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The Impact of Minimum Inhibitory and Sub Inhibitory Concentrations of Antibiotics on *Acinetobacter baumannii* and *Pseudomonas aeruginosa* Biofilm Production

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ABSTRACT

THESE bacteria are opportunistic bacterial pathogens, it are associated with hospital acquired infection and have high propensity to acquire a wide range of antibiotic resistance determents. This study aimed to investigate the biofilm-forming capacities of *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, as well as any possible correlations between the production of biofilms and exposure to different sub-lethal antibiotic dosages. The selected isolates were taken from the University of Mosul's bacterial culture collections and assessed qualitatively as well as quantitatively using standard procedures. Antimicrobial susceptibility, biofilm formation and inducibility were used. The biofilm cells showed an impressive increase in the minimum inhibitory concentrations (MICs) of various antibiotic groups. Most of the representative isolates treated to various sub-lethal antibiotic doses (1/8, 1/4, and 1/2 the MIC) showed a considerable induction of biofilm creation. For *A. baumanii* the induction of a biofilm formation was decrease with presence of ceftazidime, while the biofilm formation was increase in the presence of amikacin, the biofilm formation was decrease in the presence of gentamicin, biofilm formation was decrease in the presence of levofloxacin. Increased biofilm formation in the presence of meropenem and tetracyclinel, 7.8 mg/ml to 31.2 mg/ml respectively. Similar results were shown for the *P. aeroginosa* isolate, where biofilm formation was induction in the presence of concentration of the antibiotics amikacin, ceftazidime, gentamicin, and levofloxacin, while concentrations of meropenem and tetracycline showed a decrease in biofilm formation. This study suggests a connection between low-dose antimicrobial medication and an increased risk of *A. baumannii* and *P. aeroginosa* biofilm infections by highlighting a potential role for efflux pump and transport of auto-inducer molecules during biofilm production. Information from this study will be helpful in developing antibiofilm approaches.

Keywords: Antibiotics, *A. baumannii*, Biofilm formation, MIC, *P. aeruginosa*, sub-MIC.

Introduction

Clinical settings have a high prevalence of *A. baumannii* and *p. aeruginosa* which are large and troublesome opportunistic pathogens that frequently cause a variety of infections, [1, 2]. Because nosocomial isolates are becoming more resistant to widely used antimicrobial drugs, treating Acinetobacter infections is becoming more challenging. *A. baumannii* isolates that are clinically multidrug-resistant (MDR) are reported often and constitute a major threat to lives and health.

Furthermore, bacteria within biofilms can tolerate antibiotic doses greater than those reported in the planktonic cells by up to 1,000 times [3]. Because biofilms play a unique role in the colonization of bacteria and persistence, quorum sensing is responsible for many processes that lead to resistance to several medications, particularly the resistance nodulation cell division (RND) family. This trait plays multiple roles during the transition of planktonic cells to biofilm in acinetobacter [4,5]. Unfortunately, biofilm development responds to minimum inhibitory and sub-inhibitory antibiotic concentrations in a way that makes it even more diffusive; this is typically the result of therapy at low doses [6]. It has been shown that quorum sensing and biofilm development by bacteria are related [7,8]. QS molecules are transported out of the cells by the efflux pump system, which is involved in multidrug resistance [9,10]. AdeIJK, AdeFGH, and AdeABC efflux pumps are extensively present in clinical isolates of bacteria and are members of the resistance nodulation cell division (RND) family. According to earlier research, a range of different substances, including nutrients, oxygen, antibiotics, and pH, is formed during the development of biofilms; as a result, the cells in the inner layer of the biofilm have

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restricted access to antibiotics and are subjected to antibiotics at sub-inhibitory concentrations, Furthermore, numerous investigations have shown that certain antibiotics can change bacterial functions such as adhesions, biofilm formation and transcription of pathogenicity-causing agents at subminimum inhibitory concentrations (MIC).

The two major opportunistic bacteria that cause infection in hospital and chronic fibrosis patients are *P. aeruginosa* and *A. baumannii*, have been used as a model to discuss in deeper detail the impact of antibiotics at sub-lethal concentrations on bacterial physiology and virulence. People suffering from a wide range of infections have long-term *P. aeruginosa* and *A. baumannii* those results in the death of the patient. Pathogens grow in the presence of gradients generated by widely varying antibiotic concentrations since these patients are typically receiving antibiotic treatment. Understanding the biological reactions of these bacteria in patients undergoing therapy thus necessitates learning how *P. aeruginosa* and *A. baumannii* responds to antibiotics at sub-inhibitory concentrations. To this purpose, the current study evaluated the impact of different antibiotics classes on *A. baumannii* and *P. aeruginosa's* ability to form biofilms at their sub-MIC levels.

Material and Methods

Selection of the isolates: *A. baumannii* clinical isolates were obtained from the previous study of some authors [11] which were identified using VITEK-2 system and characterized the AI synthase abaI gene. *P. aeruginosa* clinical and identified isolates were obtained from Microbiology Laboratory, College of science.

Susceptibility test for antibiotics: By disk diffusion (Kirby Bauer's) method, testing for antimicrobial susceptibility was conducted on Mueller–Hinton agar (Merck, German) for all isolates. The isolated colonies were touched by a sterile inoculation loop and suspended in two milliliters of sterile saline solution. A McFarland standard of 0.5 was achieved by diluting the cultures ($\sim 10^7$ cells/mL). Then cultivate on Mueller Hinton agar for 24 h at 37 ◦C. Standard antibiotics were used depend on (CLSI) 2022 (Table 1).

TABLE 1. Antibiotics used in Susceptibility test.

NO.	ANTIBIOTIC	ABBREVIATION	CON.(µg)	COMPANY	ORIGIN
1	CIPROFLOXACIN	CIP	5 µg	TM Media	INDIA
2	TETRACYCLIN	TE	30 µg	TM Media	INDIA
3	KANAMYCIN	K	30 µg	TM Media	INDIA
4	LEVOFLOXACIN	LE	5 µg	TM Media	INDIA
5	VANCOMYCIN	VA	30 µg	TM Media	INDIA
6	AMIKACIN	AK	30 µg	TM Media	INDIA
7	GENTAMYCIN	HLG	10 µg	TM Media	INDIA
8	CEFTAZIDIME	CAZ	30 µg	TM Media	INDIA
9	CEFEPIME	FEP	10 µg	Bioanalyse	TURKEY
10	TOBRAMYCIN	TOB	10 µg	Bioanalyse	TURKEY
11	MEROPENEM	MEM	10 µg	Bioanalyse	TURKEY
12	CARBENICILLIN	PY	10 µg	Bioanalyse	TURKEY
13	CLINDAMYCIN	DA	10 µg	Bioanalyse	TURKEY

Prepare antibiotic stock solutions

Table (2) shows the various types of antibiotics utilized in current study. Before being used, the stock solution of each antibiotic was made in sterile distilled water and kept at -20°C. And Description of powders antibiotics utilized in the present study in table (3).

TABLE 2. Various kinds of antibiotics used in the study

NO.	ANTIBIOTICS	CLASS	INHIBITOY MECHANISM
1	MEMROPENEM 1 g	β- Lactam	Synthesis of the cell wall inhibition
2	CEFTAZIDIME	β- Lactam	Synthesis of the cell wall inhibition
3	GENTAMYCIN	Aminoglycosides	Interrupting protein synthesis
4	AMIKACIN	Aminoglycosides	Interrupting protein synthesis
5	TETRACYCLINE	Tetracycline	Interrupting protein synthesis
6	LEVOFLOXACIN	Fluoroquinolone	Inhibition DNA Synthesis

TABLE 3. Description of powders antibiotics used in the study.

NO.	ANTIBIOTIC	CONCENTRATION	COMPANY	ORIGIN
1	LEVOFLOXACIN	500 mg /100 ml	ANTIOCH	TURKEY
2	MEROPENEM	1 g	IBRAHIM	TURKY
3	CEFTAZIDIME	1 g	GSK	USA
4	GENTAMYCIN	80 mg /2 ml	BRAWN	INDIA
5	AMIKACIN	500 mg /2 ml	BRAWN	INDIA
6	TETRACYCLIN	250 mg	SDI	IRAQ

Microtiter Plate Biofilm Production Assay

Biofilm formation test was performed in a Microtiter 96-well plate as described previously by [12] After activation all the isolates in brain heart infusion broth, after diluting the cultures to a 0.5 McFarland standard (about 107 cells/mL), 200 µL aliquots of the cultures were placed onto microtiter plate wells and incubated for 24 hours at 37 °C. As controls, some wells held just sterile brain heart infusion broth. For 24 hours at 37 °C, the plates have been incubated aerobically. The culture within the well was removed and the plates underwent three rounds of washing with 200 µL of 0.9% saline to eliminate any non-adherent cells. Subsequently, the plates were dried upside-down. 200 µL of 1% crystal violet solution in water was used to stain each well for 20 minutes. After aspirating out the unbound crystal violet, 200 µL of 0.9% saline was used to wash the wells three times. To do a quantitative assessment of biofilm development, 200 µL of 95% ethanol was added, which caused the bound dye to release, intensive dye proportion directly associated with forming. A microtiter plate reader was used to measure each well's optical density (OD) at 630 nm after 100 microliters from each well were transferred to a fresh microtiter plate. All isolates were tested in triplicates and the mean values were used. The more resistant and strongest biofilm formation isolates of *A. baumannii* and *P. aeruginosa* were selected for the upcoming assessments.

Minimum inhibitory concentration (MIC)

The biofilm cells' antibiotic Susceptibility has been assessed using the Clinical and Lab Standards Institute (CLSI) Protocol [13]. Briefly, the final concentrations of the antibiotic stock solutions table (2) were achieved by serial double dilution, with controls, positive that contain bacterial growth only and negative that contain antibiotics only. Isolates *A. baumannii* isolate 23, *P. aeruginosa* isolate 12 and ATCC 19106 cells were reduced to 0.5 McFarland standard and used to prepare the inoculum. All inoculated plates were incubated for 20 h at 37°C, and then the MIC was determined at which there was 90% growth inhibition.

Antibiotic-induction biofilm production test

Following the procedure of [12], the tests on antibiotic-induced biofilm development were carried out on 96-well Microtiter plates. The isolates of *A. baumannii*, *P. aeruginosa*, and ATCC 19106 were cultured in to brain heart infusion broth at 0.5 McFarland standard and varied sub-lethal antibiotic doses (1/8, 1/4, and ½ the MIC). For a full 24 hours, inoculated plates were incubated under aerobic conditions at 37°C. Next, the optical density (OD Planktonic) at 630 nm was determined by transferring the supernatant from each well to the matching well in a fresh plate. Each well's intact biofilm was stained for 30 minutes at 37°C using a 1% crystal violet solution. Following two sterile distilled water washes, then it was left to air dry. The OD at 630 nm was determined across the biofilm cells that have been stained and were treated with 95% ethanol (ODCV Biofilm). The CV-stained biofilm cells' backgrounds stains were reduced by using the negative control (ODcv Control). A biofilm formation index was used to express the capacity to produce a biofilm: [BFI = ($OD_{CV\ Biofilm}$ - $OD_{cv\ Control}$)/$OD_{Planktonic}$]. The relative biofilm formation capacity was determined using the A. baumannii ATCC 19606 as a positive standard for the production of biofilm. (relative biofilm formation index [RBFI] = $BFI_{isolate}$/$BFI_{A.\ baumannii\ ATCC\ 19606}$).

Results

Antimicrobial susceptibility test

Each isolate underwent testing to determine its susceptibility to antibiotics. *A. baumannii* isolates exhibited high resistance to tetracycline by ratio 80%, moderate 12%, 8%, sensitive, for meropenem by ratio 88%, moderate 4%, 8%, sensitive, levofloxacin by ratio 60%, moderate 32%, 8%, ceftazidime by ratio 100%, isolates were resistance for gentamicin by ratio 92%, moderate 4%, sensitive 4%, for amikacin resistance by ratio 100% As show in Table (4). Sensitivity test for *P. aeruginosa* isolates were as following, resistance for tetracycline by ratio 100%, for meropenem 18.5 % resistance, moderate 9%, sensitive 72.5%, resistance for levofloxacin by ratio 90%, sensitive 10%, isolates were resistance for ceftazidime by ratio 100%, for amikacin were resistance by ratio 72%, sensitive 27%, and resistance for gentamicin by ratio 45.5%, moderate 9%, sensitive 36.5% As show in Table (5).

TABLE 4. Percentage antibiotics sensitivity test for *A. baumannii*

NO.	ANIBIOTICS	RESISTANC%	INTERMRDIATE%	SNSITIVE%
1	Ciprofloxacin 5ug	92	4	4
2	Tetracycline 30ug	80	12	8
3	Kanamycin 30ug	100		
4	Levofloxacin 5ug	60	32	8
5	Vancomycin 30ug	100		
6	Amikacin 30ug	100		
7	Gentamicin 120ug	92	4	4
8	Ceftazidime 30ug	100		
9	Cefepime 10ug	100		
10	Tobramycin 10ug	84	4	12
11	Meropenem 10ug	88	4	8
12	Carbincillin 25ug	100		
13	Clindamycin 10ug	100		

TABLE 5. Percentage antibiotics sensitivity test for *P. aeruginosa*

NO.	ANIBIOTICS	RESISTANC%	INTERMRDIATE%	SNSITIVE%
1	Ciprofloxacin 5ug	90		10
2	Tetracycline 30ug	100		
3	Kanamycin 30ug	90	10	
4	Levofloxacin 5ug	90		10
5	Vancomycin 30ug	100		
6	Amikacin 30ug	72		27
7	Gentamicin 120ug	45.5	9	36.5
8	Ceftazidime 30ug	100		
9	Cefepime 10ug	100		
10	Tobramycin 10ug	90		10
11	Meropenem 10ug	18.5	9	72.5
12	Carbincillin 25ug	100		
13	Clindamycin 10ug	100		

Determination of Minimum Inhibition Concentration (MIC)

(MICs) of antibiotics against planktonic cells were used to assess the antibiotic susceptibilities of the isolates. The MICs test of antibiotics against *Acinetobacter baumanii* 23 isolate fluctuate with consecutive antibiotic concentration test, the MIC test was 1.25 mg/ml for levofloxacin, 1000 mg for meropenem, 250,000 mg for ceftazidime , 40 mg/2ml for gentamicin, 15.6 mg/ml for Amikacin, 62.5 mg/ml for tetracyclin. The MICs test for *P. aeroginosa* was 62.5 mg/ml for levofloxacin, 7800 mg/ml for meropenem, 125,000 mg for ceftazidime, 40 mg/2ml for gentamicin, 15.6 mg/ml for Amikacin, 31.25 mg/ml for tetracyclin.

Antibiotic inducibility of biofilm formation.

As shown in Figure (1), The biofilm formation in the absence (0 mg/ml) and in the presence of antibiotics levofloxacin, meropenem, ceftazidime, gentamycin, amikacin and tetracycline with various sub-lethal concentrations (1/8, 1/4, and ½ the MIC) of antibiotics., the RBFIs ranged from 0.5 to 6 for *A.baumanii* and 0.5 to 4.2 for *P. aeruginosa*, showing a significant difference in the biofilm production ability.

In the absence of antibiotics (0 mg/ml) the highest ability to produce a biofilm was seen in both bacteria, for *A.baumanni* isolate 23 the induction of biofilm was not obviously detected with isolate in presence of ceftazidime and levofloxacin while the biofilm formation capacity was significantly increase in presence of amikacin, gentamicin, meropenem and tetracycline , this culture show the biofilm induction. For *P.aeroginosa* isolate 12 the induction of biofilm was not obviously detected in presence of gentamicin and tetracycline while the biofilm formation was significantly increase in presence of amikacin, ceftazidime, levofloxacine and meropenem Figure (2).

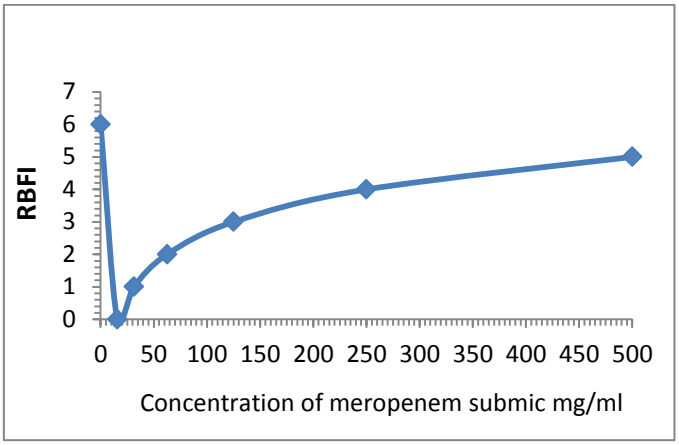

Fig.1-A. Effect meropenem on biofilm formation in Acinetobacter

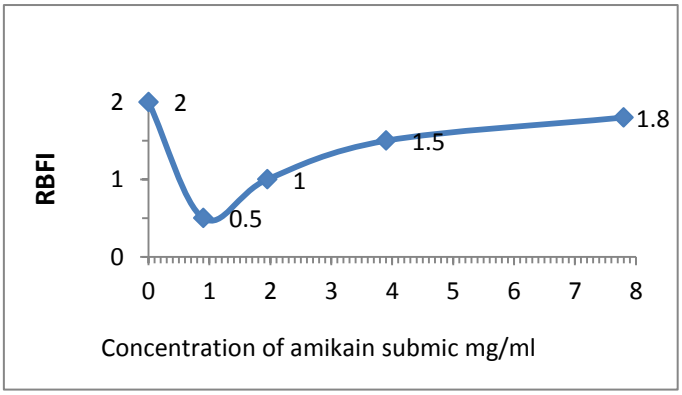

Fig.1-B. Effect amikacin on biofilm formation Acinetobacter

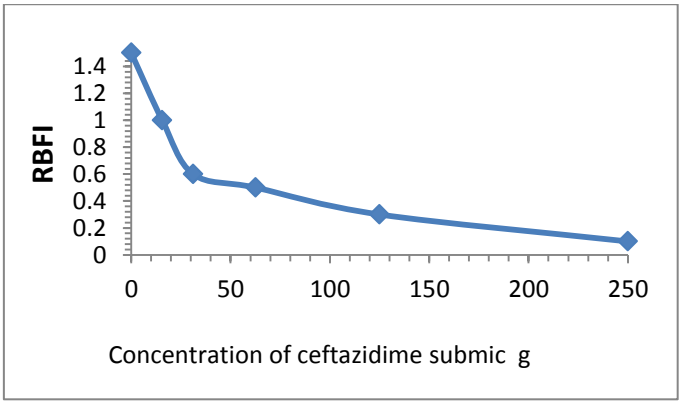

Fig. 1-C. Effect ceftazidime on biofilm formation in Acinetobacter

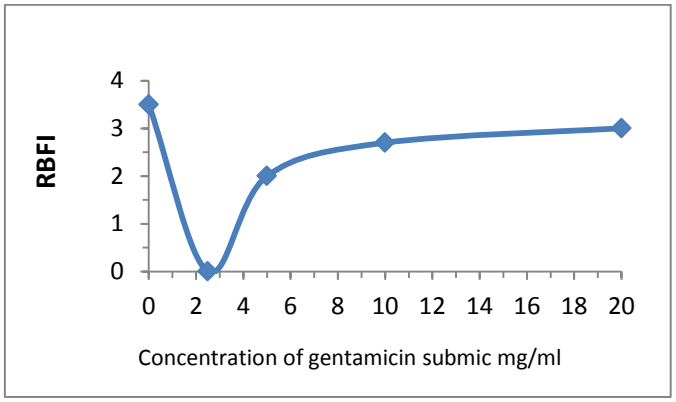

Fig.1- D. Effect gentamicin on bioflim formation Acinetobacter

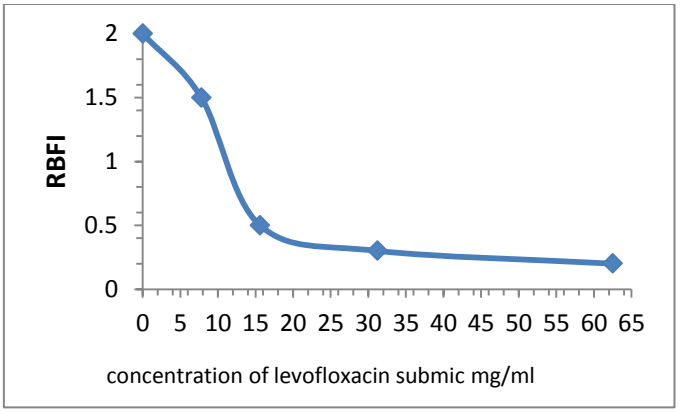

Fig.1-E. Effect levofloxacin on biofilm formation in acinetobacter

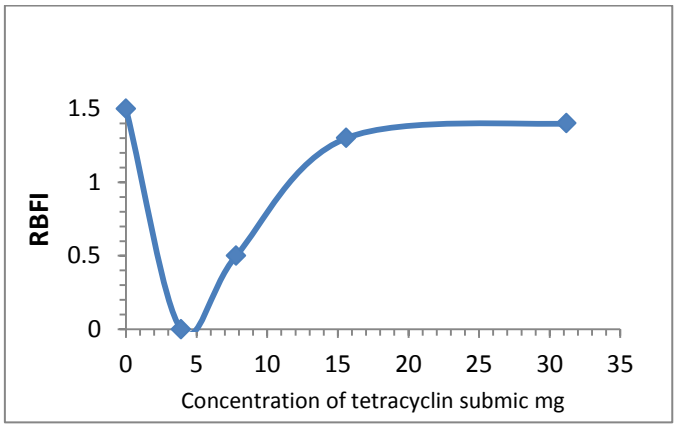

Fig.1-F. Effect tetracyclin on biofilm formation in Acinetobacter

Fig. 1(A-F). Bacterial biofilm formed by *A.baumanii* in presence of sub inhibitory concentrations of antibiotics, (amikacin, gentamicin, ceftazidime, levofloxacin, meropenem and tetracycline).

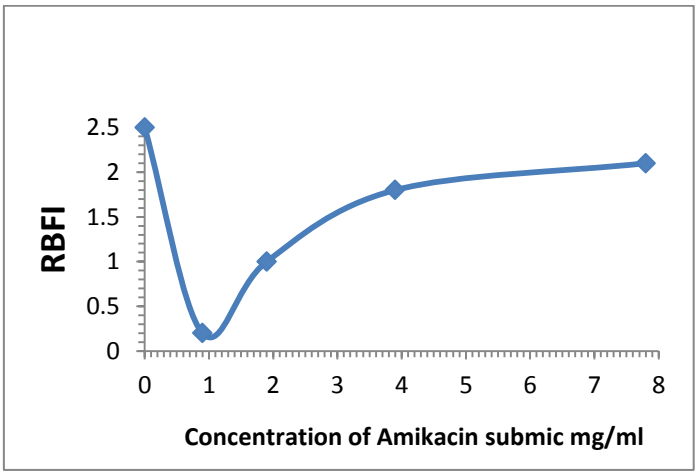

Fig.2-A. Effect of amikacin on biofilm formation of Pseudomonas

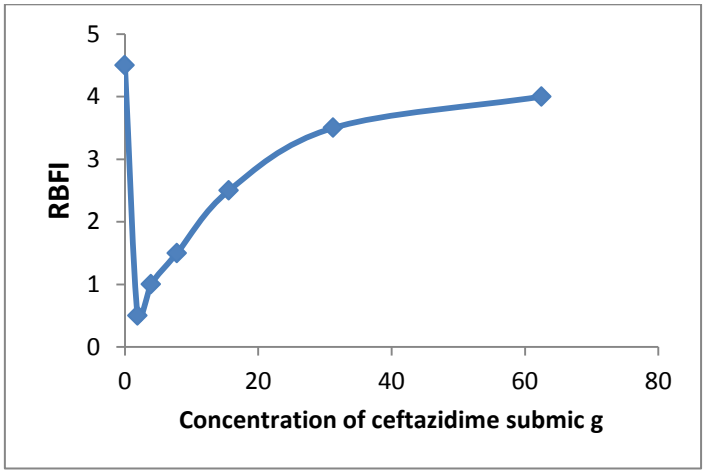

Fig.2. B. Effect ceftazidime on biofilm formation of pseudomonas

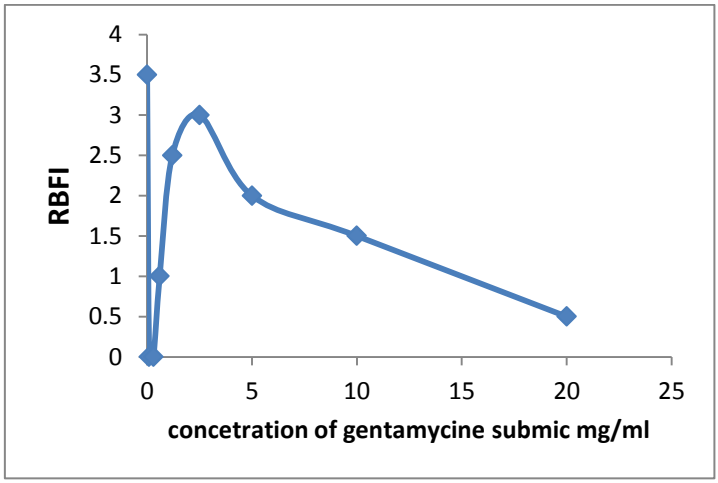

Fig. 2.C. Effect gentamycine on biofilm formation Pseudomonas

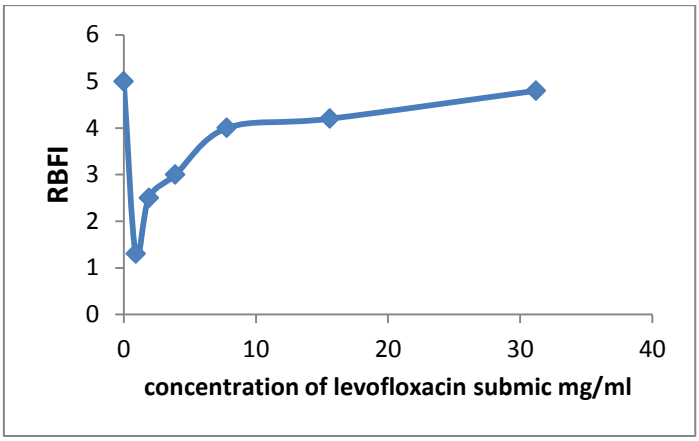

Fig. 2-D. Effect levofloxacin on biofilm Pseudomonas

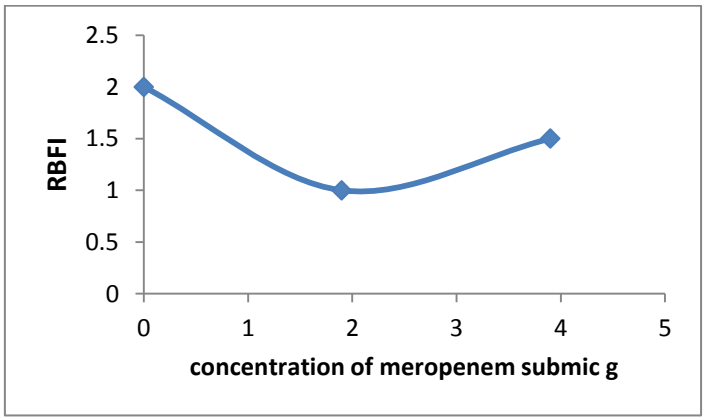

Fig.2-E. Effect meropenem on biofilm formation Pseudomonas

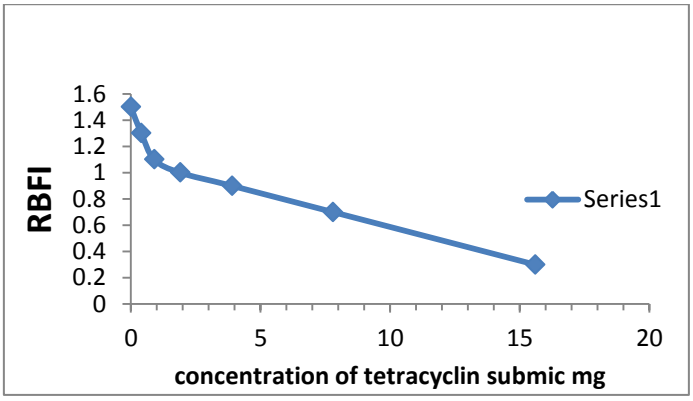

Fig.2-E. Effect tetracyclin on biofilm formation of Pseudomonas

Fig. 2(A-E). Bacterial biofilms formed by *P.aeroginosa* isolate 12 in the presence of sub inhibitory concentrations of (amikacin, gentamicin, ceftazidime, levofloxacin, meropenem and tetracycline).

Discussion

In this study, we described the isolates of *A. baumannii* and *P.aeroginosa* that were isolated from clinical samples. The isolates were previously isolated, identified, and obtained from the biology department, college of science, University of Mosul. These *A. baumannii* and *P. aeroginosa* isolates' planktonic cells showed distinct patterns of antibiotic resistance and an MDR phenotype to β-lactams, aminoglycosides, tetracyclines, and fluoroquinolones. Therefore, it represents a significant health problem. Pathogenic bacterial resistance patterns typically vary between countries as well as within them over time. Due to these variances, nationwide resistance surveys are required to support proper empirical drug therapy selections. Programs that keep track of pathogenic bacterial resistance also forecast epidemics. The identification of certain resistance patterns may indicate ongoing epidemics. Not many studies have researched the impact of antibiotics at sub MICs on biofilm development of *A. baumannii* and *P.aeroginosa*, despite the majority of earlier research [14, 15] investigating the different mechanisms of antibiotic resistance in *A. baumannii* and *P. aeroginosa* isolates. Thus, this study's goal was to investigate the effects of six antibiotics at sub-lethal concentrations of meropenem, amikacin, gentamicin, tetracyclin, ceftazidim and levofloxacin on the biofilm formation capacity in *A. baumannii* and *P.aeroginosa*.

The results indicate that the biofilm cells of these representative isolates were significantly resistant to the antibiotics, the MICs for all the antibiotics with the biofilm cells of these isolates were all relatively high values. The findings show that biofilm formation is directly linked to a considerable rise in bacterial antibiotic resistance, which is consistent with an earlier study. Antibiotic resistance in biofilm-forming cells may arise due to physiological alterations in membrane permeability and metabolism [16]. In this study, for *A. baumanii* the induction of biofilm formation was decrease with presence of ceftazidime from the first concentration (15600 mg/ml) to(250,000 mg/ml), while the biofilm formation was decreased with the presence of amikacin at the concentrations from (1.95 mg/ml to 7.8 mg/ml), the biofilm formation in the presence of gentamicin decrease in the first concentration (2.5 mg/ml) and was increase from (5 mg/ml to 20 mg/ml) concentrations, decrease biofilm formation in the presence of levofloxacin from (7.8 mg/ml to 62.5 mg/ml). Meropenem and tetracycline at concentrations (31200 mg/ml to 500,000 mg/ml) cause an increase in biofilm development (7.8 mg/ml to 31.2 mg/ml), respectively.

Similar results were shown for the *P. aeroginosa* isolate, where biofilm formation was induction in the presence of concentrations of the antibiotics amikacin, ceftazidime, gentamicin, and levofloxacin, while concentrations of meropenem and tetracycline showed a decrease in biofilm formation. Along with *P. aeruginosa*, reports of antibiotic-induced biofilm development have also been made with fluoroquinolones [17] and *Escherichia coli* [18]. It has been revealed that *P. aeruginosa's* mexXYoprM and mexABoprM multidrug efflux pump systems are implicated in both acquired and intrinsic resistance, whereas the other two systems solely mediate acquired resistance [19].

It has been stated that *P. aeruginosa's* inherent resistance to β-lactams, chloramphenicol, tetracycline, novobiocin, macrolides, and quinolones is mostly due to the system of mexAB-oprM efflux pump [20]. In addition to resistance, these efflux pump systems also have an interaction in stress virulence and response [21]. These efflux pump genes contribute to the production of *P. aeruginosa* biofilms in addition to drug extrusion, according to [20]. Accordingly, efflux pumps in this organism might have a dual function. During biofilm formation, genes encoding efflux pumps and drug transporters are significantly increased. Research revealed that based on a reanalysis of the global transcription patterns obtained during the biofilm formation phase of E. Coli strains 83972 and VR50 and a comparison with planktonic growth results, it was found that 128 of 600 upregulated genes encoded efflux pumps and transporters, Numerous efflux systems responsible for the elimination of harmful compounds from the cells, including various antibiotics, were significantly increased during biofilm formation, and several of these genes were among the most substantially up-regulated genes overall. The stimulation of several stress-related genes and the overexpression of these genes encoding drug and metabolite transport systems suggest that the biofilm development mode does put a lot of strain on the cells, causing metabolic disruption as a result of intracellular stress conditions [22].

The study found a significant correlation between the sub minimal inhibitory concentrations of antibiotics and the stimulation of biofilm development when planktonic cells migrate to biofilm cells, suggesting a relationship between biofilm formation and QS. According to recent research, multi-drug efflux pumps play a role in drug resistance, cell division, pathogenicity, and QS signal release [23]. Notably, *A. baumannii's* ability to consistently upregulate abaI and adeG resulted in the most widespread biofilm induction, which raises the possibility that this combination may affect how well *A. baumannii* develops biofilms. The generation and movement of (AHLs) in *A. baumannii* during the biofilm formation process are presumably sped up by

the overexpression of *AdeFGH*. Therefore, deactivating the *AdeFGH* efflux pump may be a useful strategy to stop the development of *A. baumannii* biofilms when an infection is present [24].

Conclusion

This research indicates a possible role for the AdeFGH efflux pump in the production and movement of autoinducer molecules when a biofilm is developing, suggesting a link between low-dose antibiotic therapy and an elevated clinical risk *A. baumannii* and *P. aeroginosa* biofilm infections. The study's findings will be useful in the development of antibiofilm strategies.

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Conflict of Interest: None

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Author`s contribution:

All researchers participated in designing the research. The first researcher carried out the practical aspect. The second researcher completed the task of supervising, statistical analysis, making tables, and writing.

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تأثير الحد الأدنى وتحت الادنى لتراكيز المضادات الحيوية المثبطة لإنتاج الأغشية الحيوية في بكتيريا *Acinetobacter baumannii* و *pseudomonas aeruginosa*

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الخلاصة:

هدفت الدراسة الحاليه الى تقييم تكوين الاغشيه الحيويه من قبل بكتريا *pseudomonas aeruginosa* and *acinstobacter baumannii* بالإضافة الى أي علاقات محتملة بين إنتاج الأغشية الحيوية والتعرض لجرعات مختلفة بتراكيز تحت القاتلة من المضادات الحيوية . تم الحصول على العزلات المختاره من بنك السلالات العزلات في جامعة الموصل وتم تقييمها نوعياً وكمياً باستخدام الطرق القياسية. تم استخدام فحص الحساسية للمضادات الحيوية، وتشكيل وتحفيز الاغشيه الحيويه. اظهوت خلايا الأغشية الحيوية زيادة في التراكيزات المثبطة الدنيا (MICs) لمجموعات المضادات الحيويه المختلفه . أظهرت معظم العزلات المعالجة بجرعات تحت الادنى من المضادات الحيوية (8/1، 4/1، و2/1 MIC) تحفيزا كبيرا لتكوين الأغشية الحيوية. بالنسبة لـ *A. baumani* انخفض تكوين الغشاء الحيوي مع وجود المضاد الحيوي (ceftazidime) من التركيز الأول (15600 ملجم/مل) إلى (250.000 ملجم/مل)، في حين زاد تكوين الغشاء الحيوي مع وجود المضاد الحيوي (amikacin) عند التراكيز من (1.95 ملغم/مل إلى 7.8 ملغم/مل)، ينخفض تكوين الغشاء الحيوي مع وجود المضاد الحيوي (gentamicin) في التركيز الأول (2.5 ملغم/مل) ويزداد من (5 ملغم/مل إلى 20 ملغم/مل)، ينخفض تكوين الغشاء الحيوي مع وجود مضاد (levofloxacin) من (7.8 مجم/مل إلى 62.5 مجم/مل). زيادة تكوين الأغشية الحيوية مع وجود المضاد الحيوي (meropenem and tetracyclin) بتركيزات (31200 مجم/مل إلى 500000 مجم/مل)، (7.8 مجم/مل إلى 31.2 مجم/مل) على التوالي. وأظهرت نتائج مماثلة لعزلة *P. aeroginosa*، حيث تم تكوين الغشاء الحيوي مع وجود تراكيز من المضادات الحيوية amikacin، ceftazidim، gentamicin، levofloxacin، في حين أظهرت تراكيز meropenem وtetracyclin انخفاضا في تكوين الغشاء الحيوي. تشير هذه الدراسة إلى وجود صلة بين الأدوية المضادة للميكروبات بجرعات منخفضة وزيادة خطر الإصابة بعدوى الأغشية الحيوية *A. baumannii* و*P. aeroginosa* من خلال تسليط الضوء على الدور المحتمل لمضخة التدفق ونقل جزيئات المحفز الذاتي أثناء إنتاج الأغشية الحيوية. المعلومات الواردة في هذه الدراسة ستكون مفيدة في تطوير أساليب المضادات الحيوية.