Egyptian Journal of Medical Microbiology Volume 26 / No.3 / July 2017 145-152

ORIGINAL ARTICLE

Biofilm Forming Bacteria Isolated from Intrauterine Devices and Their Susceptibility to Antibiotics

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Key words:
Biofilm, Intrauterine Devices, Antibiotic Susceptibility

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ABSTRACT

Background: *A biofilm is a complex aggregation of microorganisms in which cells adhere to a surface and form colonies. Biofilms have been shown to develop on medical device surfaces like IUD. A biofilm has greater resistance to environmental stresses, including antibiotics.* ***Objective****: The aim of our study is isolation of microorganisms formed on the IUDs removed from complaining females attending the Gynecological Clinic of Zagazig University Hospitals, assessment of the ability of the identified bacterial isolates to form biofilm and finally testing the antibiotic susceptibility of the biofilm forming bacteria to different antibiotics in both the biofilm and planktonic forms.* ***Methodology:*** *This study includes 72 females visiting gynecological outpatient clinic in Zagazig university hospitals for removing their intrauterine devices (IUDs). The intrauterine devices were removed with aseptic techniques. All samples were subjected to the following, microscopic examination of direct smears stained with Gram stain,cultivation on the suitable culture media, identification of isolates by API. Assessment of biofilm formation among the bacterial isolates were done by tissue culture plate method. Antibiotic susceptibility testing for planktonic cells and for biofilm forming bacteria were done by broth microdilution method.* ***Results:*** *This study showed that IUD cultures were positive in 70 (97.2%) women and negative in 2 (2.8%) women. Our study showed that the most common bacterial species isolated from 56 bacterial and mixed IUDs culture results were Coagulase negative staphylococci (32.1%), Escherichia coli (25.0%), Pseudomonas aeruginosa (21.4%), Klebsiella spp. (12.5%) and Staphylococcus aureus (9%). In our study, we found that among 56 bacterial isolates, 10 (17.8%) were moderate biofilm forming bacteria, 37 (66.1%) were weak biofilm forming bacteria and 9 (16.1%) were non biofilm forming bacteria. Results of testing antimicrobial susceptibility of biofilm forming bacteria to different antibiotics both in the planktonic and biofilm forms showed that biofilm cells had higher resistance than planktonic cells to these antibiotics.* ***Conclusion:*** *Infection of IUD by biofilm forming bacteria is relatively high. All the biofilm forming bacterial isolates in the biofilm form show higher resistance to all the antibiotics than their planktonic counterparts.*

INTRODUCTION

A biofilm is a complex aggregation of microorganisms in which cells adhere to a surface and form colonies. These cells are protected by an extracellular polysaccharide-rich matrix and are physiologically distinct from single cells of the same organism. Biofilm-associated bacteria replicate at slower rates and have reduced antibiotic susceptibility and are therefore much more difficult to eradicate with conventional antibiotic doses[1].

Biofilms have been shown to develop on medical device surfaces, and dispersal of single and clustered cells implies a significant risk of microbial dissemination within the host and increased risk of infection. Measures such as antimicrobial coating and surface alterations of medical devices provide promising opportunities in the prevention of biofilm formation on medical devices[2].

One of these medical devices is the intrauterine device which provides an extremely effective, long-term form of contraception that has the benefit of being reversible. Historically, the use of certain intrauterine devices was associated with increased risk of pelvic inflammatory disease. More recent evidence suggests that newer devices do not carry the same threat; however, certain risk factors can increase the possibility of infection[3]. Recently, biofilms exhibit high degree of antibiotic resistance, therefore efforts to discover new strategies for treatment of infections related to biofilms[4].

The aim of our study is isolation of microorganisms formed on the IUDs removed from complaining females attending the Gynecological Clinic of Zagazig University Hospitals, assessment of the ability of the identified bacterial isolates to form biofilm and finally testing the antibiotic susceptibility of the biofilm forming bacteria to different antibiotics in both the biofilm and planktonic forms.

METHODOLOGY

Cases:

This research work was carried out in Medical Microbiology & Immunology Department and Obstetrics & Gynecology Department, Faculty of Medicine, Zagazig University, during the period from August 2016 to January 2017.

This study included 72 females visiting Gynecological Outpatient Clinic in Zagazig University Hospitals for removing their intrauterine devices (IUDs). Cases were selected according to pre-set inclusion and exclusion criteria. The inclusion criteria included adult females more than 18 years old, complaining from vaginal discharge, from symptoms suggesting pelvic inflammatory disease (PID), backache or pelvic pain only.

The females were excluded if they were belonging to any of the following categories; females removing IUDs for getting pregnant, samples that were not taken under complete aseptic conditions, patients removing IUDs due to menestrual troubles and females who became pregnant on IUD.

Patients were subjected to full history taking including personal history, symptoms of vaginal discharge, and treatment taken by the patient.

Sample collection:

The intrauterine devices were removed under complete aseptic techniques. The removed IUD was transported in a sterile container to the laboratory of Microbiology and Immunology Department within one hour[5].

All samples were subjected to the following:

- ***Microscopic examination of Gram stained smears.***
- ***Cultivation on the suitable culture media.***

These include nutrient agar (Oxoid, UK), 5% blood agar, MacConkey's agar (Oxoid, UK), and Brain heart infusion (BHI) broth (Oxoid, UK).

Identification of isolates by the following methods:

Colonial morphology, microscopic examination of gram stained films. API 20 and API 20 E Strep system (Bio-Merieux.Marcy L Etoile. France) were done for identification of the isolates according to the manufacturer's instructions.

Assessment of biofilm formation *tissue culture plate method:*

Overnight cultures of tested isolates were adjusted to 0.5 McFarland. 200 µl of the previously prepared suspensions were added to the wells of sterile flat-bottomed 96-well clear polystyrene tissue culture treated microtiter plates. Three wells for each strain were used (strain tested in triplicate) and three wells in every plate were used as negative control (broth only was contained in negative control wells). The plates were covered and incubated for 24 hours at 37°C. The contents of the tissue culture plates were gently removed and the wells were washed three times with sterile phosphate buffered saline (PBS, pH 7.2). The plates were drained in an inverted position. 200 µl of 99% methanol were added to the wells for twenty minutes to fix adherent bacteria. The tissue culture plates were emptied and left to dry in air at room temperature. The wells stained with 200 µl crystal violet (1%) for 15 minutes at room temperature and the unattached stain was washed by sterile distilled water. After air drying of the plates, the attached stain was eluted by aliquots of 150 µl of 95% ethanol. The plate was covered with the lid (to minimize evaporation) and left at room temperature for thirty minutes. Optical density of the films were measured with ELISA reader at 630 nm. The cut-off value (ODc) was detected[6].

Non biofilm producer = OD ≤ODc
Weak biofilm producer = ODc <OD ≤2×ODc,
Moderate biofilm producer = 2×ODc <OD≤4×ODc
Strong biofilm producer = 4×ODc <OD

Antibiotic susceptibility testing for planktonic cells of biofilm forming bacteria *broth microdilution method.*

The used antimicrobial agents against Gram-negative bacteria except *pseudomonas aeruginosa* were cefotaxime and ampicillin-sulbactam. The antimicrobial agents used against Gram-positive bacteria were gentamicin and ciprofloxacin. The antimicrobial agents used against *pseudomonas aeruginosa* were gentamicin and ceftazidime. Antibiotics used were provided as standard powders for antibiotic (El.Nasr Co., Cairo, Egypt). *Dilutions of antibiotics were done according to CLSI (2016)*[7].

The MICs of the tested antimicrobial agents for the biofilm producers (56 isolates) were estimated by the broth microdilution technique consistent with CLSI (2012)[8]. The MIC was defined as the lowest concentration that did not show growth CLSI (2013)[9]. Collected data from the susceptibility tests were interpreted according to CLSI (2016)[7] as follow: The strains which have gentamicin MIC value ≤ 4 µg/ml, ceftazidime MIC value ≤ 8 µg/ml , cefotaxime MIC value ≤ 1 µg/ml ,ciprofloxacine MIC value ≤ 1 µg/ml and ampicillin-sulbactam MIC value ≤ 8/4 µg/ml were accepted as susceptible and which have gentamicin MIC values ≥ 16 µg/ml, ceftazidime MIC value value ≥ 32 µg/ml, cefotaxime MIC value ≥4µg/ml, ciprofloxacin MIC value ≥4µg/ml and ampicillin-sulbactam ≥ 32/16µg/ml were accepted as resistant.

Antibiotic susceptibility testing of biofilm for biofilm forming bacteria:

This was done by turbidimetric method according to Cernohorská and Votava[10]. The tissue culture plates were incubated at 37°C for 20-24 hours, after incubation with antibiotics, the tissue culture plates were rinsed three times by sterile PBS then 100 µl of CAMHB was added to each well and were incubated at 37°C for another 24 hours. After last incubation, minimal biofilm

eradication concentration (MBEC) was determined, corresponds to the lowest concentration of antibiotic that inhibit visible growth in the wells[11]. Collected data from the susceptibility tests were interpreted according to CLSI (2016)[7].

RESULTS

During this study, 72 different IUDs samples were obtained from 72 females visiting gynecological outpatient clinic in Zagazig university hospitals with their ages ranged from 25-50 years. The mean age of these females was (35.23± 6.16).

IUDs were removed due to different IUD-related side effects including: backache in 30 (41.7%) women, symptoms suggesting PID (vaginal discharge +fever +pelvic pain) in 20 (27.8%) women, abnormal vaginal discharge only in 13 (18.1%) women and pelvic pain only in 9 (12.5%) women. (Figure 1)

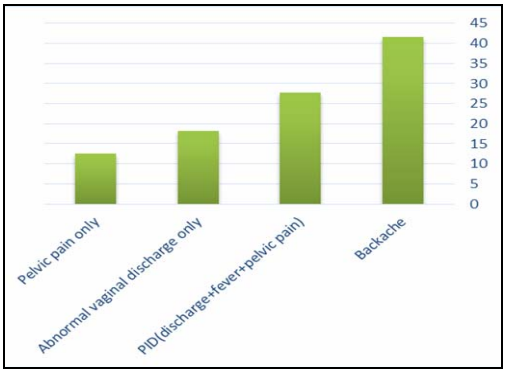

Fig. 1: Percentage of different causes of removal of IUD.

In this study the duration of use of IUD was less than 5 years in 41 (56.9%) women, from 5 to10 years in 24 (33.3%) women and for more than 10 years in 7 (9.7%) women.

Our study showed that IUD cultures were positive in 70 (97.2%) women and negative in 2 (2.8%) women. Types of infection resulting from IUDs culture were bacterial infection in 52 (72.2%) women, fungal (*Candida spp.*) infection in 14 (19.4%) women, mixed bacterial and fungal infection in 4 (5.6%) women (three of these mixed infections were *staphylococcus aureus* and *candida spp.* and one case was *coagulase negative staphylococci* and *candida spp.*). Negative results were obtained in 2 (2.8%) women only. (Table 2)

Table 1: Frequency and percentage of different types of infection resulting from IUDs culture.

Type of infection on IUD	Frequency	Percent %
Bacterial	52	72.2
Fungal (*Candida spp.*)	14	19.4
Mixed bacterial and fungal	4	5.6
Non	2	2.8
Total	72	100.0

Our study showed that there was statistically highly significant difference ($p<0.001$) in the type of infection on IUD in relation to the cause of its removal. The negative IUD cultures were found only in 2 women complaining from abnormal vaginal discharge only with percentage (100%). The mixed bacterial and fungal infection was found only in 4 women complaining from symptoms suggesting PID including vaginal discharge, fever and pelvic pain with percentage (100%). The highest percentage of isolated bacterial or isolated fungal infection was found in women complaining from backache and the lowest percentage of isolated bacterial or fungal infection was found in women complaining from pelvic pain only.

In this study 48 (66.7%) women had history of antimicrobial therapy while 24 (33.3%) women were untreated. Our study showed that there was no statistically significant difference ($P>0.05$) regarding the relation between type of infection found on IUD and history of previous antimicrobial therapy.

In this study there was statistically highly significant difference ($p<0.001$) in the type of infection on IUD in relation to the duration of its use. The negative IUD cultures were found only in 2 women using IUDs less than 5 years (100%), while in women using IUDs either from 5 to 10 years or more than 10 years all IUD cultures were positive. The mixed bacterial and fungal infection was found only in 4 women using IUDs more than 10 years (100%) and not found in women using IUDs either less than 5 years or from 5 to 10 years. In women using IUD less than 5 years, isolated bacterial infection was found in 33 women (63.5%) while the isolated fungal infection was found in 6 women (42.9%). In women using IUD from 5 to 10 years, isolated bacterial. (Table 2)

Table 2: Percentage of type of infection on IUD in relation to duration of use of IUD.

Duration of use of IUD		Type of infection on IUD				Total	X^2	P
		Bacterial	Fungal	Mixed	Non			
<5 years	Count	33	6	0	2	41	46.74	0.00**
	%	63.5%	42.9%	0.0%	100.0%	56.9%		
5-10 years	Count	19	5	0	0	24		
	%	36.5%	35.7%	0.0%	0.0%	33.3%		
>10 years	Count	0	3	4	0	7		
	%	0.0%	21.4%	100.0%	0.0%	9.7%		
Total	Count	52	14	4	2	72		
	%	100.0%	100.0%	100.0%	100.0%	100.0%		

X^2= chi square test *Significant p <0.05 **High significant p <0.001

Our study showed that the most common bacterial species isolated from 56 bacterial and mixed IUDs culture results were *Coagulase negative staphylococci* (32.1%), *Escherichia coli* (25.0%), *Pseudomonas aeruginosa* (21.4%), *Klebsiella spp.* (12.5%) and *Staphylococcus aureus* (9%). (Figure 2)

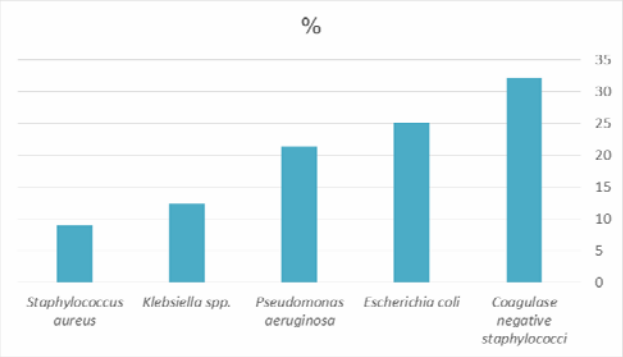

Fig. 2: Frequency of isolated bacterial species from 56 bacterial and mixed IUD cultures.

In our study, we found that among 56 bacterial isolates, 10 (17.8%) were moderate biofilm forming bacteria, 37 (66.1%) were weak biofilm forming bacteria and 9 (16.1%) were non biofilm forming bacteria. The non-biofilm forming bacteria were 3 *klebsiella spp*., 3 *E.coli* and 3 *CoNS* Isolates.(figure 3)

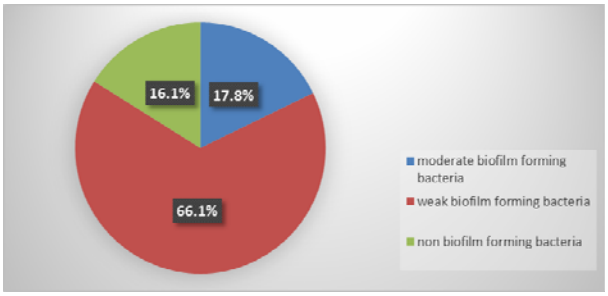

Fig. 3: Percentage of biofilm formation by bacterial isolates

Our study showed that there was statistically highly significant difference ($p<0.001$) in the ability and the grade of biofilm formation by bacterial isolates on IUD in relation to the duration of its use. The highest percentage of non-biofilm forming bacteria were found in women using IUDs less than 5 years (21.2%), while the highest percentage for moderate biofilm forming bacteria were found in women using IUDs more than 10 years (100%) and not found in women using IUDs less than 5 years. Weak biofilm forming bacteria were found in women using IUDs either for less than 5 years or from 5 to 10 years and not found in women using IUDs more than 10 years. (Figure 4)

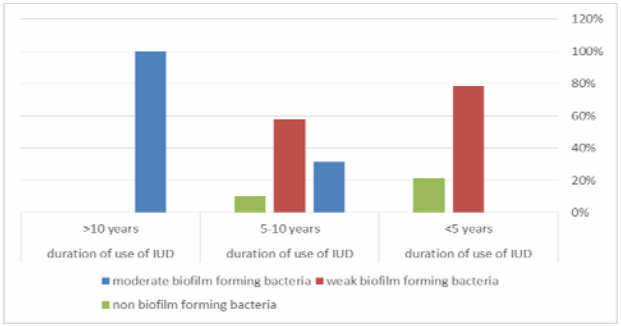

Fig. 4: Percentage of biofilm formation on IUD in relation to duration of its use.

Results of testing antimicrobial susceptibility of biofilm forming *coagulase negative staphylococci* and *staphylococcus aureus* to ciprofloxacin and gentamycin antibiotics both in the planktonic and biofilm forms showed that biofilm cells had higher resistance than planktonic cells to gentamycin and ciprofloxacin antibiotics. For ciprofloxacin minimal biofilm eradication concentration/Minimum inhibitory concentration (MBEC/MIC) (1-512) folds and for gentamycin MBEC/MIC (1-512) folds. Also, testing antimicrobial susceptibility of biofilm forming *pseudomonas aeruginosa* to gentamycin and ceftazidime antibiotics both in planktonic and biofilm forms showed that biofilm cells had higher resistance than planktonic cells to gentamycin and ceftazidime antibiotics. For ceftazidime MBEC/MIC (4-256) folds and for gentamycin MBEC/MIC (2-256) folds.

Our study showed that there was high statistically significant difference ($P<0.001$) between values of MBEC and MIC of biofilm +ve *coagulase negative staphylococci (CoNS)* and *staphylococcus aureus (S.aureus)* to ciprofloxacin antibiotic. Isolates in the biofilm form (MBEC) showed the highest degree of resistance as 15(100%) of *CoNS* and 5 (100%) of *Staph. aureus* isolates showed resistance to ciprofloxacin antibiotic. The planktonic counterpart of these isolates (MIC) showed that 14(93.3%) of *CoNS* were sensitive and 1(6.6%) of *CoNS* was resistant to ciprofloxacin. (table 3)

Table 3: Pattern of ciprofloxacin antibiotic resistance in biofilm forming *coagulase negative staphylococci* and *staphylococcus aureus* isolates in the biofilm and planktonic forms.

			Coagulase negative staphylococci	*Staphylococcus aureus*	X^2	P
Ciprofloxacin MBEC	R	Count	15	5		
		%	100.0%	100.0%		
Ciprofloxacin MIC	R	Count	1	0	0.23	0.62
		%	6.6%	0.0%		
	S	Count	14	5		
		%	93.3%	100.0%		
Total		Count	15	5		
		%	100.0%	100.0%		
P			0.00**	0.00**		

X^2= chi square test **High significant p<0.001
MIC: Minimal inhibitory concentration MBEC: Minimal biofilm eradication concentration

In this study, we found that there was high statistically significant difference between values of MBEC and MIC of biofilm +ve *coagulase negative staphylococci, Staphylococcus aureus & P. aeruginosa* to gentamycin antibiotic. Isolates in the biofilm form (MBEC) showed the highest degree of resistance as 15(100%) of *CoNS*, 5 (100%) of *S.aureus* and 12(100%) of *P. aeruginosa* isolates were resistant to gentamycin antibiotic. The planktonic counterpart of these isolates (MIC) showed that 14(93.3%) of *CoNS* were sensitive and 1(6.6%) of *CoNS* was resistant, 2(16.7%) of *P. aeruginosa* were resistant and 10(83.3%) of *P. aeruginosa* were sensitive, while all planktonic cells of *S.aureus* isolates 5(100%) were sensitive to gentamycin. (table 4)

Table 4: Pattern of gentamycin antibiotic resistance in biofilm forming *coagulase negative staphylococci, staphylococcus aureus* and *pseudomonas aeruginosa* isolates in the biofilm and planktonic forms.

			Coagulase negative staphylococci	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	X^2	P
Gentamycin MBEC	R	Count	15	12	5		
		%	100.0%	100.0%	100.0%		
Gentamycin MIC	R	Count	1	2	0	1.39	0.49
		%	6.6%	16.7%	0.0%		
	S	Count	14	10	5		
		%	93.3%	83.3%	100.0%		
Total		Count	15	12	5		
		%	100.0%	100.0%	100.0%		
P			0.00**	0.00**	0.001**		

X^2= chi square test **High significant p<0.001
MIC: Minimal inhibitory concentration MBEC: Minimal biofilm eradication concentration

Our study represented that there was high statistically significant difference (P<0.001) between values of MBEC and MIC of biofilm +ve *P. aeruginosa* to ceftazidime antibiotic. Isolates in the biofilm form (MBEC) showed the highest degree of resistance as 12(100%) of *P.aeruginosa* isolates were resistant to ceftazidime antibiotic. The planktonic counterpart of these isolates (MIC) showed that 2(16.7%) of *P.aeruginosa* were resistant and 10(83.3%) of *P.aeruginosa* were sensitive to ceftazidime. (table 5)

Table 5: Pattern of ceftazidime antibiotic resistance in biofilm forming *Pseudomonas aeruginosa* isolates in the biofilm and planktonic forms.

			Pseudomonas aeruginosa
Ceftazidime MBEC	R	Count	12
		%	100.0%
Ceftazidime MIC	R	Count	2
		%	16.7%
	S	Count	10
		%	83.3%
Total		Count	12
		%	100.0%
P			0.00**

**High significant p<0.001 MIC: Minimal inhibitory concentration
MBEC: Minimal biofilm eradication concentration

Results of testing antimicrobial susceptibility of biofilm forming *E. coli* and *klebsiella spp*. to cefotaxime and ampicillin/sulbactam antibiotics both in the planktonic and biofilm forms showed that biofilm cells showed higher resistance than planktonic cells to cefotaxime and ampicillin/sulbactam antibiotics. For cefotaxime MBEC/MIC (8-512) folds and for ampicillin/sulbactam MBEC/MIC (8-256) folds.

This table shows that there is statistically significant difference between values of MBEC and MIC of biofilm +ve *E.coli* and *klebsiella spp.* to cefotaxime antibiotic. Isolates in the biofilm form (MBEC) showed the highest degree of resistance as 11(100%) of *E.coli* and 4 (100%) of *Klebsiella* isolates were resistant to cefotaxime antibiotic. The planktonic counterpart of these isolates (MIC) showed that 9(81.8%) of *E.coli* were sensitive and 2(18.2%) of *E.coli* were resistant, while 3(75%) of *Klebsiella* were sensitive and 1(25%) of *Klebsiella* was resistant to cefotaxime. (Table 6)

Table 6: Pattern of cefotaxime antibiotic resistance in biofilm forming *E. coli* and *Klebsiella spp.* isolates in the biofilm and planktonic forms.

			Escherichia coli	*Klebsiella spp.*	X^2	P
Cefotaxime MBEC	R	Count	11	4		
		%	100.0%	100.0%		
Cefotaxime MIC	R	Count	2	1	0.76	0.38
		%	18.2%	25%		
	S	Count	9	3		
		%	81.8%	75%		
Total		Count	11	4		
		%	100.0%	100.0%		
P			0.00**	0.02*		

X^2= chi square test *Significant p <0.05 **High significant p <0.001
MIC: Minimal inhibitory concentration
MBEC: Minimal biofilm eradication concentration

Our study showed that there was statistically significant difference between values of MBEC and MIC of biofilm +ve *Escherichia coli* and *klebsiella spp.* to ampicillin/sulbactam antibiotic. Isolates in the biofilm form (MBEC) showed the highest degree of resistance as 11(100%) of *E.coli* and 4 (100%) of *Klebsiella* isolates were resistant to ampicillin/sulbactam antibiotic. The planktonic counterpart of these isolates (MIC) showed that 9(81.8%) of *E.coli* were sensitive and 2(18.2%) of *E.coli* were resistant, while 3(75%) of *Klebsiella* were sensitive and 1(25%)of *klebsiella* was resistant to ampicillin/sulbactam. (Table7)

Table 7: Pattern of ampicillin/sulbactam antibiotic resistance in biofilm forming *E. coli* and *Klebsiella spp.* isolates in the biofilm and planktonic forms.

			Escherichia coli	*Klebsiella spp.*	X^2	P
Ampicillin/sulbactam MBEC	R	Count	11	4		
		%	100.0%	100.0%		
Ampicillin/sulbactam MIC	R	Count	2	1	0.65	0.41
		%	18.2%	25%		
	S	Count	9	3		
		%	81.8%	75%		
Total		Count	11	4		
		%	100.0%	100.0%		
P			0.00**	0.02*		

X^2= chi square test *Significant p <0.05 **High significant p <0.001
MIC: Minimal inhibitory concentration MBEC: Minimal biofilm eradication concentration

DISCUSSION

Biofilms are surface-attached groups of microbial cells encased in an extracellular matrix that are significantly less susceptible to antimicrobial agents than non-adherent, planktonic cells. Biofilm-based infections are, as a result, extremely difficult to cure. This is caused by several mechanisms. Alone, each of these mechanisms partially increases the antimicrobial resistance in biofilms. But acting in concert, these defenses help to ensure the survival of biofilm cells in the face of even the most aggressive antimicrobial treatment regimens [12].

There is a strong correspondence between biofilm construction and medical indwelling devices. One of these devices are IUDs which are widely used as contraception method worldwide. It is well known that the development of biofilms on these devises is due to immigration of the microorganisms to the uterus from vagina. Vagina contains large number of fungi and bacteria[13].

The immigration of these microorganisms to the uterus usually leads to infection and discomfort. Clinical observations and researches showed obviously that treatment with antibiotics alone is frequently inadequate to treat these infections[14].

In this work, 72 IUDs were removed from 72 women. By history taking, duration of use of these IUDs was estimated to be less than 5 years in 41 (56.9%) women, from 5 to 10 years in 24 (33.3%) women and more than 10 years in 7 (9.7%) women. This is almost in agreement with Abdel-Hafeez *et al.* who reported that the duration of use of IUDs was less than 5 years in 25 (50%) women, from 5 to10 years in 20 (40%) women and for more than 10 years in 5 (10%) women.

In this study, out of 72 IUDs obtained from 72 complaining women, IUDs cultures were positive in 70 (97.2%) cases and negative in 2 (2.8%) cases. This is almost in agreement with another Egyptian study that reported IUDs positive cultures in 48 (96%) of cases and negative cultures in 2 (4%) of cases[15]. However, Al-Kattan *et al.* (2013) reported positive cultures in (84%) IUDs only and negative cultures in (16%) of cases[3]. This high incidence of microbial infection on IUDs can be attributed to the thread attached to the tail of the IUD which may be one of the routes of microbial migration from the vagina to the uterus[16].

The current study showed that the most common isolated bacteria from cultures of the removed IUDs in the included females was coagulase negative staphylococci in 18 (32.1%) of cases followed by E-coli in 14 (25%) of cases then pseudomonas aeruginosa in 12 (21.4%) of cases then klebsiella species in 7 (12.5%) of cases and finally staphylococcus aureus in 5 (9%) of cases. These findings are almost in agreement with Abdelhafeez et al. who reported that the most common isolated microorganisms from IUD culters were coagulase-negative Staphylococci in 16 (32%) of cases and klebsiella spp. was found in 9(18%) of cases[15].

In our study, out of 56 bacterial strains isolated from IUDs positive cultures, there were 47 (83.6%) bacterial isolates that formed biofilm, while 9 (16.1%) bacterial isolates were non biofilm forming. This result is in consistence with Al-Kattan *et al.* who detected biofilm forming isolates in (84%) of cases while non biofilm forming isolates were detected in (16%) of cases[3].

In our study, there was statistically highly significant relationship ($p<0.001$) between the ability and the grade of biofilm formation by bacterial isolates on IUD and the duration of its use. The percentage of women who showed positive biofilm formation on the IUD rose significantly with the duration of its use. Similar results were obtained by Abdel Hafeez et al.[15]. This results can be explained by the fact that microorganisms should attach to the device long time to produce irreversible attachment[17].

We have studied the antibiotic susceptibility of biofilm forming Gram positive bacterial isolates to ciprofloxacin and gentamycin by broth micro dilution method for their planktonic cells and biofilm cells. The results showed that biofilm cells of biofilm forming *coagulase negative staphylococci* and *staphylococcus aureus* demonstrated higher resistance than planktonic cells to gentamycin and ciprofloxacin antibiotics. For ciprofloxacin MBEC/MIC (1-512) folds and for gentamycin MBEC/MIC (1-512) folds.

There was high statistically significant difference ($P<0.001$) between values of MBEC and MIC of biofilm +ve *CoNS* and *Staph. aureus* to ciprofloxacin and gentamycin antibiotic. These results are almost in agreement with Antunes *et al.*[18].

Testing the antibiotic susceptibility of biofilm forming *P. aeruginosa* isolates to ceftazidime and gentamycin by broth micro dilution method for their planktonic cells and biofilm cells revealed that biofilm cells of biofilm forming *P. aeruginosa* had higher resistance than planktonic cells to gentamycin and ceftazidime antibiotics. For ceftazidime MBEC/MIC (4-256) folds and for gentamycin MBEC/MIC (2-256) folds.

There was high statistically significant difference ($P<0.001$) between values of MBEC and MIC of biofilm +ve *P. aeruginosa* to gentamycin and ceftazidime antibiotics. These results are almost in agreement with Perez *et al.*[19].

In this work, we also studied the antibiotic susceptibility of biofilm forming Gram negative bacterial isolates other than *P. aeruginosa (E.coli and klebsiella spp.)* to cefotaxime and ampicillin/sulbactam antibiotics by broth micro dilution method for their planktonic cells and biofilm cells. The obtained results revealed that biofilm cells of biofilm forming

Escherichia coli and klebsiella spp. demonstrated higher resistance than planktonic cells to cefotaxime and ampicillin/sulbactam antibiotics. For cefotaxime MBEC/MIC (8-512) folds and for ampicillin/sulbactam MBEC/MIC (8-256) folds.

There was high statistically significant difference ($P<0.001$) between values of MBEC and MIC of biofilm +ve Escherichia coli and klebsiella spp. to cefotaxime and ampicillin/sulbactam antibiotics. Similar results also were achieved by Naves et al.[20].

So, most recent studies showed that in general, bacterial biofilms show more resistance to a lot of antimicrobials and the mechanisms underlying this resistance are likely multifactorial. Some of these mechanisms are failure of antibiotic penetration of the biofilm, the slow growth rate, altered metabolism, role of persister cells and other molecular mechanisms[21].

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