

Comparative Study Assessing the Effect of Tigecycline and Moxifloxacin in Prevention of *Acinetobacter baumannii* Biofilm

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Abstract: Background & Aim of work: In recent years there has been an increase in life-threatening infections caused by *Acinetobacter baumannii* with multiple antibiotic resistance, especially, in case of biofilm formation. This study aimed at assessing the rate of multidrug resistance (MDR) among *A. baumannii* isolates, incidence of biofilm formation and evaluating the role of, recently produced medications from 2 different antibiotic classes; tigecycline (glycylcyclines) and moxifloxacin (fluroquinolones), in prevention of *Acinetobacter* biofilm formation.

Methods: The current investigation was carried out on 30 strains of *A. baumannii* isolated from different samples at King Khalid University Hospital. Strains were identified and characterized for their antibiotic sensitivity. The MICs tests were conducted to all yields for tigecyclin and moxifloxacin antibiotics. The frequency of biofilm formation and strength was determined by modified microtitre plate method. Finally, prevention of biofilm formation was done using 1X MIC, 2X MIC and 0.5X MIC concentrations of tigecyclin and moxifloxacin.

Results: All *A. baumannii* isolates were found to be MDR strains, resistance to tested antibiotic discs were found to be 100% in 23.3% of the tested strains, 90% of them were biofilm formers. MIC to tigecyclin was found to be 100% sensitive to all yields with MIC₉₀ and MIC₅₀ equal to 0.5 µg/ml and 0.25 µg/ml respectively. However, 83.3 % of the strains were resistant to moxifloxacin with MIC₉₀ and MIC₅₀ equal to 32 µg/ml. In testing their ability in avoidance of biofilm formation, unexpectedly, the reduction in biofilm formation were more noticed in the tested concentrations of moxifloxacin with high significance compared with controls in the 3 concentrations tested with ($p < 0.001$). While, tigecycline gave a gradual reduction; double MIC, MIC, 0.5 MIC concentrations showed the significance of ($p < 0.001$, < 0.01 and < 0.05) respectively.

Conclusion and Recommendations: Most *A. baumannii* isolates are MDR isolates with high tendency of biofilm formation, tigecyclin was the most effective tested antibiotic used on the planktonic cells while its effect on biofilm was exceeded by moxifloxacin. Further investigation is needed to understand the molecular basis of such an interesting finding.

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1. Introduction

Acinetobacter species are primarily associated with nosocomial infections in severely ill patients, particularly with ventilator associated pneumonia, wound infection and bacteraemia (Cisneros *et al.*, 1996). Multidrug-resistant *Acinetobacter baumannii* is a rapidly emerging pathogen in the health care setting, where it causes infections that include bacteremia, pneumonia, meningitis, urinary tract infection, and wound infection. The organism's ability to survive under a wide range of environmental conditions and to persist for extended periods of time on surfaces make it a frequent cause of outbreaks of infection and an endemic, health care-associated pathogen (Fournier *et al.*, 2006 and Jawad *et al.*, 1996).

The management of *Acinetobacter baumannii* infections can be difficult, due to the increasing

number of isolates exhibiting resistance to multiple classes of antibacterial agents (Fagon *et al.*, 1993 and Cisneros *et al.*, 1996). Because multidrug-resistant *Acinetobacter* infection usually occurs in severely ill patients in the ICU, the associated crude mortality rate is high, ranging from 26% to 68% (Seifert *et al.*, 1996, Sunenshine *et al.*, 2007 and Kwon, 2007).

The potential ability of *A. baumannii* to form biofilms could explain its outstanding antibiotic resistance and survival properties. This possibility is supported by a very limited number of publications which showed that a clinical isolate of this bacterium is able to attach to and form biofilm structures on glass surfaces (Vidal *et al.*, 1996, Vidal *et al.*, 1997 and Epsinal *et al.*, 2012). Bacterial biofilms, arrangements in which the cells are morphologically, metabolically and physiologically different from their planktonic

counterparts (Stoodley *et al.*, 2002), have been found on the surface of medical devices such as intubation tubes, catheters, artificial heart valves, water lines and cleaning instruments (Donlan & Costerton, 2002).

Moreover, many studies have tried newly produced antibiotics in treatment of MDR strains of *Acinetobacter* species. Tigecycline, a relatively new glycolcycline agent, has been found to have bacteriostatic activity against *Acinetobacter* species (Pachon-Ibanez *et al.*, 2004 and Seifert *et al.*, 2006). *Acinetobacter baumannii* pathogen has been shown to be susceptible to tigecycline in large-scale microbiological studies (Petersen *et al.*, 2002, Bradford *et al.*, 2005 and Betriu *et al.*, 2006). Tigecycline confers its activity by reversibly binding to the 30S subunit of the ribosome. It acts by blocking the incorporation of transfer RNA into the A site of the ribosome, thus inhibiting protein synthesis (Garrison *et al.*, 2005). In addition, newer fluoroquinolones, such as moxifloxacin, have shown increased activity against *Acinetobacter baumannii* *in vitro* in comparison with older agents such as ciprofloxacin (Vila *et al.*, 2002 and Wisplinghoff *et al.*, 2003). Moxifloxacin is a broad spectrum 8-methoxyquinolone which interacts preferentially with DNA gyrase in Gram-negative organisms (Zhanel *et al.*, 1999).

Therefore, the current study aimed at assessing the rate of multidrug resistance (MDR) among *A.baumannii* isolates, incidence of biofilm formation and evaluating the role of, recently produced medications from 2 different antibiotic classes; tigecycline (glycolcyclines) and moxifloxacin (fluoroquinolones), in prevention of *Acinetobacter* biofilm formation.

2. Subjects and Methods:

1. Bacterial isolates:

In the current study, isolates of *A.baumannii* were obtained from King Khalid Hospital Microbiology Laboratory, KSU, Riyadh, KSA, from various clinical samples. Clinical data were collected including; sex, age, site of sample as well as departments involved. Further processing of the isolates was performed at microbiology laboratories at College of pharmacy, King Saud University and Faculty of Medicine, Fayoum University.

2. Confirming the identity of *Acinetobacter* strains:

Verifying the identity of the yields was conducted by the various conventional methods including; culture on selective media, colony morphology, microscopic examination and oxidase test. Oxidase-negative Gram-negative Bacilli were further identified by Microbact (12A) Gram-negative identification system (Oxoid, Basingstoke, UK) to separate *A. baumannii*.

3. Antimicrobial susceptibility tests:

Disc diffusion test: Antibiotic sensitivity of the isolates was determined using the Kirby-Bauer antibiotic testing (KB testing or disk diffusion

antibiotic sensitivity testing) on Mueller-Hinton agar as recommended by the Clinical and Laboratory Standards Institute (CLSI, previously called NCCLS) (2006 guidelines). Susceptibility to the following antimicrobial agents was performed: amoxicillin + clavulanic acid, ampicilline, colistine, cefotaxime, ceftriaxone, cefepime, ciprofloxacin, meropenem, sulfamethoxazole, piperacillin + tazobactam, ceftazidime, imipenem, tetracycline, gentamicin, and amikacin.

MIC test: In addition, MICs of tigecyclin and moxifloxacin antibiotics were determined for each isolate using 0.5 McFarland standard. Serial two-fold dilutions of the two antibiotics were distributed in 96 microtiter plate. The inoculum suspension and standardization is done according to Clinical and Laboratory Standards Institute. The bacterial inoculum is then inoculated into the wells and incubated at 37°C overnight. MICs were then determined. Isolates were considered susceptible if the MIC of tigecyclin was ≤ 2 $\mu\text{g/mL}$ and resistant if the MIC was ≥ 8 $\mu\text{g/mL}$, while, for moxifloxacin susceptible if ≤ 1 $\mu\text{g/mL}$ and resistant if the MIC was ≥ 2 $\mu\text{g/mL}$. *Escherichia coli* (strain ATCC 25922) was used as the control strain for disc susceptibility testing and MICs. (CLSI, 2006)

4- Biofilm formation

Biofilm formation was determined as follows. Overnight cultures were diluted to 0.5 McFarland using Brain Heart Infusion broth supplemented with 3% sucrose as a growth medium (Oxoid, Madrid, Spain), deposited in 96-well plates and incubated at 37°C for 48 h without shaking. Biofilm was stained with 0.4% Crystal Violet (w/v) and quantified at 590 nm after solubilization with 95% ethanol. The experiment was performed in triplicates. OD590 values for each well were subtracted from those of the blank, which only contained Brain Heart Infusion broth without inoculum (Stepanovic *et al.*, 2000, Stepanovic *et al.*, 2007 and Yanti *et al.*, 2009). *A. baumannii* ATCC 19606 strain was employed as the positive control.

Biofilm calculation: The optical density (ODs) of each strain was obtained by the arithmetic mean of the absorbance of three wells and this value was compared with the mean absorbance of negative controls (ODnc). The following classification was used for the determination of biofilm formation: no biofilm production (ODs=ODnc), weak biofilm production (ODnc<ODs \leq 2.ODnc), moderate biofilm production (2.ODnc<ODs \leq 4.ODnc) and strong biofilm production (4ODnc<ODs). (Rodrigues *et al.*, 2010)

5. Assess the effect of Tigecycline and Moxifloxacin antibiotics on biofilm prevention:

The strongest 10 biofilm former isolates were selected to evaluate biofilm prevention. Using tigecyclin and moxifloxacin antibiotics in 2X MIC, 1X MIC, and ½X MIC concentrations, they were distributed in 96 microtiter plate, each in triplicates.

The bacterial inoculum was then inoculated into the wells and incubated at 37°C for 2 and 4 days. Degree of biofilm formation was detected using XTT/menadione reagent, incubated in the dark for 2 hrs at 37 °C and quantified at 490 nm. The experiment was performed in duplicates to detect settling cells quantity. Furthermore, the strongest two biofilm formers were subjected for further investigation to assess biofilm prevention per time period. XTT assay were done at 1, 2, 4, 6, 48 and 96 hours and planktonic cells were observed by inverted microscope at same time period (Yanti *et al.*, 2009).

6- Statistical analysis:

Data were expressed as means \pm S.D. For multi-variable comparisons, one-way ANOVA was conducted, followed by Bonferroni testing using the GRAPHADA INSTAT (ISI Software) computer program. Differences were considered significant at $P < 0.05$.

3. Results:

The current study included thirty *Acinetobacter baumannii* isolates, collected from different clinical specimens in King Khalid Hospital, Microbiology Laboratory, Rhiyadh, SA. The patients included in the study their ages ranged between 1-94 years, with a mean of 43.9 ± 28.9 years. They were 20 males representing 66.7% of the cases and 10 females representing 33.3 %. Most of the isolates were collected from different ICU wards within the hospital being 13 cases representing 43.3%. Moreover, the most frequent specimens were from wound infections representing 56.7% (17 specimens) of the cases followed by bone infections 13.3% (4 specimens), catheter borne infections 10% and then by ear infection, respiratory tract infection and urinary tract infection 6.6% each.

Twenty three percent of the total strains were resistant to 100% antibiotics, 33.33% of the strains were resistant to 75 – 90% antibiotics and 66.66% were resistant to 50 – 75% as shown in table 2. Susceptibility distribution is shown in table 1.

Table 1. Antimicrobial susceptibility results (percent) of *Acinetobacter baumannii* isolates

Antibiotics	Frequency of resistance	Intermediate	Sensitive
Amikacin	15 (50%)	-	15 (50%)
Gentamicin	12 (40%)	1 (3.3%)	17 (56.7%)
Amoxicillin + clavulanic acid	23 (76.7%)	-	7 (23.3%)
Piperacillin +Tazobactam	21 (70%)	3 (10%)	6 (20%)
Ampicillin	30 (100%)	-	-
Ceftazidime	20 (66.7%)	-	10 (33.3%)
Ceftriaxone	20 (66.7%)	2 (6.66%)	8 (26.66%)
Cefotaxime	23 (76.7%)	1 (3.3%)	6 (20%)
Cefepime	23 (76.7%)	-	7 (23.3%)
Imipenem	22 (73.3%)	-	8 (26.7%)
Meropenem	27 (90%)	-	3 (10%)
Ciprofloxacin	25 (83.3%)	-	5 (16.7%)
Sulfamethoxazole	14 (46.7%)	-	16 (53.3%)
Colistin	7 (23%)	-	23 (76.66%)
Tetracycline	16 (53.33%)	5 (16.66%)	9 (30%)

Table 2. Percentage of resistant strains against the used antibiotics

Antibiotics	Number of isolates showing resistance	Percent
AK, GM, AMC, PTZ, AMP, CAZ CRO, CTX, FEP, IMP, MEP, CIP, SXT, COL, TCN	7	23.33
GM, AMC, PTZ, AMP, CAZ, CRO, CTX, FEP, MEP, CIP, COL, TCN	10	33.33
AMP, AMC, CRO, CTX, FEP, MEP, CIP, TCN	20	66.66
AMP, FEP, MEP, CIP	23	76.66

AK- Amikacin, GM- Gentamicin, AMC- Amoxicillin + clavulanic acid, PTZ- Piperacillin +Tazobactam, AMP- Ampicillin, CAZ- Ceftazidime, CRO- Ceftriaxone, CTX- Cefotaxime, FEP- Cefepime, IMP- Imipenem, MEP- Meropenem, CIP- Ciprofloxacin, SXT- Sulfamethoxazole, COL- Colistin, TCN- Tetracycline.

One hundred percent of the isolates were sensitive to tigecycline with MIC₉₀ and MIC₅₀ equal to 0.5 µg/ml and 0.25µg/ml respectively. However, 83.3 % of the

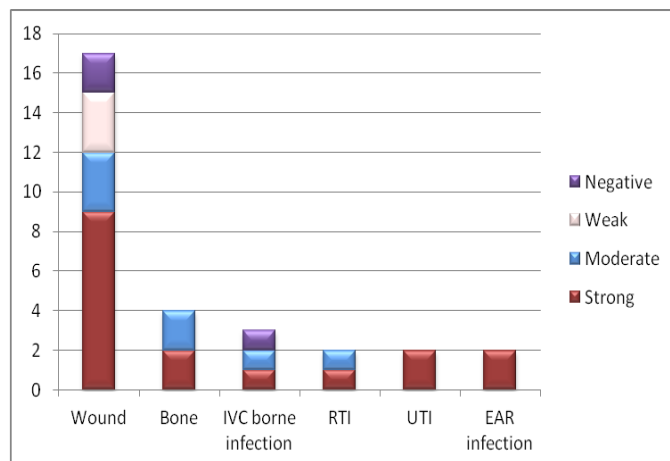
strains were resistant to moxifloxacin with MIC₉₀ and MIC₅₀ equal to 32 µg/ml as shown in table 3.

Table 3. MICs of Tigecycline and Moxifloxacin in the tested isolates

	MIC ₉₀	MIC ₅₀	Maximum MIC	Minimum MIC	MIC mean
Tigecycline	0.5	0.25	0.5	0.06	0.34
Moxifloxacin	32	32	64	0.125	28

In the current study, **90%** of strains were biofilm former (**56.7%** strong, **23.3%** moderate, **10%** weak biofilm former). There were no significant difference (p value > 0.05) found related to the site of sample collection shown their distribution in figure 1.

Moreover, there were no relationship detected between the degree of biofilm formation and ability of the organisms to show multidrug resistance, in fact, negative and weak biofilm formers showed the highest resistance as shown in table 4.

**Figure 1:** Distribution of isolates biofilm strength among different sample sites**Table 4.** Relationship between biofilm strength and antimicrobial resistance among the tested yields

Antibiotics	Resistance				
	Biofilm strong isolates (n=17)	Biofilm intermediate isolates (n=7)	Biofilm weak isolates (n=3)	Biofilm negative isolates (n=3)	Resistance of all isolates (n=30)
Amikacin	4 (23.5%)	5 (71.4%)	3 (100%)	3 (100%)	15 (50%)
Gentamicin	4 (23.5%)	4 (57.1%)	3 (100%)	1 (33.33%)	12 (40%)
Amoxicillin + clavulanic acid	15 (88.24%)	5 (71.4%)	2 (66.66%)	1 (33.33%)	23 (76%)
Piperacillin + Tazobactam	12 (70.56%)	5 (71.4%)	2 (66.66%)	2 (66.66%)	21 (70%)
Ampicillin	17 (100%)	7 (100%)	2 (100%)	3 (100%)	30 (100%)
Ceftazidime	11 (64.7%)	4 (57.12%)	3 (100%)	2 (66.66%)	20 (66.66%)
Ceftriaxone	11 (64.7%)	4 (57.12%)	3 (100%)	2 (66.66%)	20 (66.7%)
Cefotaxime	13 (76.47%)	5 (71.4%)	3 (100%)	2 (66.66%)	23 (76.7%)
Cefepime	12 (70.56%)	5 (71.4%)	3 (100%)	3 (100%)	23 (76.7%)
Imipenem	10 (58.82%)	7 (100%)	3 (100%)	2 (66.66%)	22 (73.3%)
Meropenem	15 (88.24%)	6 (85.71%)	3 (100%)	3 (100%)	27 (90%)
Ciprofloxacin	14 (82.35%)	5 (71.4%)	3 (100%)	3 (100%)	25 (83.3%)
Sulfamethoxazole	8 (47.05%)	2 (28.57%)	2 (66.66%)	2 (66.66%)	14 (46.7%)
Colistin	3 (17.64%)	3 (42.85%)	1 (33.33%)	0	7 (23%)
Tetracycline	10 (58.82%)	3 (42.85%)	2 (66.66%)	1 (66.66%)	16 (53.33%)

In testing the ability of tigecycline and moxifloxacin in prevention of biofilm formation, unexpectedly, the reduction in biofilm formation were more noticed in the tested concentrations of moxifloxacin with high significance ($p <$

0.001) compared with controls in the 3 concentrations tested with. While, tigecycline gave a gradual reduction; double MIC, MIC, 0.5 MIC concentrations showed the significance of ($p < 0.001$; < 0.01 and < 0.05) respectively as shown in Figure 2, 3.

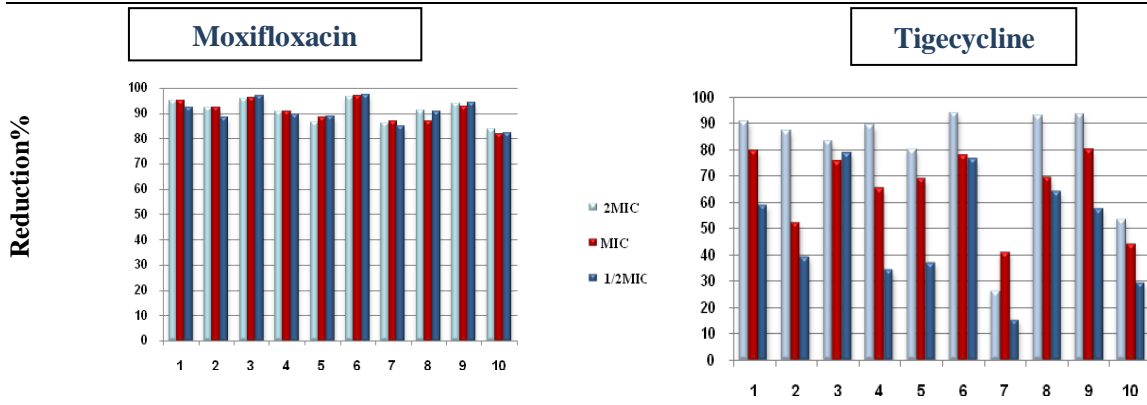


Figure 2. The effect of moxifloxacin and tigecycline in the 3 concentrations tested among the ten isolates investigated



Figure 3: The minimum inhibitory concentration shown of several isolates indicating the higher sensitivity of tigecyclin over moxifloxacin in the tested isolates

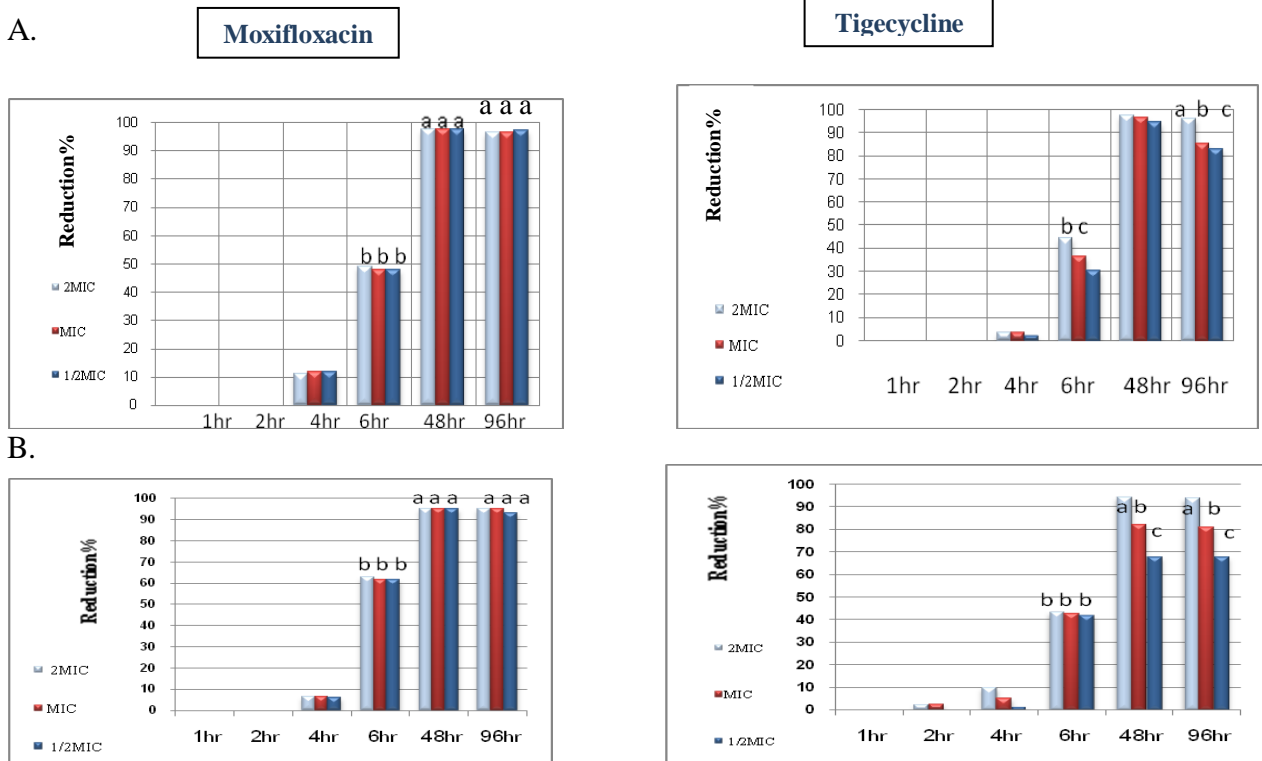


Figure 4: Reduction Charts of two isolates at different time periods per hour. The values are expressed in % of biofilm reduction . ^aP < 0.001 ^bP < 0.01, ^cP < 0.05 compared to control strains.

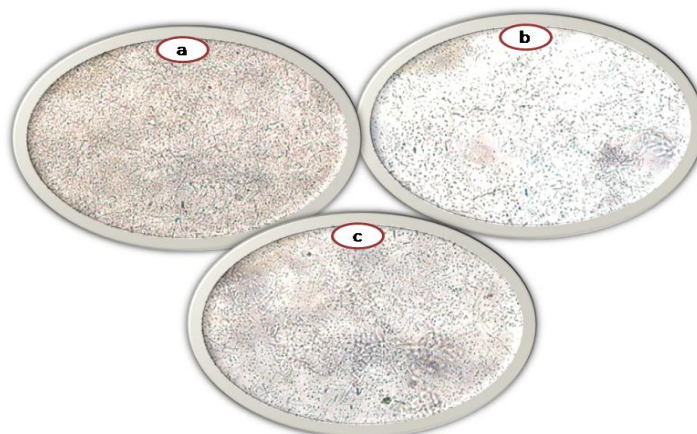


Figure 5: *A. baumannii* under inverted microscope X 385 a- control strain after 48 hours , b- tigecycline effect on the planktonic cells in 2XMIC concentration, c- moxifloxacin effect on the planktonic cells in 2XMIC concentration.

4. Discussion:

A. baumannii is an opportunistic pathogen that is particularly successful at colonizing and persisting in the hospital environment, able to resist desiccation (Jawad *et al.*, 1996, Cappelli *et al.* and 2003, Dima *et al.*, 2006) and survive on inanimate surfaces for months (Jawad *et al.*, 1997). It is among the most common causes of device-related nosocomial infection which result when the organism is able to resist physical and chemical disinfection, often by forming a biofilm (Thomas *et al.*, 2008).

The age of the patients included in the present study ranged between 1 and 94 years, with a mean of 43.9 ± 28.9 years. They were 20 males representing 66.7% of the cases and 10 females representing 33.3%. These findings were in agreement with Gaber *et al.* (2010) who reported that no specific age for acinetobacter infected patients and found that the mean age was 43.6 ± 21.2 years (ranging between 10 and 90 years) and the higher incidence of infection was observed in males (19/30) than in females (11/30). Also Husni *et al.* (1999) found that the mean age of the patients was 50 (ranging between 21 and 84 years), and 79% of them were males. Most of the patients included were inpatients in different ICU wards being 13 cases representing 43.3%. Similar finding was reached by Fournier and Richet (2006), they found 29 out of 44 cases were isolated from ICU wards. Gaber *et al.* (2010) reported that critically ill patient in ICUs at a higher risk of nosocomial infections due to prolonged ICU stay, underlying chronic diseases, disruption of barriers (endotracheal intubation, tracheostomy, urinary catheterization and CVC) and prolonged use of antimicrobial therapy.

Moreover, the most frequent specimens were collected from wound infections representing 56.7% of the cases followed by bone infections 13.3%, catheter borne infections 10% and then ear infection, respiratory tract infection and urinary tract infection

6.6% each. In agreement with present study Iregbu *et al.* (2002), Joshi *et al.* (2003) and Joshi *et al.* (2006) reported higher incidence of acinetobacter spp. was found in wound infections but this followed by urinary tract infections. In contrast to our findings, Lee *et al.* (2004) found that the most frequently isolated yields were from respiratory secretions representing 57%. Other sites of isolation included wound (19%), blood borne (4%), urine (3%), and bile (3%). These difference in rate and type of infections may be due to variation of infection control protocols in ICUs in some researches or isolation of acinetobacter spp. from different wards of the hospitals not only from ICUs in other researches.

In the current study, 30 *A. baumannii* strains were isolated, 100% of which were MDR isolates. Disc diffusion method was employed to assess the sensitivity of the isolates to different antibiotics, 23.3% were found to be resistant to 100% of the tested antibiotics. MICs by microdilution method were conducted to assess the effect of tigecycline and moxifloxacin on all yields. 100% of the isolates were sensitive to tigecycline with MIC₉₀ and MIC₅₀ equal to 0.5 µg/ml and 0.25 µg/ml respectively. However, 83.3% of the strains were resistant to moxifloxacin with MIC₉₀ and MIC₅₀ equal to 32 µg/ml. Comparable results were found by Henwood *et al.* (2002) who found that more than 90% of the isolates were sensitive to tigecyclin by the broth microdilution method, with MIC₉₀ and MIC₅₀ equal to 2 µg/ml and 0.5-1 µg/ml respectively. Moreover, the study conducted by Soussy *et al.* (2003) was in agreement with the effect of moxifloxacin on *A. baumannii* in the present study, they found that moxifloxacin displayed poor activity against *A. baumannii* whereas it was more active against *A. lwoffii* and other *Acinetobacter* species (MICs between 0.032mg/L and 0.5mg/L).

In the current study, 90% of strains were biofilm formers (56.6% strong, 23.3% moderate, 10% weak

biofilm former). These findings were comparable with the results of Wroblewska *et al.* (2008) who found that 100% of strains were biofilm formers in their study, however, only 12% were strong biofilm formers, 47% moderate and 41% were weak. Moreover, there was no correlation found between degree of biofilm formation and antibiotic resistance (Table 4). Several studies had similar conclusion, they found that weak biofilm formers were presenting high antibiotic resistance pattern among their isolates (Rodriguez-Ban *et al.*, 2008 and Epsinal *et al.*, 2012). Nevertheless, some others correlated between antibiotic resistance and strong biofilm formation (Wendt *et al.*, 1997, Lee *et al.*, 2008; and Rao *et al.*, 2008).

In addition, there were no relationship detected between the degree of biofilm formation and site of sample collection (Figure 1). These results agreed with Wroblewska *et al.* (2008) who found that *A. baumannii* site of isolation had no significant effect on their ability to produce biofilm.

In testing the avoidance of biofilm formation, unexpectedly, the reduction in biofilm formation was more noticed in the tested concentrations of moxifloxacin with high significance compared with controls in the 3 concentrations tested with p value of < 0.001 . Pompilio *et al.* (2010) found comparable results; biofilm production was significantly lower ($p < 0.001$) in the presence of 0.036X and 0.066X MIC on a different tested organism. No statistically significant differences were observed between inhibition levels caused by 0.066X and 0.036 X MIC exposures for tested strains. Biofilm inhibition by moxifloxacin may be explained through different mechanisms; inhibition of the synthesis or expression of adhesins on the bacterial cell surface or modifying the bacterial shape in such a way that interferes with the ability of the micro-organisms to approach host cell-surface receptors (Lorian & Ernst, 1987; Lorian *et al.*, 1989). Fluoroquinolones were found in many studies to reduce the virulence of some bacteria (Gram-negative bacilli, in particular) by inducing an elongation of the cell soma along its longitudinal axis, a phenomenon also known as filamentation. These morphologically altered cells generally show reduced pathogenicity in terms of lower levels of adhesion, altered susceptibility to phagocytosis and decreased release of bacterial enzymes (Labro *et al.*, 1987; Chen *et al.*, 2005). In addition, Drago *et al.* (2005) observed that moxifloxacin subMICs (0.125 \times and 0.06 \times MIC) induced filamentation in a remarkable portion of *Klebsiella pneumoniae* in an animal experimental model of pulmonary infection.

However, some previous studies on *E. coli* found the effect of fluoroquinolones was limited at concentrations equal to or not less than 0.125 \times MIC on biofilm prevention (Baskin *et al.*, 2002; Wojnicz & Jankowski, 2007).

On the other hand, tigecycline gave a gradual reduction on biofilm formed by the isolates; double MIC, MIC, 0.5 MIC concentrations showed significant growth reduction with values of $p < 0.001$; < 0.01 and < 0.05 respectively. These results were consistent with the work of Maestre *et al.* (2012); who found that biofilm reduction was highly significant on their tested strains, however, there were no difference in biofilm reduction ($p < 0.001$) for the 2 tested concentrations (0.25 \times MIC, and 0.5 \times MIC) of tigecycline.

Conclusion and Recommendations:

All thirty *Acinetobacter* isolates were multidrug resistant organisms, 90% of which were biofilm formers, and 57% of the tested strains were strong biofilm formers. Tigecycline was the most effective tested antibiotic against *Acinetobacter* yields (100% sensitivity), while, moxifloxacin showed much lower activity (83.3% were resistant). However, though tigecyclin was effective against biofilm formation, its effect was exceeded by moxifloxacin in the tested concentrations in prevention of biofilm formation. Further investigation is needed to understand the molecular basis of such an interesting finding.

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