Biofilm Formation and Its Association with Multiple Drug Resistance among Clinical Isolates of *Acinetobacter baumanii*

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ABSTRACT

Background: *Biofilm formation is a developmental process with intercellular signals that regulate growth. Microorganisms growing in a biofilm are associated with chronic and recurrent human infections and are highly resistant to antimicrobial agents e.g., Amoxycillin 93.3%, Aztreonam 86% Amoxyclav 83.3%, Piperacillin 83.3%, Ceftriaxone 80%, Ceftazidime 80% and Amikacin 80%.* ***Objectives:*** *The aim of this work was to determine biofilm production among clinical isolates of Acinetobacter baumanii, (A. baumanii, to determine the antibiotic susceptibility pattern to different antibiotics and to associate between biofilm production and multidrug resistance (MDR).*

Methods: In a prospective study a total of 30 isolates of A. baumannii were included in this study; these isolates were obtained from clinical specimens like urine, sputum, blood, wound swab and tracheal aspirate of patients admitted to critically care units of Suez Canal University Hospitals. All isolates of A. baumanii were screened for biofilm production by both tube method (TM) and tissue culture plate method (TCP). Biofilm production is demonstrated with TM, in which bacterial film lining a culture tube stained with a cationic dye and visually scaled. In the second TCP method, the optical density of the stained bacterial film is determined spectrophotometrically. Antibiotic susceptibility test for all A. baumannii clinical isolates were done using the Kirby- Bauer disc diffusion method. ***Results:*** *Thirty strains of A. baumannii were isolated from different clinical specimens; 12 (40 %) from tracheal aspirate, sputum 6 (20%), urine 5(16.7%), blood 5 (16.7%) and Wound swab 2 (6.6%) respectively. Both TCP and TM screened 18 (60%) strains of A. baumannii as biofilm producers. Antibiotic susceptibility tests revealed higher resistance among biofilm producers strains of A. baumanii to most of the generally used antibiotics compared with non-biofilm producers strains. Also we demonstrated a higher difference between frequency of MDR producers strains of A. baumanii [72.2% for penicillins, cephalosporins (including inhibitor combinations), fluoroquinolones and amino glycosides] and (61.2% for Imipenem, Amikacin and Aztreonam); in compare to [41.7% for penicillins, cephalosporins (including inhibitor combinations), fluoroquinolones and amino glycosides] and (33.3% for Imipenem, Amikacin and Aztreonam) among non biofilm producers strains of A. baumanii respectively; and this difference was statistically non significant (P>0.05). The high rate of in vitro antibiotic resistance of the A. baumanii strains indicate the importance of controlled antibiotic usage and appliance of proper hospital infection control measures.*

Key words: Acinetobacter baumanii, tissue culture plate, tube method, biofilm detection, multidrug resistance.

INTRODUCTION

Acinetobacter baumannii is the most relevant human pathogen within the Acinetobacter genus; referred to as a pleomorphic aerobic Gram- negative bacillus (similar in appearance to Haemophilus influenzae on Gram stain). The organism is commonly isolated from the hospital environment and hospitalized patients.[1]Most *A. baumannii* isolates are considered as MDR, containing in their genome small isolated islands of alien (meaning transmitted genetically from other organisms), DNA and other cytological and genetic material; a properties that confers more virulence to this organism.[2] The definition of MDR among clinical isolates of *A. baumannii* vary in literature, the most common two definitions of the term MDR are carbapenem resistant or resistant to ≥ three classes of antimicrobials; including all penicillins and cephalosporins (including inhibitor combinations), fluoroquinolones and aminoglycosiges.[3,4] The term panresistant is used to describe strains of acinetobacter that is resistant to all standard antimicrobial agents tested except colistin.[5]

Hospital-acquired infection with acinetobacter occurs through catheters, breathing tubes and open wounds. It usually infects those with compromised immune system, such as the wounded, the elderly, children, or

those with immune diseases. Colonization poses no threat to people not already ill, but colonized healthcare workers and hospital visitors can spread the bacteria into neighboring wards and other medical facilities.[6]The number of Nosocomial infections (hospital-acquired infections) caused by *A. baumannii* has increased in recent years; as have most other Nosocomial pathogens, e.g., Methicillin resistant Staphylococcus aureus(MRSA), Vancomycin resistant Staphylococcus aureus(VRSA),Vancomycin resistant enterococcus(VRE) etc.[7, 8] One of the reasons is that the bacteria can live up to five months on undisturbed surfaces, depending on humidity levels.[9] Furthermore this organism frequently causes infections associated with medical devices, e.g., vascular catheters, Foley catheters, Cerebrospinal fluid shunts etc.[10]

Nosocomial *A. baumannii* bacteremia may cause severe clinical disease that is associated with an elevated mortality rate.[11] *A. baumannii* infection often prove difficult to treat due to high level of resistance to multiple antibiotics as a result of both intrinsic and extrinsic mechanisms.[12] The ability of some *A. baumannii* isolates to produce biofilms might also explain its outstanding antibiotic resistance, survival properties and increased its pathogenicity.[13] Recently some studies showed a positive correlation between biofilm formation and presence of extended spectrum of β- lactamase (ESBL) among the *A. baumannii* isolates.[14] Biofilm formation is a well known pathogenic mechanism in device related infections in hospitals.[15] Moreover, the environmental survival of some Nosocomial pathogens including *A. baumannii* may be facilitated by biofilm formation on abiotic surface.[16]

Therefore, the present work was undertaken on clinical isolates of *A. baumannii* from different clinical specimens and devices associated infections to determine the frequency of biofilm production among the isolates by using different detection methods. Also, we aimed at finding a possible association between biofilm formation and the occurrence of MDR among clinical isolates of *A. baumanii*.

MATERIALS AND METHODS

A prospective study was conducted from June 2010 to April 2011 at Microbiology Department, Faculty of Medicine, and Suez Canal University. A total number of 30 isolates of *A. baumannii* were included in this study; these isolates were isolated from different clinical specimens like urine, sputum, blood, wound swab and tracheal aspirate of patients admitted to critically care units of Suez Canal University Hospital. All the specimens were sent to microbiology laboratory for routine culture, isolation identification, screening for biofilm production and for antibiotic sensitivity testing. The *A. baumannii* bacterium was isolated and identified on standard bacteriological techniques like culture of each specimen on both Blood and MacConkey media then incubation at 37cº for 24-48 hours, colonial growth identification by its morphology, Gram stain; further identification of colonies through a battery of biochemical tests which included Catalase, Oxidase, Citrate, Nitrate and Urease tests. Also by carbohydrate fermentation tests for Glucose, lactose, Sucrose, and Mannitol; beside OF dextrose test and growth at 44 cº.[17] The resulting growth was also identified and confirmed up to the species level by using API 20 NE test (BioMérieux, Durham, NC); according to the instruction manual provided with the kit.

1) Detection of biofilm.

a) Tube method (TM).

A qualitative test for biofilm formation was performed according to Christensen and his associates.[18] In brief; a loopful of test organism was inoculated in 10 mL of trypticase soy broth with 1% glucose in test tubes. The tubes were incubated at 37 cº for 24 hours, after incubation, the content of each tube was decanted and washed with phosphate buffered saline (pH 7.3) and dried. Tubes were then stained with 0.1% crystal violet. Excess stain was washed with distilled water. Tubes were dried in inverted position. The scoring for tube method was done according to the results of the control strains (Reference strain of positive biofilm producer *Staphylococcus epidermidis* ATCC 35984 and negative biofilm producer *Staphylococcus epidermidis* ATCC 12228). Biofilm formation was considered positive when a visible film lined the wall and the bottom of the tube. The amount of biofilm formed was scored as: - I) weak/None, II) Moderate and III) High/strong. The experiment was performed in triplicate and repeated three times.

b) Tissue culture plate method (TCP).

Also a quantitative test described by Christensen and his coworkers.[20] was performed as a gold-standard method for biofilm detection[19] According to this method; organisms isolated from fresh agar plates were inoculated in10 mL of trypticase soy broth with 1% glucose. Broths were incubated at 37 cº for 24 h. The cultures were then diluted 1:100 with fresh medium. Individual wells of sterile 96 well- flat

bottom polystyrene tissue culture plates (Sigma-Aldrich, Costar, USA) were filled with 200 µL of the diluted cultures. The control organisms were treated the same way as the test organisms also incubated, diluted and added to tissue culture plates. Negative control wells contained inoculated sterile broth. The plates were incubated at 37 cº for 24 h. After incubation, the contents of each well were removed by gentle tapping. The wells were washed with 0.2 ml of phosphate buffered saline (pH 7.3) four times to remove free floating bacteria. The adhered biofilm formed by bacteria was fixed by 2% sodium acetate and stained by 0.1% crystal violet. Excess stain was removed with distilled water and plates were kept for drying. The optical density (OD) of stained adherent biofilm was obtained by using micro ELISA autoreader (model 680, Biorad, UK) at wavelength 570 nm (figure -1).

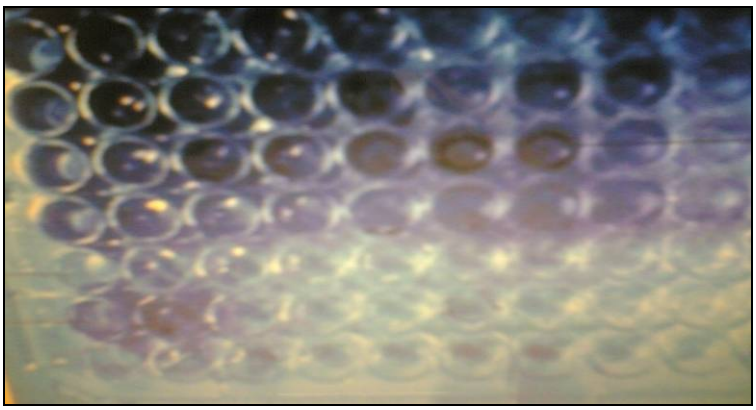

Figure (1): TCP for Detection of Biofilm Formation among Clinical Isolates of *A. baumannii*.

The experiment was performed in triplicate and repeated three times. The interpretation of biofilm production was done according to the criteria of Stepanovic et al., [21] as each isolate was classified belonging to its biofilm production according to the data of the following table (1).

Table (1) Interpretation of biofilm production.[21]

Average OD value	*Biofilm production*
OD ≤ ODc (< 0.09)	**Non –adherent (0)**
ODc < OD ≤ 2 x ODc (0.09 - 0.18)	**weakly adherent (+ or 1)**
2×ODc < OD ≤ 4×ODc (0.18-0.36)	**Moderate (++ or 2)**
4 x ODc < OD (0.36)	**Strong (+++or 3)**

Optical density cut-off value (ODc) = average OD of negative control+3xstandard deviation (SD) of negative control.

2) Antimicrobial susceptibility testing:

Antibiotic susceptibility test for all isolates using the Kirby-Bauer disc diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI).[22] Inoculate were prepared by suspending the isolates in normal saline equal to the turbidity of 0.5 McFarland turbidity standard (10^6 CFU/mL) and applied on Mueller Hinton agar (Oxoid, UK) plates. The following antibiotic discs were used to detect MDR among *A. baumannii* isolates e.g. Amoxicillin (20µg), Amoxyclav (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), Cefepime (30µg), Amikacin (30µg), Aztreonam (30µg), Doxycyline (30µg), Netilmycin (30µg), Piperacillin (100 µg), Piperacillin/tazobactam (100/10µg), Co-trimoxazole 1.25µg + 23.75 µg, Imipenem (10µg), Gentamicin10µg, Ciprofloxacin (5µg) and Ofloxacin (5µg). All antibiotic discs were obtained from (Oxoid, UK). The results were interpreted according to criteria set by Clinical and Laboratory Standards Institute. [22]

RESULTS

During the period of our study (ten months from June 2010 to April 2011), 30 isolates of *A. baumannii* were isolated. Of these 12 strains were isolated from tracheal aspirates (40%), 6 (20%) from sputum, 5 (16.7%) from both urine and blood samples separately and 2 (6.6) from wound swab (table 2).

Table (2) Source of *Acinetobacter baumannii* isolates.

Culture	*No (%)*
tracheal aspirate	12 (40%)
Sputum	6 (20%)
Urine*	5 (16.7%)
Blood	5 (16.7%)
Wound swab	2(6.6%)
Total	30(100%)

**All urine samples were taken from catheterized patients.*

Both quantitative TCP and qualitative TM methods for screening of biofilm production have been used in this study. Only 18 (60%) out of the 30 isolates were found to be biofilm producers by both methods (table 3). Of the 18 biofilm producers, 15 were found to be high biofilm producers and 3 moderate biofilm producers by the TCP method. On the other hand 12 and 6 isolates were considered as high and moderate biofilm producers by the TM respectively. The majority of biofilm production among *A. baumannii* bacteria 17 (94.4%) were isolated from all tracheal aspirates and urine specimens; but only one (5.6%) from sputum.

Table (3). Screening of *Acinetobacter baumannii* isolates for Biofilm Formation by TCP and TM.

	Biofilm Formation*	***TCP n (%)***	***TM n (%)***
No of A. baumannii isolates (30)	High	15 (50%)	12 (40%)
	Moderate	3 (10%)	6 (20%)
	weakly adherent	----	-----
	Non –adherent	12 (40%)	12 (40%)
Total		30 (100%)	30 (100%)

** The majority of biofilm production among Acinetobacter baumannii bacteria 17 (94.4%) were isolated from all tracheal aspirate and urine specimens; but only one (5.6%) from sputum.*

Concerning antibiotic sensitivity observed among all *A. baumannii* isolates, Amoxycillin was the least sensitive antibiotic (6.7 %) followed by Aztreonam (13.3%) and Amoxyclav and Piperacillin (16.7%**)** for each**.** On the other hand, Netilmycin was the highest sensitive antibiotic (70%) followed by Imipenem (50%), Ciprofloxacin (40%) and Doxycyline (40%). The sensitivity pattern for the rest of the antibiotics is demonstrated (table 4).

Table (4) Antibiotic susceptibility results of *Acinetobacter baumannii* isolates.

Antibiotic	***N/30 (% sensitive)***
Amoxycillin (20µg)	2 (6.7 %)
Amoxyclav (30µg)	5 (16.7%)
Ceftriaxone (30µg)	6 (20%)
Ceftazidime(30µg)	6 (20%)
Cefepime (30µg)	9 (30%)
Amikacin (30µg)	6 (20%)
Aztreonam (30µg)	4 (13.3%)
Doxycyline 30 µg	12 (40%)
Netilmycin 30 µg	21 (70%)
Piperacillin (100µg)	5 (16.7%)
Piperacillin/tazobactam (100/10µg)	15 (50%)
Co-trimoxazole 1.25µg +23.75 µg	9 (30%)
Imipenem (10µg)	15 (50%)
Gentamicin (10µg)	6 (20%)
Ciprofloxacin (5µg)	12 (40%)
Ofloxacin (5µg)	10 (33.3%)

For antibiotic resistance pattern among both positive and negative biofilm producing *A. baumannii* isolates, we could demonstrate higher antibiotic resistance pattern among biofilm producers of *A. baumannii* isolates compared to the negative biofilm producers isolates. For example, 100% resistance pattern was observed among positive biofilm producing *A. baumannii* isolates for amoxicillin, Amoxyclav, Ceftriaxone and Ceftazidime, compared to 83%, 58%, 50% and 50% resistance pattern for the same antibiotics among the negative biofilm producing *A. baumannii* isolates (table 5).

Table (5) Antibiotic susceptibility results of both biofilm positive and negative *A. baumannii* Isolates.

Antibiotic	*Resistance Biofilm positive isolates N =18(%)*	*Resistance Biofilm negative isolates N =12(%)*	*Resistance of all isolates N =30 (%)*
Amoxycillin (20µg)	18 (100%)	10(83.3%)	28(93.3%)
Amoxyclav (30µg)	18 (100%)	7(58.3%)	25(83.3%)
Ceftriaxone (30µg)	18 (100%)	6(50%)	24(80%)
Ceftazidime(30µg)	18 (100%)	6(50%)	24 (80%)
Cefepime (30µg)	17 (94.4%)	4(33.3%)	21 (70%)
Amikacin (30µg)	16 (88.9%)	8(66.7%)	24(80%)
Aztreonam (30µg)	16 (88.9%)	10(66.7%)	26(86.7%)
Doxycyline 30 µg	12 (66.7%)	6(50%)	18(60%)
Netilmycin 30 µg	7 (38.9%)	2(16.7%)	9(30%)
Piperacillin (100µg)	17 (94.4%)	8 (66.7%)	25(83.3%)
Piperacillin/tazobactam(100/10µg)	13 (72.2%)	2 (16.7%)	15(50%)
Co-trimoxazole (1.25µg +23.75 µg)	13 (72.2%)	8 (66.7%)	21(70%)
Imipenem (10µg)	11 (61.1%)	4 (33.3%)	15(50%)
Gentamicin (10µg)	17(94.4%)	7 (58.3%)	24(80%)
Ciprofloxacin (5µg)	13 (72.2%)	5 (41.7%)	18(60%)
Ofloxacin (5µg)	14(77.8%)	6 (50%)	20(66.7%)

For MDR patterns among both biofilm positive and negative isolates of *A. baumannii*, we found that 13 out of 18 (72.2%) positive biofilm producing *A. baumannii* isolates were considered as MDR to all penicillins, cephalosporins (including inhibitor combinations), fluoroquinolones and amino glycosides compared to 5 (41.7%) MDR among non producers ones (table 6). On the other hand Imipenem resistance with or without other anti microbial resistance was noticed in 11 out of 18 (61.1%) positive biofilm producing *A. baumannii* isolates, While only 4 out of 12 (33.3%) of negative biofilm producing *A. baumannii* isolates were found Imipenem resistant. All these differences had none statistically significant results as the P value > 0.05 (table 6).

Table (6) MDR patterns of both positive & negative biofilm producer among *A. baumanni* isolates.

Multiple drug combinations	*MDR Biofilm positive Isolates N = (%) 18*	*MDR Biofilm negative Isolates N = (%) 12*
All penicillins, cephalosporins (including inhibitor combinations) fluoroquinolones and amino glycosides	13 (72.2%)*	5 (41.7%)
***P value > 0.05 (not significant)**		
Imipenem, Amikacin and Aztreonam ****P value > 0.05 (not significant)**	11 (61.1%) **	4 (33.3%)

DISCUSSION

Acinetobacter baumannii is emerging as an important pathogen, especially in intensive care units (ICUs). The increasing development of multiple antimicrobial resistances in this pathogen has severely restricted the therapeutic options available for infected patients, and increased the length of stay in ICUs and mortality.[3,23] Despite intensive efforts, Nosocomial acquisition of MDR *A.* baumannii is still a problem due to the great ability of *A. baumannii* to disseminate from and colonize human and environmental reservoirs.[24.25] It is also considered the most common causes of device related Nosocomial infection that results when the organism is able to resist physical and chemical disinfection, often by forming a biofilm.[26] Biofilm exhibit resistance to antibiotics by various methods like restricted penetration of antibiotic into biofilms, decreased growth rate and expression of resistance genes.[27]

In this study, the isolation rates of *A. baumanii* reached 40% from tracheal aspirates and 20 % from sputum samples. These results were in parallel with the results performed in India that revealed isolation rates of about 24 % from tracheal aspirates and 16 % from sputum sample.[28] On other hand our results were not parallel with another study done in Taiwan that showed a very high isolation rate of *A. baumanii* recovered from sputum 71%.[29]

For biofilm production, both qualitative (TM) and quantitative (TCP) methods showed 18 *A. baumanii isolates* (60%) as biofilm producers; and the majority of them were from tracheal aspirates and urine specimens 17/18 (94.4%). These results were in agreement with the results of Donlan who reported a strong association between biofilm producing bacteria and urinary catheters; [30] the results were also comparable with the results of two separate studies done in India that showed 60 and 62% of their isolates as biofilm producers.[28, 31]

Also in our study, Imipenem resistance of *A. baumannii* isolates was 50 %; a result in agreement with other studies reporting 53% and 54% Imipenem resistance among *A. baumannii* isolates. [28.32]. On the contrary, one study done in Pondicherry India showed 100 % resistance to Imipenem.[31], and another one in Korea revealed 17% Imipenem-resistant.[33] The discrepancy of previous results related to Imipenem resistance of *A. baumannii* isolates may be due to regional variation to the pattern of antimicrobial use and other many risk factors. The important risk factors for the acquisition of Imipenem-resistant *A. baumannii* include previous carbapenem use, longer duration of hospital stay until infection, ICU stay, urgent surgery, total parenteral nutrition, having a central venous catheter, endotracheal tube and urinary catheter or nasogastric tube.[34]

In the 30 *A. baumanii* isolates used in our study, the highest sensitivity was seen in Netilmycin 70%, then both Imipenem and Piperacillin/tazobactam each one 50%, followed by Doxycyline and Ciprofloxacin 40% for each one; then Ofloxacin 33.3%, Cefepime and Co-trimoxazole 30% for each one; but on the other hand the least sensitivity was seen in Gentamicin, Amikacin, Ceftazidime and Ceftriaxone 20% for each one followed by Amoxyclav and Piperacillin 16.7% for each one then Aztreonam 13.3%, and lastly Amoxycillin 6.7%. These results more or less were parallel with a study conducted in UK showed the highest sensitivity was seen in Imipenem 93% then Amikacin 79%, Piperacillin /tazobactam 61%, Gentamicin 57%, Ciprofloxacin 54%, Ceftazidime 54%, Piperacillin 28%, respectively.[35]

Also our results demonstrated a higher difference between frequency of MDR among positive biofilm producers strains of *A. baumanii* (72.2% for penicillins, cephalosporins, (including inhibitor combinations), fluoroquinolones and amino glycosides) and (61.2% for Imipenem, Amikacin and Aztreonam); in compare to (41.7%) for penicillins, cephalosporins, (including inhibitor combinations), fluoroquinolones and amino glycosides) and (33.3% for Imipenem, Amikacin and Aztreonam) among non biofilm producers strains of *A. baumanii* respectively; but this difference was statistically non significant as the P value 0.05; This statistically non significant result may return to the small number of studied isolates (30 *A. baumanii* isolates only). On other hand these results were in agreement with another study was conducted in USA regarding the MDR, the results showed, about 79.5% (66/83) were MDR *A. baumanii*; among these 62 (74.7%) were resistant to Ceftazidime and 66 (79.5%) were resistant to Imipenem. The Imipenem resistant *A. baumannii* isolates 79.5% (66/83) was also resistant to Kanamycin, Amikacin, Gentamicin, Streptomycin, Tetracycline, Ciprofloxacin and Nalidixic acid.[36] Also in our study positive biofilm producers of *A. baumannii* clinical isolates was showed increased resistance to Ceftazidime 100%, Cefepime 94.4% and Piperacillin 94.4%; but on other hand negative biofilm producers showed increased resistance only for Amoxycillin 83.3%; these results were

correlated with a recent study showed significantly higher resistance to Cephotaxime, Amikacin, Ciprofloxacin and Aztreonam among positive biofilm producers; but negative biofilm producers showed increased resistance only for Ofloxacin.[31]

In conclusion, continuous surveillance of the in-vitro antimicrobial susceptibility of *A. baumannii* isolates in various geographical areas is necessary in order to generate data useful for optimizing empirical antimicrobial treatment of patients with infections that are likely to be caused by this pathogen. Our investigation reveals a simultaneous emergence of resistance to many antimicrobial agents when the organisms produce biofilm and represents a severe threat in the treatment of hospitalized patients. It is a challenge to treat and also to eradicate this MDR organism because the overall healthcare costs which are attributed to the treatment of biofilm associated infections will be much higher. The high rate of in vitro antibiotic resistance of the *A. baumanii* strains indicate the importance of controlled antibiotic usage and appliance of proper hospital infection control measures. In this study we could not demonstrate significant statistically association between biofilm producers strains of *A. baumanii* and MDR; this could be attributed to the small size of the sample; therefore we recommend a further study using a bigger sample size and continuous research based on genomic levels to confirm or exclude this association.

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تكويـــن البيوفيـلم وعلاقتـــه بمقـاومة المضادات الحيـوية المختلفـة بيـن سـلالات مـــيكروب *A. baumanii* والمعـزول من العينـات الطبيـة المختلفـة

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لقد اثبت بالدراسة ان تكوين البيوفيلم بين سلاله ميكروب *A. baumanii* المعزولة من العينات الطبية المختلفة تخضع لعملية تكوين مستمر للبيوفيلم تحت تاثير اشارات حسيه داخليه مسؤله عن تنظيم نمو هذه السلاله وسلالات اخري من الميكروبات و نمو مثل هذه الميكروبات داخل طبقه البيوفيلم يكون مرتبط بتكرار العدوي الجرثومبه وتحوبلها الي عدوي مزمنه وايضا اكساب مثل هذه السلالات الميكروبيه مناعه ضد المضادات الحيويه وتتميز سلالات ميكروب *A. baumanii* بالقدرة الفائقة علي التكوين المستمر للبيوفيلم وكان الغرض من هذه الدراسه هو دراسه مدي قدرة سلالات ميكروب *A. baumanii* المعزولة من مختلف العينات الطبية علي انتاج وتكوين طبقة البيوفيلم وعلاقة هذا البيوفيلم بالمقاومة للمضادات الحيوية المختلفة وخصوصا مقاومتة لمجموعه من المضادات الحيوية مجتمعه ولقد أجريت هذه الدراسه بمعمل الميكروبيولوجي التابع لكلية الطب جامعة قناة السويس في الفترة ما بين يونيو ٢٠١١ الي ابريل ٢٠١١ وشملت الدراسة علي التعرف والعزل البكتريولوجي لثلاثون سلالة من ميكروب *A. baumanii* تم جمعها من مختلف العينات الطبية للمرضي مثل البول, والبصلق, والدم, ومسحه من الجروح, وايضا من الافرازات الماخوذة من انبوبة الحنجرة الموضوعة لبعض المرضي وكانت تلك العينات الطبية للمرضي المحجوزين بوحدات الرعاية الحرجة بمستشفيات جامعة قناة السويس, ولقد تم فحص جميع هذه السلالات البكتريه بالنسبه لقدرتها علي تكوين و انتاج طبقة البيوفيلم وذلك عن استخدام كل من طريقة الانبوبة وتعتبر هذة الطريقة تحديد كيفي لانتاج وتكوين البكتريا لطبقة البيوفيلم وايضا بطريقة طبق الزرع النسيجي وتعتبر هذة الطريقة تحديد كيفي وكمي لانتاج وتكوين البكتريا لطبقة البيوفيلم, واخيرا تم عمل اختبار الحساسية لجميع سلالات ميكروب *A. baumanii* المعزولة بالنسبة لاقراص المضادات الحيويه المختلفة وذلك عن طريق استخدام Disc Diffusion Method .

ولقد اسفرت النتائج علي ان كل من طريقه الانبوبة وايضا طريقة طبق الزرع النسيجي لها نفس القدرة علي تحديد تكوين و انتاج طبقة البيوفيلم حيث قد تم تحديد تكوين انتاج طبقة البيوفيلم بين ثمانية عشر سلالة من ميكروب *A. baumanii* بكلتا الطريقتين بنسبة ٦٠%, وايضا اثبتت الدراسة علي ان سلالات ميكروب *A. baumanii* لها القدرة علي مقاومة المضادات الحيوية المختلفة امثلة الاموكسسلين ٩٣.٣%, والاموكساكلاف ٨٣.٣%, وسيفتدازديم ٨٠%, وسيفوبيم٧٠%, وبيبرسيلين٨٣.٣%, واميكسين٨٠%, واخيرا قد وجد ان السلالات من ميكروب *A. baumanii* المنتجة والمكونة لطبقة البيوفيلم بتكون اكثر مقاومة للمضادات الحيوية المختلفة مجتمعة مع بعضها عن السلالات من ميكروب *A. baumanii* الغير منتجة ومكونة لطبقة البيوفيلم,وايضا ابرزت هذة الدراسة علي ان السلالات المرضية مـــن ميكروب *A. baumanii* *المنتجة* لطبقة البيوفيلم تمثل مشكلة كبيرة وخصوصا تجاه مقاومة المضادات الحيوية المختلفة مجتمعة مع بعضها ويرجع ذلك لطبقة البيوفيلم ,حيث وجد ان البيوفيلم له علاقة قوية في احداث المقاومة للمضادات الحيوية بين سلالات ميكروب *A. baumanii*. .