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Biofilm Formation of Pathogenic Bacterial Species Isolated From Urinary Catheters

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ABSTRACT

Many bacterial species can produce biofilm on medical device surfaces. Biofilm formation increases the persistence of infection and antimicrobial agents' resistance as well as a healthcare-associated infection. The urinary catheter is one of the medical devices that pathogenic bacterial species can colonies and form biofilm on their surfaces leading to recurrent and persistent urinary tract infections. This study aimed to detect biofilm formation of different gram-positive and gram-negative bacterial species isolated from rubber and silicone urinary catheters.

Evaluation of biofilm formation was performed by using 0.1% of crystal violet and pieces of 1 cm long of rubber and silicone catheters. After washing catheter pieces three times with phosphate buffer saline finally washed with ethanol. The optic density of alcohol wash was measured at 450nm. Biofilm production was evaluated according to the mean of OD as following: 0.120; weak biofilm-forming, 0.120-0.240 moderate biofilm-forming, and 0.240 strong biofilm forming.

The results of this study reported 62.3% of tested isolates were strong biofilm producers. Gram-negative bacteria were more potent in biofilm formation than gram-positive bacteria (78.9% and 32.7% respectively). The highest species strongly formed biofilm in gram-positive was E. faecalis in rubber urinary catheter while Staph. epidermidis was the highest in silicon urinary catheters. Most gram-negative species were strong biofilm producers. The high prevalence of biofilm-forming bacterial species among collected isolates is considered a risk factor that might lead to recurrent or persistent urinary tract infection and healthcare-associated infection.

قدرة السلالات البكتيرية المعزولة من على اسطح انابيب القسطرة البولية المستعملة لمرضى الكلى على تكوين الطبقة الحيوية (البيوفلم)

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الكلمات المفتاحية:
انابيب القسطرة البولية
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الملخص

العديد من الانواغ البكتيرية قادرة على تكوين البيوفلم على اسطح الاجهزة الطبية، تكون اليوفلم يزيد من قدرة البكتيريا على مقاومة العوامل المضادة لها، تعتبر أنابيب القسطرة البولية احد انواع الاجهزة الطبية المهمة لتكوين البيوفلم الامر الذي يتسبب في حدوث التهابات المسالك البولية. هدفت هذه الدراسة الى معرفة قدرة الانواع البكتيرية الموجبة والسالبة لصبغة جرام المعزولة من على اسطح انابيب القسطرة البولية على تكوين البيوفلم. تم تقدير البيوفلم المتكون على قطع من انابيب القسطرة البولية المصنوعة من كلاً من السيلكون والمطاط بطول 1سم وذلك باستخدام تركيز 0.1% صبغة الجنشيان البنفسجي، تم غسل البيوفلم مرتين ومن تم تم استخدام الايثانول 70% لاجل امتصاص الصبغة من خلايا البكتيريا، باستخدام جهاز قياس الطيف الضوئي عند الطول

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الموجي 450 نانوميتر تم تقييم قدرة العزلات على انتاج البيوفلم وفق المعايير المعترف بها. بينت نتائج الدراسة قدرة حوالي 62% من العزلات على انتاج البيوفلم بدرجة عالية، كما أن الأنواع البكتيرية السالبة كانت قادرة على انتاج البيوفلم بدرجة أعلى من الأنواع موجبة الصبغ، بينت الدراسة أن بكتيريا E. faecalis كانت الأعلى تكويناً للبيوفلم على اسطح انابيب المطاط بينما كانت بكتيريا Staph. Epidermidis الأعلى تكويناً على اسطح انابيب السيلكون من بين انواع البكتيريا الموجبة لصبغة جرام، بينما كانت البكتيريا السالبة لصبغة جرام اعلى قدرة على تكوين البيوفلم مقارنة بالبكتيريا الموجبة لصبغة جرام.

Introduction

Biofilm is a mechanism of protection acquired by a group of Microbial cells of prokaryotic and/or eukaryotic cells surrounded by matrix structured from different materials produced entirely or partially by the microbial community (1). Biofilm is a multistage process that starts with microbial cell adhesion to the surface followed by production and accumulation of extracellular matrix consisted of one or more polymeric substances like protein, extracellular DNA, EPS, and peptides required in quorum sensing (QS) (2). Bacterial cells embedded in biofilm will have protection from the effective concentration of antimicrobials and disinfectant, more nutrient availability, and source of microbial infection (3).

Biofilm plays a major role in healthcare-associated infection associated with medical device implantation (4). Bacterial adhesion and biofilm formation recorded as risk factors preluding to bacteriuria and bacteremia (5). Medical device-associated infection increases health care financial costs and enhances patients' morbidity and mortality (6). Gram-negative and gram-negative bacteria as well as candida species can form biofilm in medical devices (7). Bacteria commonly isolated from urinary catheters include gram-positive *Enterococcus faecalis* (*E. faecalis*), *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), and *Streptococcus viridans* (*Strep. viridans*); and the gram-negative *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumonia*), *Proteus mirabilis* (*Pr. mirabilis*), and *Pseudomonas aeruginosa* (*P. aeruginosa*) (7). Urinary tract infection (UTI) relapse was more associated with biofilm-forming isolates in urinary tract catheterized patients (8). Microbial biofilm on urinary catheters was a major factor in persistent UTI (9). The stone formation was reported as a sequel of biofilm-forming *Proteus mirabilis* (*P. mirabilis*) on Foley catheters (10). Bacterial isolates obtained from urine samples of UTI patients showed the different capabilities of biofilm formation. Isolates most commonly identified were; *S. aureus* (24%), *P. aeruginosa* (18%), *E. faecalis* (14%) and others (44%) (11). A study found 73.4% of catheter-associated bacterial isolates were biofilm-forming while 26.6% were non-biofilm-forming isolates. *E. coli* were the highest identified among collected isolates (52.3%) and *Enterobacter cloacae* (*Enter. Cloacae*) were found the strongest in biofilm formation (87.5%) (12). This study aimed to evaluate biofilm formation by bacterial isolates associated with catheterized UTI patients.

Methods

Study setting

This study was conducted in private Alnokhba hospital, Misurata city, Libya; as a part of a large study investigated bacterial isolates associated with the urinary catheters (internal and external parts as well as urine accumulated in the urinary bag)[(13)(in press)].

Bacterial isolate Identification

Standard microbiological methods were performed to identify collected bacterial isolates included in the previous study investigated pathogenic bacteria associated with urinary catheters (data not published). Briefly, performed tests included were; gram stain, oxidase test API 20 E biochemical test. Catalase and coagulase tests were performed to identify gram-positive isolates. Esculin hydrolysis was used to differentiate *E. faecalis* from Streptococcus species.

Tested bacterial isolates

Gram-positive bacterial species (n=49); *S. aureus* (n=14), *S. epidermidis* (n=5), *Strep. spp* (n=3), and *E. faecalis* (n=27).

Gram-negative bacterial species (n=57); *E. coli* (n=18), *K. pneumonia* (n=13), *Citrobacter freundii* (*Cit. freundii*) (n=2), *P. mirabilis* (n=4), *Enter. cloacae* (n=1), serratia species (n=7), *Acinetobacter baumannii* (*Acin. baumannii*) (n=5), and *P. aeruginosa* (n=7).

Biofilm assay

Isolates biofilm formation detection was performed by using 0.1% of crystal violet solution as described by Zhang et al; 2004 (12). Stored tested bacterial isolates were activated by inoculation on blood agar and incubated at 37°C for 24 hours. Three pieces of the urinary catheter (1 centimeter (cm) long) were impregnated in one tube of nutrient broth that was previously inoculated with 3 colonies of activated bacterial isolate and incubated at 37°C for 24 hours. Soaked catheter pieces were removed from the nutrient broth and each piece placed on a separate glass tube and washed three times with one ml of phosphate buffer saline. After washing, 0.6ml of 0.01% Crystal violet solution was added into tubes and stayed for 15 min without shaking. Crystal violet was washed 2 times with phosphate buffer saline followed by the addition of 5 ml of absolute ethanol and stayed at room temperature for 10 minutes. The optic density (OD) of collected ethanol was estimated at 540 nm. The Control group was included by using the same steps without bacterial isolates in each experiment. Biofilm formation was divided according to obtained OD mean value; <0.120; weak biofilm-forming, 0.120-0.240 moderate biofilm-forming and >0.240 strong biofilm forming the value was estimated as previously explained (14).

Statistical data analysis

Data analysis was performed by SPSS 22 Inc. Chicago, USA. Chi-square test was used to estimate the correlation between biofilm formation and bacterial group. Results were considered significant when *P*-value ≤ 0.05.

Results and discussion

In this study, of 106 included isolates, strong biofilm formation on rubber catheter was reported in 66 (62.3%) of tested isolates; 16 (32.6%) gram-positive and 50 (87.7%) gram-negative). On silicone catheter, 57.5% of isolates were strongly from biofilm; 16 (32.7%) gram-positive and 45 (78.9%) gram-negative as shown in table1 and figure 1. The ability of tested gram-negative bacterial isolates was significantly higher than gram-positive tested isolates on rubber and silicone catheter ($P< 0.05$). In agreement with this, in a study conducted in Algeria, only gram-negative bacilli were identified in samples obtained from biofilm formed on the inner surface of silicone urinary catheter (15). A contradictory result reported in a study that investigated biofilm formation in a urethral stent which reported gram-positive bacteria was higher than gram-negative bacteria (16). This contradiction may be related to biomaterial surface differences between urinary stent and urinary catheter and different sources of bacterial isolate reveal the variability of biofilm-producing ability.

Table 1. Gram-positive and gram-negative isolates biofilm formation on rubber and silicone urinary catheter

Gram stain Grouping N= 106		Strong positive N (%)	Weak positive N (%)	Non adherent N (%)
Gram positive (49)	Rubber	16 (32.7)	14 (28.6)	19 (38.7)
	Silicone	16 (32.7)	18 (36.7)	15 (30.6)
Gram negative (57)	Rubber	50 (87.7)	7 (12.3)	0
	Silicon	45 (78.9)	4 (7)	8 (14.1)
Total 106	Rubber	66 (62.3)	21(19.8)	19 (17.9)
	Silicon	61 (57.5)	22 (20.8)	23 (21.7)

N; number of isolates

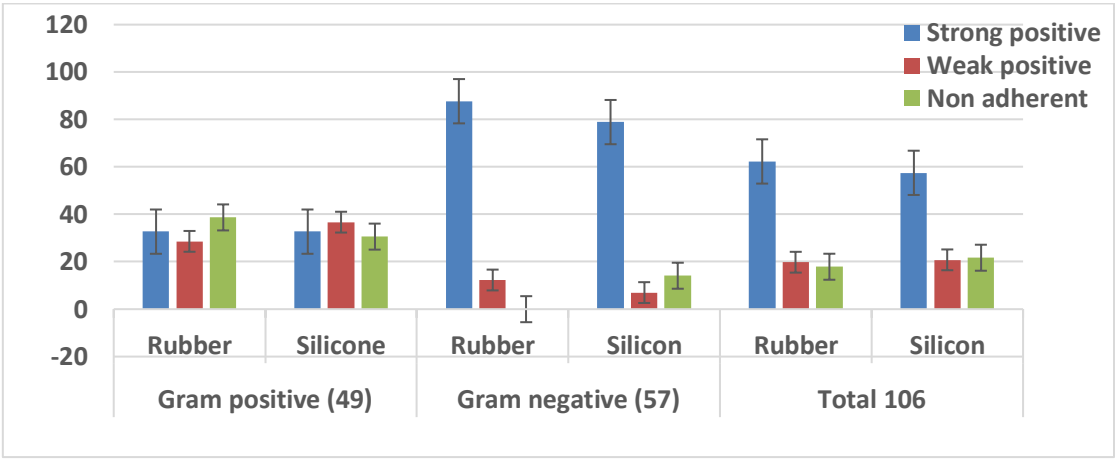

Figure 1. Biofilm formation in Gram-Negative and Garm-Positive Groups

In gram-positive bacteria, *E. faecalis* was the highest isolate strongly formed biofilm on rubber catheter (81.5%), while was the lowest on silicone catheter (14.8%). *S. epidermidis* strong positive biofilm formation group was the highest on silicon catheter (100%) (table2).

Table 2. Gram positive isolates biofilm formation

Bacterial species (N)		Strong positive N (%)	Weak positive N (%)	Non adherent N (%)
S. aureus (14)	Rubber	9 (64.3)	2 (14.3)	3 (21.4)
	Silicone	8 (57.1)	4 (28.6)	2 (14.3)
S. epidermidis (5)	Rubber	4 (80)	1 (20)	0
	Silicone	5 (100)	0	0
E. faecalis (27)	Rubber	22 (81.5)	3 (11.1)	2 (7.4)
	Silicone	4 (14.8)	5 (18.5)	18 (66.7)
Streptococcus spp (3)	Rubber	0	3 (100)	0
	Silicone	2(66.7)	1 (33.3)	0
Total = 49	Rubber	36 (73.4)	6 (12.2)	7 (14.3)
	Silicone	17 (34.6)	12 (24.5)	20 (40.8)

N; number of isolates

The highest bacterial isolates among the gram-positive group were *S. epidermidis* followed by *E. faecalis.* The high prevalence of *E. faecalis* with its ability to produce biofilm and high resistance to antimicrobial poses a menace to patients with UTI. It was reported the pathogenicity of *E. faecalis* is not associated with specific virulence factor but more linked to its multi-resistance to the antimicrobial factors and biofilm production (17).

Tested gram-negative isolates of *E. coli*, *K. pneumonia*, *P. aeruginosa* and *Acinetobacter baumannii* revealed 100% strong biofilm formation on rubber catheter, Whereas *K. pneumonia*, *P. aeruginosa*, Acinetobacter *baumannii* and *Serratia spp* reported 100% as strong positive biofilm forming on silicon catheter. Strong biofilm formation of *E. coli* was more associated with rubber urinary catheter (100%) than silicone urinary catheter (33.3%) (table 3). In contrast, *P. mirabilis* and *Serratia spp* strong biofilm formation was more associated with silicon urinary catheter. Similar results were reported in Pakistan, whereas *Enter. Cloacae* and *E. coli* were the dominant bacterial isolates identified as biofilm producing organisms obtained from indwelling urinary catheter of patients suffering from UTI (18). These results were revealed, some bacterial species had a strong ability to form biofilm on specific biomaterial type of urinary catheter and had a weak biofilm production on another urinary catheter made of different biomaterial types.

Table 3. Gram-negative isolate biofilm formation

Bacterial species (N) N= 57		Strong positive N (%)	Weak positive N (%)	Non adherent N (%)
E.coli (18)	Rubber	18 (100)	0	0
	Silicone	6 (33.3)	4 (22.2)	8 (44.4)
K. pneumonia (13)	Rubber	13 (100)	0	0
	Silicone	13 (100)	0	0
P. aeruginosa (7)	Rubber	7 (100)	0	0
	Silicone	7 (100)	0	0
Serratia spp (7)	Rubber	3 (42.9)	4 (57.1)	0
	Silicone	7 (100)	0	0
Acinetobacter baumannii (5)	Rubber	5 (100)	0	0
	Silicone	5 (100)	0	0
Proteus merabilis (4)	Rubber	1 (25)	3(75)	0
	Silicone	4 (100)	0	0
Citrobacter freundii (2)	Rubber	2(100)	0	0
	Silicone	2(100)	0	0
Enterobacter cloacae (1)		1(100)	0	0
		1(100)	0	0
Total =	Rubber	50(87.7)	7(12.3)	0
	Silicone	45(78.9)	4(7.1)	8(14)

N; number of isolates

In conclusion, the high prevalence of strong biofilm-producing isolates (62.3%) on urinary catheter surfaces is a major concern as it was reported as a factor predisposing to bacteriuria and bacteremia and increasing bacterial resistance to the antimicrobials. This study showed the biofilm production was higher in gram-negative than gram-positive bacteria in both rubber and silicone urinary catheters. Among gram-positive bacteria, *E. faecalis* was the strongest biofilm formed species on the rubber catheter and *Staph epidermidis* was the strongest on the silicon catheter.

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