Original Research Article

Analysis the Antibiogram Profiles of Biofilm Forming *Staphylococcus aureus* and *Escherichia coli*

ABSTRACT

Background and Objectives: Bacteria attach to surfaces and produce polymeric matrix resulting in the formation of biofilms are involved in a wide range of human infections. Biofilms forming *Staphylococcus aureus* and *Escherichia coli* are considered to be highly resistant to antibiotics.This study was aimed to analysis theantibiogram profile of biofilmforming *S. aureus* and *E. coli*in Mukalla city, Hadhramaut,Yemen.**Methods:**A total of 60 clinical isolates of *S. aureus* and *E. coli* were isolated and identified by standard bacteriological procedures, then subjected to biofilm detection by tissue culture plate (TCP) method. The antibiotics susceptibility test was performed by Kirby-Bauer disc diffusion method. Chi- square test used to analyze the data and*P*value< 0.05 was taken as significant.**Results:**Of the total isolates *S. aureus* and *E. coli*, TCP method detected 33(55%%) as strong, 15(25%) as moderate and 12(25%) as weak/non-biofilm producers. Biofilm forming *S. aureus* developed significantly higher degree of resistance againstamoxicillin/clavulanic acid100%, ceftazidime 95.8%, cefotaxime62.5%, cefadroxil 45.8%, ciprofloxacin 41.7% and ceftriaxone 25% with statistically significant correlation of amoxicillin/clavulanic acid and ceftazidime resistance and bacterial biofilm production (*P-value*< 0.05). The rates of antibiotics resistance biofilm *E. coli* were 100%, 91.7%,75%, 70.8%, 66.7%, 62.5% and 33.3%for amoxicillin/clavulanic acid, cefadroxil, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin and co-trimoxazole respectively with statistically significant correlation of cefadroxil resistance (*P-value* < 0.05).**Conclusion:**TCP method showed that *S. aureus* and *E. coli* isolates have high degree of biofilm forming ability. A high antibiotics resistance was observed in biofilm producers than non-biofilm producers.

Key words: Biofilm formation, *Escherichia coli*,Multi-drug resistance, *Staphylococcus aureus*, Tissue culture plate

INTRODUCTION

Bacterial biofilm defined as an organized bacterial community embedded in an extracellular polymeric matrix attached to biotic or abiotic surfaces[1]. Bacterial biofilms are usually pathogenic and causing infections. Among all microbial and chronic infections, about 65% are associated with biofilm formation[2], whereas biofilm protects the microorganism from host defenses and impedes delivery of antibiotics which may cause impairment in wound healing[3].The ability of bacteria to aggregate and form biofilm is strictly related to the capacity of producing an extracellular mucoid substance such as the slime whose main component of polysaccharide nature and consists of glycosaminoglycans[4].Theextracellular polymeric matrix that can restrict the diffusion of substances and binding to antibiotics and this will provide effective resistance for biofilm cells[5]. Biofilm formations also help in the spread of antibiotic resistant traits in bacterial pathogens by increasing mutation rates and by the exchange of genes which are responsible for antibiotic resistance[6].

Staphylococcus aureus (*S. aureus*) and *Escherichia coli* (*E. coli*) are the most common etiologicalagent causing bothcommunity and hospital- acquired infections[7,8].*E. coli*infections leading to serioussecondary health issues worldwideand tends to form microcolonies in mucosalining of urinary bladder known as biofilm[8]. Thesebiofilms make the bacterium to resist the host immuneresponse, more virulent and lead to the evolution ofantibiotics resistance by enclosing them in anextracellular biochemical matrix[9].The ability of *S. aureus* to form biofilm is considered to be a major virulence factor influencing its survival and

persistence in both the environment and the host[10].*S. aureus* biofilms have been associated with a variety of persistent infections which respond poorly to conventional antibiotic therapy [11].

Biofilmproducing bacteria show higher resistance to standard antibiotics used for the treatment of bacterial infections.Therefore, this study aimed to analysis theantibiogram profile of biofilmforming *S. aureus* and *E. coli*.

MATERIALS AND METHODS

Bacteriological testing

A total of 60 isolates of *S. aureus* and *E. coli* wereisolated from different samples of wound swabs and midstream urinethen analyzed and identified by the standard methods for bacterial culture growth, Gram staining and biochemical tests[12].Antibiotics susceptibility testing was done using Kirby-Bauer method disc diffusion according to the guidelines of the Clinical Laboratory Standard Institute (CLSI)[13].

Biofilm formation detection by tissue culture plate (TCP) method

Quantitative TCP method was performed as described by Yadavet al.[14]. In briefly, subcultures of the isolates in nutrient agar were inoculated in 10mL of trypticase soy broth with 1% glucose and incubated for 24 hours at 37ºC, then the cultures were diluted 1:100 with fresh medium. The wells of sterile 96 polystyrene microtiter plates were filled with 0.2ml aliquots of the diluted cultures. Negative control wells were maintained by adding broth without culture. After incubation for 24 hours at 37ºC, the wells removed by gentle tapping and washed with 0.2mL phosphate buffer saline (pH 7.3) three times to remove free floating planktonic bacteria. Thewells then were dried for 1 hour and stained with crystal violet (0.1% w/v) and the excess stains removed using deionized water, then the plates were kept for drying. Quantitative analysis of biofilm production was performed by adding 150µl of 95% ethanol to destain each well. After 30 min, optical density (OD) of stained adherent biofilm was obtained by using microtiter plate ELISA reader at wave length 630 nm. The experiment was done in triplicate and repeated three times.Optical density cut-off value (ODc) calculated as average OD of negative control + 3x standard deviation (SD) of negative control.The bacterial species tested were classified into four categories as follows: $OD \leq ODc$ no biofilm producer; $ODc < OD \leq 2 \times ODc$ weak biofilm producer; $2 \times ODc < OD \leq 4 \times ODc$ moderate biofilm producer; $4 \times ODc < OD$ strong biofilm producer.

Statistical analysis

Data analysis were conducted using the software of Statistical Package for Social Sciences (SPSS) version 25. A Chi-square test was used to study distribution andchanges in antibiotics resistance profiles. Statistical significance determined at *P-value* <0.05.

RESULTS

Biofilm detection by tissue culture plate (TCP) method

TCP method detectedbiofilm formation in 33(55%%) as strong, 15(25%) as moderate and 12(25%) as weak/non-biofilm producers.Among *S. aureus* isolates, 18 were strong biofilm producers, 6isolates were moderate biofilm producers and 6isolates were weak/non-biofilm producers. Of *E. coli* isolates showed 15were strong biofilm producers, 9isolates were moderate biofilm producers, andweak/non-biofilm producers isolates identified in6 isolates. There was no significant statistical analysis of TCP method for screening biofilm production (*P-value* = 1.000) as shown in table (1).

Table (1): Analysis of *S. aureus* and *E. coli* for biofilm formation by TCP method

Biofilm formation by TCP method		χ^2 test value	*P-value*
Result	No. (%)		
Strong	33 (55)	0.00	1.000
Moderate	15 (25)		
Weak/None	12 (20)		
Total	60 (20)		

Relationship theantibiogram profiles with biofilm and non-biofilm producing *S. aureus* and *E. coli*

Among 60 *S. aureus* and *E. coli* isolates, biofilm producers isolates by TCP method showed high resistance rates to antibiotics used compared to non-biofilm producers isolates.*S. aureus* biofilm producing isolates found highly resistant to amoxicillin/clavulanic acid, ceftazidime, cefotaxime, cefadroxil, ciprofloxacin and ceftriaxone in a rate of 100%, 95.8%, 62.5%, 45.8%, 41.7% and 25% respectively. There was significant statistical correlation of antibiotic resistance of amoxicillin/clavulanic acidand ceftazidime and bacterial biofilm production (*P-value*< 0.05)as show in table (2).

Table (2). Antibiogram profiles of biofilm and non-biofilm producing *S. aureus*

Antibiotic	Biofilm producer 24(80%)			Non-biofilm producer 6(20%)			χ^2 test value	*P-value*
	S	I	R	S	I	R		
Ciprofloxacin	14	0	10	4	0	2	0.139	0.709
Co-trimoxazole	22	0	2	6	0	0	0.536	0.464
Ceftriaxone	8	10	6	3	2	1	0.590	0.745
Cefotaxime	2	7	15	2	3	1	4.766	0.092
Amoxicillin/clavulanic acid	0	0	24	1	0	5	4.138	0.042*
Amikacin	19	2	3	6	0	0	1.500	0.472
Cefadroxil	5	8	11	3	1	2	2.149	0.342
Ceftazidime	0	1	23	2	0	4	8.704	0.013*

**P-value*< 0.05 is considered statistically significant

Biofilm producing *E. coli* isolates had increased resistance profiles of the antibiotics amoxicillin/clavulanic acid, cefadroxil, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacinand co-trimoxazole, 100%, 91.7%, 75%, 70.8%, 66.7%, 62.5% and 33.3% respectively with significant statistical correlation of antibiotic resistance of cefadroxil (*P-value* < 0.05)as show in table (3).

Table (3). Antibiogram profiles of biofilm and non-biofilm producing *E. coli*

Antibiotic	Biofilm producer 24(80%)			Non-biofilm producer 6(20%)			χ^2 test value	*P-value*
	S	I	R	S	I	R		
Ciprofloxacin	8	1	15	4	0	2	2.304	0.316
Co-trimoxazole	16	0	8	4	0	2	0.00	0.694
Ceftriaxone	6	2	16	3	1	2	2.222	0.329
Cefotaxime	5	1	18	2	1	3	1.875	0.392
Amoxicillin/clavulanic acid	0	0	24	0	0	6	-	-
Amikacin	18	4	2	4	1	1	0.379	0.827
Cefadroxil	2	0	22	4	0	2	10.208	0.007*
Ceftazidime	5	2	17	2	1	3	0.967	0.617

**P-value*< 0.05 is considered statistically significant

DISCUSSION

In this study, we investigated the ability of *S. aureus* and *E. coli* isolates to form biofilm in vitro by phenotypic TCP method because they can be performed in most laboratories' settings.Bacterial biofilms are most of the time associated with long-term persistence of bacteria in various environmental conditions[15].More than 50% of all microbial infections have now been associated with the biofilm formation, and several bacterial surface structures are known to be involved in biofilm creation[16].

TCP was most reliable and easy method for detection of biofilm and it can be used as a general screening method for detection of bacterial biofilm producing[17,18,19].In contrast, statistical analysis of biofilm formation indicated that TCP method was the most sensitive and specific method for screening biofilm production[20].

In the present study, among all isolates *S. aureus* and *E. coli*TCP method detected biofilm formation 80% with no significant statistics (*P-value* = 1.000). According to these results, similar researches revealed TCP method detected 81%bacterial isolates biofilm producer[21]. Other study found that TCP detected 64% as bacterial biofilm producer[22], whereas another study showed that TCP detected 27% as bacterial biofilm producers[23]. A study revealed that 76% were bacterial biofilm producers detected by TCP method[24]. Other study reported biofilm producer identified by TCP method 22%[25]. Also,several studies showed similar results for the detection of biofilm production[26,27].

Bacterial biofilm display dramatically increased resistance to antibiotics[15]. In this study, we analyzed the antibiotics resistance profiles of biofilm and non-biofilm producing of all isolates *S. aureus* and *E. coli*. Biofilm forming isolates demonstrated increased resistance to the commonly used antibiotics compared to non-biofilm producers.*S. aureus* isolates biofilm producing in our study found highly resistant to amoxicillin/clavulanic acid, ceftazidime, cefotaxime, cefadroxil, ciprofloxacin and ceftriaxone in rate of 100%, 95.8%, 62.5%, 45.8%, 41.7% and 25% respectively with significant statistical correlation of antibiotic resistance of amoxicillin/clavulanic acidand ceftazidime and bacterial biofilm production (*P-value*< 0.05). This profilesof resistance coincides with the study findings reported highly resistant biofilm produced *S. aureus* to co-trimoxazole 66.7% and ciprofloxacin 60%[3]. Other studyshowed resistance to ciprofloxacin and co-trimoxazole 83.3% and 28.6% respectively[28]. Other research reported that resistance toward erythromycin and co-trimoxazole was increased due to the excessive use of these drugs for the treatment of both minor and more serious

staphylococcal infections[3]. Other study found that the Gram-positive bacteria had high resistance to ciprofloxacin40% and co-trimoxazole 30%[17].Our study results revealed that biofilm producing *E. coli* isolates had increased resistance profilesof the antibiotics amoxiclav100%, cefadroxil91.7%, cefotaxime75%, cetazidime70.8%, ceftriaxone66.7%, ciprofloxacin 62.5% and co-trimoxazole33.3% with significant statistical correlation of antibiotic resistance of cefadroxil (*P-value*< 0.05). This profileof resistance agreed with the study findings reported high resistant biofilm producing *E. coli* to amoxicillin/clavulanic acid, ceftriaxone, ciprofloxacinand amikacin 77.61%, 71.48%, 71.48%and 7.58% respectively[29], other study showed biofilm producing *E. coli* were resistance to ceftaxime, ceftriaxone, and amoxicillin/clavulanic acid65.6%, 50% and 40.6%respectively[30]. While other studyshowed less rate resistance biofilm producing *E. coli* to co-trimoxazole, ciprofloxacin andceftaxime47.4%, 47% and42.5%respectively[31]. Gram negative bacteria had high resistance to ciprofloxacin, co-trimoxazole, amikacin and ceftriaxone 95%, 90%, 64% and 58% respectively[17], another study found resistance of biofilm forming *E. coli* isolates to ciprofloxacin and amikacin 95% and 65% respectively[26].The increased antibiotics resistance among bacterial biofilm producers is due to slow growth rate and the presence of the protective covering of exopolysaccharide which alters the penetration of antibiotics through the biofilm and hinders the activity of antibiotics against the bacterial cells[3,31].

CONCLUSION

S. aureus and *E. coli* isolates have high degree of biofilm forming ability detection by TCP method. Highly antibiotics resistance was observed in biofilm producers than non-biofilm producers.Antibioticstherapiesrecommended areamoxicillin/clavulanic acid, cefadroxil, cefotaxime and ceftazidime were the least active antibiotics, whereas co-trimoxazole and amikacin were found as the most effect for *S. aureus* and *E. coli* biofilm producers.

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CONFLICT OF INTEREST

No conflict of interest associated with this work.

AUTHOR'S CONTRIBUTION

The manuscript was prepared, written and approved in collaboration with all authors.

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