

## Virulence Determinants of *Acinetobacter baumannii* Isolated from Different Infections in Baghdad Hospitals

Sajad Numan\*, Fitua Al-Saedi\*, Israa Burhan\*, Mojtaba Mohammadzadeh Vazifeh\*\*

\*Clinical Laboratory Sciences, Pharmacy college, Mustansiriyah University, Iraq

\*\* Department of Microbial Biotechnology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran

Article Info:

DOI : <https://doi.org/10.32947/ajps.v24i3.1009>

Abstract :

Received Mar 2023

Revised Sept 2023

Accepted Oct 2023

Corresponding Author email:

[fituaaziz@uomustansiriyah.edu.iq](mailto:fituaaziz@uomustansiriyah.edu.iq)

orcid: <https://orcid.org/0000-0003-0734-8192>

*Acinetobacter baumannii* is an essential pathogenic bacteria cause nosocomial infections worldwide. The current work aimed to characterize several virulence factors in *Acinetobacter baumannii* isolated from diverse clinical specimens in Baghdad.

Clinical samples including sputum, blood, urine, CSF and wound swabs were collected from patients who were admitted to hospitals in Baghdad. Identification of *Acinetobacter baumannii* isolates was performed using Vitek 2 system. Phospholipase (plcN) and elastase (lasB) genes were identified using Polymerase chain reaction (PCR) technique. Other virulence factors such as motility, biofilm formation, hemolysin production were screened.

Sixty-nine isolates were identified as *Acinetobacter baumannii*. The isolates were screened for Phospholipase (plcN) and elastase (lasB) production. The results of amplification revealed that out of the total isolates, 18 (26.6%) isolates included both (plcN) and (lasB) genes, 18 (26.6%) isolates included only (plcN) and 23 (33.3%) isolates have only (lasB).

Sixty (86.9%) isolates were positive for biofilm formation, 8 (11.6%) isolates indicated intermediate biofilm producers and 52 (75.4%) isolates were weak biofilm producers. Sixty six *A.baumannii* isolates were motile on Nutrient agar (0.3%). Sixteen isolates were intermediate motile and 50 were highly motile. Positive association was found between the biofilm formation capacity and surface associated motility.

Fifty-seven isolates showed hemolysis on Blood agar on the second day of the incubation. Of these, 48 (69.56%) isolates show  $\beta$  –hemolysis and 9 (13.04%) isolates show  $\alpha$ -hemolysis.

**Keywords:** *Acinetobacter baumannii*, antibiotics resistance, virulence factors.

محددات الضراوة لبكتيريا *Acinetobacter baumannii* المعزولة من الإصابات المختلفة في مستشفيات بغداد

سجاد عبد العظيم نعمان\*، فتوة منور عزيز\*، اسراء برهان\*، مجتبی محمد زاده وظیفه\*\*

\*فرع العلوم المختبرية السريرية-كلية الصيدلة –الجامعة المستنصرية

\*\* قسم التقنيات الاحيائية كلية العلوم المساندة والتقنيات المتقدمة في علوم الحياة – جامعة العلوم والثقافة طهران- إيران



**الخلاصة:**

تعد بكتيريا *Acinetobacter baumannii* من البكتيريا المسببة للأمراض الأساسية التي تسبب عدوى المستشفيات في جميع أنحاء العالم. يهدف العمل الحالي إلى توصيف العديد من عوامل الضراوة في *Acinetobacter baumannii* المعزولة من مختلف العينات السريرية في بغداد. تم جمع العينات السريرية بما في ذلك البلغم والدم والبول وسائل النخاع الشوكي ومسحات الجروح من المرضى الذين تم إدخالهم إلى مستشفيات بغداد. شخّصت عزلات *Acinetobacter baumannii* باستخدام نظام Vitek 2. تم التعرف على جينات الفوسفوليبيز (plcN) والايلاستيز (lasB) باستخدام تقنية تفاعل البلمرة المتسلسل (PCR). تم فحص عوامل الضراوة الأخرى مثل الحركة، وتشكيل البيوفيلم، وإنتاج الهيمولايسين. تم تشخيص تسعة وستين عزلة على أنها *Acinetobacter baumannii*. تم فحص العزلات لاحتوائها على جينات الفوسفوليبيز (plcN) والايلاستيز (lasB). أظهرت النتائج أنه من إجمالي العزلات، 18 (26.6%) عزلة تضمنت جينات كلا من (plcN) و (lasB) و 18 (26.6%) عزلة متضمنة جين (plcN) و 23 (33.3%) عزلة متضمنة جين (lasB). ستين عزلة (86.9%) كانت موجبة لتكوين الأغشية الحيوية، بينما أشارت 8 عزلات (11.6%) إلى تكوين وسيط للغشاء الحيوي و 52 عزلة (75.4%) كانت منتجة ضعيفة للغشاء الحيوي، 66 عزلة *A.baumannii* متحركة على (Nutrient % 0.3 a gar (16) عزلة كانت متوسطة الحركة و 50 عزلة عالية الحركة. تم العثور على ارتباط إيجابي بين قدرة تكوين الأغشية الحيوية والحركة السطحية المرتبطة. أظهرت 57 عزلة انحلال دم على أجار الدم في اليوم الثاني من الحضنة. من بين هذه العزلات، أظهرت 48 عزلة (69.56%) انحلال الدم و 9 عزلات (13.04%) أظهرت انحلال الدم ألفا.

**الكلمات المفتاحية:** *Acinetobacter baumannii*, مقاومة المضادات الحيوية، عوامل الضراوة

**Introduction**

*Acinetobacter baumannii* is a significant pathogenic bacteria cause nosocomial infections worldwide (1). This pathogen is responsible for opportunistic infections (2). *A. baumannii* have virulence factors that responsible for their survival, pathogenicity and antibiotics resistance (3, 4). Virulence factors induces bacterial attachment, colonization and host cells invasion (5, 6). Enzymes and toxins production, Biofilm formation and motility are essential factors that contribute to *A. baumannii* pathogenicity and survival in different environments.

It has been reported that mutation of virulence factors related genes displayed a diminished of bacterial virulence.

Several enzymes and toxins are known as a virulence factor of *A. baumannii* includes: Phospholipases, elastase and hemolysin. Phospholipases (PLs) are a various set of enzymes that cleaves phospholipid ester bonds. They are divided into three categories

depend on the cleavage site; phospholipase A (PLA), phospholipase C (PLC), and phospholipase D (PLD). Phospholipase A cleaves fatty acids from the glycerol frame, while phospholipase C hydrolyzes the phosphorylated base group. Phospholipase D remove the head group. Phospholipases (PLs) are produced from different pathogenic bacteria causing various infectious diseases. They are considered as important virulence factors contributing to bacterial persistence or spreading without causing host cells damage. In addition, the potential role of PLs as virulence factors is to change host immune response and effect on host cellular signaling. (7, 8) Phospholipase enzymes are essential virulence features in *A. baumannii* pathogenesis. *A. baumannii* has phospholipase C (PLC), and phospholipase D (PLD) (9, 10).

Elastases are another essential virulence factors that can damage elastin and contribute to the damage of host defense or tissue in a



variety of means (11) This enzyme expressed by the *lasB* gene, which was found in *A. baumannii* isolates, which can breakdown elastin and lead to tissue damage.

The expression of adhesion factors results in bacterial attachment, a key step towards infection (12,13) and biofilm formation. Biofilms exhibit a good defense against host immune system, antimicrobials, and different un- bearable conditions than the planktonic cells (14). It has been reported that 65–80% of infectious disease are produced via biofilm- producing pathogens (15).

Another virulence factor that may aids in the pathogenicity of *A. baumannii* is motility. Though, *A. baumannii* was known as non-motile due to the lack of flagella, it is capable to move in two means: twitching motility and surface-associated motility. It has been revealed that the twitching motility is based on type IV pili ( 16, 17). For Surface-associated motility in *A. baumannii*, it has been shown that different factors affect it, such as light (18), quorum sensing ( 19), iron availability (20,21) production of lipopolysaccharides ( 22) and at least to five of the six identified efflux pump superfamilies' ( 23).

In literatures, it has been reported that a rise in bacterial motility is related to an increase in bacterial infection (24). However, *A. baumannii* mutant's incapable to motile displayed an diminished virulence (25, 23).

This study aimed to isolate *A. baumannii* from diverse clinical samples and detect several virulence factors, which induce pathogenicity and related to bacterial survival.

## Methods

### 1- Isolation and Identification of *A. baumannii* isolates.

Five hundred of various clinical samples involve sputum, blood, wound swabs, cerebrospinal fluid (CSF) and urine were collected from patients of (ALImamain AL-Kadhimein Medical City /bacteriology laboratory, Baghdad Teaching Hospital, Nu'man Teaching Hospital and Child Central Teaching Hospital) in Baghdad.

Bacterial identification was done using the VITEK-2 compact system (26).

### 2-Detection of *IasB* and *PlcN* genes

Genomic DNA was extracted from *A. baumannii* isolates using (ZR Fungal/Yeast/Bacterial DNA MiniPrep Kit (ZYMO). The extraction process was done according to the manufacture's instructions. The phospholipase (*plcN*) and elastase (*IasB*) genes were detected using specific primers (Table 1). To amplify *IasB* and *PlcN* genes, 25 µl of PCR mixture was used as shown in table 2. The amplification conditions are shown in table 3.

**Table1: Primers pairs used for the amplification of *IasB* and *PlcN* genes**

| Primer description       | Sequence                      |
|--------------------------|-------------------------------|
| <b>IasB</b> gene Forward | 5'-GGAATGAACGAAGCGTTCTC - 3'  |
| <b>IasB</b> gene Reverse | 5'-GGTCCAGTAGTAGCGGTTGG - 3'  |
| <b>PlcN</b> gene Forward | 5'-GTTATCGCAACCAGCCCTAC - 3'  |
| <b>PlcN</b> gene Reverse | 5'-AGGTCTGAACACCTGGAACAC - 3' |

**Table 2: Mixture of the specific interaction for diagnosis gene**

| Components            | Concentration |
|-----------------------|---------------|
| <b>Taq PCR PreMix</b> | 5µl           |



|                       |                                  |
|-----------------------|----------------------------------|
| <b>Forward primer</b> | 10 picomols/ $\mu$ l (1 $\mu$ l) |
| <b>Reverse primer</b> | 10 picomols/ $\mu$ l (1 $\mu$ l) |
| <b>DNA</b>            | 1.5 $\mu$ l                      |
| <b>Distill water</b>  | 16.5 $\mu$ l                     |
| <b>Final volume</b>   | 25 $\mu$ l                       |

**Table 3: The optimum conditions of detection gene**

| No. | Phase                | Tm (°C) | Time    | No. of cycle |
|-----|----------------------|---------|---------|--------------|
| 1-  | Initial Denaturation | 95°C    | 10 min. | 1 cycle      |
| 2-  | Denaturation -2      | 95°C    | 30 sec  | 35 cycles    |
| 3-  | Annealing            | 55°C    | 30 sec  |              |
| 4-  | Extension-1          | 72°C    | 30 sec  |              |
| 5-  | Extension -2         | 72°C    | 10 min. | 1 cycle      |

### 3-Biofilm detection assay (Tissue culture plate method)

*A.baumannii* was grown at 37° C for 24 hours in 10 mL Trypticase soy broth (TSB). 20  $\mu$ L of the overnight culture was added to each well of a 96-well flat-bottomed polystyrene tissue culture plate (3 wells for each strain). 180  $\mu$ L of sterile TSB medium was added in to each well. TSB broth was used as a negative control. The plates were covered and incubated at 37°C for 24 hours. Then, the content of wells was removed and the wells were washed 3X with sterile phosphate buffer saline (pH 7.2). 200  $\mu$ L of methanol was added to each well for 15 minutes. The plates were poured and left to dry. Then, 200  $\mu$ L of 2% crystal violet was added for 7 minutes. Plates were washed carefully with tap water and left to dry. To soluble the adherent cells, 160  $\mu$ L of 33% glacial acetic acid was added to each well. The optical density (OD) was measured at 630 nm using ELISA reader.

Average OD value Biofilm production

$OD \leq OD_c$  Non-adherent

$OD_c < OD \leq 2 \times OD_c$  Weakly adherent

$2 \times OD_c < OD \leq 4 \times OD_c$  Moderately adherent

$4 \times OD_c < OD$  Strongly adherent

Optical density cut-off value ( $OD_c$ ) = average OD of negative control + 3x standard deviation (SD) of negative control (28).

### 4-Motility assay

To investigate bacterial motility, a pure colony of *A. baumannii* was grown in N. broth at 37°C overnight. The bacterial suspension turbidity was adjusted to 0.5 McFarland. Ten microliters of bacterial suspension were inoculated on the center of Nutrient agar plates of 0.3%. The plates were incubated at 37°C for 24 hours. The growth area was measured using ruler. The bacterial motility was characterized depend on the criteria defined previously. These criteria involved (1) negative (-) if the growth area diameter was <5 mm; (2) intermediate (+) if the growth area diameter was 5-20 mm; and (3) highly motile (++) if the growth area diameter was >20 mm (28).

### 5-Hemolysin production

To investigate hemolytic activity, a pure colony of bacterial isolates was grown in N.broth at 37°C overnight. The suspension turbidity was adjusted to 0.5 McFarland. Ten microliters of bacterial suspension were inoculated on 5% blood agar plates. The

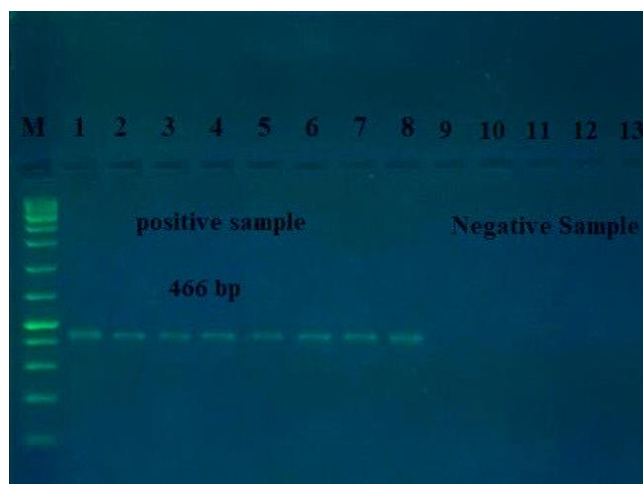


plates were then incubated at 37°C for 6 days (28)

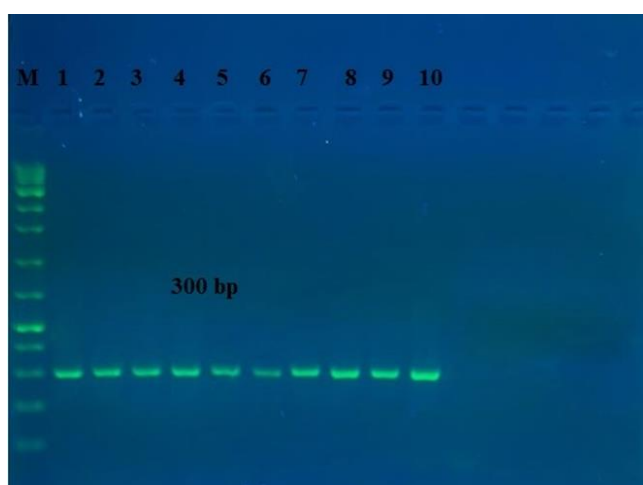
## Results

Sixty-nine isolates were identified as *Acinetobacter baumannii*. The isolates were screened for Phospholipase (plcN) and elastase (lasB) production using PCR technique. The PCR products were tested by

agarose gel electrophoresis. The results shown the presence of the expected bands. A band size of 466 bp for plcN (Figure 1), and of 300 bp for lasB gene (Figure 2). The results of amplification revealed that out of the total isolates, 18 (26.6%) isolates included both (plcN) and (lasB) genes, 18 (26.6%) isolates included only (plcN) and 23 (33.3%) isolates have only (lasB).



**Figure 1: Detection of the phospholipase (plcN) of the *A. baumannii* isolates. The figure shows results of the gel electrophoresis for detection of the phospholipase (plcN) of the *A. baumannii* isolates. Lane M: DNA Marker. Lane 1-8: plcN(466 bp).**



**Figure 2: Detection of elastase (lasB) of the *A. baumannii* isolates. The figure shows the results of the gel electrophoresis for detection of elastase (lasB) of the *A. baumannii* isolates. Lane M: DNA Marker. Lane 1-10: lasB gene (300pb).**

Sixty (86.9%) isolates were positive for biofilm formation, 8 (11.6%) isolates indicated intermediate biofilm producers and 52(75.4%) isolates were weak biofilm producers. 66 *A.baumannii* isolates were motile on Nutrient agar (0.3%). 50 isolates were highly motile and 16 were intermediate. The data of correlation revealed a significant correlation between motility and biofilm formation among *A. baumannii* isolates (Correlation coefficient =0.69,  $P<0.0001$ ).

Fifty-seven isolates showed hemolysis on Blood agar on the second day of the incubation. Of these, 48 (69.56%) isolates show  $\beta$  –hemolysis and 9 (13.04%) isolates show  $\alpha$ -hemolysis.

## Discussion

Recently, studies have stated that *A. baumannii* is a nosocomial pathogenic bacterium that spreads in hospitals. The importance of *A. baumannii* depend on their persistence in clinics and the resistance to the available antibiotics (29, 30,31). In addition to the presence of different virulence factors (32).

The results of amplification shown that out of the total isolates, 18 (26.6%) isolates included both (*plcN*) and (*lasB*) gene, 18(26.6%) isolates included only (*plcN*) and 23 (33.3%) isolates have only (*lasB*). Kareem et al., showed that (23.3%) of the Iraqi *A. baumannii* isolates were positive by PCR for *plcN* and (53.3%) for *lasB* (33). Aliramezani et.al. showed that of 100 *A. baumannii* isolates, 20 isolates included *plcN* and four isolates included *lasB* (29).

Biofilm is a critical virulence factor contributes to *A. baumannii* infections. The capability of *A. baumannii* to produce biofilm facilitates its survival in different environments (33, 34), and reduced susceptibility to antibiotics (36,37, 26).

In *A. baumannii*, several determinants regulate the biofilm-forming ability. Among

them, the biofilm-associated protein expressed by the *bap* gene (38, 39). The outer membrane protein A (*OmpA*) also play a significant role in biofilm formation (39). In addition to *blaPER-1* gene. It has been reported that *blaPER-1* gene contributes in *A. baumannii* adherence and biofilm forming (41, 42). Pili also contributes to *A. baumannii* biofilms formation and bacterial attachment—the chaperon/usher system required for the assembly and production of pili. The genes are clustered together in the form of a *csu* operon, they encode for a pilus-like bundle structure in *A. baumannii* (43). Therefore, the *csuE* gene has showed to be an essential factor in *A. baumannii* biofilm formation (44).

In the present study, 60 (86.9%) isolates were positive for biofilm formation, 8 (11.6%) isolates indicated intermediate biofilm producers and 52(75.4%) isolates were weak biofilm producers. A study reported that 60% *A. baumannii* isolates produced biofilm (45). The results of the current study demonstrated that 66 *A.baumannii* isolates were motile (surface associated motility). Motility ability was detected by measuring the diameter of bacterial movement across the nutrient agar (0.3%) plates. We found only three isolates isolated from blood were none motile. However, all other isolates were motile. Various studies have revealed the presence of the surface associated motility in *A. baumannii* (46, 47). It has been shown that surface-associated motility of *A. baumannii* ATCC 17978] was related to *rec A* protein (48), the *CheA/Y*-like protein *A1S\_2811*(49) and *CheW*-like protein, constituents of a hypothetical chemotactic signal transduction system( 48).

Surface associated motility is affected by different factors such as experimental conditions, origin of clinical isolates and environmental factors. In clinical *A. baumannii* isolates, the surface-associated



motility is common and depend on 1,3-diaminopropane (DAP)(46). Another study revealed that *A. baumannii* motility based on the source of clinical isolates. Vijayakumar et al., shown that the bacterial isolates isolated from blood samples were more motile than the bacteria isolated from sputum (49).

Based on our results, the data of correlation shown a significant correlation between motility and biofilm formation among *A. baumannii* isolates. Previous studies provide evidence of the association between the biofilm formation capacity and surface associated motility in *A. baumannii* (50, 51).

## Conclusions

Multiple virulence factors might be expressed by *A. baumannii* isolates. In the current work different virulence factors, such as motility, biofilm, hemolysin, Elastase, and phospholipase were detected among *A. baumannii* isolates. A significant correlation between biofilm formation and motility has been found.

The findings of the current study showed that multivirulent *A. baumannii* isolates might cause serious infections.

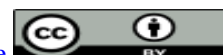
**Acknowledgement:** The authors would like to thank Mustansiriyah University

(www.uomustansiriyah.edu.iq) Baghdad-Iraq for its support in the present work.

**Funding:** Self-funding

## References

- 1- Lin, M. F., and Lan, C. Y. Antimicrobial resistance in *Acinetobacter baumannii*: from bench to bedside. *World J. Clin. Cases.* 2014; 2, 787–814.
- 2- Peleg, A. Y., Seifert, H., and Paterson, D. L. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin. Microbiol. Rev.* 2008; 21, 538–582.
- 3- Gaddy JA, Actis LA. Regulation of *Acinetobacter baumannii* biofilm formation. *Future Microbiol* 2009; 4:273-8.
- 4- Eze EC, Chenia HY, El Zowalaty ME. *Acinetobacter baumannii* biofilms: effects of physicochemical factors, virulence, antibiotic resistance determinants, gene regulation, and future antimicrobial treatments. *Infect Drug Resist* 2018; 11:2277-99.
- 5- McConnell MJ, Actis L, Pachón J. *Acinetobacter baumannii*: human infections, factors contributing to pathogenesis and animal models. *FEMS Microbiol Lett* 2013; 37:130-55.
- 6- Harding CM, Hennon SW, Feldman MF. Uncovering the mechanisms of *Acinetobacter baumannii* virulence. *Nat Rev Microbiol* 2018; 16:91-102.
- 7- Selvy PE, Lavieri RR, Lindsley CW, Brown HA. Phospholipase D: enzymology, functionality, and chemical modulation. *Chem Rev.* 2011; 111: 6064–6119.
- 8- Chen CL, Dudek A, Liang YH, Janapatla RP, Lee HY, Hsu L, et.al. d-mannose-sensitive pilus of *Acinetobacter baumannii* is linked to biofilm formation and adherence onto respiratory tract epithelial cells. *Journal of Microbiology, Immunology and Infection.* 2021; 10(2):24-38
- 9- Camarena L, Bruno V, Euskirchen G, Poggio S, Snyder M. Molecular mechanisms of ethanol-induced pathogenesis revealed by RNAsequencing. *PLoS Pathog.* 2010; 6: e1000834. doi: 10.1371/journal.ppat.1000834
- 10- Stahl J, Bergmann H, Göttig S, Ebersberger I, Aeverhoff B. *Acinetobacter baumannii* virulence is mediated by the concerted action of three phospholipases



- D. PLoS ONE 2015; 10:e0138360. doi: 10.1371/journal.pone. 0138360
- 11- Lee J, Choi C, Kang Y, et al. Differences in phenotypic and genotypic traits against antimicrobial agents between *Acinetobacter baumannii* and *Acinetobacter* genomic species 13TU. The Journal of antimicrobial chemotherapy. 2007; 59(4):633-639. Doi:10.1093/jac/dkm007.
- 12- Al-Saedi, F. Strategies in anti-adhesion therapy: A review article. Al Mustansiriyah Journal of Pharmaceutical Sciences, 2021; 21(1).
- 13- AL-Araji KM. Attachment of *Proteus mirabilis* to human urinary sediment epithelial cells in vitro. Al Mustansiriyah Journal of Pharmaceutical Sciences, 2005; 2 (2).
- 14- Gunn JS, Bakaletz LO, Wozniak DJ. What's on the outside matters: the role of the extracellular polymeric substance of Gram-negative biofilms in evading host immunity and as a target for therapeutic intervention. J Biol Chem. 2016;291(24):12538-12546.
- 15- Ramos-Gallardo G. Chronic wounds in burn injury: a case report on importance of biofilms. World J Plast Surg. 2016;5(2):175.
- 16- Wilharm G, Piesker J, Laue M, Skiebe E . DNA uptake by the nosocomial pathogen *Acinetobacter baumannii* occurs during movement along wet surfaces. J Bacteriol. 2013;195(18):4146–4153.
- 17- Harding CM, Tracy EN, Carruthers MD, Rather PN, Actis LA, Munson RS Jr. *Acinetobacter baumannii* strain M2 produces type IV pili which play a role in natural transformation and twitching motility but not surface-associated motility. MBio. 2013; https://doi.org/10.1128/mBio.00360-13
- 18- Mussi MA, Gaddy JA, Cabruja M, Arivett BA, Viale AM, Rasia R, Actis LA The opportunistic human pathogen *Acinetobacter baumannii* senses and responds to light. J Bacteriol. 2010;192(24):6336–6345.
- 19- Clemmer KM, Bonomo RA, Rather PN. Genetic analysis of surface motility in *Acinetobacter baumannii*. Microbiology. 2011;157(Pt 9):2534.
- 20- Eijkelkamp BA, Hassan KA, Paulsen IT, Brown MH Investigation of the human pathogen *Acinetobacter baumannii* under iron limiting conditions. BMC Genomics (2011) 12:126.
- 21- McQueary CN, Kirkup BC, Si Y, Barlow M, Actis LA, Craft DW, Zurawski DV. Extracellular stress and lipopolysaccharide modulate *Acinetobacter baumannii* surface-associated motility. J Microbiol. 2012 ;50 :434-43
- 22- McQueary CN, Kirkup BC, Si Y, Barlow M, Actis LA, Craft DW et al. Extracellular stress and lipopolysaccharide modulate *Acinetobacter baumannii* surface-associated motility. J Microbiol. 2012;50(3):434–443.
- 23- Eijkelkamp BA, Stroehrer UH, Hassan KA, Elbourne LD, Paulsen IT, Brown MH. H-NS plays a role in expression of *Acinetobacter baumannii* virulence features. Infect Immun. 2013;81(7):2574–2583.
- 24- Pérez-Varela M, Corral J, Vallejo JA, Rumbo-Feal S, Bou G, Aranda J, Barbé J. Mutants in the  $\beta$ -subunit of the RNA polymerase impairing the surface-associated motility and virulence of *Acinetobacter baumannii*. Infect Immun. 2017; 85:8.
- 25- Pérez-Varela M, Corral J, Aranda J, Barbé J. Roles of efflux pumps from



- different superfamilies in the surface-associated motility and virulence of *Acinetobacter baumannii* ATCC 17978. *Antimicrob Agents Chemother.* 2019;63(3).
- 26- Bazzi AM, Rabaan AA, Fawarah MM, Al-Tawfiq JA. Direct identification and susceptibility testing of positive blood cultures using high speed cold centrifugation and Vitek II system. *J Infect Public Health.* 2017;10(3):299-307
- 27- Babapour E, Haddadi A, Mirnejad R. , Angaji, S , & Amirmozafari N. Biofilm formation in clinical isolates of nosocomial *Acinetobacter baumannii* and its relationship with multidrug resistance. *Asian Pacific Journal of Tropical Biomedicine.* 2016;6 (6).
- 28- Aliramezani A, Soleimani M, Fard RMN, Nojoomi F. Virulence determinants and biofilm formation of *Acinetobacter baumannii* isolated from hospitalized patients. *Germs.* 2019 Sep 2; 9(3):148-153
- 29- Doughari HJ, Ndakidemi PA, Human IS, Benade S. The ecology, biology and pathogenesis of *Acinetobacter* spp.: an overview. *Microbes Environ* 2011; 26:101-12.
- 30- Asif M, Alvi IA, Rehman SU. Insight into *Acinetobacter baumannii*: pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. *Infect Drug Resist* 2018; 11:1249-60.
- 31- Nocera FP, Attili AR, De Martino L. *Acinetobacter baumannii*: Its Clinical Significance in Human and Veterinary Medicine. *Pathogens.* 2021;27;10(2):127.
- 32- Eze EC, Chenia HY, El Zowalaty ME. *Acinetobacter baumannii* biofilms: effects of physicochemical factors, virulence, antibiotic resistance determinants, gene regulation, and future antimicrobial treatments. *Infect Drug Resist* 2018; 11:2277-99.
- 33- Kareem SM, Al-Kadmy IMS, Al-Kaabi MH, et.al. *Acinetobacter baumannii* virulence is enhanced by the combined presence of virulence factors genes phospholipase C (plcN) and elastase (lasB). *Microb Pathog* 2017; 110:568-72.
- 34- Gaddy JA, Tomaras AP, Actis LA. The *Acinetobacter baumannii* 19606 OmpA protein plays a role in biofilm formation on abiotic surfaces and in the interaction of this pathogen with eukaryotic cells. *Infect. Immun.*2009;77, 3150–3160.
- 35- Donlan RM. Costerton JW. Biofilms: Survival mechanisms of clinically relevant microorganisms. *Clin. Microbiol. Rev.* 2002;15, 167–193.
- 36- Yang CH, Su PW, Moi SH, Chuang LY. Biofilm formation in *Acinetobacter baumannii*: Genotype-phenotype correlation. *Molecules.* 2019; 24, 1849
- 37- Gedefie A, Demsis W, Ashagrie M, Kassa Y, Tesfaye M, Tilahun M, et.al., *Acinetobacter baumannii* Biofilm Formation and Its Role in Disease Pathogenesis: A Review. *Infect Drug Resist.* 2021;10; 14:3711-3719.
- 38- Fattahian Y, Rasooli I, Gargari M, Rahbar MR, Astaneh SDA, Amani J. Protection against *Acinetobacter baumannii* infection via its functional deprivation of biofilm associated protein (Bap). *Microb. Pathog.* 2011;51, 402–406.
- 39- Aliramezani A, Douraghi M, Hajihasani A, Mohammadzadeh M, Rahbar M. Clonal relatedness and biofilm formation of OXA-23-producing carbapenem resistant *Acinetobacter baumannii* isolates from hospital environment. *Micobial. Pathog.* 2016; 99, 204–208.
- 40- Loehfelm T.W, Luke NR, Campagnari AA. Identification and characterization of



- an *Acinetobacter baumannii* biofilm-associated protein. *J. Bacteriol.* 2008, 190, 1036–1044.
- 41- Brossard KA, Campagnari AA. The *Acinetobacter baumannii* biofilm-associated protein plays a role in adherence to human epithelial cells. *Infect. Immun.* 2012, 80, 228–233.
  - 42- Lee HW, Kim J, Lee JC, LeeYC, Seol SY. Capacity of multidrug-resistant clinical isolates of *Acinetobacter baumannii* to form biofilm and adhere to epithelial cell surfaces. *Clin. Microbiol. Infect.* 2008, 14, 49–54.
  - 43- Cincaro L, Polansky O, Babak V, Kulich P, Kralik P. Changes in the Expression of biofilm-associated surface proteins in *Staphylococcus aureus* food-environmental isolates subjected to sublethal concentrations of disinfectants. *Biomed. Res. Int.* 2016, 4034517.
  - 44- Tomaras AP, Flagler MJ, Dorsey CW, Gaddy JA, Actis, LA. Characterization of a two-component regulatory system from *Acinetobacter baumannii* that controls biofilm formation and cellular morphology. *Microbiol.* 2008, 154, 3398–3409.
  - 45- Leung E.C.M, Leung P.H.M, Lai R.W. Emergence of carbapenem-resistant *Acinetobacter baumannii* ST195 harboring blaOxa-23 isolated from bacteremia in Hong Kong. *Microb. Drug Resist.* 2019, 25, 1199–1203.
  - 46- Skiebe E, de Berardinis V, Morczinek P, Kerrinnes T, Faber F, Lepka D, et al. Surface-associated motility, a common trait of clinical isolates of *Acinetobacter baumannii*, depends on 1,3-diaminopropane. *Int J Med Microbiol.* 2012, 302 (3):117–28.
  - 47- U, Skiebe E, Wilharm G. Novel Genes Required for Surface-Associated Motility in *Acinetobacter baumannii*. *Curr Microbiol.* 2021, 78 (4):1509-1528.
  - 48- Corral J, Pérez-Varela M, Barbé J, Aranda J. Direct interaction between RecA and a CheW-like protein is required for surface-associated motility, chemotaxis and the full virulence of *Acinetobacter baumannii* strain ATCC 17978. *Virulence.* 2020, 11 (1):315-326.
  - 49- Vijayakumar S, Rajenderan S, Laishram S, Anandan S, Balaji V, Biswas I. Biofilm formation and motility depend on the nature of the *Acinetobacter baumannii* clinical isolates. *Front Public Health* 2016; 4:105
  - 50- Giles S K, Stroehrer UH, Eijkelkamp BA, Brown MH. Identification of genes essential for pellicle formation in *Acinetobacter baumannii*. *BMC Microbiol.* 2015, 6;15:116.
  - 51- Chen R, Lv R, Xiao L, Wang M, Du Z, Tan, Y., et al. A1S\_2811, a CheA/Y-like hybrid two-component regulator from *Acinetobacter baumannii* ATCC17978, is involved in surface motility and biofilm formation in this bacterium. *Microbiologyopen.* 2017; 6 (5).

