Section A-Research paper
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Study of Virulence Genes in *Acintobacter baumannii* Isolated from Pediatric Patients with Hospital Acquired Bacteremia

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Abstract

Background: *Acintobacter baumannii* (*A. baumannii*) associated with hospital acquired sepsis in pediatric patients represent health care problem. The multidrug resistance of *A. baumanni* is a risk factor associated with this organism.

Objective: The present study aims were to i. study the presence of virulence factors of *A. baumannii* isolated from bacteremia from children in ICUs, ii. Study antibiotics resistance of isolated *A. baumannii* regarding ESBL and carbapenemase, iii. Study the association between the virulence factors, extended-spectrum-*β*-lactamase (ESBL) and crabapenemase activity.

Method: The study was a retrograde cross-sectional study that included one hundred children with hospital acquired bacteremia admitted to intensive care units. The isolates were subjected to polymerase chain reaction (PCR) to detect virulence genes bap (biofilm-associated protein), phospholipase (plcN), elastase (lasB), biofilm-associated protein **(bap) and** outer membrane protein A **(Omp) genes. The sensitivity to antibiotics was determined by discs diffusion method, ESBL and crabapenemase activity was determined by double discs diffusion and combined disc diffusion method respectively.**

Results: The isolated *A. baumanni* were ESBL producers (40%) and carbapenemase producers (96%). The isolates of *Acintobacter baumannii* had high frequency of bap and OmpA genes (95%, 70% respectively) and lower frequency of picN and laspB genes (26%, 8% respectively). The *A. baumannii* isolates with ESBL activity had significant association with OmpA genes (90%, P=0.001) and 57.5% of the isolates had significant association with picN gene (P=0.001). There was significant association between MDR of *A. baumannii* and the presence of OmpA gene and ***plcN*** gene (P=0.048, P=0.001 respectively**.**

The present study highlight the presence of virulence genes bap, OmpA, picN and laspB in clinical isolates of *A. baumannii* isolated from pediatric patients with sepsis. Almost all the

isolates had bap gene and more than half isolates have OmpA gene. The ESBL resistance and multi- drug resistance of *A. baumannii* was significantly associated with the presence of OmpA and picN genes.

Keywords: *Acintobacter baumannii*, ESBL, pediatric patients, sepsis, virulence genes.

Introduction

Acinetobacter baumannii (A.baumannii) is a Gram negative bacilli associated with hospital acquired infections. There are several infections linked to *Acinetobacter baumannii* such as pneumonia (1), bacteremia (2), cystitis (3), and meningitis (4). Risk factors for invasive infection with *A. baumannii* include prior hospitalization, underlying etiology of the disease, previous infection an antimicrobial therapy, and the presence of central venous or urinary catheters (5).

The major threat associated with *Acinetobacter baumannii* is marked antibiotics resistance pattern due to broad spectrum antibiotics misuse (6, 7).The common antibiotics resistance of *Acintobacter baumannii* is due to horizontal gene transfer that leads to resistance of β-lactams antibiotics (8). Moreover, the presence of adaptive genes in the genome of *Acintobacter baumannii* leads to its prolonged persistence in the hospital environment (9, 10).

The mechanisms of antibiotics resistance of *Acinetobacter* spp. are mediated through different mechanisms such as the production of *β*-lactamases, alterations in outer membrane proteins, production of penicillin-binding proteins, and enhanced activity of efflux pumps (11). The *β*-lactamases in *Acintobacter* spp. include extended-spectrum-*β*-lactamases (ESBLs), metallo-*β*-lactamases (MBLs), and oxacillinases (12).

The pathogenesis of *Acintobacter baumannii* depends upon the expression of various virulence factors that play a vital role in adherence of the organism to the host cells. These virulence factors are bap (biofilm-associated protein), OmpA (outer membrane protein A), phospholipaseD, the Csu (chaperone-usher type pilus), the Acinetobacter trimeric autotransporter (Ata), and the acinetobactin-
mediated iron acquisition system (13-15).

The virulence genes mediate their actions principally through adherence to the host cells. The bacterial cell surface expressing OmpA leads to cytotoxicity by binding to cell surface death receptors (16). The translocation of OmpA in the mitochondria leads proapoptotic signals, through the activation of Bcl-2 family proteins, the release of cytochrome C and apoptosis-inducing factor (17). OmpA can also be translocated to the nucleus courtesy of its self-encoded nuclear localization signal (KTKEGRAMNRR) leading to the degradation of the host DNA in a DNase I-like manner (18-20). In addition to its cytotoxic properties, OmpA is associated with other virulence attributes, including resistance to alternate complement-mediated killing through factor H binding and promoting adhesion to extracellular matrix proteins, including fibronectin, which is important for lung epithelial colonization (21, 22).

The Bap virulence factor promotes cell adhesion, biofilm formation (23, 24). Csu Type 1 chaperone-usher pili also play a vital role in adhesion and biofilm formation which establish

persistence of *Acintobacter baumannii* in hospital environment and leads to difficulty in eradication of the infections (23, 24).

The phospholipases aids in the invasion of *Acintobacter baumanni*. The trimeric autotransporter adhesin Ata are known as important virulence factor that regulate adhesion, the formation of biofilm, immune evasion, angiogenesis or cell death (25-28).

There are limited studies about infections of pediatric patients in intensive care units (ICUs) with *Acintobacter baumannii* and the risk factors associated with this infection, antibiotics resistance and virulence genes.

Therefore, the present study aims were to i. study the presence of virulence factors of *A. baumannii* isolated from bacteremia from children in ICUs, ii. Study antibiotics resistance of isolated *A.baumannii* regarding ESBL and carbapenemase, iii. Study the association between the virulence factors, ESBL and crabapenemase activity.

Material and Method

The study was a retrograde cross-sectional study that included children with hospital acquired bacteremia admitted to ICUs at Mansoura University Hospital from January 2020 till January 2022. The diagnosis of hospital acquired bacteremia was based upon the criteria of center of diseases control (CDC) criteria (29). The included children were with age below 18 years admitted to hospital with duration ≥ 48 hours with positive blood culture for *Actinobacter baumannii*. The study was approved by Mansoura Faculty of Medicine ethical committee-(R.22.09.1851) and the study was performed according to Helsinki guidelines.

Medical history was taken for each child and clinical examination was performed.

Blood Culture

Eight militer blood samples were obtained from each child under complete sterile conditions. Blood samples were subjected to automated blood culture by Bact/alert system (Biomerieux-USA). The positive blood culture bottles were subjected to subculture on blood agar, and chocolate agar and incubated for 24 hours at 37ºC. The sub cultured colonies were identified by Gram stain and Gram negative bacilli were further identified by biochemical reaction by API 20 E. The final confirmation was performed by detection of bla OXA-51 carbapenemase gene by PCR (29).

Antibiotics susceptibility by Disc Diffusion Method

The sensitivity of *Acintobacter baumannii* to antibiotics was determined by the use of antibiotics discs diffusion method according to Clinical and laboratory Standard Institute (CLSI) guidelines (30). The antibiotics discs were ceftazidime (30 μg), cefepime (30 μg), imipenem (10 μg), meropenem (10 μg), gentamicin (10 μg), amikacin (30 μg), sulfamethoxazole/trimethoprim (1.25/23.75 μg), and piperacillin/tazobactam (100 μg/10 μg) and cefotaxime (30).

Combined Discs Test for Detection of carbapenemase

Isolates resistant to imipenem and/ or meropenem were further studied by double disc containing imipenem and EDTA. *Acintobacter baumannii* isolates were diluted with Muller-Hinton broth to obtain optical density equal to 0.5 McFarland opacity standard and sub cultured on Mueller-Hinton agar and two 10 μg imipenem discs were placed on the culture plate with

20 mm distance from center to center of the discs and 10 μl of 0.5 M ethylenediaminetetraacetic acid (EDTA)was added to one of the imipenem discs. The plate was incubated at 37ºC for 18 hours. The increase in the sensitivity diameter ≥7 mm around the disc of imipenem-EDTA disc compared to the imipenem disc were considered as carbapenemase producers (30).

Detection of ESBL Producing Isolates

The detection of ESBLs production was performed according to CSLI guidelines by double discs diffusion method. Isolates resistant to ceftazidime and / or cefotaxime were diluted with Muller-Hinton broth to obtain optical density equal to 0.5 McFarland opacity standard and sub cultured on two Mueller-Hinton agar plates and on one plate double discs were added one cetazidime disc and the other disc was cetazidime with clavulinic acid(30/10 μg) on the other plate two discs were added one disc of cefotaxime acid and the other disc was cefotaxime (30 μg) + clavulanic (10 μg) , then the plates were incubated at 37ºC for 18 hours. The increase of the inhibition zone t ≥5 mm around the ceftazidime/clavulanic acid disc and cefotaxime/clavulanic disc compared to ceftazidime and cefotaxime was defined as a ESBLs producing isolate (30).

Polymerase Chain Reaction for Detection of Virulence Genes

DNA Extraction

Colonies from *Acintobacter baumannii* were used for DNA extraction by ready to use QIAamp DNA Micro Kit (Qiagen –Germany). The extraction was performed according to the manufacturer instruction. The extracted DNA was kept frozen at -20ºC till amplification.

Molecular detection of bap (biofilm-associated protein), phospholipase (*plc*N) and elastase (*las*B) and *bap* and Omp genes

The used primers for the amplification were listed in table 1. The amplification was performed by the use of Qiagen amplification mixture with 20 µL of master mix containing with added 2 microns of extracted DNA and 10 pM reverse and forward primers (1 µL each). The amplification procedures included denaturation at 95ºC for 10 minutes, followed by 35 cycles' 0f heating at 95ºC for 30 seconds then 55ºC for 30 seconds and 72ºC for 30 seconds. The final step was elongation at 72°C for 10 minutes. The amplified products were subjected to electrophoresis at 1.5 gel with ethidium bromide for 20 minutes (31).

Table (1): The sequences of the used primers and the amplified products base pair(bp)

Gene	Primers	bp
bla$_{\text{oxa-51-like}}$	5'-TAATGCTTTGATCGGCCTTG-3' 5'-TGGATTGCACTTCATCTTGG-3'	353
bap	5'-ATGCCTGAGATACAAATTATTGCCAAGGATAATC-3' 5'-AGGTGCTGAAGAATCATCATCATTAC-3'	**560**

plcN	5'-GTTATCGCAACCAGCCCTAC-3' 5'-AGGTCGAACACCTGGAACAC-3'	466
lasB	**5'-GGAATGAACGAAGCGTTCTC-3'** **5'-GGTCCAGTAGTAGCGGTTGG-3'**	300
OmpA	**TGGTCACTTGAAGCCTGCTG** **TGCAGTAGCGTTAGGGTATTCAG**	**208**

Statistical Analysis

The data of the study was analyzed by SPPS22. The qualitative data was expressed as number and percentages. The comparison was performed by Chi-square test and P was considered significant if <0.05. The numerical data was expressed as mean and standard deviation for parametric data and expressed as minimum, maximum and median if it was non parametric.

Results

The study included 100 pediatric patients with hospital acquired bacteremia associated with *A. baumannii*. *Acintobacter baumannii* was identified by biochemical reactions and PCR. The age of the patients were from 1.00 month up to 192.00 months with median age 24 months. The patients were 51% males and 49% females. The hospital used devices 'were urinary tract catheter, peripheral venous catheter and central venous catheter in 84%, 78% and 27% of the patients respectively. The common signs were fever, bradycardia and hypothermia in 40%, 48% and 26% respectively, table 2.

The isolated *A. baumanni* were ESBL producers (40%) and carbapenemase producers (96%). The isolates had high resistance to sulfamethoxazole/trimethoprim (46%), imipenem (96%), meropenem (95%), amikacin (52%), gentamicin (55%) cefotaxime (42%), ceftazidime (38%) and cefepime (34%). MDR resistance *A. baumannii* with resistance≥ three antibiotics classes, were 55%, table 3

The isolates of *Acintobacter baumannii* had high frequency of bap and OmpA genes (95%, 70% respectively) and lower frequency of picN and laspB genes (26%, 8% respectively), table 4.

The *A. baumannii* isolates with ESBL activity had significant association with OmpA genes (90%, P=0.001) and 57.5% of the isolates had significant association with picN gene (P=0.001).), table 5.
There was significant association between MDR of A. baumannii and the presence of OmpA gene and ***plcN gene (*P=0.048, P=0.001 respectively, table 6.**

Table (2): Demographic and clinical data of the studied patients

Parameter	
Sex **Male** **Female**	 51% 49%
Age –months Minimum Maximum Median	 1.00 192.00 24.00
Central venous catheter (CVC)	27%
Urinary catheter	84%
Peripheral venous cannula	78%
Fever	40%
Hypothermia	26%
Apnea	25%
Bradycardia	46%
Oliguria	13%

Table (3): Antibiotics resistance of isolated *A. baumannii*

Antibiotics	%
ceftazidime	38
cefepime	34
imipenem	96
meropenem	95
gentamicin	55
amikacin	52
sulfamethoxazole/trimethoprim	46
piperacillin/tazobactam	28
Cefotaxime	42
Multidrug resistance	55
ESBL	40

Carbapenemase	96

Table (4): Virulence genes detected in isolated *A. baumannii*

Gene	**%**
bap	**95**
OmpA	**70**
plcN	**26**
lasB	**8**

Table (5): Association of virulence genes, ESBLs and carabamanse in *A. baumannii*

	ESBL		**Carbapenemase**	
	Positive (n=40) No. %	**Negative (n=60) No. %**	**Positive (n=96) No. %**	**Negative (n=4) No. %**
Bap				
Positive	**40 100%**	**55 91.7%**	**91**	**4**
Negative	**0 0%**	**5 8.3%**	**5**	**0**
P	**0.06**		**0.64**	
OmpA				
Positive	**36 90%**	**34 56.7%**	**67**	**3**
Negative	**4 10%**	**26 43.3 %**	**29**	**1**
P	**0.001**		**0.82**	
plcN				
Positive	**23 57.5%**	**3 5%**	**24**	**2**
Negative	**17 42.5%**	**57 95 %**	**72**	**2**
P	**=0.001**		**0.25**	
lasB				
Positive	**3 7.5%**	**5 8.3%**	**8**	**0**
Negative	**37 92.5%**	**55 91.7%**	**88**	**4**
P	**0.9**		**0.55**	

Table (6): Association of virulence genes and MDR resistance in A. baumannii

	MDR (n=55)	Non MDR (n=45)
	No. %	No. %
Bap		

Positive	53 96.4%	42 93.3%
Negative	2 3.6%	3 6.7%
P	0.5	
OmpA		
Positive	43 78.2%	27 60%
Negative	12 21.8%	18 40%
P	0.048	
plcN		
Positive	23 41.8%	3 6.7%
Negative	32 58.2%	42 93.3%
P	0.001	
lasB		
Positive	4 7.3%	4 8.9%
Negative	51 92.7%	41 91.1%
P	0.8	

Discussion

Health care associated infections represent major health problem with safety risk of hospitalized children. These infections increase morbidity mortality and prolonged hospitalization with higher health costs (32, 33).

In the present study among 100 pediatric patients with bacteremia, the urinary catheter and CVC were common devices used in pediatric patients. This finding was online with previous reports, these devices were predisposing factors for HAIs among pediatric patients (34, 35). Previous study from United states of America revealed improvement in infections associated with urinary catheter while the improvement was not reported for central venous catheter associated infections, reflecting the need for novel approach for prevention measures (36). There is a need for adherence to the central catheter insertion and maintenance bundles (37). Moreover, there may be a need for different approach in maintenance of central venous catheter in children such as the use of ethanol locks, inclusion of oral care to reduce the risk of translocation from mucositis and inclusion of new criteria for surveillance of central venous catheter bacteremia after 48 hours of admission (38). For prevention of urinary catheter associated infections strict adherence infection prevention bundle is needed with extending beyond aseptic technique and emphasizing appropriate indications for placement and timely removal of indwelling urinary catheters (39).

In the present study, the use of peripheral venous cannula was detected in 78% of the infected pediatrics. There are well known complications associated with peripheral venous cannula such as hematoma, phlebitis, extravasation and bruising (40). Systemic review claimed that the blood stream infections might be a possible complication for prolonged use of peripheral cannula (41). Previous study determined blood stream infections association with insertion of peripheral

cannula and this infection may be attributed to catheter-dressing disruptions and proximal insertion of the cannula as predisposing factors to these infections (43).

Infections by *Acinobacter baumanii* had increased due to use of medical devices and improper use of antibiotics a (43).

The isolates of *A. baumannii* had high resistance to sulfamethoxazole/trimethoprim (46%), imipenem, meropenem, cefotaxime and cefepime amikacin,gentamicin and multi drug resistance was detected in 45% of isolated *A. baumannii*.

The resistance rates of *A. baumannii* varied between different studies from 31.8 to 92.1% to ceftazidime; 8.8 to 89.9% vs imipenem, from 12.2 to 89.9% vs piperacillin / tazobactam, from 28.8 to 91.6% vs fluoroquinolones and 30 to 90.3% vs aminoglycosides (44-46). This variation of resistance rates can be attributed to the difference in the antibiotics prescription policy.

The isolated *A. baumanni* were ESBL producers (40%) and carbapenemase producers (96%). The rates of ESBL production in *A. baumannii* ranged from 22% up to 59% % (47, 48). Similar rate of carbapenemase production was reported previously from Egypt (97.5%) (49).

The differences in the reporting frequency may be attributed to the difficulty of ESBL identification in *Acinetobacter* spp. due to the lack of the standard protocols (50).

Other hazard associated with *A. baumannii* is the presence of virulence genes such as bap and omp (51).

In the present study, more than half isolates of *Acintobacter baumannii* had high frequency of bap and OmpA genes and lower frequency was reported for picN and laspB genes. These rates were similar to previous studies (52. 53).

In general, the increasing number of *A. baumannii* infections due to the bacterial persistence in hospital environments and acquiring of virulence factors by the bacteria are major health concerns (53).

In the present study, there was significant association between OmpA and picN genes and ESBL activity. Moreover, there was significant association between MDR of isolated *A. baumannii* and both genes. The exact mechanism of association between OmpA gene and antibiotics resistance may be attributed to extrusion of antibiotics from the bacterial cells through outer membrane and couples with inner membrane efflux systems, such as major facilitator superfamily efflux pumps or RND systems lacking the OMP component (54). The other mechanism that may be associated with virulence genes leading to antibiotics resistance is biofilm formation. The biofilm formation leads to decrease of antibiotics diffusion, slow rate of bacterial growth and adaptive mechanisms to antibiotics by the formed biofilm (55).

The finding of such association between ESBL and virulence genes may suggest the use of anti-virulence agents for management of antibiotics resistant infections associated with A. baumannii (56).

Conclusion

The present study highlight the presence of virulence genes bap, OmpA, picN and laspB in clinical isolates of *A. baumannii* isolated from pediatric patients with sepsis. Almost all the isolates had bap gene and more than half isolates have OmpA gene. The ESBL resistance and

multi- drug resistance of *A. baumannii* was significantly associated with the presence of OmpA and picN genes.

Reference

1-Jung, S.Y.; Lee, S.H.; Lee, S.Y.; Yang, S.; Noh, H.; Chung, E.K.; Lee, J.I. Antimicrobials for the treatment of drug-resistant
acinetobacter baumannii pneumonia in critically Ill Patients: A systemic review and bayesian network meta-analysis. Crit. Care
2017, 21, 319. [CrossRef] [PubMed]

2- Eliopoulos, G.M.; Maragakis, L.L.; Perl, T.M. Acinetobacter baumannii: Epidemiology, antimicrobial resistance, and treatmentoptions. Clin. Infect. Dis. 2008, 46, 1254–1263. [CrossRef]

3-Jiménez-Guerra, G.; Heras-Cañas, V.; Gutiérrez-Soto, M.; Del Pilar Aznarte-Padial, M.; Expósito-Ruiz, M.; Navarro-Marí, J.M.; Gutiérrez-Fernández, J. Urinary tract infection by Acinetobacter baumannii and pseudomonas aeruginosa: Evolution of antimicrobial resistance and therapeutic alternatives. J. Med. Microbiol. 2018, 67, 790–797. [CrossRef] [PubMed]

4-Siegman-Igra, Y.; Bar-Yosef, S.; Gorea, A.; Avram, J. Nosocomial acinetobacter meningitis secondary to invasive procedures: Report
of 25 cases and review. Clin. Infect. Dis. 1993, 17, 843–849.

5-Ozdemir H, Kendirli T, Ergün H, Ciftçi E, Tapisiz A, Güriz H, Aysev D, Ince E, Doğru U. Nosocomial infections due to Acinetobacter baumannii in a pediatric intensive care unit in Turkey. Turk J Pediatr. 2011 May-Jun;53(3):255-60. PMID: 21980805.

6-N. Duan, L. Sun, C. Huang, H. Li, and B. Cheng, "Microbial distribution and antibiotic susceptibility of bloodstream infections in different intensive care units," *Frontiers in Microbiology*, vol. 12, Article ID 792282, 2021.

7- J. Zhao, Y. Zhu, J. Han et al., "Genome-scale metabolic modeling reveals metabolic alterations of multidrug-resistant *Acinetobacter baumannii* in a murine bloodstream infection model," *Microorganisms*, vol. 8, no. 11, p. 1793, 2020. View at: Publisher Site | Google Scholar

6- M. D. Zilberberg, M. H. Kollef, and A. F. Shorr, "Secular trends in *Acinetobacter baumannii* resistance in respiratory and blood stream specimens in the United States, 2003 to 2012: a survey study," *Journal of Hospital Medicine*, vol. 11, no. 1, pp. 21–26, 2016.

7-Noori M., Karimi A., Fallah F., et al. High prevalence of metallo-beta-lactamase producing *Acinetobacter baumannii* isolated from two hospitals of Tehran, Iran. *Archives of Pediatric Infectious Diseases*. 2014;2(3)

8- Cerqueira G. M., Peleg A. Y. Insights into Acinetobacter baumannii pathogenicity. *IUBMB Life*. 2011;63(12):1055–1060. doi: 10.1002/iub.533.

9-Riess T, Andersson SGE, Lupas A, et al. *Bartonella* adhesin a mediates a proangiogenic host cell response. *J Exp Med*. 2004;200:1267–1278.

10- Raghunathan D, Wells TJ, Morris FC, et al. SadA, a trimeric autotransporter from *Salmonella enterica* serovar Typhimurium, can promote biofilm formation and provides limited protection against infection. *Infect Immun*. 2011;79:4342–4352.

11-Cerqueira G. M., Peleg A. Y. Insights into Acinetobacter baumannii pathogenicity. *IUBMB Life*. 2011;63(12):1055–1060. doi: 10.1002/iub.533. [PubMed] [CrossRef] [Google Scholar]
12- Noori M., Karimi A., Fallah F., et al. High prevalence of metallo-beta-lactamase producing *Acinetobacter baumannii* isolated from two hospitals of Tehran, Iran. *Archives of Pediatric Infectious Diseases.* 2014;2(3)]
13-Smani Y, McConnell MJ, Pachon J. Role of fibronectin in the adhesion of Acinetobacter baumannii to host cells. PLoS One. 2012;7(4). e33073. doi: 10.1371/journal.pone.0033073. [PubMed: 22514602]. [PubMed Central: PMC3326023
14-El Tahir Y, Skurnik M. YadA, the multifaceted *Yersinia* adhesin. *Int J Med Microbiol*. 2001;291:209–218.
15-Pearson MM, Laurence CA, Guinn SE, et al. Biofilm formation by *Moraxella catarrhalis in vitro*: roles of the UspA1 adhesin and the Hag hemagglutinin. *Infect Immun*. 2006;74:1588–1596.
16-Ahmad T. A., Tawfik D. M., Sheweita S. A., Haroun M., El-Sayed L. H. (2016). Development of immunization trials against *Acinetobacter baumannii*. *Trials Vaccinol.* 5, 53–60. 10.1016/j.trivac.2016.03.001 [CrossRef] [Google Scholar]
17-Hee C. C., Young L. E., Chul L. Y., In P. T., Jung K. H., Hee H. S., et al. (2005). Outer membrane protein 38 of *Acinetobacter baumannii* localizes to the mitochondria and induces apoptosis of epithelial cells. *Cell. Microbiol.* 7, 1127–1138. 10.1111/j.1462-5822.2005.00538.x
18-Hee C. C., Hee H. S., Young L. J., Sik L. J., Seok L. Y., Ae K. S., et al. (2008). *Acinetobacter baumannii* outer membrane protein A targets the nucleus and induces cytotoxicity. *Cell. Microbiol.* 10, 309–319. 10.1111/j.1462-5822.2007.01041.x
19-Choi C. H., Hyun S. H., Kim J., Lee Y. C., Seol S. Y., Cho D. T., et al. (2008a). Nuclear translocation and DNAse I-like enzymatic activity of *Acinetobacter baumannii* outer membrane protein A. FEMS Microbiol. Lett. 288, 62–67. 10.1111/j.1574-6968.2008.01323.x
20-Rumbo C., Tomás M., Fernández Moreira E., Soares N. C., Carvajal M., Santillana E., et al.. (2014). The *Acinetobacter baumannii* Omp33-36 porin is a virulence factor that induces apoptosis and modulates autophagy in human cells. Infect. Immun. 82, 4666–4680. 10.1128/IAI.02034-14, PMID: [
21-Kim S. W., Choi C. H., Moon D. C., Jin J. S., Lee J. H., Shin J.-H., et al.. (2009). Serum resistance of *Acinetobacter baumannii* through the binding of factor H to outer membrane proteins. FEMS Microbiol. Lett. 301, 224–231. 10.1111/j.1574-6968.2009.01820.x,
22-Smani Y., McConnell M. J., Pachón J. (2012). Role of fibronectin in the adhesion of *Acinetobacter baumannii* to host cells. *PLoS One* 7:e33073. 10.1371/journal.pone.0033073, PMID:
23-Loehfelm T. W., Luke N. R., Campagnari A. A. (2008). Identification and characterization of an *Acinetobacter baumannii* biofilm-associated protein. *J. Bacteriol.* 190, 1036–1044. 10.1128/JB.01416-07,
24-Brossard K. A., Campagnari A. A. (2011). The *Acinetobacter baumannii* biofilm associated protein (Bap) plays a role in adherence to human epithelial cells. *Infect. Immun.* 80, 228–233.

10.1128/IAI.05913-11
25-Riess T, Andersson SGE, Lupas A, et al. *Bartonella* adhesin a mediates a proangiogenic host cell response. *J Exp Med*. 2004;200:1267–1278
26-Raghunathan D, Wells TJ, Morris FC, et al. SadA, a trimeric autotransporter from *Salmonella enterica* serovar Typhimurium, can promote biofilm formation and provides limited protection against infection. *Infect Immun*. 2011;79:4342–4352.
27- El Tahir Y, Skurnik M. YadA, the multifaceted *Yersinia* adhesin. *Int J Med Microbiol*. 2001;291:209–218
28-[13] Pearson MM, Laurence CA, Guinn SE, et al. Biofilm formation by *Moraxella catarrhalis in vitro*: roles of the UspA1 adhesin and the Hag hemagglutinin. *Infect Immun*. 2006;74:1588–1596.
29-Turton J. F., Woodford N., Glover J., Yarde S., Kaufmann M. E., Pitt T. L. Identification of Acinetobacter baumannii by detection of the bla OXA-51-like carbapenemase gene intrinsic to this species. *Journal of Clinical Microbiology*. 2006;44(8):2974–2976. doi: 10.1128/JCM.01021-06.
30-Clinical and Laboratory Standards Institute (CLSI) M100-S23. Wayne, Pa, USA: CLSI; 2013. Performance standards for antimicrobial susceptibility testing; twentythird informational supplement. [
31-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. doi: 10.18683/germs.2019.1171. PMID: 31646145; PMCID: PMC6783638.
332-Miller MR, Niedner MF, Huskins WC, et al; National Association of Children's Hospitals and Related Institutions Pediatric Intensive Care Unit Central Line–Associated Bloodstream Infection Quality Transformation Teams. Reducing PICU central line-associated bloodstream infections: 3-year results. Pediatrics. 2011;128(5):e1077-e1083. doi:10.1542/peds.2010-3675PubMedGoogle ScholarCrossref
33-Septimus EJ, Moody J. Prevention of device-related healthcare-associated infections. F1000Res. Published online January 14, 2016. Accessed September 16, 2019. https://f1000research.com/articles/5-65/v1
. 34-Mobley RE, Bizzarro MJ. Central line-associated bloodstream infections in the NICU: successes and controversies in the quest for zero. Semin Perinatol. 2017;41(3):166-174.
35-Letica-Kriegel AS, Salmasian H, Vawdrey DK, et al. Identifying the risk factors for catheter-associated urinary tract infections: a large cross-sectional study of six hospitals. BMJ Open. 2019;9(2):e022137. doi:10.1136/bmjopen-2018-022137PubMedGoogle Scholar
-3636-Hsu HE, Mathew R, Wang R, et al. Health Care–Associated Infections Among Critically Ill Children in the US, 2013-2018. JAMA Pediatr. 2020;174(12):1176–1183. doi:10.1001/jamapediatrics.2020.3223
37- Furuya EY, Dick AW, Herzig CT, Pogorzelska-Maziarz M, Larson EL, Stone PW. Central line–associated bloodstream infection reduction and bundle compliance in intensive care units: a national study. *Infect Control Hosp Epidemiol*. 2016;37(7):805-810.

doi:10.1017/ice.2016.67PubMedGoogle ScholarCrossref
38-Dantes RB, Abbo LM, Anderson D, et al. Hospital epidemiologists' and infection preventionists' opinions regarding hospital-onset bacteremia and fungemia as a potential healthcare-associated infection metric. *Infect Control Hosp Epidemiol*. 2019;40(5):536-540. doi:10.1017/ice.2019.40
40- Siegel BI, Figueroa J, Stockwell JA. Impact of a daily PICU rounding checklist on urinary catheter utilization and infection. *Pediatr Qual Saf*. 2018;3(3):e078. doi:10.1097/pq9.0000000000000078
41- Mermel LA. Short-term peripheral venous catheter-related bloodstream infections: a systematic review. *Clin Infect Dis.* 2017;65(10):1757–1762. doi: 10.1093/cid/cix562
42- Buetti N, Marschall J, Drees M, Fakih MG, Hadaway L, Maragakis LL, Monsees E, Novosad S, O'Grady NP, Rupp ME, Wolf J, Yokoe D, Mermel LA. Strategies to prevent central line-associated bloodstream infections in acute-care hospitals: 2022 Update. Infect Control Hosp Epidemiol. 2022 Apr 19;43(5):1-17
43- 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 Aug 21;11:1249-1260
44- Xu J, Sun Z, Li Y, Zhou Q. Surveillance and Correlation of Antibiotic Consumption and Resistance of Acinetobacter baumannii complex in a Tertiary Care Hospital in Northeast China, 2003–2011. Int J Environ Res Public Health. 2013;10:1462–1473.
45-Al-Mously N, Hakawi A. Acinetobacter baumannii bloodstream infections in a tertiary hospital:Antimicrobial resistance surveillance. Int J Infect Control. 2013;9(2):1–8. [Google Scholar]
46-Jaggi N, Sissodia P, Sharma L. Acinetobacter baumannii isolates in a tertiary care hospital: Antimicrobial resistance and clinical significance. J Microbiol Infect Dis. 2012;2(2):57–63
47-Khan MFK, Rashid SS, Ramli ANM, Ishmael UC, Maziz MNH. Detection of ESBL and MBL in *Acinetobacter* spp. and their plasmid profile analysis. Jordan J Biol Sci 2019;12:373–378.
48-Abd El-Baky RM, Farhan SM, Ibrahim RA, Mahran KM, Hetta HF. Antimicrobial resistance pattern and molecular epidemiology of ESBL and MBL producing Acinetobacter baumannii isolated from hospitals in Minia, Egypt. Alexandria J Med 2020;56:4–13.
49-Alkasaby NM, El Sayed Zaki M. Molecular Study of Acinetobacter baumannii Isolates for Metallo-β-Lactamases and Extended-Spectrum-β-Lactamases Genes in Intensive Care Unit, Mansoura University Hospital, Egypt. Int J Microbiol. 2017;2017:3925868. doi: 10.1155/2017/3925868. Epub 2017 May 8. PMID: 28567057; PMCID: PMC5439075.
50-Batra P, Khurana S, Govindaswamy A, Aravinda A, Bajpai V, Ayyanar M, et al. Antibiotic resistance profile and co-production of extended spectrum beta lactamases and AmpC in *Acinetobacter* spp. in a level 1 trauma center from India. J Lab Physicians 2019;11:128–132
51-Liu C, Chang Y, Xu Y, Luo Y, Wu L, Mei Z, Li S, Wang R, Jia X. Distribution of virulence-associated genes and antimicrobial susceptibility in clinical *Acinetobacter baumannii* isolates.

Oncotarget. 2018 Apr 24;9(31):21663-21673. doi: 10.18632/oncotarget.24651. PMID: 29774093; PMCID: PMC5955172.

52-Uwingabiye J, Frikh M, Lemnouer A, Bssaibis F, Belefquih B, Maleb A, Dahraoui S, Belyamani L, Bait A, Haimeur C, Louzi L, Ibrahimi A, Elouennass M. Acinetobacter infections prevalence and frequency of the antibiotics resistance: comparative study of intensive care units versus other hospital units. Pan Afr Med J. 2016 Apr 15;23:191. doi: 10.11604/pamj.2016.23.191.7915. PMID: 27347280; PMCID: PMC4907753.

53- 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. doi: 10.18683/germs.2019.1171. PMID: 31646145; PMCID: PMC6783638.

54- Smani Y, Fàbrega A, Roca I, Sánchez-Encinales V, Vila J, Pachón J. Role of OmpA in the multidrug resistance phenotype of Acinetobacter baumannii. Antimicrob Agents Chemother. 2014;58(3):1806-8. doi: 10.1128/AAC.02101-13. Epub 2013 Dec 30. PMID: 24379205; PMCID: PMC3957889.

55- Zeighami H, Valadkhani F, Shapouri R, Samadi E, Haghi F. Virulence characteristics of multidrug resistant biofilm forming Acinetobacter baumannii isolated from intensive care unit patients. BMC Infect Dis. 2019 Jul 17;19(1):629. doi: 10.1186/s12879-019-4272-0. PMID: 31315572; PMCID: PMC6637494.

56- Huang W, Zhang Q, Li W, Chen Y, Shu C, Li Q, Zhou J, Ye C, Bai H, Sun W, Yang X, Ma Y. Anti-outer Membrane Vesicle Antibodies Increase Antibiotic Sensitivity of Pan-Drug-Resistant *Acinetobacter baumannii*. Front Microbiol. 2019 Jun 18;10:1379.