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Effect of Glucose On Biofilm Formation In *Acinetobacter Baumannii* Isolated From Clinical Sources

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ABSTRACT

Background : *Acinetobacter baumannii* is emerging as an important nosocomial pathogen, multidrug resistance as will as ability to withstand environmental stresses.

Result : Total of 120 specimens from various clinical sources (wounds ,burns ,urine, sputum) were collected from patients suffering from different infections reviewed

Teaching hospitals in al Ramadi city ,21 isolates of *Acinetobacter baumannii* were diagnosed and augmented with the Vitek 2 device. Ten antibiotics were used to assess the susceptibility of these bacterial isolates from different clinical sources, results of Antibiotic susceptibility test indicate that all isolate 100% were resistant to Piperacillin and Rifampicin, 85.5% resistance to Trimethoprim, 80% resistance to Amikacin, 76% were resistant to Levofloxacin, Gentamicin and Ciprofloxacin, while the resistance rate was 66.66%, 47% and 33.33% of isolates were resistant to Tobramycin , Meropenem and Imipenem respectively. Result of adding 1% glucose enhanced the biofilm formation in 19 isolates of *A. baumannii*, 7 out of these isolate formed the biofilm better than without glucose. furthermore adding of 5% glucose into (T.S.B) medium enhanced biofilm formation in another 7 out of 19 isolates formed biofilm at this concentration of glucose, meanwhile result of adding 8% glucose inhibited biofilm formation in 12 isolates , since 4 out of 12 isolates lost it′s capability of biofilm formation , as well as rest 8 isolates formed biofilm less than 1% glucose and 5% glucose .

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INTRODUCTION

A. baumannii is Coccobacilli Gram-negative, non-motile, strict aerobic, non-fermented, positive for the catalase test and negative for the oxidase test[1]. This bacteria are opportunistic pathogens responsible for 2% –10% of all infections in hospital.[2]

The increased medical interest in *A. baumannii* is due to its ability to cause many infections for people staying in hospitals; this species causes nosocomial infections and several diseases, including urinary tract infection, respiratory tract infection, skin inflammation, endocarditis, meningitis, bacteremia and pneumonia, in immunosuppressed subjects[3].

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A. baumannii is one of the bacteria that have been given the term ESKAPE (an acronym that combines the scientific names of six highly pathogenic, antibiotic-resistant pathogens, namely, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp*.) [4]. This group can avoid or eliminate the effect of many common antibiotics due to their increasing MDR , this bacteria are the leading cause of life-threatening or nosocomial infections in immune compromised patients and critically ill patients who are at a high risk .[5]

Biofilms are bacterial communities that have accumulated in a matrix and is an extracellular polymeric material made up of polysaccharides, lipids, proteins and nucleic acids[6]. Biofilm development is a complex process in which microorganisms shift their growth pattern from planktonic to sticky and is affected by a variety of environmental conditions, such as surface porosity, fluid flow and nutrient availability, biofilms are combinations of diverse microbial communities and

polymers that protect bacteria from antibiotic treatment by acting as a physical barrier.[7]

The biofilm goes through four stages during formation

First stage - bacterial attachment to the surface

Second stage - microcolony formation

Third stage - biofilm maturation

Fourth stage - detachment stage [8]

A biofilm is a protection for bacterial cells that enable them to withstand several factors, including nutrient deficiency and low pH, and provide the necessary protection for bacterial populations from host defenses , biofilm formation inside the laboratory depends on a number of physical and chemical factors, including the contents of the culture medium, temperature, pH and oxygen, biofilm formation is a means for bacteria to continue infecting [9].

Factors such as ethanol, glucosamine, temperature, and sub-inhibitory concentrations of some antibiotics have been found to influence extracellular matrix expression and biofilm formation in vitro. Glucose shows multiple effects on bacterial growth and biofilm formation.[10]

(Pan, Y., et al. 2010)[11] reported that glucose combined with sodium choired showed synergistic effect on bromating *listeria monocytogenes* biofilm formation through the accumulation of extracellular polymeric substances rather by increasing the number of viable biofilm cell . You et al (2014)[10] found that glucose induced *staphylococcus aureus* biofilm formation through the accessory protein GbaAB in a polysaccharide intracellular adhesion – dependent montk .As a carbon source and a metabolite glucose shows multiple effect on bacterial growth and biofilm formation .

MATERIALS AND METHODS

In this study, 120 different clinical samples were collected from patients who visited Al Ramadi teaching hospitals . 117 of these samples included wound , burns , sputum and UTIs , as well as 3 sample from walls and floors of operations room and pleas in tubes containing read made media to maintain the swab wet during transferring to laboratory . Each specimen was immediately inoculated on blood agar and MacConkeyś agar and blood agar plates at 37c◦ for 24 -48 hour .Identification of of *A. baumannii* studied according to microscopical , biochemical test [12] then conformed with Vitec 2 device.

Antibiotic Sensitivity

The susceptibility of *A. baumannii* isolate were determined by antibiotic disk diffusion method and compare with zone of inhibition determined by (CLSI, 2022).[13]. And to decide the susceptibility of bacteria to antimicrobial agents , whether being resistant or sensitive [14].

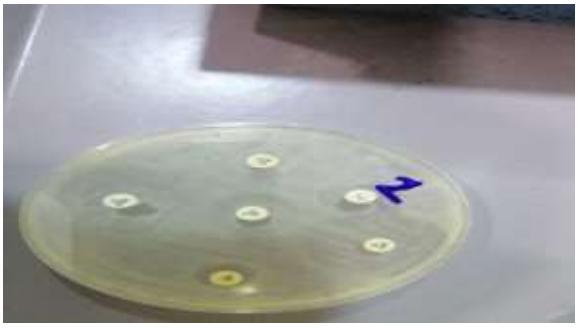

Figure (2) disc diffusion method

Biofilm formation assay using microtiter plate (MTP) .[15]

Bacterial cells obtained after a 24 hour of cultivation in (T.S.B.) + GL , were separated by Vortex Shaker . Dilute isolates by adding medium to a dilution identical to McFarland, then adding 200 µl (1 µL = 10^{-6} L = 10^{-3} ml) of sterile (T.S.B) medium to the first and second holes of the plat and Adding 200 µl of bacterial dilution to the isolates under study (two holes for each isolate .Incubate the plate was at 37 °C for 24 hours .Dispose the unrelated bacterial suspension by washing the pits with distilled water three times and leaving the plate to dry for 15 minutes.Added 200 µl of 0.1% crystal violet dye to the pits for 45 minutes. Then remove excess dye by washing the etching three times with distilled water and leaving the plate to dry for 15 minutes . After that it is added absolute ethanol to the pits and read the absorbance with an ELISA device at a wavelength of 630 nm . Compare the average reading for each two pits with the average reading for the first and second pits to estimate the biofilm formation of each isolate , the reading of the biofilm composition is according to the equations.

Effect of glucose on biofilm formation :

Briefly, 1% glucose was added to the medium (T.S.B.) which was then sterilized for 15 minutes at 121

°C and distributed to transparent tubes. Meanwhile, 5% and 8% glucose was separately added to the growth medium (T.S.B), which was monitored for 15 minutes and then distribute to transparent tubes. Afterwards, 100 µl of activated isolates were added to the tubes containing the medium (T.S.B.) and different glucose concentrations.

After cultivation of *A. baumannii* isolate for each concentration of glucose at 37c◦for 24 hour ,biofilm investigated by the absorption of crystal violet dye method in 96- hole of microtiter plate

Results and discussion

Diagnosis of *A. baumannii*

Table (1) showed that 21 isolates out of 120 sample were return to *A. baumannii* bacteria, included 9 isolates from wound and skin infection, 6 isolates from UTI, 3 isolates from respiratory tract infection (sputum), 2 isolates from burn infection and 1 from walls and floors (environment) .

Table(1) Number and sources of *A. baumannii* isolates

Source of sample	number of samples	number of isolate *A. baumannii*	percentage
Wound	37	9	42.85%
Urine	40	6	28.57%
Sputum	30	3	14.28%
Burns	10	2	9.76%
walls and floors	3	1	4.76%

Results of this study was corresponded to the results of the study [16] , whom they found that wound infections were the highest percentage of 25.20% followed by burns infections with19.81% urinary tract infection with 12.82%, respiratory infections(sputum) with 11.18% and bacteremia with 12.72%.

Results Table (2) indicate the results of antibiotic susceptibility test for *A. baumannii* . showed that all *A. baumannii* (100%) were resistant to piperacillin and rifampicin, whereas 85.5% showed resistance to trimethoprim , (80%) from isolates resistant to amikacin. A total of (76%) of the isolates were resistant to levofloxacin, gentamicin and ciprofloxacin, whereas (66.66%) , (47%) and (33.33%) resistant to tobramycin, meropenem and imipenem respectively. *A. baumannii* isolates in this study showed the lowest percentage (33.33%) of resistance to imipenem antibiotic , therefore, imipenem corded is the antibiotic of choice for the *A. baumannii* infection .

Table(2) showing antibiotic susceptibility testing according to CLS 2022

	AK	PRL	TOB	LEV-5	CN-10	TMP-10	CIP-10	RA-5	IPM-10	MEM-10
1-	R	R	R	R	R	R	R	R	R	R
2-	R	R	I	R	R	R	R	R	S	R
3-	R	R	R	S	R	R	R	R	S	I
4-	R	R	R	I	S	S	S	R	R	I
5-	R	R	R	R	S	R	R	R	I	R
6-	S	R	S	S	R	R	R	R	S	R
7-	R	R	I	R	I	R	R	R	R	I
8-	R	R	I	I	I	I	R	R	S	S
9-	S	R	R	S	R	R	R	R	I	S
10-	R	R	R	R	R	R	R	R	I	R
11-	S	R	R	R	R	R	R	R	S	R
12-	R	R	R	R	R	R	R	R	R	I
13-	R	R	I	I	R	S	I	R	I	I
14-	R	R	S	R	R	R	S	R	R	R
15-	R	R	R	R	R	R	R	R	S	S
16-	I	R	R	R	R	R	R	R	S	S
17-	R	R	R	R	S	R	R	R	R	R
18-	R	R	R	R	R	R	S	R	S	S
19-	R	R	I	R	R	R	R	R	R	R
20-	R	R	R	R	R	R	S	R	S	I
21-	R	R	R	R	R	R	R	R	S	R
%	80%	100%	66.6%	%76	76%	85.5%	76%	100%	33.4%	47%

Where AK= Amikacin, PRL= Piperacillin 100, TOB= Tobramycin, LEV-5= Levofloxacin, CN-10= Gentamicin, TMP-10= Trimethoprim, CIP-10= Ciprofloxacin, RA-5= Rifampicin, IPM-10= Imipenem, MEM-10= meropenem.

EFFECT OF GLUCOSE TO BIOFILM FORMATION:

Table No. (3) indicated the absorbance reading (A.R.) of biofilm by *A. baumannii* isolates after cultivated in (T.S.B.) medium supplemented severally with 1%, 5% and 8% of glucose incubated at 37c◦for 24 hour ,results after take into account that 0.094 was absorbance reading (A.R.) level for control wells in experiments showed that adding 1% glucose enhanced formation of biofilm in 7 isolates out of 19 *A. baumannii* isolates better than without glucose when formed superior biofilm , included 2 isolates (no. 14 and 18) which formed strong biofilm with A.R. reached to 0.625 and 0.462 respectively while it's A.R. were 0.178 and 0.259 without glucose and isolates (no.2,11 and 13) formed moderate biofilm at 1% glucose with A.R. reached to 0.188,0.204 and 0.280 respectively while these isolate formed weak biofilm without glucose when it's A.R. were 0.118, 0.145 and 0.183 respectively . Also isolate no.1 and 9 formed moderate and weak biofilm with A.R. reached to 0.294 and 0.130 at 1% glucose while not formed biofilm without glucose.

Table (3) showing the ELISA scan rate at a wavelength of 630 nm for biofilm detection

Isolate	Biofilm reading after activation of bacterial isolates on medium (T.S.B.) without the addition of glucose	Biofilm reading after activation of bacterial isolates on (T.S.B.) medium after adding 1% glucose	Biofilm reading after activation of bacterial isolates on (T.S.B.) medium after adding 5% glucose	Biofilm reading after activation of bacterial isolates on (T.S.B.) medium after adding 8% glucose
1	0.090	0.294	0.412	0.241
2	0.118	0.188	0.126	0.139
3	0.122	0.103	0.190	0.068
4	0.122	0.104	0.190	0.252
5	0.141	0.109	0.149	0.202
6	0.128	0.081	0.086	0.083
7	0.158	0.133	0.192	0.072
8	0.082	0.084	0.086	0.062
9	0.084	0.130	0.147	0.082
10	0.182	0.128	0.226	0.132
11	0.145	0.204	0.129	0.158
12	0.155	0.150	0.224	0.097
13	0.183	0.280	0.202	0.117
14	0.178	0.625	0.182	0.182
15	0.185	0.118	0.140	0.060
16	0.172	0.095	0.098	0.166
17	0.21	0.101	0.247	0.143
18	0.259	0.462	0.231	0.109
19	0.285	0.352	0.295	0.192
20	0.399	0.389	0.289	0.127
21	0.164	0.171	0.151	0.1335

Furthermore adding 5% glucose to the (T.S.B.) medium also enhanced formation biofilm in another 7 isolate of *A. baumannii* which included one isolate (no. 1) formed strong biofilm with A.R. reached to 0.412 while it negative without glucose and 6 isolates (no. 3,4,7,10,12 and 17) formed moderate biofilm at 5% glucose with A.R. reached to 0.190 , 0.190 , 0.192 , 0.226 , 0.224 , and 0.247 respectively while previous isolate formed weak biofilm at 1% glucose as well as without glucose except isolate no. 17 which formed moderate biofilm .

Table (4) showing the biofilm of the isolates after comparing the absorbance readings of the ELISA device with the absorbance readings of the control pits.

Isolate	Biofilm reading after activation of bacterial isolates on medium (T.S.B.) without the addition of glucose	Biofilm reading after activation of bacterial isolates on (T.S.B.) medium after adding 1% glucose	Biofilm reading after activation of bacterial isolates on (T.S.B.) medium after adding 5% glucose	Biofilm reading after activation of bacterial isolates on (T.S.B.) medium after adding 8% glucose
1	not configured	medium component	Powerful component	medium component
2	weak component	medium component	weak component	weak component
3	weak component	weak component	medium component	not configured
4	weak component	weak component	medium component	medium component
5	weak component	weak component	weak component	medium component
6	weak component	not configured	not configured	not configured

7	**weak component**	**weak component**	**medium component**	**not configured**
8	**not configured**	**not configured**	**not configured**	**not configured**
9	**not configured**	**weak component**	**weak component**	**not configured**
10	**weak component**	**weak component**	**medium component**	**weak component**
11	**weak component**	**medium component**	**weak component**	**weak component**
12	**weak component**	**weak component**	**medium component**	**weak component**
13	**weak component**	**medium component**	**medium component**	**weak component**
14	**weak component**	**Powerful component**	**weak component**	**weak component**
15	**weak component**	**weak component**	**weak component**	**not configured**
16	**weak component**	**weak component**	**weak component**	**weak component**
17	**medium component**	**weak component**	**medium component**	**weak component**
18	**medium component**	**Powerful component**	**medium component**	**weak component**
19	**medium component**	**medium component**	**medium component**	**medium component**
20	**Powerful component**	**Powerful component**	**medium component**	**weak component**
21	**weak component**	**weak component**	**weak component**	**weak component**

Results of this study were in agreed with the results pengfei et al [17] when they found that adding 2% and 4% of glucose significantly enhance biofilm formation in *pseudomonas aeruginosa* in time 8 – 24 hour treatment when compared to no. glucose addition and they found that glucose increase extrasellar polymeric substance (EPS) production upregulating psLA gen expression .

Meanwhile result of adding 8% glucose inhabited biofilm formation in 12 isolates of *A. baumannii* isolate when 4 of these isolate (no. 3,7 , 9 and 15) lost its capability of biofilm formation , and the rest 8 isolate (no.1,2,10,12, 13,17,18 and 20) formed biofilm less than at 5% glucose , when all these isolate expect no.1 formed weak biofilm at 8% glucose while it moderate at 5% glucose .

CONCLUSION:

This study showed that 1% and 5% glucose enhanced biofilm formation as positive stress factor . while 8% glucose inhibited biofilm formation made glucose a negative stress for *A. baumannii* isolates to forming biofilm.

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تأثير الكلوكوز على تكوين الغشاء الحيوي *لبكتريا Acinetobacter baumannll* المعزولة من مصادر سريريه مختلفة

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الخلاصة:

Acinetobacter baumannii تعد من الممرضات المهمة في المستشفيات، وتتميز بمقاومتها للأدوية المتعددة، فضلاً عن قدرتها على تحمل الضغوط البيئية وهي بكتيريا مكونة للغشاء الحيوي. في هذه الدراسة، تم تشخيص 21 عزلة تعود لهذا الجنس البكتيري من 120 عينة سريرية مختلفة جمعت من مستشفى الرمادي العام التعليمي وشخصت العزلات بجهاز Vitek 2. أظهرت نتائج اختبار حساسية المضادات الحيوية أن جميع العزلات (100٪) قاومت المضادين Piperacillin وRifampicin ، بينما أظهرت 85.5٪ من العزلات مقاومة للمضاد Trimethoprim و80٪ من العزلات أظهرت المقاومة للمضاد Amikacin. و كانت 76% من العزلات قيد الدراسة مقاومه للمضادات Levofloxacin و Gentamicin و Ciprofloxacin ، بينما كانت نسبة المقاومة 66.66٪ ، 47٪ ، 33.33٪ لكل من Tobramycin وMeropenem و Imipenem.على التوالي.

أشارت النتائج دراستنا إلى أن عزلات *A. baumannii* النامية على وسط (T.S.B) معزز بـ 1٪ كلوكوز عززت تكوين الغشاء الحيوي في 19 عزلة أقوى من نتائج تكوين الغشاء الحيوي عند تنمية العزلات على وسط بدون جلوكوز ، بحيث كانت 3 مكونه للغشاء الحيوي بشكل قوي و5 عزلات مكونة بشكل متوسط، في حين أن 11 عزله كونت غشاء بشكل ضعيف. أظهرت نتائج الغشاء الحيوي بعد تنمية العزلات قيد الدراسة على وسط (T.S.B) معززب 5٪ كلوكوز أن 19 عزلة كونت الغشاء الحيوي، كانت 10 عزلات مكونه للغشاء الحيوي بشكل متوسط و(9) عزلات كونت الغشاء الحيوي بشكل ضعيف. وعد إضافة تركيز 8٪ الجلوكوز إلى وسط التنمية (T.S.B) للعزلات مثبط لتكوين الغشاء الحيوي. حيث كونت 17 عزلة فقط الغشاء الحيوي، في حين 3 عزلات كونت الغشاء الحيوي بشكل متوسط، 14 عزلة كونت الغشاء الحيوي بشكل ضعيف و 4 عزلات لم تكون الغشاء الحيوي.