Molecular Identification of epsA and ompA genes for *Acintobacter baumannii* Isolated from Burn Wounds

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Abstract

This study was designed to molecular detection of *epsA* and *ompA* genes for *Acinetobacter baumannii* Isolates that isolated from clinical. Burn wounds samples from a total of 80 clinical different Wounds, samples were collected from Ghazi Al Hariri Hospital and Baghdad hospital in Baghdad / Iraq. The results of the current study showed that seventy (87.5%) were clinical wounds positive samples, while the rest (10) were negative wounds samples (12.5 %). From 70 samples inoculated on the Chromagar Acinetobacter, 10 isolate were grown on the medium, *A. baumannii* isolates on the Chromagar appeared as bright red colonies after 24h and incubation at 37 C . The results of *epsA* and *ompA* genes detection clarify that 8 isolate (80%) of *A. baumannii* isolates gave a positive result and carrying *epsA* and *ompA* genes while 2 (20%) of *Acintobacter baumannii* isolates were lack the gene and no cross reactivity could be observed with any of the AS-3 and AS-9 isolates. The identification of *A. baumannii* isolates were depended on culturing these isolates on CHROM agar Acinetobacter, MacConkey, blood, nutrient agar, biochemical tests and API NE 20 test VITEK 2 . Compact were carried out the confirms. Results showed the amplification of *epsA* and *ompA* genes for eight. *A. baumannii* isolates were AS-1, AS-2, AS-4, AS-5, AS-6, AS-7, AS-8 and AS-10. PCR product 531 bp of *ompA* gene and 451 bp of *epsA* gene.

Keywords: *Virulence genes, OmpA, EpsA, Acinetobacter baumannii*

Introduction

Acinetobacter baumannii is the most prevalent infection-causing organism in the hospital environment. *Acinetobacter baumannii* causes hospital acquired infections, such as ventilator-associated pneumonia, bacteremia, urinary tract infections, meningitis, and surgical wound infections [(1)] . *Acinetobacter baumannii* is a Gram-negative bacillus that is aerobic, pleomorphic and non-motile bacteria; it has a high incidence among immuncommpromised *Acintobacter baumannii* is referred to as ''Iraqibacter'' due to its seemingly sudden emergence in military treatment facilities during the Iraq War . *Acintobacter baumannii* produces high molecular weight capsular polysaccharide which surrounds the outer membrane [(2)]. Capsular polysaccharide forms a discrete layer on the bacterial surface providing protection from diverse environmental conditions, assisting in evasion of host immune defenses, and increasing resistance to a number of antimicrobial compounds [(3)]. *Acintobacter baumannii* is capable of entering and persisting inside host cells, it adheres to host cells, then translocate into nucleus. After killing host cells, it disseminates in bloodstream and tissues [(4)]. As a class of important virulence factors in bacteria, outer membrane proteins (OMPs) have attracted much more attention. Among those OMPs of *A. baumannii*, *OmpA* is the most deeply studied virulence factor which plays key roles in regulating the adhesion, aggressiveness, and biofilm formation of *A. baumannii* and immune response of host. Other virulence factors such as exopolysacharide also have an important role in the invasion of these bacteria . *Acinetobacter baumannii* produces a polysaccharide export outer membrane protein, called exopolysacharide or EPS, which is encoded by EpsA. EPS accumulates on the cell surface and provides protection to the cells against the harsh external environment [(5)] . The present work was aimed to molecular detection of some virulence genes (*OmpA* Gene and *EpsA* Gene) of *Acinetobacter* baumannii isolates

Materials and Methods

Sample collection

This study was conducted during the period from December 2019 to April 2020, During the study period, eighty clinical burn wounds swabs samples were taken. Burn wounds samples from a total of 80 clinical different Wounds, samples were collected from *Ghazi Al Hariri Hospital* (40 samples) and Baghdad hospital (40 samples) in Baghdad / Iraq.

Biochemical test

Biochemical test as Oxidase, Indole, Methyl red, Lactose fermentation, Citrate utilization, Catalase production and Urease test. were performed on the isolates to confirm their identification *Acintobacter baumannii*. All the tests were according to [6] .

API (Analytical Profile Index)

This test is used to identify *Acintobacter baumannii* bacterium, also used to determine and differentiate several types of Gram-negative bacteria .

Identification Using Vitek 2 System

The VITEK 2 is an automated microbiology system utilizing growth-based technology, according to the manufacturer's instructions (BioMerieux-France).

Molecular study

DNA Extraction

Genomic DNA was isolated from bacterial growth according to the protocol of ABIOpure . Primer sets used are shown in table (1) .

Table (1): Primer sets used in the present study

Primer name	Sequence	Annealing Temp. °C	Product Size (bp)	
epsA-F	5`-AGCAAGTGGTTATCCAATCG-3`	50	415	415
epsA-R	5`-ACCAGACTCACCCATTACAT-3`			
ompA-F	5`-CGCTTCTGCTGGTGCTGAAT-3`		531	531
ompA-R	5`-CGTGCAGTAGCGTTAGGGTA-3`			

Quantitation of DNA

Quantus Fluorometer was used to detect the concentration of extracted DNA in order to detect the goodness of samples for downstream applications. For 1 µl of DNA, 199 µl of diluted QuantyFlour Dye was mixed. After 5 min. incubation at room temperature, DNA concentration values were detected.

Polymerase chain reaction to check the quality of DNA

Polymerase chain reaction master mix was prepared (with final volume 20 µl per one reaction) containing 10µM forward and reverse primers, 2 X of master mix, and 5µl of nuclease-free water was added until the volume reached to 17 µl. Then, 3 µl of DNA was added to mixture .

Results and Discussion

According to table (2) , Seventy (87.5%) were clinical wounds positive samples, while the rest (10) were negative wounds samples (12.5 %).

Table (2): Total number of burn wounds samples used for the isolation of bacteria

Clinical wounds samples	Positive (growth)	Negative (no growth)
80	70	10
Percentage	87.5%	12.5 %

The increasing body of literature on this subject suggested that *Acinetobacter* infections occur mainly in four patient populations and settings . At least 25% of healthy individuals may carry *Acinetobacters* as part of their normal skin flora, but carriage of *Acinetobacter* spp. By healthy subjects at other body site is normally low, in contrast, high colonization rate of the throat, skin, respiratory tract or digestive tract of hospitalized patients with clinically significant strains have been reported during outbreaks of infection [7] .

The majority of infections caused by *A. baumannii* are contracted in hospitals, most often in critically ill patients hospitalized in intensive care or surgery *Acintobacter baumannii* can resist dehydration, detergents, UV radiation and common chemical sanitizers, making it extremely difficult to eradicate [8] .

The emergence of multidrug resistance *A. baumannii* and carbapenam-resistant *A. baumannii* have cause hospital outbreaks and threat in treatment due to its wide variety of resistance mechanisms and high survival rate on a biotic surfaces [9] .

A. baumannii traumatic wound infections have become a topic of recent interest with reports of increasing incidences of outbreaks among victims of combat injuries and natural disasters . Recent data from Iraq and Afghanistan identify highly resistant strains of *A. baumannii* to be some of the most common organisms causing severe and often lethal wound infections [10] .

A. baumannii infection occurs when the immunological barriers of the host are breached (e.g. mechanical ventilation) and is hence considered an opportunistic pathogen . *A. baumannii* causes various types infections complicating burn wounds [11] .

From 70 samples inoculated on the Chromagar *Acinetobacter*, 10 isolate grew on the medium . CHROMagar Acinetobacter is a recently developed medium for selective and rapid identification of *Acinetobacter* spp. [12] .

Acinetobacter baumannii is rod-shaped which grows well on MacConkey agar (without salt). Although officially classified as not lactose-fermenting ,they are often partially lactose-fermenting when grown on MacConkey agar . Growth and purity of cultures of *Acinetobacter baumannii* were determined by culture on MacConkey agar and Blood agar. On MacConkey agar it's formed pale coloured, Non lactose fermenting colonies and on Blood Agar it's formed non-hemolytic colonies. There was only one type of colonies attesting to its purity [13] .

Ten isolates of *A. baumannii* grow at 44°C were positive on blood agar medium after 24 hrs of incubation. A appropriate temperature for the growth of most *Acinetobacter* spp were 37ºC whereas *A. baumannii* can grow well at high a temperature of 44 °C [14] .

The suspected all isolates of *A. baumannii* and were then subjected to the related biochemical tests. All isolates of *A. baumannii* were found to be catalase positive and oxidase / indole negative. Tests on Lactose fermentation and motility test gave negative results. The positive results for the test appeared in methyl red and Citrate utilization, finally we used the urease test which give negative results for *Acintobacter baumannii* . The isolates were identified as *A. baumannii* and have been confirmed by using Api 20 and VITEK 2 Compact, figure (1) .

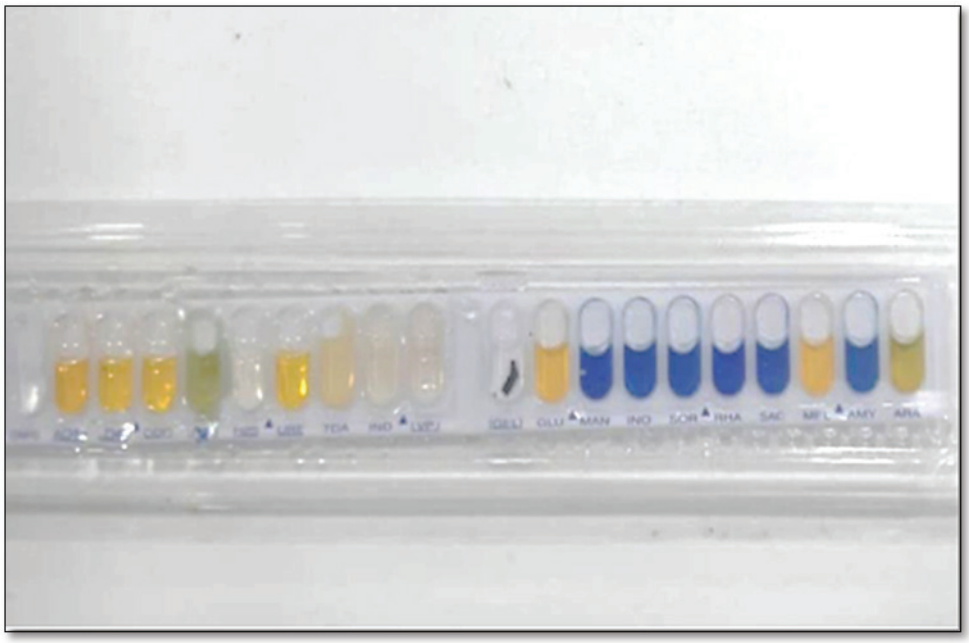

Figure. 1 : API test for identification of *Acintobacter bumannii*.

Acinetobacter was identified as non motile, oxidase negative and catalase positive. *Acinetobacter baumannii* is positive for citrate. Citrate in simmon citrate medium is important to detect weather the bacteria isolates able to grow on it as a unique carbon and energy source [15] .

Non-lactose fermenting and non-motile of *Acinetobacter baumannii* isolates [16] . Urease test was positive for *Acinitobacter baumannii* urease enzyme catalyzes the breakdown of urea, and the bacteria that can produce this enzyme is able to detoxify the waste products and to drive metabolic energy from its utilization which change the medium color from yellow to purple-pink, indicating urease positive test [6] .

Acinetobacter baumannii isolates were oxidase negative and catalase positive [17] . API NE 20 test VITEK 2 Compact were carried out confirms the biochemical tests regarding the species identification of the *A.baumannii* isolates. results showed that all 30 isolates were identified as *A.baumannii*, as shown in Figure (4). API 20 NE is a standardized system for the identification of non-fastidious, non-enteric Gramnegative rods, combining 8 conventional tests which include potassium nitrate (NO3), L-tryptophane (TRP), D-glucose (GLU), L-arginine (ADH), urea (URE), esculin ferric citrate (ESC), gelatin (GEL), and 4-nitrophenyl-β-D-galactopyranoside (PNPG), and also the 12 assimilation tests which include Dglucose (GLU), L-arabinose (ARA), D-mannose (MNE), D-mannitol (MAN), N-acetyl-glucosamine (NAG), D-maltose (MAL), potassium gluconate (GNT), capric acid (CAP), adipic acid (ADI), malic acid (MLT), trisodium citrate (CIT), and the phenylacetic acid PAC [18] .

Genomic DNA was extracted from the most 10 isolates of *Acintobacter baumannii* using genomic DNA kit. Figure (2). The result shows that the recorded range of DNA concentration was 50 ng / µl and the DNA purity was 1.8-2.0.

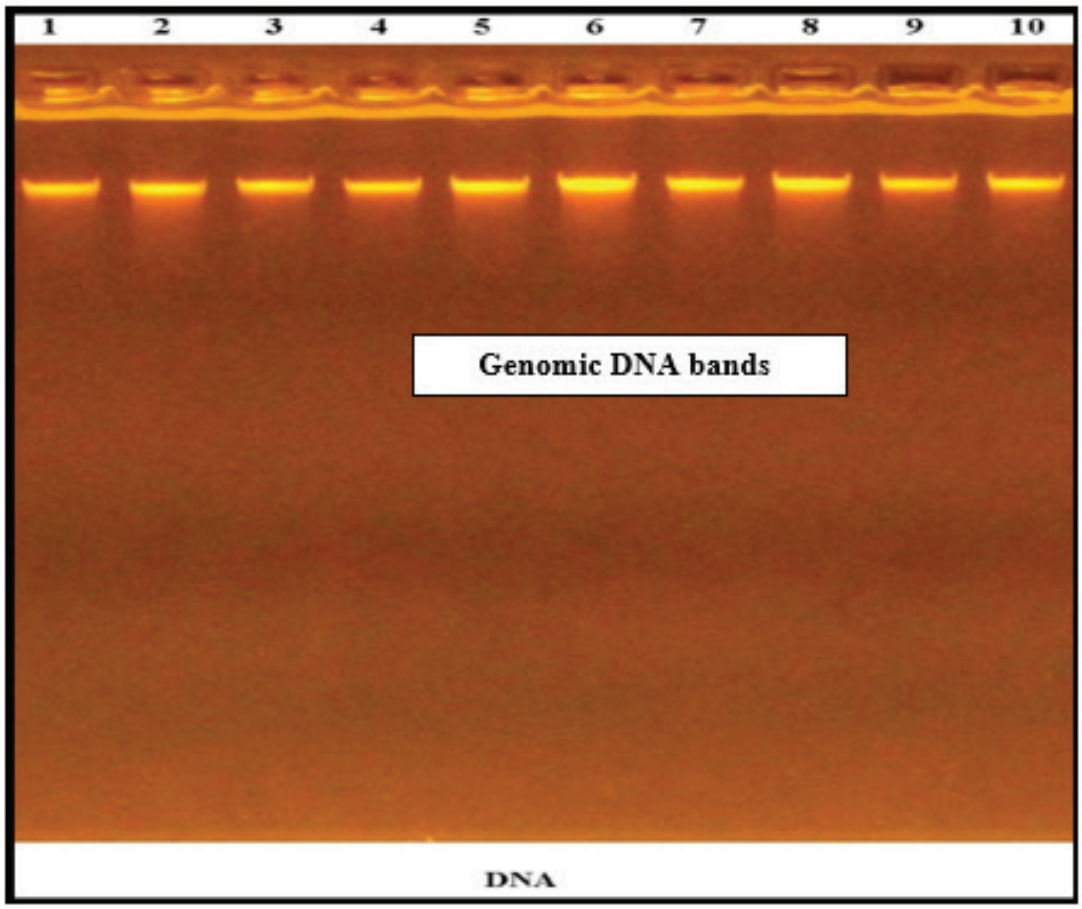

Figure. 2 : Genomic DNA bands extracted from *A. baumannii* isolates on (1%) agarose,75V, for 30 min stained with ethidium bromide. Lane 1-10: DNA of Lane.

The results of *epsA* and *ompA* genes detection clarify that 8 isolate (80%) of *A. baumannii* isolates gave a positive result and carrying *epsA* and *ompA* genes while 2 (20%) of *Acintobacter baumannii* isolates were lack the gene and no cross reactivity could be observed with any of the AS-3 and AS-9 isolates.

The results showed the amplification of *epsA* and *ompA* genes for eight *Acintobacter baumannii* isolates were AS-1, AS-2, AS-4, AS-5, AS-6, AS-7, AS-8 and AS-10. PCR product 531 bp of *ompA* gene figure (3) and 451 bp of *epsA* gene figure (4). The infection symptoms caused by *Acintobacter baumannii* are closely associated with its virulence factor. But the virulence genes distribution in clinical *A. baumannii* was rarely reported, except for *bap, omp* 33-36 and *traT* [19] .

This study examined the distribution of two virulence genes (*epsA* and *ompA* genes) in clinically collected *A. baumannii* isolates. The molecular investigation was performed to detect the amplification plots of the target genes and reference gene in order to find the threshold cycle value for each them as in figures (6) and (7).

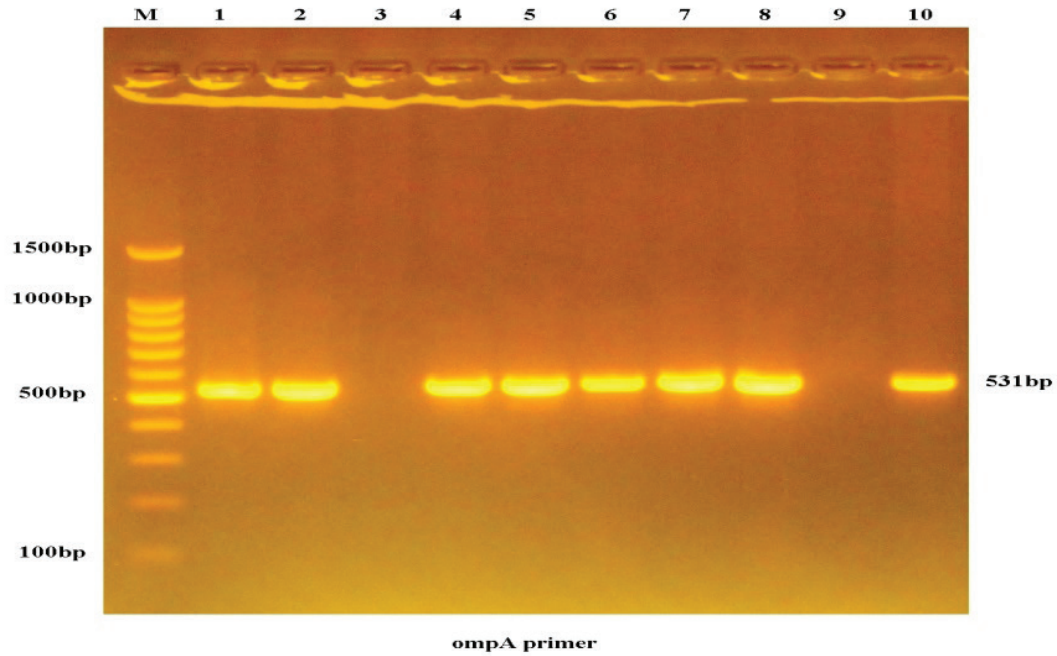

Figure. 3 : Results of the amplification of *ompA gene* of *Acinetobacter baumannii* samples were fractionated on 1% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-10 resemble 531bp PCR products.

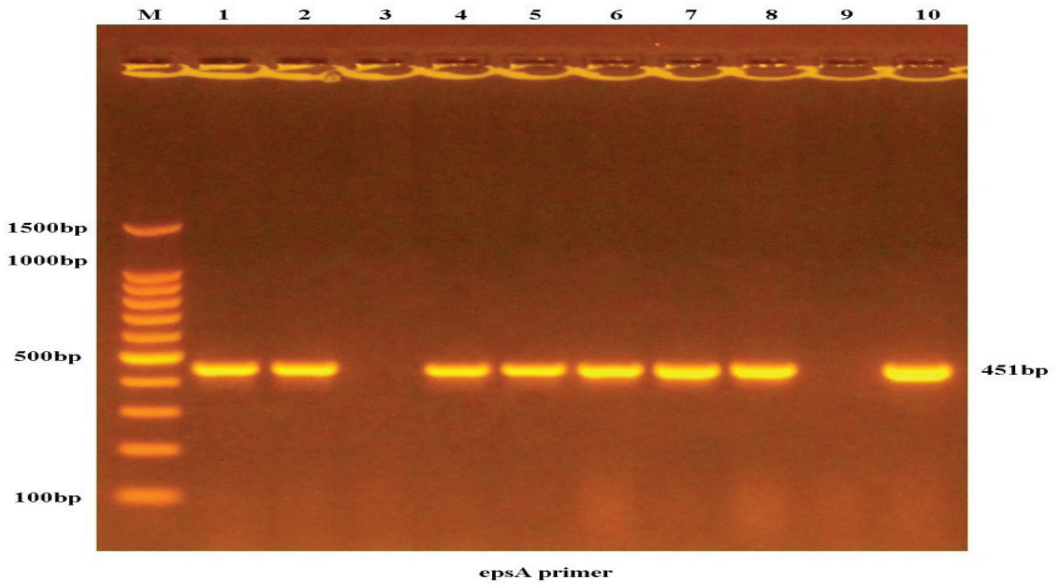

Figure. 4 : Results of the amplification of *epsA gene* of *Acinetobacter baumannii* samples were fractionated on 1% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-10 resemble 451bp PCR products.

Several virulence factors involved in bioflm formation of *A. baumannii* such as the outer membrane protein A (*OmpA*), biofilm associated protein (*Bap*)chaperon-usher pilus (*Csu*), extracellular exopolysaccharide (*EPS*). The *OmpA* of *A. baumannii* is essential for the attachment to human epithelial cells, development of biofilms and antimicrobial resistance [20] .

Previous results showed that all *A. baumannii* isolates carried at least one biofilm related gene. The most prevalent gene was *csuE* (100%), followed by *pgaB* (98%), *epsA* and *ptk* (95%), *bfmS* (92%) and *ompA* (81%). 98% of isolates carried more than 4 biofilm related genes, simultaneously [21] .

Ethical Clearance: Taken from Al-Nahrain University ethical committee

Source of Funding : Self

Conflict of Interest : Nil

References

(1) AL-Kadmy, S.; Ali, A.; Salman, I. and Khazaa, S. Molecular characterization of Acinetobacter baumannii isolated from Iraqi hospital environment. New Microbes and New Infections . 2018 ; 21: 51-57.

(2) usso TA, Luke NR, Beanan JM, Olson R, Sauberan SL, MacDonald U, Schultz LW, Umland TC, Campagnari AA. The K1 capsular polysaccharide of Acinetobacter baumannii strain 307-0294 is a major virulence factor. Infect. Immun. 2010 ; 78 : 3993–4000.

(3) Geisinger, E., and Isberg, R. R. Antibiotic modulation of capsular exopolysaccharide and virulence in Acinetobacter baumannii. PLoS Pathog. 2015; 11: e1004691.

(4) Parra-Millan R, Guerrero-Gomez D, Ayerbe-Algaba R, Pachon-Ibanez ME, Miranda-Vizuete A, Pachon J, Smani Y. Intracellular Trafficking and Persistence of Acinetobacter baumannii Requires Transcription Factor EB. mSphere. 2018 ; 3 : e00106–18.

5) Sánchez-Encinales V, Álvarez-Marín R, Pachón-Ibáñez ME, Fernández-Cuenca F, Pascual A, Garnacho-Montero J, Martínez-Martínez L, Vila J, Tomás MM, Cisneros JM, Bou G, Rodríguez-Baño J, Pachón J, Smani Y. Overproduction of Outer Membrane Protein A by Acinetobacter baumannii as a Risk Factor for Nosocomial Pneumonia, Bacteremia, and Mortality Rate Increase. J Infect Dis. 2017 ; 215(6) : 966-974.

(6) Forbes, B.; Sahm, D. and Weissfeld, A. Baily and Scott Diagnostic Microbiology. (9th ed.). Mosby Company. Baltimore, USA, 2002 .

(7) Towner K. The Genus *Acinetobacter*. Prokaryotes. 2006 ; 6 : 746 –758. Zeighami, H. ; F. Valadkhani, R. Shapouri, E. Samadi, and F. Haghicorresponding. Virulence characteristics of multidrug resistant biofilm forming *Acinetobacter baumannii* isolated from intensive care unit patients, BMC Infectious Diseases. 2019 ; 19 : 629 .

(8) AL-Saleem, N. H. Genotyping Relatedness of Acinetobacter baumannii isolated from Medical City/Baghdad. PH.D. dissertation. Biology department. College of Sciences. Baghdad University, 2013 .

(9) Aimsaad, L.; Diraphat, P.; Utrarachkij, F.; Thunyaharn, S.; Samakoses, R. and Siripanichgon, K. Epidemiological Characteristics of Acinetobacter baumannii Infections at Phramongkutklao Hospital. Journal of the Medical Association of Thailand. 2009 ; 92 (7):164-172.

(10) Be NA, Allen JE, Brown TS, Gardner SN, McLoughlin KS, Forsberg JA, Kirkup BC, Chromy BA, Luciw PA, Elster EA, Jaing CJ. Microbial profiling of combat wound infection through detection microarray and next-generation sequencing. J Clin Microbiol. 2014 ; 52(7) : 2583-94.

(11) Howard, A. , M. O'Donoghue, A. Feeney, R.D. Sleator. *Acinetobacter baumannii*: an emerging opportunistic pathogen Virulence. 2012 ; 3 : 243-250 .

(12) Gordon NC and Wareham DW. Evaluation of CHROMagar Acinetobacter for detection of enteric carriage of multidrug-resistant *Acinetobacter baumannii* in samples from critically ill patients. J Clin Microbiol. 2009 ; 47 : 2249–2251.

(13) Shrikant N. Biofilm formation and Immunomodulation by *Acinetobacter baumannii* on endotracheal tubes: In vitro study. Dissertation (Master) , India, 2017 .

(14) Scott, P. T., K. Petersen, J. Fishbain, D. W. Craft, A. J. Ewell, K. Moran, et al. *Acinetobacter baumannii* infections among patients at military medical facilities treating injured U.S. service members, 2002–2004. Morb. Mortal. Wkly. Rep. 2004 ; 53 : 1063–1066.

(15) Cisneros J. M., Rodriguez- Bano J . Nosocomial bacteremia due to *A. baumanii*: epidemiology,

clinical features and treatment. Clin Microbiol Infect. 2002 ; 8 (11) : 687-91.

16) Hussein, N. H., Al-Mathkhury, H. J. F. and Sabbah, M. A. Serological relatedness among clinical and environmental *Acinetobacter baumannii* isolates isolated from hospitals in Baghdad. Iraqi Journal of Science. 2013 ; 54(3) : 553-559.

(17) Doughari, H. J., Ndakidemi, P. A., Human, I. S. and Benade, S. The ecology, biology and pathogenesis of *Acinetobacter* spp.: an overview. Microbes and environments. 2011 ; 26(2) :101-112 .

(18) Daef E. A, Mohamad IS, Ahmad A S, El-Gendy S G, Ahmed E H, Sayed I M. Relationship between clinical and environmental isolates of Acinetobacter baumannii in Assiut University hospitals. J Am Sci. 2013 ; 9(11s) : 67–73.

(19) Sung J. Y, Koo S. H, Kim S, Kwon G. C. Persistence of Multidrug-Resistant *Acinetobacter baumannii* Isolates Harboring blaOXA-23 and bap for 5 Years. J Microbiol Biotechnol. 2016 ; 26 : 1481–9.

(20) Ghasemi E, Ghalavand Z, Goudarzi H, Yeganeh F, Hashemi A, Dabiri H, Mirsamadi ES, Foroumand M. Phenotypic and Genotypic Investigation of Biofilm Formation in Clinical and Environmental Isolates of *Acinetobacter baumannii*. Arch Clin Infect Dis. 2018 ; 13(4) : e12914.

(21) Zeighami, H. ; F. Valadkhani, R. Shapouri, E. Samadi, and F. Haghicorresponding . Virulence characteristics of multidrug resistant biofilm forming *Acinetobacter baumannii* isolated from intensive care unit patients, BMC Infectious Diseases. 2019 ; 19 : 629 .