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The bacteriological study of *Acinetobacter baumannii* isolated from burn patients in Al- Nasiriyah city - Iraq.

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Safaa M.kathom **Intidhaar N. Abid***
***Dep. Pathological analysis –College of Science - University of Thi-Qar**
Emails: Intidhaar12ih-pa@sci.utq.edu.iq
alhasnawymary@gmail.com

Abstract

The aim of present study was to investigated some virulence factors of *Acinetobacter baumannii* as well as study their tolerance to some environmental factors. It was identified 20 isolates of *A.baumannii*. burn patients in Al-Hussien Teaching Hospital and other burn care units in Al-Nasiriyah city . The specimens were collected during period from August 2018 to February 2019. It were taken by swabs from pus of the burned area and then inoculated on blood agar and MacConkey agar. The isolates were diagnosed by microscopic examination, biochemical tests , API 20 E and Vitek- 2 system . some virulence factors of bacteria detected such as capsule , biofilm formation , colony factor antigens and gelatin liquification , the results revealed that100%, 85% ,75% and 65% of isolates were positive to capsule, biofilm formation , colony factor antigens producing and gelatin liquification respectively .The effect of some environmental factors on bacterial growth are studied .It is found that the optimal range of pH for bacterial growth is 6-10 ,and the optimal growth of bacteria in a concentration 5% of NaCl , as well as , it shows that all bacterial isolates have the ability to grow in 44 °C. Due to the spread of these bacteria in our hospitale ,especially in the burns unite , this study was doen o detecated its properties and virulence factors .

Keywords: *Acinetobacter baumannii* , Virulence Factors , environmental factors , burn patients

Introduction

Acinetobacter baumannii became wide spread particularly in intensive care units and burn care units because its considered normal flora in human body and change to opportunistic pathogen which have resistance to antibiotics (Poirel *et al.*, 2010 ; Abdel-El-Haleem, 2003) . The most infection that causes by these bacteria are included meningitis , urinary tract infection, peritoneal dialysis , heart disease , arthritis , osteomyelitis , eye infection and skin injuries(Braun,2008 ; Abston , 2000) .

Microbial colonization on open wounds and causes a local infection , it spread to deeper tissues lead to systemic infections, in most time the normal flora that opportunistic pathogens of burn injury (Ahmad *et al.*, 2006).

*Acinetobacter*spp. have thin hair structures on the surface. Pilli are thinner and shorter than the flagella, it facilitates adhesion and colons in host and it considered importing virulent factor of bacteria (Brooks *et al* ., 2004 ; Crouzet *et al*., 2014).

Acinetobacter spp. have two types of pilli ,thick pilli help in motile and thin pilli use by bacteria to adhesion on surface of host (Ishii *et al* .,2004 ; Gohl *et al* .,2006).

The pilli are consisting of lactin and its purified from Enterobacteriaceae , its protein structure consisted of subunit of polypeptide polymers ,these subunits called pillin , pillin is responsible of linking between the pilli and its specific receptor on the host (Gohl*et al* ., 2006).

Capsule the most of important virulence factors in *A. baumannii*, which protects from phagocytosis, dehydration,and make the bacteria more resistant to antibiotic and hosts defense ,this explains the ability of bacteria to survive in medical devices and hospital environment (Tomaras *et al*., 2003; Zhang *et al.*, 2003 ; Braun and Vidotto, 2004).

Although the *Acinetobacter* lives in single form, in most time it form aggregations with others and results mass that resist inappropriate environment and escape from the immune system , these masses are called biofilm, it can adhere to both living and non-living surfaces (King *et al* .,2009 ; Lewis, 2010).

The biofilm formation assist the cell to tolerate many factors such as pH reduction, nutrient deficiencies, and the host defenses, therefore, it is important for bacteria to continuation causes injury (Sharma *et al.*, 2014).

Several studies indicated that the fimbriae and capsule contributed to forming biofilm membrane , therefore ,these bacteria show resistance against both the host immunity and antibiotics in high degree because the transfer same virulence plasmids that have antibiotic-resistant agents between bacteria cells (Klausen *et al*., 2003) . *A.baumannii* can form the biofilm membrane , therefor it has ability to colonize on medical devices and causes resistance to many antibiotics (Rao *et al.*, 2008). Other studies show the *A.baumannii* have evaluate biofilm production when isolated from 86 different hospitals and from various clinical specimens (Davey and Otool , 2000 ; Henwood *et al* ., 2002).

The gelatin enzyme can analysis gelatin , hemoglobin , casein and the collagen during injury that found in subcutaneous tissue, therefore it can destroy the host cell (Kanemistsu *et al.*, 2001). Other studies show the *A.baumannii* have gelatinase activity when isolated this bacteria from 86 different hospitals and from various clinical specimens (Cevahir., 2008).

The growth of bacteria at 44 °C is a physiological characteristic of the species *A. baumannii* which distinguish them from other species of genus Acinetobacter (Peymani *et al*., 2011 ; Doughari *et al*., 2011).These bacteria are tolerant to a high salt concentration of NaCl and it can grow in the pH range between 5.5 -8.5 (Hood *et al* ., 2010)

Materials and Methods

Specimens collections

Atotal of 205 burn patients of Al-Hussien Teaching Hospital and other burn care units in Al-Nasiriyah city were collected . The specimens were collected during the period from August / 2018 to February /2019. Swabs inoculated on blood agar and MacConkey agar. (Himedia , India) , isolates identification was performed by routine laboratory methods

including API 20 E system(BioMerieux , France) . andVitek- 2 system (GN-ID card to Gram negative identification) (BioMerieux , France)

1-Virulence factors detection

Gelatinase liquefaction

This test was used to detect the ability of bacteria to produce the gelatinase,the gelatin media tubes were seeded with the bacterial inoculms and then incubated at 37 ° C for 24 - 48 hours , after that , the tubes were cooled in refrigerator at 4 °C for 1-2 hours , the gelatin liquification was indicated to positive result (Colleeet *al*., 1996).

Biofilm formation

This test was performed by inoculating trypton soya broth with bacterial growth by the loop an it was incubated at 37 ° C for 48 hours, after that the media were decanted carefully and stained by 1% of crystal violet for 30 minutes, then washed with D.W. and left to dry at room temperature . The results were compared with negative control. The appearance of biofilm layer on tubes surface was indicated to positive result (MacFaddin, 2000).

Detection of Colony factor antigens (CFA)

The agglutination of RBC method was used according to Collee *et al*.,(1996) as the following :

1- RBC suspension was prepared from human blood (group O) in anticoagulant tubes and it was centrifuged at 2000 rpm for 5 minutes , it was washed with normal saline for three times by centrifuge 2000 rpm for 5 minutes , and then discarded the suspension.

2-The precipitate RBC was suspended with 3% normal saline,

3- It was prepared bacterial suspension by taking half of bacterial growth from TSA medium and mixing with 1 ml of 0.15 of NaCl , one drop of bacterial suspension was mixed with one drop of the RBC suspension on a glass slide and mix by stick.The control slides were prepared by using two slides ,one of them was contained bacterial suspension with normal saline and the other slid was contained RBC suspension with normal saline .

4- The agglutination of RBC with bacteria was detected after 0.5 -2 minutes at room temperature .

Detection of capsule

The Indian ink method was used to detect the ability of bacterial isolates to the formation of the capsule according to MacFaddin(2000).

2-Detection the effect of some environmental factors on bacterial growth

Salt tolerance test

This test was performed by using a series of NaCl salt concentration from 0.5% to 6% in nutrient broth tubes and one tube was control without NaCl for comparison, these tubes were

inoculated by bacteria and incubated at 37°C for 24 hours, after that it was determined the NaCl concentration of bacterial growth (Hood *et al* ., 2010).

Test the tolerate of pH change

this test was carried out by using a series tubes of nutrient broth in different values of pH from 1 to 14 , the pH was edited by using HCl and NaOH, these tubes were inoculated by bacterial growth and incubated at 37° C for 24 hours, after incubated period , it was determined the value of the pH in which the bacteria can grow (Garrity *et al* .,2005) .

Test thetolerate of high temperatures

All isolated bacteria of *A.baumannii* were cultured on MacConkey agar media and incubated at44°C for 24 hours (MacFaddin, 2000).

Results

Identification of *Acinetobacterbaumannii* .

*Acinetobacter baumannii*is isolated from bus of burn patients using blood agar and MacConkey agar , the isolates appear white to gray colonies , 2-3 mm in diameter and non-hemolytic ,when they are cultured on blood agar , on MacConkey agar , bacteria isolates appear small , smooth with a high creamy colonies .

*Acinetobacter baumannii*mi croscopically appear as gram-negative ,coccobacilli , non-motile , and encapsulated .

Biochemical test and API20 E kit are used to identification of *Acinetobacter baumannii*,the results show that *Acinetobacter baumannii*isolates are non motile , and give negative result to oxidase , indole ,methel red ,vogesproskauer and avirable to urease and gelatinase . All isolates are positive to catalase and simmons citrate , triple-sugar-iron test was AL-kaline / no change.

It is found that all isolates give negative result to β-galactosidase , arginine dihydrolase ,lysine decarboxylase ,ornithine decarboxylase , production of hydrogen sulfide , tryptophan deaminase , , fermentation of manors , inositol, sorbitol , rhamnose , sucrose ,and amygdalin ,and all isolates give positive results to fermentation of glucose, melibiose , and arabinose by Api20 E test . VITEK-2 system is used to confirmation , to identification of the bacterial isolates .

1-Investigation of some virulence factors of *A.baumannii* (biofilm ,Colony factor antigens and gelatin liquification)

The present study shows that the capacity of bacteria to forming the biofilm appears in 17 (85%) of isolates and 3 (15%) give negative result (Figure 1).

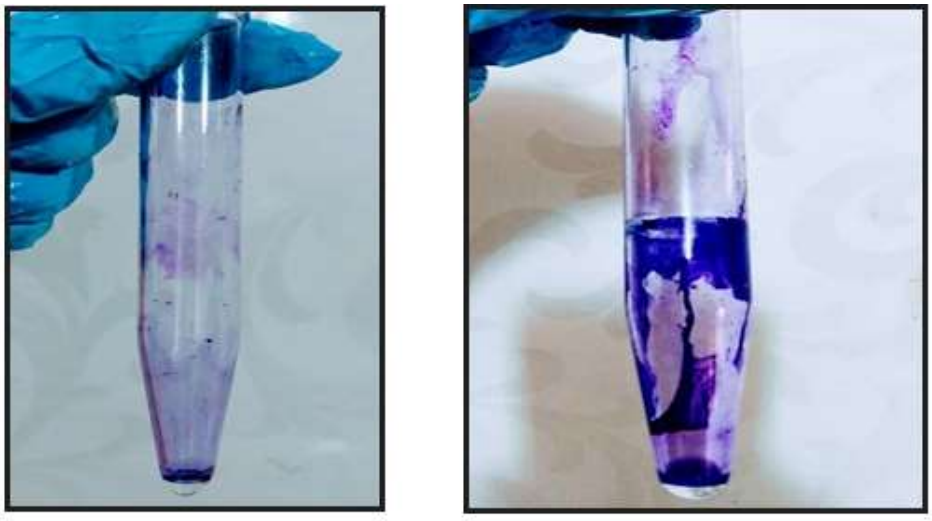

Figure (1): Biofilm formation in *A.baumannii* , the left tube shows the negative result , the right tube shows the positive result.

The results show that the 15 (75%) of isolates have the ability to clotting the RBC (have Colony factor antigens) and 5(25%) of isolates give negative result .

Table (1) shows13 that (65%) of isolates have the ability to gelatin liquification while 7(35 %) are negative to this test , and all isolates (100%) produce the capsule.

Table (1): Production of biofilm ,colony factor antigens and gelatin liquification of *Acinetobacterbaumannii*

Virulence factor	No .& (%) of positive isolates	No .& (%) of negative isolates
Biofilm	17 (85%)	3 (15%)
Colony factor antigens	15 (75%)	5(25%)
Gelatin liquification	13 (65%)	7(35 %)
Capsule	20(100%)	0(0%)

2-Effect of some environmental factors on bacterial growth .

The isolates of *Acinetobacterbaumannii* are tested for their ability to grow in different degrees of pH are ranged between 1-14 ,the results show that , the optimal range of growth bacteria is between 6-10 .

When tested the isolates to tolerance of different concentration of NaCl , the results show that ,the optimal growth of *Acinetobacterbaumannii*is at 5% of NaCl .

From the results, it is appeared that all bacterial isolates have the ability to grow in high temperature (44 °C) for 24 hr(Table 2).

Table (2): Effect pH, NaCl and temperature on growth of *Acinetobacterbaumannii.*

The factor	Optimal degree or range of bacterial growth	No. & (%)of positive isolates
PH	6-10	20 (100%)
NaCl	5%	20(100 %)
Temperature	44°C	20 (100%)

4.Discussion

The epidemiology of*Acinetobacter baumannii* was different from hospital to another, but in the most cases it became at the fifth rate of epidemiological bacteria in burns patients after *Pseudomonas aeruginosa, Staphylococcus aureus ,E. coli* and *Enterobacter*spp. (Karah ,2011) .

The current study shows 85% of *A. baumannii* to have the ability to produce biofilm , these results agree with those obtained by Nahar *et al*., (2012) who found 75% of *A. baumannii*that are able to formation biofilm , Cevahir*et al*. (2008) show that 61 from 86 of *Acinetobacter*spp. are forming biofilm. The reason for the differences in the biofilm formation may be due to several factors, including humidity, temperature and oxygen (Gotz, 2002). Many studies reported that ,the ability of bacteria to biofilm formation are assist in resistance to antibiotics, phagocytosis, opsonisation and various environmental conditions (Abdulla *et al.*, 2015).

The colony factor antigens are considered from pathogencity factors that aid to adherence of bacteria on mucous surface and epithelial cell of respiratory tract , urinary , genital and gastric tract (Brooks*et al* ., 2004). The results demonstrate that 75% of bacterial isolates are possessed these factors , these percentage are higher than that in results of Cevahir*et al*. (2008) who found 22 out of 86 isolates of *A.baumannii* are able to produce CFA.

Bacteria are capable to adherence on the surface of host cells as well as protecting them from inappropriate environmental conditions. This explains the long-term survival of bacteria in the hospital environment and causes the nosocomial infections (Ramos *et al.*, 2013).

The gelatinase is an important virulence factor of bacteria that uses lysis gelatin and collagen in subcutaneous tissue during wound infection,and assists bacteria to penetrate the cell membrane where infected humans and animals (Abdulla , 2015).

The results of this study show that 65% of *A.baumannii* is a positive for production the gelatin enzyme .These results were agreement with the results of Valli and Gopinath (2016) who found that the percentage of the gelatin enzyme production were 60% while the results

were disagree with studies of Cevahir*et al.* (2008) and Nahar*et al.* (2012) they founded the production of gelatinase by their isolates of *A.baumannii* were 14% and 0% respectively .

The results show that , *A.baumannii* can grow in pH ranged between 6-10 .These results identical with Hood *et al* ., (2010) show the *Acinetobacter*spp. can grow in the pH range between 5.5 -8.5 , Garrity*et al* .,(2005) , founded the *Acinetobacter*spp. can grow in the pH ranged between 5 -8.

In palcosol, Hrenovic*et al* .,(2014) show that the isolates of *Acinetobacter*spp. able to survive in a low pH (3.37), desiccation and in a high temperature (50°C).

This difference in results may be due to the acquired the new mutations by bacteria in recent years (Hood *et al* ., 2010) , or the other studies are included other species of *Acintobacter*and the current study was included *A. baumanniii* .

The recent study is founded *A. baumannii* isolates grow at 44°C , The growth of bacteria at 44 °C is a physiological characteristic of the species *A. baumanniii* which distinguish them from other species of genus *Acinetobacter* (Peymani*et al*., 2011).

These bacteria are tolerant to a high salt concentration of NaCl, which has reached 5%. These results similar with both Hrenovic and Ivankovic (2009) and Jasim (2014) who observed that the level of growth is decreased when the NaCl concentration increased . The tolerance of bacteria to high concentration of salts makes it highly resistant to many antibiotics (Hood *et al* ., 2010).

This study shows that the *A. baumannii* isolates are possess many virulence factors such as hemolysin , gelateinase production , Colony factor antigens , capsule and biofilm . *A. baumannii* tolerates some environmental factors such as pH ,NaCl , and a high temperatue . Therefor other studies are necessary to determine other virulence factors in bacteria.

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الخلاصة

تهدف الدراسة الحاليه الى عزل وتشخيص بكتريا *Acinetobacter baumannii* المعزولة من مرضى الحروق والكشف عن بعض عوامل الضراوة وتأثير بعض العوامل البيئية على النمو البكتيري .شملت هذه الدراسة جمع 205 مسحة سريرية من مرضى الحروق في مستشفى الحسين التعليمي ووحدات رعاية مرضى الحروق في مدينة الناصرية خلال الفترة الزمنية من اغسطس2018 الى شباط 2019 تم أخذ المسحات من قيح المناطق المحروقة للمريض ومن ثم زراعتها على أجار الدم وأجار ماكونكي. وقد تم تشخيص العزلات البكتيريه عن طريق الفحص المجهري ،الاختبارات الكيميائية الحيوية ،API 20 E و VITEK – 2.اظهرت الدراسة الحالية وجود 20 عزلة بكتيرية تابعة الى الجنس *A.baumannii*. تمت دراسة العديد من عوامل الضراوة لبكتيريا *A.baumannii* ومن بينها تكوين الغشاء الحيوي ،وإنتاج مستعمرات عوامل المستضدات ،تسييل الجيلاتين ووجود المحفظة ، وقد كانت النتائج 85 ٪ و 75 ٪ و 65 ٪ 100 ٪ على التوالي. تم دراسة تأثير بعض العوامل البيئية على نمو البكتيريا ومن بينها الرقم الهيدروجيني وتحمل الملوحة ودرجات الحرارة العالية . فقد وجد أنها تنمو في مدى يتراوح 6- 10 رقم هيدروجيني، وتنمو حتى تركيز 5 ٪ من كلوريد الصوديوم ، وكذلك لديها القدرة على النمو في درجات حرارة عالية تصل الى 44 درجة مئوية.

الكلمات المفتاحية : *Acinetobacter baumannii* مرضى الحروق , عوامل البيئة , عوامل الضراوة,