Molecular Identification of rblB, Cnf1 and CsuE.genes for Acintobacter baumannii Isolated from Burn, Wounds infection and and Environmental Samples

Sarah Y. AL-falahat[1], Wathiq Abbas Al-Draghi[2]
[1,2] Institute of Genetic Engineering and Biotechnology for postgraduate studies, University of Baghdad, Baghdad, Iraq
Email: sarahyousifali1234@gmail.com

Abstract

Multidrug-resistant Acinetobacter baumannii has emerged as a major problem around the world, posing a serious hazard to patients with burn and wound infections, In this study. Two hundred and fifty samples were collected from various sites in Baghdad/Iraq hospitals (Al-Yarmouk Teaching, burn center of Medical City, and Kamal Al Samray Hospitals) and divided into two groups based on their source: clinical samples (150) and environmental samples (100), collected and tested from October 2021 to March 2022. These samples were cultured on culture medium, and seven -three A. baumannii isolates were identified by microscopic examination and biochemical tests. The identification of (73) isolates was verified using the VITEK-2 system and a molecular approach based on the rplB gene. In addition, antibiotic susceptibility testing was performed on all bacterial isolates, and the findings revealed that 36 isolates had Multi-Drug Resistance (MDR). This study found that 14 isolates carrying the csuE gene, and in all 16 A. baumannii isolates are not carried Cytotoxic necrotizing factor (cnf1 gen) , Sequencing analysis was applied to the PCR product of 14 positive samples from 16 isolates to csuE gene the isolates detect Sequencing and similarity of genetic relationship between locally isolates and high identity global isolates in gene bank.

Keywords: Multi-Drug Resistance, Burns *rblB*, 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 biofilm 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 (2). 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 (3). Acinetobacter baumannii contain rplB housekeeping gene that encode to the 50S ribosomal protein L2. It is essential for ribosome activity and is a major component of the peptidyl transferase This protein included in process activity (peptidyl transferase activity and binds to functionally important domains of 23S rRNA), one of the rRNA binding proteins, and contacts with the 16S rRNA in the 70S ribosome (4,5), Therefore *rplB* gene is very important housekeeping gene(6). As a class of important virulence factors in bacteria the Csu (chaperone-usher type pilus) A pili-like structure encoded by the location of csu, which is widely distributed among clinical strains, can reasonably help primary cell adhesion. Pilus, which aids biofilm development and adhesion, is crucial to A. baumannii's ability to produce biofilms. csu E is a member of the usher-chaperone system in a similar way. Opera csu is a gene cluster that produces pili-like bundle structures in bacteria. This gene has been shown to have a role in A. baumannii biofilm development (7). The cytotoxic necrotizing factor (cnf) is a member of the necrotizing toxin family found in the epidermis, Because of the toxin toxicity factor, which has the ability to inhibit wound healing and is created by bacteria associated with wounds after surgery, the bacteria that produce cytotoxic necrotizing factor have the potential to thrive at the site of infection. As a result, the toxicity factor is one of the most important virulence factors in bacteria, and it serves numerous purposes (it enhances Superoxidase production, has the ability to stick to human epithelial cells, and reduces the number of phagocytic cells in the body) (8,9). and study comparison of locally isolated with global isolates documented in gene bank of Acinetobacter baumannii isolates.

doi.org/10.31838/hiv23.01.5 Received: 09.09.22, Revised: 10.10.22, Accepted: 14.11.22

Materials and Methods Sample collection

Two hundred and fifty samples were collected from various sites in Baghdad/Iraq hospitals (Al-Yarmouk Teaching, burn center of Medical City, and Kamal Al Samray Hospitals) and divided into two groups based on their source: clinical samples (150) and environmental samples (100), collected and tested from October 2021 to March 2022. These samples were cultured on culture medium, and seven -three *A. baumannii* isolates were identified by microscopic examination and biochemical tests. The identification of (73) isolates was verified using the VITEK-2 system and a molecular approach based on the *rplB* gene.

Molecular study DNA Extraction

The estimation of the extracted DNA concentration was done by using the Quantus™ Fluorometer Single-Tube Format Protocol (Calliauw *et al.*, 2016),

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

Primer Name	Sequence 5`-3`	Annealing Temp. (˚C)	Product size (bp)
cnf1-F	AAGATGGAGTTTCCTATGCAGGAG	58	498
cnf1-R	CATTCAGAGTCCTGCCCTCATTATT		
rplB-F	GTAGAGCGTATTGAATACGATCCTAACC	50	475
rplB-R	CACCACCACCRTGYGGGTGATC		
csuE-F	CATCTTCTATTTCGGTCCC	60	168
csuE-R	CGGTCTGAGCATTGGTAA		

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

In this study, the results revealed that a total number of *A. baumannii* was included in 73 (29.2 %) of the 250 clinical and environmental samples tested. The samples were taken from various environmental and clinical sources, including wounds and burns. And environmental sources including beds, tables, sinks and floors.

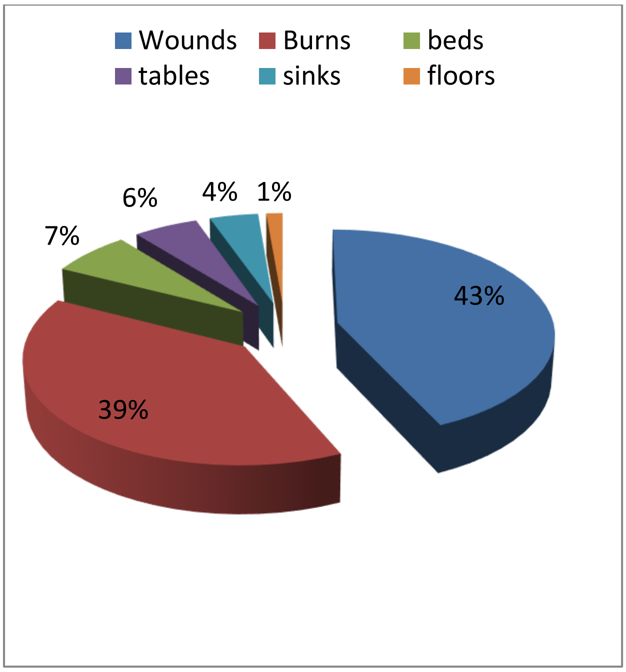


Figure 1: Total number of burn wounds samples used for the isolation of bacteria

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 250 samples inoculated on the Chromagar Acinetobacter, 73 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 VITEK 2 Compact, **Antibiotic Susceptibility Testing,**The development of *Acinetobacter baumannii* to become extensively drug-resistant and multidrug-resistant are rated as a significant curative issue which can be clarified by several theories; one of them is the incorrect use of antibiotics, besides, the extensive use of antibiotics. This made these bacteria to be the focus of the researchers' attention. Susceptibility to antibiotics was determined by VITEK 2 system and determined by using the disk diffusion method as illustrated in section ntimicrobial susceptibility patterns were determined by disk diffusion method on Mueller–Hinton agar (MHA) (Biolab, Hungary) according to CLSI guide- lines. The following antimicrobial disks. The Vitek 2 system automatically measures a turbidity signal for each test well containing an antibiotic, every 15 minutes for up to 18 hours. These dataare used to generate growth curves and by comparing with a control, the minimum inhibitory concentration (MIC) of each antibiotic is estimated. MIC results (µg/ml) are translated into clinical categories (Susceptible, Intermediate, and Resistant) by comparing with Breakpoints for susceptibility category determination recommended by the clinical and laboratory standards institute (CLSI) guidelines. The results showed that only 36 isolates are MDR, according to reports all eighty-three isolates are resistant to ceftazidime (94.59%) and are more sensitive to colistin (6.75%) and Trimethoprim/Sulfamethoxazole (22.97%), Also, the results showed that 30 isolates are resistant to meropenem and 20 isolates are resistant to Imipenem, and they were 38 isolates resistant to Piperacillin and 36 isolates resistant Ciprofloxacin. Detection of *rblB,cnf1*and *csuE* virulence genes by Polymerase Chain Reaction (PCR) technique PCR assay was used to determine the virulence genes in all 16 *A. baumannii* isolates. The presence of the rplB gene was confirmed by PCR analysis in all 16 (100 percent) asshown in Figure (1), A. baumannii isolates in this investigation, confirming the usual diagnostic of culture, biochemical tests, and Vitek-2 assays. Furthermore, as shown in Figure, the generated amplicons of this gene emerged clearly on agarose at 475 bp during the electrophoresis technique, In addition, a PCR assay was used to detect a single gene using particular primers. The findings of this analysis showed that 14 isolates (87.5 %) carried the chaperone-usher type pilus (*csuE* gene), asshown in Figure (2), studies show Habib et al.,that gen *csuE* gen in All A. baumannii isolates carried at least one biofilm related gene. The most prevalent gene was csuE (100%),AL-kadmy et al,. (2017) (15).Csga, virulence gene were prevalent amongthe A. baumannii strains of our clinical infections, In all 16 *A. baumannii* isolates (0.0%) are not carried Cytotoxic necrotizing factor (cnf1 gen),A study carried out for molecular characterization of *A. baumannii* isolated from Iraq hospitals environment showed Genotypic detection of some virulence genes of A. baumannii which included cnf1, showed the presence of these genes in 10 % ,(16), The results of this study were consistent with the findings of Abd Al-Mahdi et al.(2016) (17). Who showed that only two isolates (28%) of A. baumannii bacteria had the cnf1 gene from the total isolates of their study, while the findings of Momtaz et al. (2015) showed that (35%) of isolates had cnf1 gene(18).

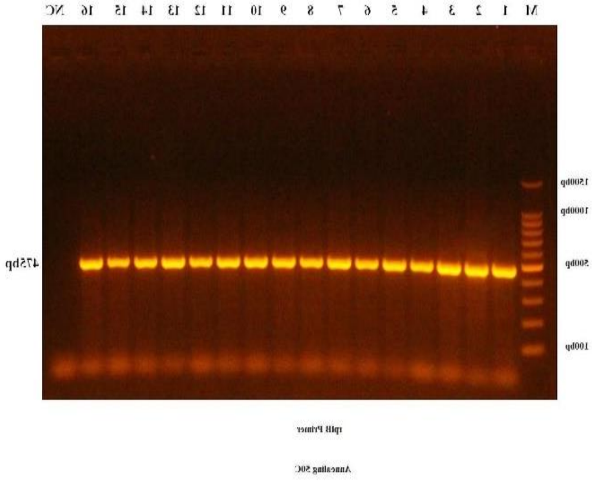


Figure (1): Results of the amplification of rplB gene of Acinetobacter baumannii samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-1S resemble 475bp PCR products.

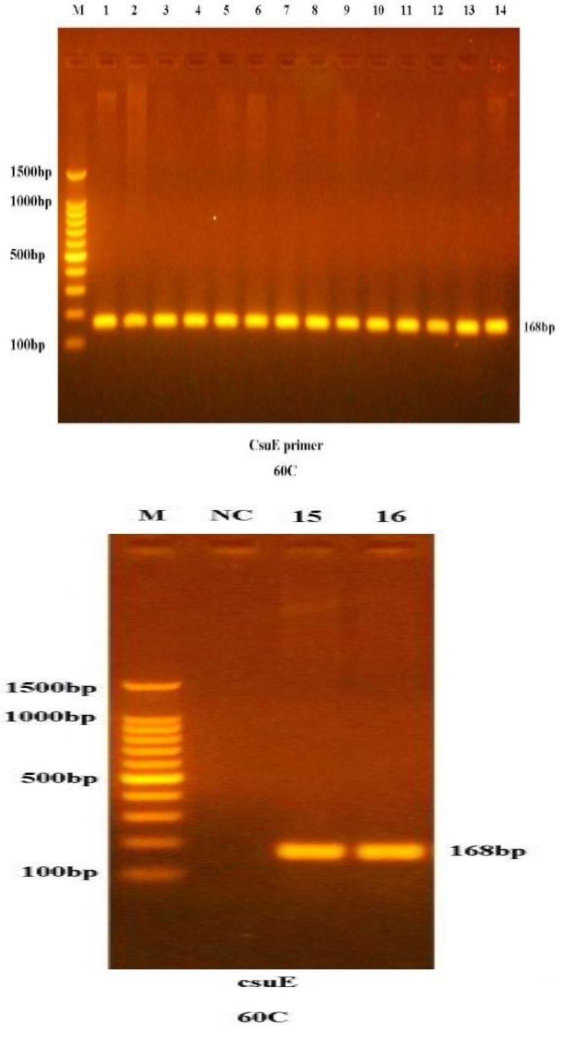


Figure (2) Results of the amplification of CsuE gene of Acinetobacter baumannii samples were fractionated on

1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-1S resemble 168bp.

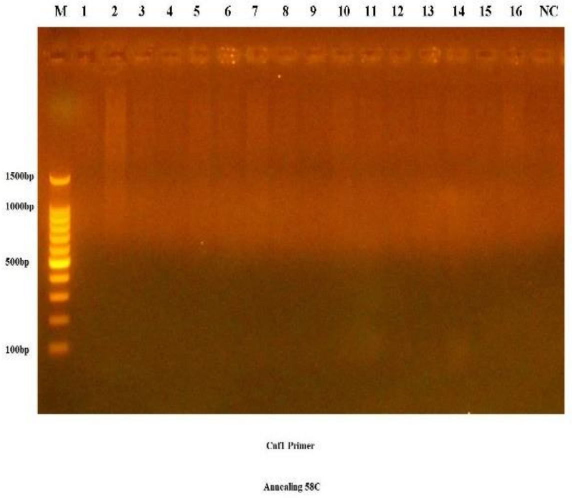


Figure (4) : Gel Electrophoresis for PCR Amplification of A. baumannii cnf1 Gene on 1.5%agarose gel, 100 V for 75 min. lane (M): DNA ladder marker, lane 1- 18: amplified products for bacterial isolates.

Sequencing Analysis

Sequencing of csuE gene Analysis

Sequencing **of *csuE*** gene of all isolates wasdone by sending PCR products of amplification of ***csuE*** gene to Macrogen Corporation-Korea using ABI3730XL, automated DNA sequencer. Then sequenced DNA in NCBI GenBanK database and the results were analyzed by using Geneious 9 software to draw phylogenetic tree. Sequencing analysis was applied to the PCR product of 14 positive samples from 16 isolates. Phylogenetic tree of the *csuE* gene carried out by using Geneious 14 software for all (16) locally isolates of multidrug resistance A. baumannii from sources of isolation included, wound, and Burns infections. The results in phylogenetic tree showed 3 groups of isolates as shown in Fig.(2) and Table (3). The isolates in group (A) Includes isolates whose similarity by sequence analysis between them100% and the group (A) Comparison of all locally *A. baumannii* isolates with high identity global isolates in gene bank under accession numbers (CP0465361, CP 054560.1, CP 044517.1, CP 051875.1, CP 054560.1, CP 044517.1, CP051875.1, CP 051869.1, CP 065051.1, CP 009256.1, CP 044519.1, CP 048827.1, CP 050385.1, Ap022836.1, CP045428.1, CP046654.1, CP045110.1) was carried out to detect similarity of genetic relationship between locally and global isolates.

Group	isolates	Description	Per Ident	Accession	New Accession
A	3,4,5.6,7,8,9, 13,14,15,16,	Acnetobacter baumanii strain CUVE-MIC596 chromosome,complete genome	100.00%	CP041148.1	LC719466.1

By credit The results in phylogenetic tree, The Group (A) include isolates (3,4,5.6,7,8,9,13,14,15,16,), the Description Acnetobacter baumanii strain CUVE-MIC596 chromosome,complete genome and Per Ident 100.00%the Accession Cp041148.1 and New Accession LC719466.1. The isolates in group (B) Includes isolates whose similarity by sequence analysis between them 100% and the group (B) Comparison of all locally *A. baumannii* isolates with high identity global isolates in gene bank under accession numbers (CP050403.1 , CP051866.1, CP027246.2, CP041035.1, CP039930.1, CP039343.1, CP039341.1, CP037870.1, CP037870.1, CP037869.1, CP023029.1, CP001172.2, CP030106.1, LS48372.1, CP027528.1).

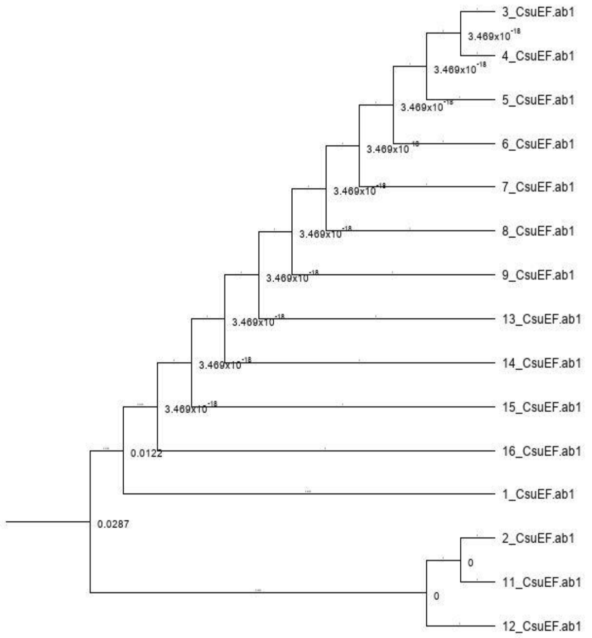
Group	isolates	Description	Per Ident	Accession	New Accession
B	2,12,11	Acnetobacter baumanii strain VB2486 chromosome,complete genome	100.00%	CP050403.1	LC719464.1

By credit The results in phylogenetic tree, The Group (B) include isolates (2,11,12),theDescription Acnetobacter baumanii strain VB2486 chromosome,complete genome and Per Ident 100.00%the Accession CP050403.1 and New Accession LC719464.1. The isolates in group (C) Includes only one number (1) isolates whose not similarity group (A and B) sequence analysis ,and the group (C) Comparison of all locally *A. baumannii* isolates with high identity global isolates in gene bank Which is similar to three recorded isolates only, which is considered the fourth and has been recorded in the gene bank under accession numbers (LC719465.1) and Comparison of all locally *A. baumannii* isolates with high identity global isolates in gene bank under accession numbers(CP046536.1) was carried out to detect similarity of genetic relationship between locally and global isolates.

Group	isolates	Description	Per Ident	Accession	New Accession
C	1	Acnetobacter baumanii strain XL380 chromosome,complete genome	100.00%	CP046536.1	LC719465.1

By The results in phylogenetic tree, The Group (C) include isolates (1),theDescription Acnetobacter baumanii strain XL380 chromosome,complete genome and Per Ident 100.00%the Accession CP046536.1 and New Accession LC719465.1 .

-TreeofcsuEgen



-

Conclusion

The present study showed that *A.baumannii* consider as one of the important causes of nosocomial infections in hospitals especially burns and wounds infections and most of them multidrug resistance, *rplB* gene is very important housekeeping gene for molecular detection of *A. baumannii* at the genus and species level, *csuE* that have been diagnosed in the collected isolates and have a role in the formation of the biofilm, which is a major cause of antibiotic resistance, and it is worth mentioning that the genetic necrosis factor1*cnF1* gene was not present in the collected isolates, sequence analysis of *csuE* showed that the genetic sequence of isolates collected from hospitals in Baghdad has the same genetic sequence found in the gene bank for *csuE* genes.

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

3- 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.

4-Von Mering C., Hugenholtz P., Raes J., Tringe SG., Doerks T., Jensen LJ., Ward N. and Bork P., Quantitative phylogenetic assessment of microbial communities in diverse environments, Science 315(15), P. 1126–1130, 2007.

5- Case RJ., Boucher Y., Dahllof I., Holmstrom C., Doolittle WF. and Kjelleberg S., Use of 16S rRNA and rpoB Alaa Salim Hamzah genes asmolecular markers for microbial ecology studies, Appl Environ Microbiol, 73(12), P. 278–288, 2007.

6- Wu X., Monchy S., Taghavi S., Zhu W., Ramos J., and Van Der Lelie D., Comparative genomics and functional analysis of niche-specific adaptation in Pseudomonas putida, FEMS Microbiol Rev, 35(10), P. 299–323, 2011.

7- Ayoub, M., C. and Hammoudi H., D., (2020). Insights into *Acinetobacter baumannii*: A Review of Microbiological, Virulence, and Resistance Traits in a Threatening Nosocomial Pathogen. *Antibiotics*. 9(3):119.

8- R. A. S. Al-Amean, M. S. Razzaq, M.A.K. Al-Saadi, Med. J. of Babylon, 8(4), 189-197 (2011).

9-Z. Petkovsek, K. Elersic, M. Gubina, D. Zgur-Bertok, S.M. Erjavec, Infections, 33,135-144(2001)

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-M. S. AL-Kadmy, A. N. M. Ali, I. M. A. Salman and S. S. KhazaalBranch of Biotechnology, Department of Biology, College of Science, Mustansiriyah

University, Baghdad, Iraq Molecular characterization of Acinetobacter baumannii isolated from Iraqi hospital environment November 2017New Microbes and New Infections 21(C) DOI: 10.1016/j.nmni.2017.10.010.

16- Rana ,M.,A., and Rasha ,Z., A., (2021). Molecular Study of the cnf1 and cnf2 (Cytotoxic Necrotizing Factors) Genes of Acinetobacter baumannii Isolated from Different Diseases. Diyala Journal for Pure Science DJPS P- ISSN:2222-8373, E-ISSN:2518-9255 Volume 17, Issue 1 , January 2021.

17- Abd Al-Mahdi Z., Bunyan I. A., Al-Shukri M. S., World J. of pharm. Res., 5(3) 157- 187(2016).

18- Momtaz H., Seifati S., Tavakol M., Inter. J. of Med. Lab, 2(2), 87-97 (2015)