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ARTICLE

Comparison between Antimicrobial Minimal Biofilm Eradication Concentration and Minimal Inhibitory Concentration in Clinical Isolates in Device Related Infections

[1]*Shereen Fawzy*, [1]*Walaa Abdel-Latif**, [2]*Rasha Gamal*

[1]Microbiology & Immunology Department, Faculty of Medicine - Ain Shams University, Egypt

[2]Microbiology & Immunology Department, Abbassia Chest Hospital, MOH, Egypt

Key words:

Biofilm, Device Associated Health Care Associated Infections (DA-HAI), Minimal Biofilm Eradication Concentration (MBEC), Minimal Inhibitory Concentration (MIC).

ABSTRACT

Background: *Biofilms have been defined as complex microbial assemblages anchored to abiotic or biotic surfaces. This microbial assemblage may harbor single or multiple microbial populations or micro colonies. Biofilms play a pivotal role in device related infections (DRIs). Formation of biofilm on medical devices is a growing problem.* ***Objectives:*** *we aimed to compare between the minimal biofilm eradication concentration (MBEC) of sessile cells of biofilm forming isolates and minimal inhibitory concentration (MIC) of their planktonic counterpart in device related infections.* ***Methodology:*** *This study was conducted on 90 patients divided into three groups according to device associated health care associated infections (DA-HAI). Group I included 30 patients with central line-associated blood stream infection (CLABSI), group II included 30 patients with catheter associated urinary tract infection (CAUTI), and group III included 30 patients with ventilator associated pneumonia (VAP). Detection of biofilm formation was done using tissue culture plate and antibiotic susceptibility of biofilm forming bacterial isolates was done by disc diffusion method. MIC and MBEC were done only for biofilm forming isolates.* ***Results:*** *Out of 79\90 bacterial isolates, 23 isolates (29%) were biofilm forming. There was statistically significant difference between MIC and MBEC of vancomycin and levofloxacin tested for gram positive biofilm forming bacterial isolates ($p<0.05$) and between MIC and MBEC of imipenem and levofloxacin. For both gram positive cocci and gram negative bacilli; isolates which were sensitive or intermediately sensitive in the MIC values showed resistance in their MBEC values.* ***Conclusion:*** *The difference between MBEC and MIC was statistically highly significant. Thus, it is recommended to detect MBEC rather than MIC to antimicrobials for treatment of DRIs.*

INTRODUCTION

Biofilms are the colonial way of life of microorganisms. Although bacteria frequently live as unicellular organisms, many spend at least part of their lives in complex communities, and some have adopted truly multicellular life styles and have abandoned unicellular growth[1]. They have been defined as complex microbial assemblages anchored to abiotic or biotic surfaces. This microbial assemblage may harbor single or multiple microbial populations or micro colonies. [2]. Biofilms typically cause chronic infections, which means that the infections persist despite apparently adequate antibiotic therapy and the host's innate and adaptive defense mechanisms [3].

****Corresponding Author:***
Walaa Abdel-Latif
Microbiology & Immunology Department. Faculty of Medicine, Ain Shams University, Egypt.
E-mail: loulla_latif@yahoo.com; Tel.: 0100622383

Consequently, biofilm-related infections are inherently challenging to treat and difficult to fully eradicate with normal treatment regimens[4]**.** Owing to frequent failure of treatment of these infections, based on conventional antimicrobial susceptibility testing, it has become more complex and difficult to treat such infections. In general, more than 100 times the antimicrobial concentrations are needed to kill biofilm-forming bacteria than to kill planktonic bacteria[5]**.** Bacteria commonly isolated from these devices include gram-positive *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus viridans*; and the gram-negative *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis,* and *Pseudomonas aeruginosa* [6]**.**

Minimal Inhibitory Concentration (MIC) has long been the standard for antibiotic susceptibility testing. The MIC measures the actions of antibiotics against planktonic organisms and serves as an important

reference in the treatment of many acute infections. Application of MICs in the treatment of chronic or device-related infections involving bacterial biofilms is often ineffective the innate tolerance of microbial biofilms to antibiotic therapy has led to problems in their eradication and in the management of patients with device-related infections. This resistance is lost once the biofilm is reverted to conditions that permit planktonic growth. This difference in antibiotic susceptibility between planktonic and biolfilm populations of the same organism may result from differences in the diffusion of antibiotics or much more complex changes in the microbial physiology of the biofilm[7].

Aim of the work: To compare the Minimal Biofilm Eradication Concentration (MBEC) of sessile cells of biofilm forming isolates with Minimal Inhibitory concentration (MIC) of their planktonic counterpart in device related infections.

METHODOLOGY

This study was conducted on 90 patients admitted to the intensive care unit (ICU) Abbassia Chest Hospital from November 2012 to June 2014. They were classified into three groups of DA-HAI: *Group I*: Thirty patients with central venous catheters (CVC) had at least one of the following sings & symptoms; fever (temperature ≥ 38 ◦C), chills, hypotension or erythyma at the site of catheter insertion as a sign of CLABSI. *Group II*: Thirty patients with indwelling urinary catheter had at least one of the following signs and symptoms; fever (>38°C), suprapubic tenderness, costovertebral angle pain or tenderness. *Group III*: Thirty mechanically ventilated patients with signs of VAP including; a chest radiograph showing new or progressive infiltrates, consolidation, cavitation, or pleural effusion plus at least two of the following criteria: fever(>38°C), purulent secretions, leukocytosis or leucopenia. A specimen obtained by endotracheal aspirate (ETA) or broncho-alveolar lavage (BAL).

Each group of patients was subjected to:

1. **Culture Methods**:

Group I: Catheter tip was cultured semi-quantitatively & concomitant peripheral blood sample was drawn through a peripheral vein and inoculated into conventional blood culture. Central line-associated blood stream infection (CLABSI) is defined as >15 CFU were grown from the catheter tip which is same organism as grown from peripheral blood [8]. ***Group II***: Urine samples were cultured semi-quantitatively, count more than 10^5 was considered significant. ***Group III***: Endotracheal aspirate (ETA) or broncho-alveolar lavage (BAL) was cultured semi-quantitatively[10]. All isolated organisms were identified by standard bacteriological techniques [9,11].

2. **Detection of Biofilm Formation [12]:**

a. **Inoculum preparation:**
The strain is transferred from the glycerol stock culture onto appropriate non inhibitory medium tryptone soya agar and incubated overnight aerobically at 35˚C–37˚C, three to four well-isolated identical colonies were suspended in broth and incubated without shaking for 18 h. After incubation, the stationary-phase culture was vortexed and the turbidity of the bacterial suspension was adjusted to obtain turbidity optically comparable to that of the 0.5 McFarland standard (~10^8 CFU/ml) then it was diluted 1:100 .and inoculated into a microtiter plate (MTP) (200 µL per well). Each test was carried out in triplicate (three wells per strain), six wells were used for the negative control. Negative control wells contain broth only: 200 ml of tryptic soy broth supplemented with 1% glucose per well. The inoculated plate was covered with a lid and incubated aerobically for 24 h at 37°C under static conditions.

b. **Washing:** After incubation, the contents of the wells were decanted into a discard container. Each well was washed three times with 300 µL of sterile saline and then plates were drained in an inverted position.

c. **Fixation:** After washing, the remaining attached bacteria were heat-fixed by using a Wise Stir® MSH-20D digital hot plate stirrer exposing them to heat at 60 °C for 60 min [13].

d. **Staining:** The adherent biofilm layer formed in each MTP well was stained with 150 µL of 2% Hucker crystal violet for 15 min at room temperature. After staining, the stain was aspirated with a pipette and excess stain was rinsed off by placing the MTP under running tap water. Crystal violet stains bacterial cells but not the slimy material. After the MTP was air dried at room temperature, the dye bound to the cells was resolubilized with 150 µL of 95% ethanol per well. Ethanol was gently added ,the addition of ethanol enables indirect measurement of bacteria attached both to the bottom and walls of the wells and thereafter the MTP covered with the lid (to minimize evaporation) and left at room temperature for 30 min without shaking [1].

e. **Interpretation of the results:**
The optical density (OD) of each well stained with crystal violet was measured at 620 nm using a MTP reader. The average OD values were calculated for all tested strains and negative controls, the cut-off value (ODc) was established. It is defined as three standard deviations (SD) above that of uninoculated medium (negative control): ODc=average OD of negative control + (3×SD of negative control).Final OD value of a tested strain was expressed as

average OD value of the strain reduced by ODc value (OD= average OD of a strain -ODc). ODc value was calculated for each MTP separately. When a negative value was obtained, it was presented as zero, while any positive value indicated biofilm production. For easier interpretation of the results, strains were divided into the following categories as shown in figure 1: Non biofilm producer (0) OD ≤ODc, Weak biofilm producer (+ or 1) = ODc <OD ≤2×ODc, Moderate biofilm producer (++ or 2) = 2×ODc <OD≤4×ODc and strong biofilm producer (+++or 3) = 4×ODc <OD [12] .

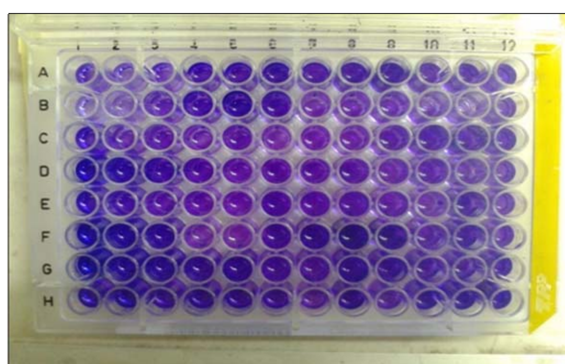

Fig. 1: MTP showing different degrees of biofilm formation.

3. **Antibiotic susceptibility testing for planktonic cells:**

a. ***Disc diffusion method:*** Antibiotic susceptibility testing was performed using susceptibility test disks (Becton Dickinson, Germany), and interpretation was done according to 2013 CLSI guidelines. Susceptibility testing was performed on Mueller–Hinton agar (bioMe´rieux, France), using overnight cultures at a 0.5 McFarland standard followed by incubation at 35 _C for 16–18 h [14].

b. ***Minimal inhibitory concentration (MIC):*** Done only for biofilm forming isolates (figure 2).

Fig. 2: MIC test using Imipenem antibiotic

Antibiotics used:

The antibiotics were selected as follows: levofloxacin and vancomycin for gram positive cocci and amikacin and imipenem for gram negative cocci. MIC was determined by broth microdilution using 96 wells MTP and results were interpreted as follows [14] : Antibiotic solution was prepared following the manufacturer's guidelines. Bacterial suspension was prepared from an overnight culture and diluted in broth to turbidity comparable to that of a 0.5 McFarland turbidity standard, this suspension was further diluted 1:100 (~10^6 CFU/mL) with broth. Two hundred µl of broth was pipetted in the sterility control well (column 12) and 100 µl in the growth control well (column 11). For each bacterial isolate tested, 100 µl of each antibiotic dilution was added into the respective well. Each well containing the antibiotic solution and the growth control well was inoculated with 100 µl of the bacterial suspension. This resulted in the final desired inoculum of 5 ×10^5 CFU /ml. All assay tubes were incubated overnight at 37°C The lowest concentration of the antimicrobial agent that inhibited the growth of the microorganism being tested as detected by lack of visual turbidity, matching with a negative control included with the test, was known as MIC.

Table 1: Antibiotics used for MIC, interpretive standards and concentrations used

Antibiotics	*MIC Resistant (µg/ml) or more*	*Interpretive Intermediate (µg/ml)*	*Standard Sensitive (µg/ml) or less*	*Concentrations used (µg/ml)*
Amikacin	64	32	16	512 - 1
Imipenem				
Enterobacteriaceae	4	2	1	128 -0,25
Pseudomonas	16	8	4	
Levofloxacin	>8	4	<2	64 – 0.125
Vancomycin				
Staph.aureus	16	4-8	2	256 - 0.5
CoNS, Enterococci	32	8-16	4	

C-Antibiotic susceptibility of biofilm (MBEC):

For the biofilm forming isolates antibiotic susceptibility of sessile cells were tested and compared to the MIC of their planktonic counterpart as follows[15]: One hundred µl of the standardized inoculum, as described in biofilm formation, were added to each well of a 96-well MTP and incubated at 37˚C for 24 h. The medium was then discarded; the wells were washed

with saline. One hundred µl of the antibiotics at two-fold dilutions was added to the established biofilms. Following overnight incubation, wells were then washed with saline and filled with 100µl of broth. The viability of the biofilm was determined after 24 h of incubation at 37˚C visually through turbidity of broth. The minimum biofilm eradication concentration (MBEC) was read as the minimum antibiotic concentration at which bacteria failed to re-grow. Sterility controls and antibiotic-free controls were included in all experiments[15] (Figure 3) .

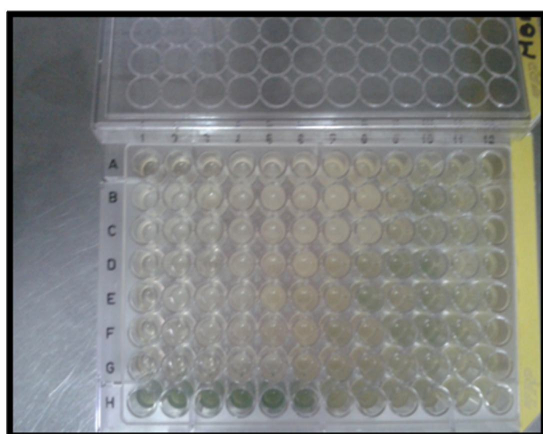

Fig. 3: MBEC test for Imipenem antibiotic.

4. **Statistical methods:** Data were coded and entered using the statistical package SPSS version 15. Data were summarized using descriptive statistics: number and percentage for qualitative values. Statistical differences between independent groups were tested using the Chi Square test for qualitative variables while dependent group comparisons were done using Cochrane and MacNemar tests.

RESULTS

As regards, the yield of growth of the culture of samples from a total 90 patients it was found that: Samples of 10/90 (11%) patients didn't yield any growth [5 in group I, 2 in group II, 3 in group III]. Samples of 6/90 (6.7%) patients have yielded bacterial growth of insignificant count [3 in group I, 2 in group II, 1 in group III]. *Candida spp.* were isolated from samples of 6/90 (6.7%) patients [4 in group II, 2 in group III]. Bacterial isolates of significant count were recovered from 68/90 (75.6%) patients. Out of these 68 patients, 11(16%) patients had mixed bacterial infection (two bacterial isolates were recovered from their samples), while the other 57(84%) patients, only one isolate was recovered from each sample. 79 bacterial isolates were recovered from 68 patients; the most frequently isolated bacteria was *Klebsiella spp.* 18/79(22.8%) then *Staphylococci spp.* 16/79 (20.3%) [*S.aureus* 8/79 (10%) and *CoNs* 8/79 (10%)], *Pseudomonas spp.*15/79 (19%), *Acinetobacter spp.* 11/79 (14%), *E.coli* 6/79 (7.6%), *Enterobacter spp*. 5/79 (6.3%), *Proteus spp*. 4/79(5%) and lastly *Enterococci spp.* and *Citrobacter spp.* 2/79 (2.5%) for each as shown in table 2.

Table 2: Distribution of Bacterial isolates in each of the three groups:

Bacterial isolates	***Group I (N= 23)*** *N (%)*	***Group II (N= 27)*** *N (%)*	***Group III (N= 29)*** *N (%)*	***Total isolates (N= 79)*** *N (%)*
S.aureus	5 (22%)	3 (11%)	0 (0%)	8 (10%)
CoNs	4 (17%)	2 (7%)	2 (7%)	8 (10%)
Enterococci spp.	0 (0%)	2 (7%)	0 (0%)	2 (3%)
Klebsiella spp.	7 (30%)	6 (22%)	5 (17%)	18 (23%)
E.coli	0 (0%)	6 (22%)	0 (0%)	6 (8%)
Citrobacter spp.	0 (0%)	0 (0%)	2 (7%)	2 (3%)
Enterobacter spp.	2 (9%)	1 (4%)	2 (7%)	5 (6%)
Proteus spp.	0 (0%)	1 (4%)	3 (10%)	4 (5%)
Pseudomonas spp.	2 (9%)	4 (15%)	9 (31%)	15 (19%)
Acinetobacter spp.	3 (13%)	2 (7%)	6 (21%)	11 (14%)

Table 2 shows the distribution of bacterial isolates regarding each group of patients as follows: In group I, *Staphylococci spp*. (39.1%) [*S.aureus* (21.7%), *CoNs* (17.4%)] were the most common isolated bacteria in CLABSI. In group II, *Klebsiella spp.* and *E.coli* were isolated equally and more frequently (22%) in CAUTI followed by *Staphylococci spp.* (18%) [*S.aureus* (11%), *CoNs (*7%)], *Pseudomaonas spp.* (15%). In group III , *Pseudomonas* spp. (31%) was the most common in VAP followed by *Acinetobacetr spp.* (21%).

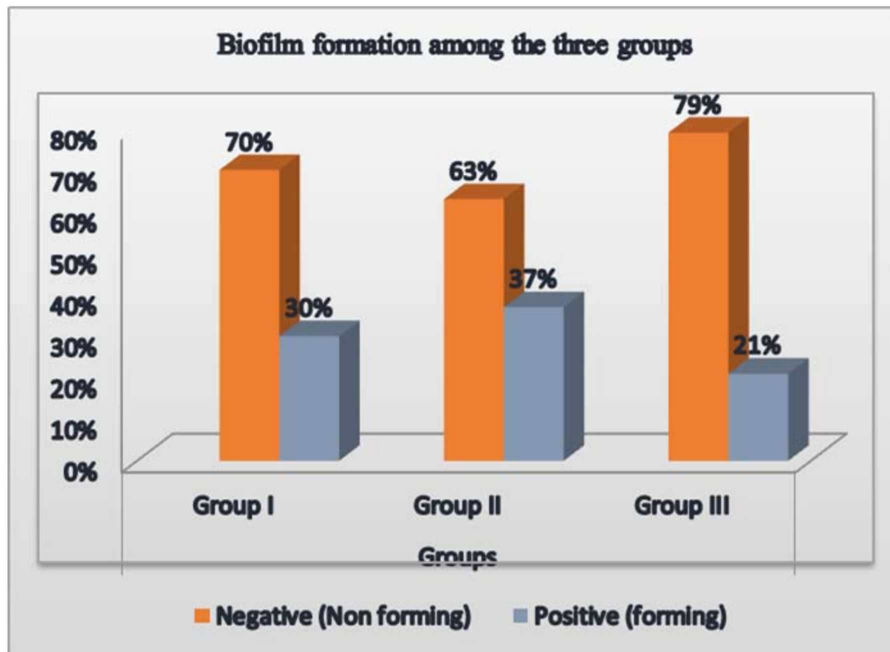

Figure 4: Biofilm forming & non-forming isolates among the three groups.

Biofilm testing for Bacterial isolates: Out of 79 bacterial isolates, 23 isolates (29%) were biofilm forming and 56 isolates (71%) were non biofilm forming. Among group I ,7/23 (30%) were biofilm forming, among group II, 10/27(37%) were biofilm forming and among group III, 6/29 (21%) biofilm forming. There were statistically insignificant differences between the 3 groups regarding biofilm testing; *p*-value (0.05) as shown in figure 4.

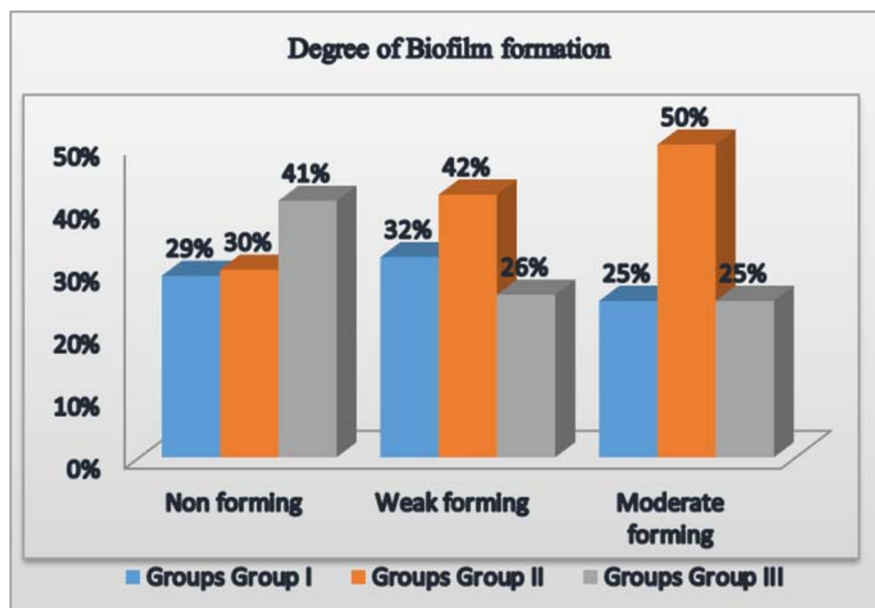

Figure 5: Difference in degree of biofilm formation between the three groups.

As regards the degree of biofilm formation; there were statistically insignificant differences between the three groups; *p*-value (>0.05) figure 5.

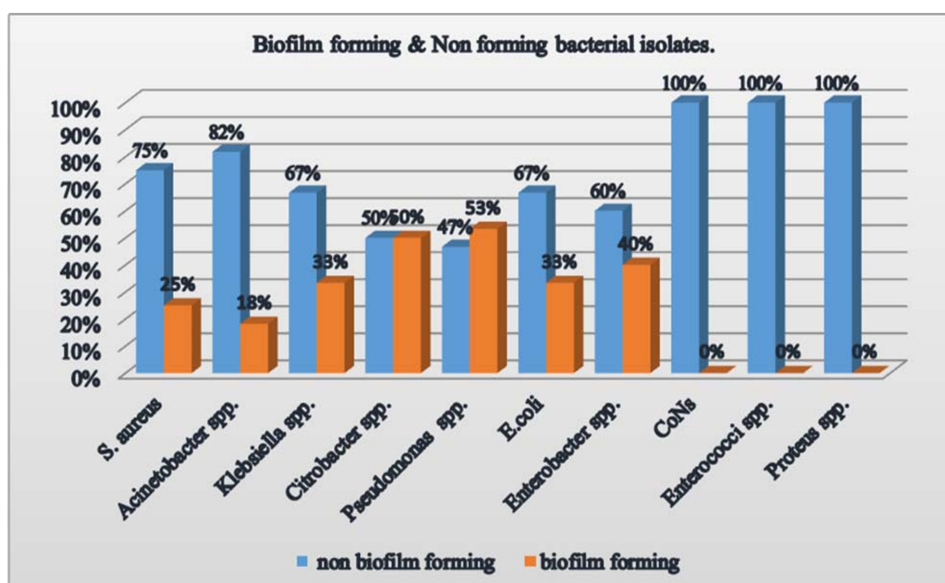

Fig.e 6: Biofilm formation among different bacterial isolates.

Figure 6 shows that there were no statistical significant difference was found between different bacterial isolates regarding the tendency for biofilm formation; *p*-value (>0.05). The bacteria with the highest tendency to form biofilm was *pseudomonas* spp as 53% of isolates were biofilm producers.

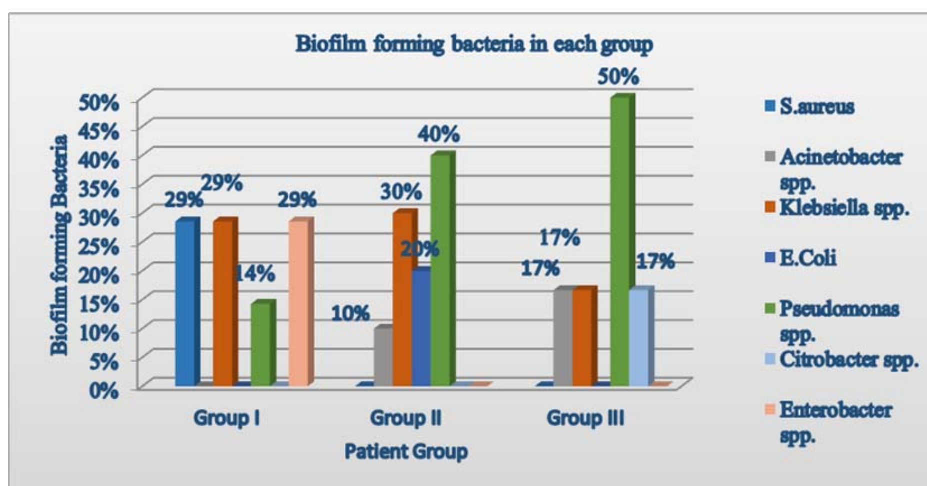

Figure 7: Difference in biofilm forming bacterial isolates among the three groups.

Regarding the distribution of biofilm forming bacterial isolates among the 3 groups; biofilm formation in group I was equal for *S.aureus*, *Klebsiella spp.* And *Enterobacter spp.* (29% for each) then *Pseudomonas spp.* (14%). Meanwhile, in group II, the most frequent biofilm forming bacterial isolate was *Pseudomonas spp.* (40%) followed by *Klebseilla spp.* (30%*),* then *E-coli* (20%) and *Acinetobacter spp.* (10%).In group III, the most frequent biofilm forming bacterial isolate was *Pseudomonas spp.* (50%) followed by, *Klebseilla spp.*, *Acinetobacter spp.* and *Citrobacter* spp. were equally biofilm forming (17% for each) (Figure 7) .

Antibiotic susceptibility testing: Regarding disc diffusion method; results for antimicrobial susceptibility testing were as follows; gram positive biofilm forming bacterial isolates showed highest susceptibility to vancomycin, levofloxacin, ampicillin/sulbactam, cefatriaxone and mefoxin, and least susceptibility to oxacillin, penicillin, erythromycin and ampicillinas. Whereas, gram negative bacterial isolates had the highest susceptibility to amikacin (81%), imipenem (76%) and gentamycin (52%), while being least susceptible to cefotaxime (5%), cefepime (5%) and ceftriaxone (10%) as shown in figure 8 and 9.

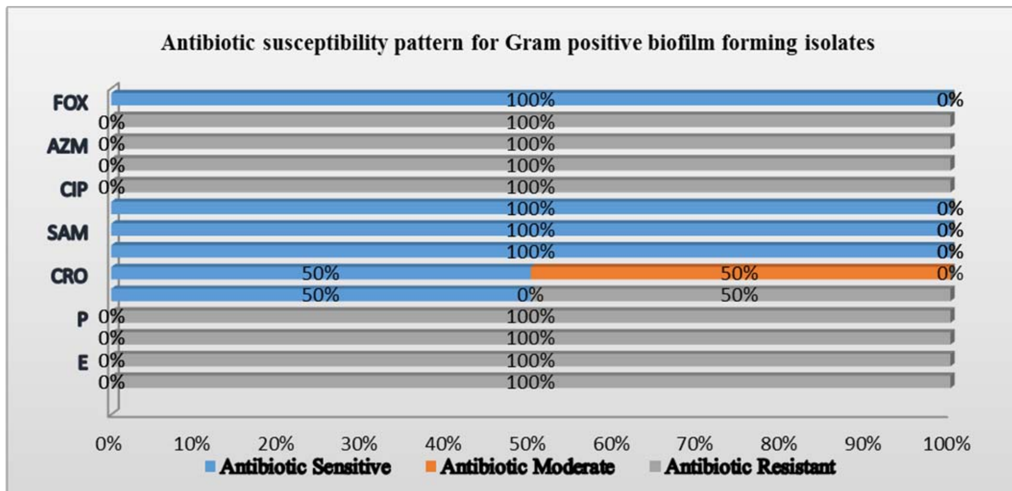

Fig. 8: Pattern of antibiotics susceptibility of Gram positive biofilm forming bacterial isolates. E=erythromycin, P=penicillin, CRO=ceftriaxone, SAM= ampicillin/sulbactam, CIP=ciprofloxacin, AZM=azithromycin, FOX=mefoxin

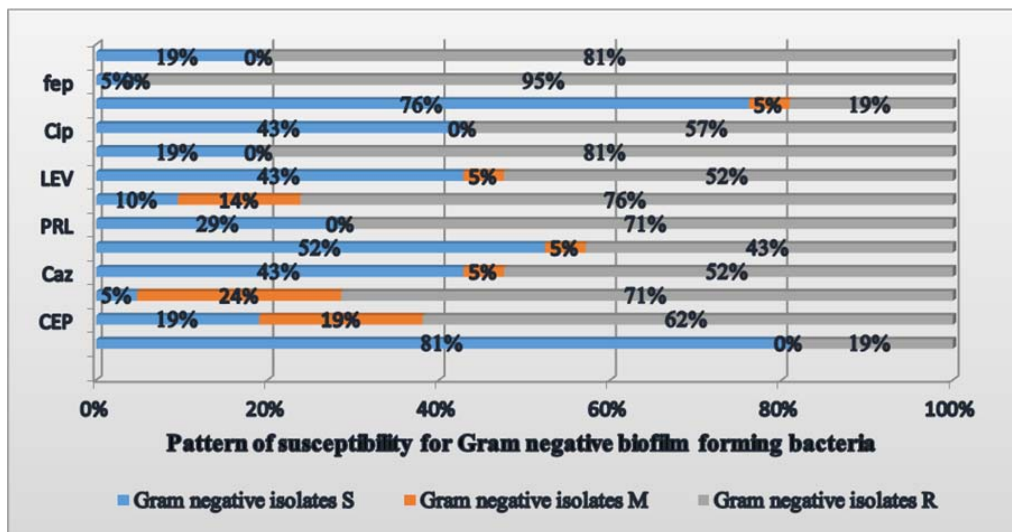

Fig. 9: Antibiotic susceptibility pattern for gram negative biofilm forming bacterial isolates. CEP=cefoperazone, Caz=ceftazidime, PRL=piperacillin, LEV=levofloxacin, Cip=ciprofloxacin, fep= cefepime.

There were statistically significant differences between MIC and MBEC of vancomycin and levofloxacin tested for Gram positive biofilm forming bacterial isolates ($p>0.05$). Isolates that were sensitive or intermediately sensitive in the MIC showed resistance in their MBEC values. There were a highly significant differences between MIC and MBEC of imipenem and amikacin tested for gram negative biofilm forming bacterial isolates ($p<0.01$); as for imipenem isolates that were sensitive or intermediately sensitive in their MIC values showed resistance in their MBEC value and for amikacin isolates that were sensitive or intermediately sensitive in their MIC value showed resistance in their MBEC values except for three isolates as two of them remained sensitive, while the third showed intermediate sensitivity as shown in table 3.

Table (3): Difference between MIC and MBEC of biofilm forming isolates:

Test Result	***MIC***			***MBEC***			$\chi 2$	**P-*value***
	S	**M**	**R**	**S**	**M**	**R**		
Levofloxacin	2(100%)	0	0	0	0	2(100%)	4.000	0.046(S)
Vancomycin	2(100%)	0	0	0	0	2(100%)	4.000	0.046(S)
Amikacin	17(81%)	0	4(19%)	2(10%)	1(4%)	18(86%)	21.751	<0.001(HS)
Imipenem	16(76%)	1(4%)	4(19%)	0	0	21(100%)	28.560	<0.001(HS)

S=sensitive *M=intermediate* *R=resistant* *HS=highly significant*

DISCUSSION

DA-HAIs in the ICUs in Egypt pose greater threats to patient safety than in industrialized countries, and infection control programs, including surveillance and guidelines, must become a priority [16]. Among CVC (group I) the most common causative organism of CLABSI was *Staphylococci spp.* (39.1%): [*Staphylococcus aureus* (21.7%), *Coagulase negative Staphylococci* (*CoNs*) (17.4%)] followed by, *Klebsiella spp.* (30.4%), *Acinetobacter spp.* (13%), and the least causative pathogens were *Pseudomonas spp.* and *Enterobacter spp.* (8.7% each). These results agreed with the results of *Martínez et al.* [17] who found that the micro-organisms identified in central venous catheters were: *CoNs*, 46.8%; *Pseudomonas aeruginosa*, 20.7%; *Candida spp*., 8.1%; *Enterobacteriaceae*, 13.5%; *Enterococcus spp.,* 3.6%; *Staphylococcus aureus*, 3.6%; and other, 3.6%. In the present study, patients using urinary catheters (group II); the most common causative organisms of CAUTI were *E.coli* and *Klebseilla spp.* (22% each) followed by *Staphylcocci spp.* (18%), *Pseudomonas spp.* (15%), *Enterococcus spp.*, *Acinetobacter spp.* (7%) each, and the least common causes were *Proteus spp.* and *Enterobacter spp.* (4%) each. These results agreed with a study by *Hussein NS*[18] who found that *E.coli* presented the highest prevalence (39%), followed by *Staphylococcus* (30%), *Klebsiella spp.* (17%), *Enterococcus* (7%) and 7% other species. Regarding our study, in mechanically ventilated patients (group III) the most common causative organism of VAP was *Pseudomonas spp.* (31%) followed by *Acinetobacter spp.* (21%), *Klebsiella spp.* (17%), *Proteus spp.* (10%), and the least common were *Staphylococci spp.*, *Citrobacter spp.*, *Enterobacter spp.* (7%) each. These results agreed with the results of *Joseph et al.* [19] who found that *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were the most common potential VAP pathogens isolated from the surveillance cultures.

In our study, out of 79 bacterial isolates; isolates (29%) isolates were biofilm forming. These results agreed with the results of *Černohorská and Votava* [20] who have studied biofilm formation and its antibiotic susceptibility compared to plankotonic population, and found that a total of 42 (32%) out of 133 isolates were biofilm forming. In the present study, the prevalence of biofilm production among culture positive devices were (30.4%) in CVCs, (43.5%) in urinary catheters and (26.1%) in mechanical ventilator. These results agreed with *Trautner and Darouiche* [21] who reported similar results. They recorded that CVC and urinary catheters are the two most commonly inserted medical devices, and they are likewise the two most common causes of bloodstream infection which is due to biofilm formation on the surfaces of both types of catheters.

In the present study the prevalence of different organisms in biofilm production was as follows; the most dominant biofilm forming organism was *Pseudomonas spp.* (53%) followed by *Citrobacter spp.* (50%), *Enterobacter spp.* (40%) then *Klebsiella spp.* and *E.coli* (33%) each, *Staphylococcus spp.* (25%) and finally *Acinetobacter spp.* (18%). Similar results were reported by *Fricks et al.* [22] who have found that Biofilm-forming ability was greatest amongst *P. aeruginosa* biofilms from newly colonized mechanically ventilated patients. Two Different results were reported by *Donlan* [23], who noted that the organisms commonly developing biofilms in urinary devices are *S.epidermidis*, *E.coli*, *P.aeruginosa*, *K. pneumonaiae* and other gram negative organisms, while in CVC are *Staphylococcus spp.*, *C.albicans*, *P. aeruginosa* and *K. pneumonaiae*.

The difference in these results may be explained by difference in locality and environmental conditions, keeping in mind that though the ability of biofilm production is controlled by a chromosomal gene, this gene can be can be transferred from one strain to another by conjugation and so can be more predominant or less predominant in different localities [24].

Concerning antibiotic susceptibility testing in the present study, the most effective antibiotics against biofilm forming gram positive bacterial isolates were cefoxitin, vancomycin, levofloxacin and ceftriaxone are the least susceptible were oxacillin, penicillin, erythromycin and ampicillin, while the most susceptible antibiotics for biofilm forming gram negative bacterial isolates were amikacin, imipenem, gentamicin, ceftazidime and ciprofloxacin and the least susceptible were cefepime, methoprime/sulfamethoxazole, ampicillin/sulbactam and ceftriaxone. Similar results were reported by *Habibi et al.* [25] who found that four of the most common gram negative isolates (*Acinetobacter, Pseudomonas, E.coi and Klebsiella*) are almost resistant to the third generation cephalosporins,

i.e. ceftazidime and cefotaxime. Additionally, both coagulase negative *Staphylococcus* and *Staphylococcus aureus* were methicillin resistant but showed in vitro sensitivity to vancomycin.

In the present work *Pseudomonas spp.* was the predominant biofilm forming bacterial isolate and showed the highest percentage (100%) of resistance to cefotaxime, ampicillin/sulbactam and ceftriaxone, cefepime and trimethoprime-sulfamethoxazole and the highest susceptibility to imipenem (75%) then amikacin (63%), piperacillin (50%) and ceftazidime (50%). This was in agreement with *Ceri et al.*[7] who found that *P. aeruginosa* biofilm was the most susceptible to the aminoglycosides, tobramycin and amikacin but was not nearly as susceptible to gentamicin. Poor sensitivity to cefotaxime was also observed by *Mathai et al.* [26]. One of the major reasons for persistence and survival of *P. aeruginosa* in the lungs is the growth of these bacteria in biofilm communities[26]. The present work showed a clear difference in antibiotic susceptibility between planktonic populations of each tested organism (MIC) and its biofilm counterpart (MBEC), with high statistical significance (P-value <0.001). Similar results reported by *Macià, et al.*[27], that have compared MIC and MBEC of aztreonam, ceftazidime, meropenem, imipenem, ciprofloxacin, tobramycin, colistin and azithromycin using *P. aeruginosa* as model organism and susceptibility parameters were defined , and found that most antibiotics show more than one twofold-dilution increase in the MBEC versus MIC. Similar results were reported by *Fricks et al.* [22] who have compared MICs and MBECs of five antibiotics tobramycin, ceftazidime, piperacillin/tazobactam, imipenem/cilastatin and levofloxacin against six *Pseudomonas* isolates from mechanically ventilated patients and have demonstrated a substantial difference in antibiotic resistance. The MBEC was found to be greater than the MIC for all antibiotics except one (i.e. the MBEC for MV5 tested against ceftazidime remained stable. The β-lactam antibiotics exhibited the greatest increase in antibiotic resistance (MBECs ≥ 256 µg/mL for five of the six isolates). Levofloxacin MBECs were found to be two to five doubling dilutions higher than the corresponding MIC; tobramycin MBECs were found to be three to five doubling dilutions higher than the corresponding MIC. Also have found significant positive correlations between MIC and biofilm formation existed for imipenem ($r = 0.83$, $P<0.001$), levofloxacin ($r = 0.57$, $P < 0.021$) and ceftazidime ($r = 0.62$, $P<0.01$), suggesting that resistance towards these antimicrobials strongly correlated with biofilm formation. Similar results were found by *Naves et al.*[28] who studied the susceptibility of *E.coli* biofilm producing strain in their planktonic and biofilm associated forms to amoxicillin, amoxicillin/clavulanic, cefotaxime, gentamycin and ciprofoxacin and found that *E.coli* biofilms were much less sensitive than their planktonic counterparts to tested antibiotics. *Antunes et al* [29] have compared the MIC and MBEC of vancomycin for *Staphylococci* isolates and found that all isolates presented higher MBEC than MIC for vancomycin. Antibiotic resistance in biofilms is complex and results from contributions of intrinsic, acquired and adaptive mechanisms. Most notably, biofilm specific features such as the differential expression of multiple gene networks, extracellular matrix, and the metabolic heterogeneity of sub-populations within a biofilm colony are major contributors to antibiotic resistance [30]. The reasons for the higher resistance of cells embedded in biofilms may include, e.g., limited diffusion of antibiotics into the biofilm or decreased bacterial growth; some antibiotics can also react with biofilm matrix and, on the other hand, the cells in biofilm can adapt and form protected phenotypes [31]. The most promising strategy is to dose antibiotics based on biofilm susceptibilities in lieu of planktonic susceptibilities [22] .

CONCLUSION AND RECOMMENDATIONS

The difference between MBEC and MIC was statistically highly significant. Thus, it is recommended to detect MBEC rather than MIC to antimicrobials for treatment of Device Related Infections.

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