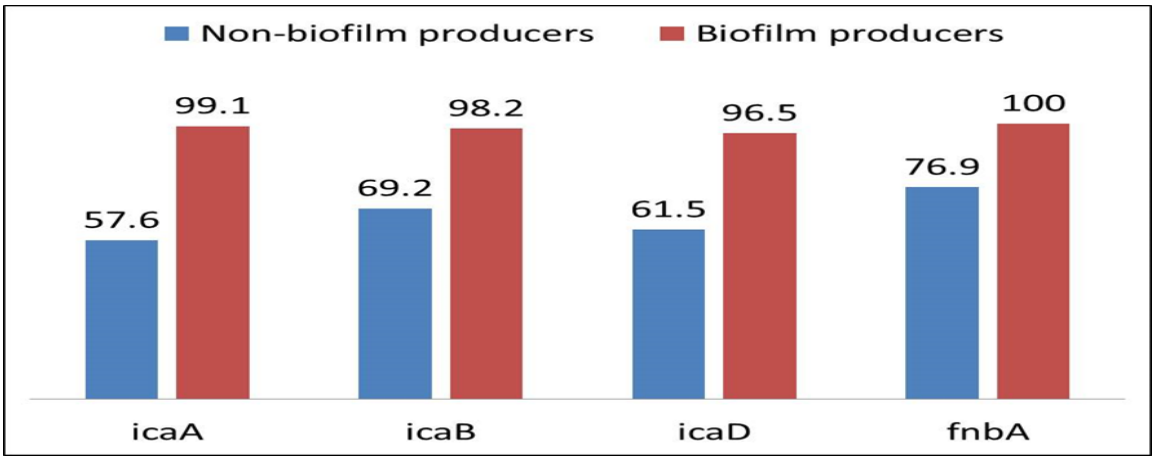
N: number, Chi square test used to determine *p* value. *: means significant *p* value.

Table 5. Comparison of the antimicrobial resistance patternsbetween wound samples of plastic surgery department (outpatients) and tracheal aspirate samples from ICU (inpatients).

Antibiotic resistance	Plastic surgery department samples (outpatient)	ICU of neurological department samples (inpatient)	*p* value
Vancomycin	48% (46/94)	48.9% (23/47)	0.5
Linezolide	30.8% (29/94)	31.9%(15/47)	0.3
Ampicillin	75.5% (71/94)	93.6% (44/47)	0.05
Amoxicillin clavulanic	35% (33/94)	87.2% (41/47)	0.00*
Ceftriaxone	86.1% (81/94)	100% (47/47)	0.006*
Erythromycin	10.6% (10/94)	93.1% (41/47)	0.000*
Teicoplanin	7.4% (7/94)	19.1% (9/47)	0.08
Cefoxitin	67% (63/94)	55.3% (26/47)	0.1
MRSA	72.3% (68/94)	93.6% (44/47)	0.002*
MDR	68% (64/94)	91.4% (43/47)	0.005*

Chi square test used to determine *p* value. *: means significant *p* value.

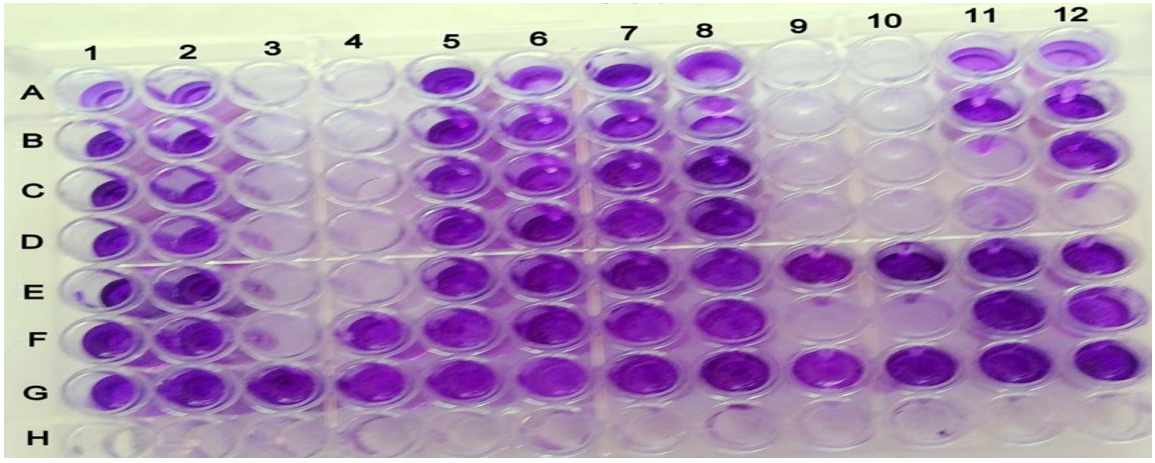
Figure 1. Percentage (%) of biofilm genes in biofilm producing and non-biofilm producing *S. aureus* strains. Biofilm genes (*icaA, icaB, icaD* and *fnbA*), *p* value= 0.001.



Supplementary figure 1. Biofilm and non-biofilm *S. aureus* colonies on CRA.

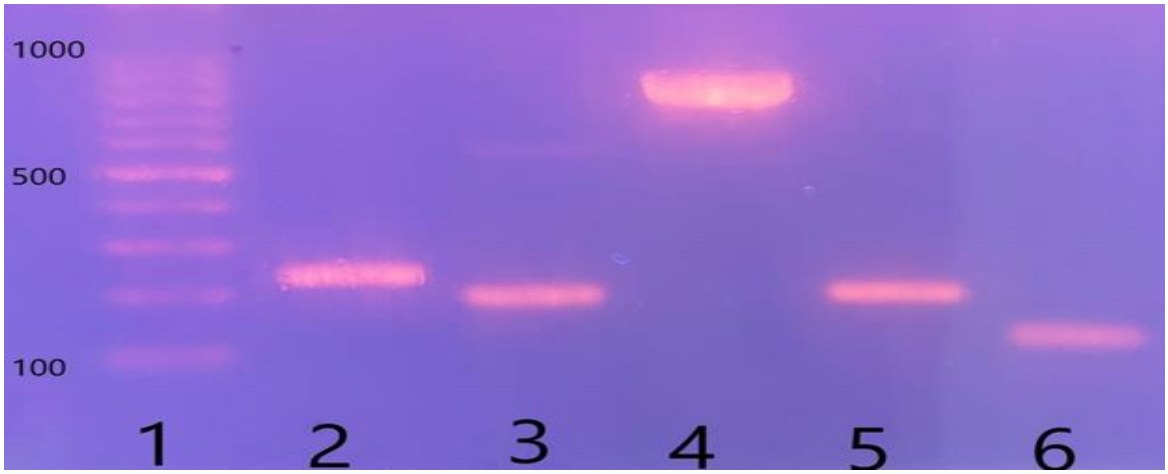
A: black colonies of biofilm forming strain. B: red colonies of non-biofilm strain.

Supplementary figure 2. Biofilm formation using micro-titer plate



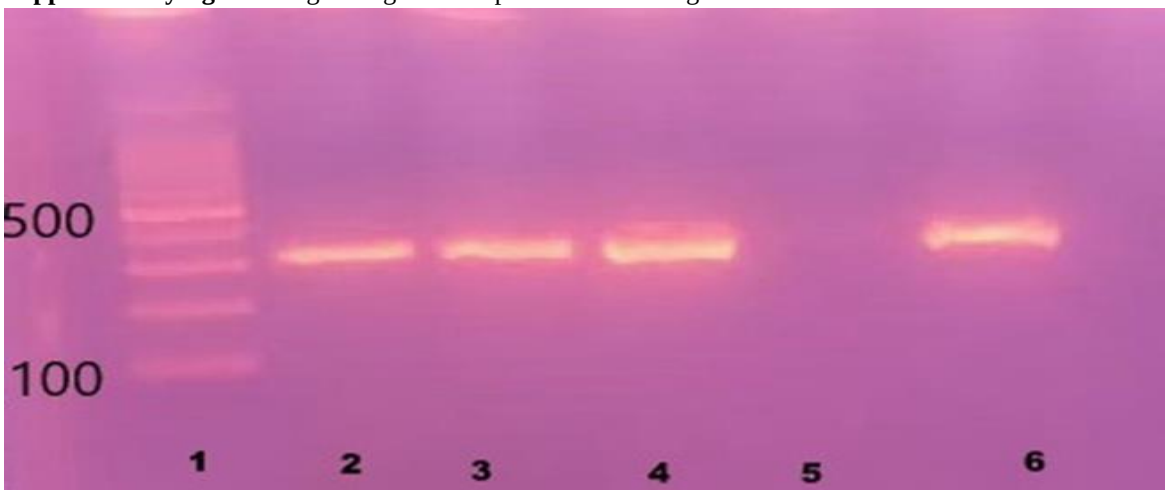
Shows rings of crystal violet stained biofilm in the wells of micro-titer plate. Color gradient is different due to different grades of biofilm formation, for example: E10 is stong, G8 moderate, G6 weak biofilm producers and F3 is non biofilm producer. Row (H) is the negative control row.

Supplementary figure 3. Agarose gel electrophoresis of *16s-rRNA* and biofilm genes



1: DNA ladder (100-1000) in base pair (bp), 2: *16s-rRNA* at 228bp, 3: *icaA* at 188 4: *icaB* at 880bp, 5: *icaD* at 198bp and 6: *fnbA* at 128bp

Supplementary figure 4. Agarose gel electrophoresis of *mecA* gene



1: DNA ladder (100-1000) in base pair (bp). 2, 3, 4 and 6: Positive band of *mecA* gene at 310bp. 5: negative sample, no band.

Discussion

In this study, the percentage of biofilm-producing *S. aureus* isolates was found to be 81.6% (115⁄141).Our result was consistent with **Karki et al.** [17] who observed that out of 110 isolates, 86.3% (95/110) were detected as biofilm producer.

In the present study, 141 *S. aureus* isolates were graded as follows: non biofilm producers (18.4%), weak (47.5%), moderate (28.4%) and strong (5.6%) biofilm producers according to the results of MTP method.

This finding is similar to the result reported by **Neopane et al.** [18]. Who investigated biofilm formation by *S.aureus* in wounds showed that the prevalence of biofilm formation was 69.8% (6.97% strong, 27.9% moderate, 34.88% weak and 30.2 non biofilm producers). However this finding was lower than the result detected by **Bimanand et al.** [19], who demonstrated that 96 % of isolates were biofilm producers, the distribution of biofilm formation between isolates was 4.2%, 54.2%, 35.4% as strong, moderate and weak, respectively. A lower rate of biofilm formation was demonstrated by **Nasr et al.** [20] in Egypt, where 46% of *S. aureus* isolates produced biofilm by MTP assay; 26% strong, 12% moderate and 8% were weak biofilm producers.

The difference in biofilm production may be attributed to differences in virulence capacity of bacteria to form biofilm and the number of bacterial cells that succeeded in adherence, type of specimen, geographical origin and the genetic makeup of the *S. aureus* isolate. Also environmental factors like growth medium, type of surface (rough/smooth), porosity and the charge of the surface affect biofilm formation.

Regarding studying possible risk factors for biofilm formation by *S. aureus*, the source of the isolates and history of antibiotic administration shows significant association with biofilm formation. Biofilm formation was significantly higher in: wound samples than tracheal aspirate samples, and higher in patients with a history of antibiotic administration. Also **Solti et al.** [21], **Thummeepak et al.** [22] and **Kord et al.** [11] demonstrated that there are some correlations between the biofilm-forming capacity of bacteria, and patient demographic characteristics.But there is no correlation between the ages or gender of the patients and biofilm formation, this finding are consistent with **Taj et al.** [23] and **Cha et al.** [24].

The prevalence of *icaA, icaB, icaD* and *fnbA among S. aureus* isolates were 91.4%, 92.9%, 90% 95.7% respectively. Our findings are similar to **Khlaf et al.** [25] who reported that the prevalence of *icaA, icaB* and *icaD* genes were 95.8%, 91.6% and 95.8%, respectively. Also **Torlak et al.** [26] and **Tekeli et al.** [27] demonstrated that *ica* genes were detected among all isolates of *S. aureus*.

All the biofilm producing isolates in our study were positive for at least two or more genes. This was consistent with **Gad et al.** [28] and **Mahmoudi et al.** [29] who detected *ica* genes in all biofilm forming *S.aureus* isolates.

In this study the prevalence of MRSA screened by Oxacillin was 79.4% (112/141). This result was less than results observed by **Gitau et al.** [30] who reported that the prevalence of MRSA was 91.97% (867/944) and higher than the results observed by **Hasan et al.** [31] and **Saeed et al**.[32] who reported a prevalence of 75% and 76% respectively. Unlike the previous results, **Dilnessa** [33] found that only 12.8% from 1912 *S. aureus* isolates were MRSA.

In the current study, the incidence of *mecA* gene was 93.6% (132/141), this incidence was lower than the results reported by **Saeed et al.** [32] who detected that *mecA* gene incidence was 33.3%.

The percentage of resistance to all antibiotics in ICU patients were higher than patients with plastic surgery, this may be explained by prolonged use of antibiotic in the ICU patients, prolonged stay in hospital and more virulent organisms in ICU.

The percentage of biofilm forming *S. aureus* isolates was higher in the plastic surgery department than ICU isolates. This may be due to the large area involved in the multiplication of *S. aureus* in wounds.

In the present study, MDRSA and MRSA were significantly higher (P<0.02) in biofilm producers (83.4%) compared with the biofilm non-producers (61.5%). These data were in accordance with the results obtained by **Moghadam et al.** [34], **Neopane et al.** [18] and **Ibrahim et al.** [35] who observed that biofilm forming isolates of *S. aureus* exhibit a high antimicrobial resistance pattern than biofilm non-producers.

Conclusions

Biofilm-producing *S. aureus* isolates exhibit high tendency to develop multidrug resistance and methicillin resistance. The percentage of resistance to all antibiotics in ICU patients was higher than the plastic surgery department patients,

while the percentage of biofilm formation was higher in the Plastic Surgery Department.

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Conflict of interests

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Ethical Approval

Written informed consents were obtained from all individuals. The study was approved by the Ethical Committee of Minia University, Faculty of Medicine.

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