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Detection of a gene responsible for virulence factors in Acinetobaterbaumannii isolated from wounds, burns and respiratory tract infections

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Abstract: The study obtained 20isolates of Acinetobacterbaumanniiout of 60 samples that collected from several pathological casesincluding wounds, burns, and respiratory tract infections. The results of detectionofiutA gene in Acinetobacterbaumannii showed that (17) isolates have iutAgeneby 85%, while (8) isolatesisolated from woundsshowedthe presence of this gene by 40% and 20% in the isolates of respiratory tract infections.The resistance of Acinetobacterbaumannii isolates against 10 of antibiotics wasdetermined; the results revealed that the isolates were resistant to Ceftazidime(100%), Ciprofloxcin and Tobramicin(80%), Gentamicin (75%), Norfloxacin, and Trimethoprim-Sulphamethoxazole (65%), Aztreonam (50%), Imipenem (40%), and Colistin (10%).

Keyword: Acinetobacterbaumannii, resistance to antibiotics, iutA gene.

Introduction

Acinetobacterbaumannii is characterized by being negative for Gram stain, Strictaaerobi, and negative for oxidase test and positive for the catalase test (Asifet al., 2018).Moreover,Acinetobacterbaumanniicauses an opportunistic nosocomial infection because it is responsible for many hospital-acquired infections and among Immunocompromised patients, wounds infections, burns, urinary tract infection, Septiccemia and endocarditis (Demirdalet al.., 2016).A. baumannii has many virulence factors that assist it in causing injury including its ability to form biofilm, capsule, and production of many enzymes including lipase, gelatinase, protase, as well as production of Colicin, polysaccharide, and iron carriers SiderphoresVijayakumaret al. 2016). Iron is an essential element for the bacterial growth as it is used as aredoxcatalystagent (oxidative agent)in proteins that contribute to the electron transfer process in the respiratory cycle, and also plays an important role in the occurrence of infection (Koga et al., 2014). A. baumannii have iron transport systems that include protein receptors with highly affinityfor iron; these receptors are present in the outer membrane of this bacteria and negative bacteria in general. These receptors expel the Ferrusiderophores complexes and transport them across the outer membrane to the peripheral plasma, so that these complexes bind to peripheral plasma proteins (Lee et al., 2010) which bind directly to ATP-dependent protein transporter and serve to transport iron into the bacterial cell (AbdAl-Mahdi et al., 2016).A. baumanniiis

Characterized with high resistant to various groups of antibiotics, which cause an increase in mortality rates for the patients; these groups of antibiotics includedβ-lactam Penicillins and Cephalosporins, Aminoglycoside,Fluoroquinolones and Trimethprim- Sulphamethoxazole. The reason for this is due to the high ability of the bacteria to produce β-lactamase broad-spectrum enzymes,modifyingenzymes,the genetic mutations as represented by topoisomerase and the genes carried on the plasmids and other mechanisms that helped them to resist antibiotics and thus the difficulty of controlling and eliminating of these bacteria (Weiner et al., 2016). The aims of study are isolation and diagnosis of Acinetobacterbaumannii from clinical cases including wounds, burns and respiratory tract infections and detection of Siderophores like aerobactin (iutA) gene that responsible for iron carriers as one of the important virulence factors.

Materials and Methods

Isolation and Identification

Sixty samples were collected from different pathological cases (wounds, burns, and respiratory tract infections) and from several hospitals in Baghdad (Baghdad Teaching Hospital, Burns and Wounds Hospital and Teaching Laboratories/Medical City, Al-Yarmouk Teaching Hospital, Imam Ali Hospital) for the period from 10/12/2019 until December 30/12/ 2019.The samples of A. baumannii were diagnosed using MacConkey agar and blood agar culture media, and the biochemical assays (Catalase, Oxidase), while the final diagnosis of isolates was performed using API20E and Vitek2 systems.

The sensitivity test and Molecular study

The sensitivity test to antibiotics was performed for 10 of antibiotics using Kirby Bauer method on Muller-Hinton agar according to the instruction of WHO (2003) using discs of the following antibiotics:Ceftazidime, Cefotaxime, Ciprofloxacin-Tobramycin, Gentamycin, Norfloxacin, Trimethoprim-Sulphamethoxazole, Imipenem, ColistinandAztreonam.The measurement of the diameter of inhibition zone (mm) around the antibiotic discs was used and compared with the tables of international measurements (CLSI, 2018).

Genomic DNA extraction of isolates was performed using (Wizard® Genomic DNA Purification Kit) according to the manufacture instructions (Promega, USA), anddetection of iutA gene, which responsible to iron carriers, inA. baumannii using a PCR technique with specific primers as shown in Table (1).

Table (1): The primer sequence of iutA

Gene		PrimerSequene (3-5)	Product size (bp)	Reference
	F	GGCTGGACATCATGGGAACTGG	300	AbdAL-Mahdi

iutA	**R**	**CGT CGG GAA CGG GTA GAA TCG**		**etal.,2016**

1- PCR amplification of iutA

The optimal conditions for detecting iutA gene are by using a PCR technique and AccuPower® PCRPremex kit (Bioneer, Korea). These condition were included 25 cycles for 12 minutes at 95° C for the initial denaturation of DNA, followed by a cycle included 30 seconds at 93° C as annealing stage for the attachment of primers to the template DNA, and 3 minutes at 68 ° C to elongate the bound primers, then only one cycle for 10 minutes and at 72° C for the elongation of amplified DNA strand. Then, 5 µL of the final product was transfer for electrophoresis on agarose gel (2%) with a voltage difference of 100V for 60 minutes.After performing the necessary tests for diagnosis the bacteria,this study obtained 20 isolates of Acinetobacterbaumannii from 60 samples collected from various pathological cases included wounds infections (9) isolates(36%), burns infections (6) isolates (30%), and respiratory tract infections (5) isolates (33.33%) as shown in Table (2).

Table (2): The source, number and the percentage of Acinetobacterbaumannii.

Source of Acinetobacterbaumannii	No. of samples	No. of Acinetobacterbaumannii isolates	Percentage (%)
Wounds	**25**	**9**	**36**
Burns	**20**	**6**	**30**
Respiratory tract Infection	**15**	**5**	**33.333**
Total	**60**	**20**	

Results and discussion

The results of this study were consistent with the findings of Al-Dulaimiet al. (2017) who found that the highest isolation rate of the studied bacteria was from wound infections with (10.8%) and (48.3%) from burns; this is due to the presence of bacteria in the disinfectant materials that are used to disinfect the operating hall because of the impermeability of the cell membrane for these antiseptics in addition to their presence in the humid environment in hospitals, resistance to dehydration, as well as their transmission by the medical staff, and burn infections especially hospitalized patients in burn halls, due to the loss of skin, which represents the first line of defense and contamination of corridors.Furthermore, the results showed high resistance of A. baumannii isolates to studied antibiotics; it was resistant to Ceftazidime and Cefotaxime (100%), Ciprofloxacin and Tobramycin (80%), Gentamicin (75%), Trimethoprim-Sulphamethoxazoleand Norfloxacin(65%), Aztreonem (50%) and Imipenem (40%), while it was less resistant to Colistin(10%) (Figure 1).

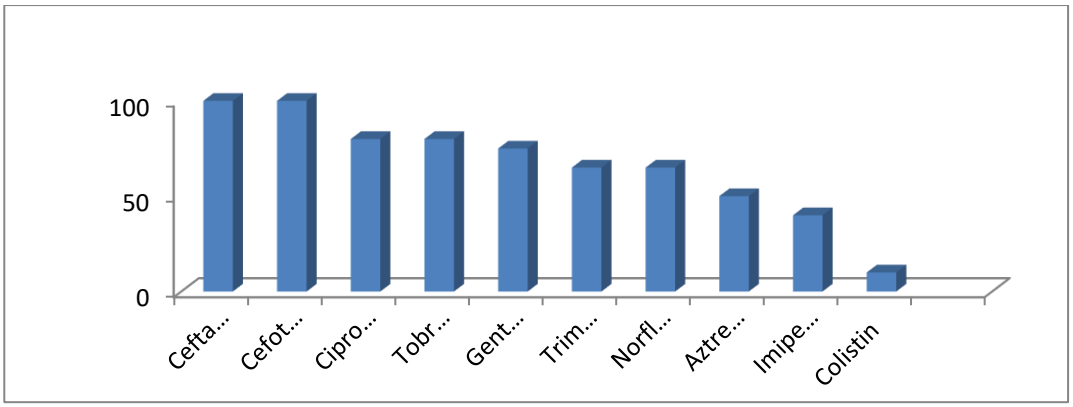

Figure (1): The percentage of resistance of A. baumanniito various types of antibiotics

The reason for the high resistance of bacteria to antibiotics is the excessive intake of antibiotics without consulting the specialists, in addition to the genetic factors carrying the resistance feature that the bacteria acquire it, as the studied isolates showed high resistance to β-lactam antibiotics represented bypenicillins and cephalosporins due to the ability of these isolates to produce the enzymes of extendedspectrum β-lactamase (EsBls) (Ibrahimagicet al., 2017).Several studies have indicated that the resistance of A. baumannii to ceftazidime and cefotaxime is due to have multiple mechanisms represented by alteration of outer membrane proteins, the presence of the efflux pumps and the modulation of the target site represented by penicillin-binding proteins (Jeonet al., 2015). Interestingly, the results of this study are in agreement with the findings of Tuwajj (2016) who conducted using (9) isolates of A. baumannii and also with the findings of Nahareet al. (2012) using (52) isolates ofA. baumanniiand found that the resistance to each of antibiotics was 100%.While the resistance of A. baumanniito aminoclycosides antibiotics is due to their ability to produce aminoglycosides-modifying enzyme, and the studied isolates of A. baumanniiin this study showed high resistance to Tobramycin by (80%) and Gentamicin by (75%). These results are in agreement with the study conducted by Karim,MaysamHailan (2019) that observed the percentage of resistance of A. baumannii isolates to Tobramyin and Gentamicin was 78.6% and 85.7%, respectively; and it is consistent with Gupta et al. (2016) study who found that A. baumannii resistance to Tobramyin and Gentamicin was 80%.Moreover, our local isolates also showed resistance to Quilones;the reason for this resistance is due to genetic mutations that occur in A. baumannii that prevent DNA replication, as well as their possession of efflux pumps and the presence of transposons genes (Zakiet al., 2018).The percentage of isolates resistance to Norfloxacinwas 65% and Ciprofloxacin was 80%; these results are consistent with the findings of Al-Hamadaniet al. (2014)who revealed the percentage of resistance to the above antibiotics was 72.5% and 52.5% respectively.A. baumanniiisolates showed resistance to Trimethoprim-Sulphamethoxazole due to their ability to possess multiple mechanisms represented by altering the permeability of the plasma membrane, Efflux pump and plasmid-borne genes (Esterlyet al., 2011). The resistance of studied isolates in this study was 65%; this is in agreement Daryanavard and Safaei (2015) who indicated that the percentage of resistance to this antibiotic was 65%.Imipenem is the best antibioticthat prevent the growth of this bacteria; although during the last years from 2010-2019, the resistance of A. baumanniitoImipenemwas increased due to its

production of Carbapenem hydrolyzing class DB-lactamase (CHDLs) enzymes (Shafigetet al.,2018). Moreover, the results of current study revealed that the resistance of A. baumanniiImipenem was 40% which is consistent with the findings of Al-Hadidet al. (2019) and Al-Dulaimiet al. (2017)who found that the resistance to Imipenem of this bacteria was 50%. Additionally, it is consistent with findings of Karim,MaysamHailan (2019) which was found the percentage of the resistance A. baumanniiisolateswere 42.8% and 50% to Imipenem andAztreonamrespectively. Therefore, Imipenem is no longer the best antibiotic for these bacteria.On the other hand, Colistin is considered as one of the most toxic antibioticsand act mainly on LPS, the main component of Gram-negative bacteria.The current results showed that the resistance of A. baumannii to this antibioticswas 10%; this is in agreement with the observation of Karim,MayssamHailan (2019)who foundthat the resistance to Colistin was 14.3%.Interestingly, due to the frequent use of Colistin in the treatment of infection with these bacteria, the resistance of A. baumannii to this antibioticwas increased (Qureshiet al., 2015). However, most studies have proven that A. baumanniiinfectioncan be treated and controlled by combining of antibiotics(Lee et al., 2017).This study investigatedthe presence of iutA gene that responsible forsiderphores encoding using PCR technique. The results revealed that (17) isolates by (85%) haveiutA gene, these isolates involved (8)isolatesby(40%) from wounds infections, (5) isolates by (25%) from burn cases, and (4) isolates by (20%) from respiratory tract infections. The molecular size of the iutA gene is 300 base pairs after electrophoresis as shown in Table (3) and Figure (2).

Table (3): The number and percentage of Acinetobacterbaumannii that possess the (iutA) gene.

Source	No. of A.baumannii isolates	The percentage (%)
wounds	**8**	**40**
Burns	**5**	**25**
Respiratory tract Infection	**4**	**20**
Total number	**17**	**85**

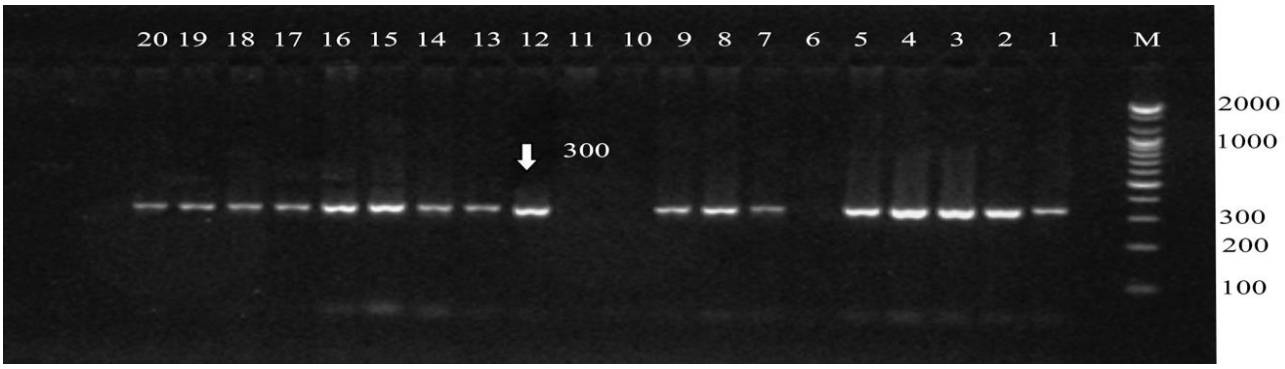

Figure (2): The electrophoresis of PCR product of iutA gene (300pb) in Acinetobacterbaumanniiisolates.

The expression of iutA gene in bacteria that encoding of Siderophores is of aerobatic type which is a protein found in the outer membrane of the bacteria that is responsible for absorbing iron; it is composed of a polypeptide with a molecular mass of 78223Da, as it can efficiently isolate iron that helps the growth of these microorganisms in host tissues and body fluids (Momtazet al., 2015). The results of this study are in agreement with findings of Landgrafet al. (2012) who found that the expression of iutA gene in A.baumanniiin wounds isolates was (38%), while the results ofAbd-AlMahdiet al. (2016) reported that he percentage of this gene in A.baumanniiin isolated from wounds infections was 57.1%.Furthermore, the current results are also in agreement with the observations of DARVISHI (2016) who found that the expression percentage of iutA gene was 15% in A. baumannii isolates isolated from respiratory tract infections from Tehran hospitals in Iran, while Johangiriet al. (2019) reported that the prevalence of iutAgenewas 10%in A. baumannii isolates.

References

AbdALMahdi, Z.; Bunyan,I,A and AL Shukri,M,S.(2016).Molecular Study for some Virulence Factors of Acinetobacterbaumanniiisolated from patients with Wound Infection in HillaCity.World Journal of pharmaceutical Research.5(3):175-187.

AL-Hadeedy, U. E.I ;Najdat,B .M and AL–jebory.(2019). Isolation and Diagnosis of AcinetobacterbaumanniiRecentlyIsolated from patients in Kirkuk Hospitals and study their Antibiotics Resistance. KUJSS .14(13):155-173.

Al-Hamadani, A. H.; ALMohana, A. M. and ALKhazaali, A. S. (2014). Emergence of Plasmid mediated aac(6)-Ib-cr Gene in Flouroquinolon-resistant Acinetobacter spp. Quality Management Journal, 10(17).

Asif, M.; Alvi, I.A. and Rehman, S.U. (2018). Insight intoAcinetobacterbaumannii: pathogenesis, global resistance,mechanisms of resistance, treatment options, and alternativemodalities. Infect Drug Resist., 11:1249-1260.

CLSI document M100-S17. Wayne, PA, USA: CLSI; 2018. Clinical and Laboratory Standards Institute. Performance Standards forAntimicrobial Susceptibility Testing; Seventeenth Informational Supplement.

DARVISHL.M. (2016).Virulence Factors profile and Antimicrobial Resistance of Acinetobacterbaumannii Strains Isolated from Various Infection Recovered from Immunosuppressivepatients.Biomedical 8 pharmacology Journal. 9 (3). 1057-1062.

Daryanavard, R. and Safaei, H. S.(2015). Virulence genes and antimicrobial resistance properties of Acinetobacterbaumanniiisolated from pediatrics suffered from UTIs. International Journal of Advanced Research in Biological Sciences, 2(11): 272–27.

Demirdal, T.; Sari, U.S. and Nemli, S.A. (2016). Is inhaled colistinbeneficial in ventilator associated pneumonia or nosocomialpneumonia caused by Acinetobacterbaumannii? Ann. Clin.Microbiol. Antimicrob., 15(1):1–6.

Esterly, J.S.; Griffith, M.; Qi, C.; Malczynski, M.; Postelnick, M.J.andScheetz, M.H. (2011). Impact of carbapenemresistanceand receipt of active antimicrobial therapy on clinical outcomes of Acinetobacterbaumanniibloodstreaminfections. Antimicrob Agents Chemother., 55(10): 4844–4849.

Gupta, R.; Malik, A.; Rizvi, M. and Ahmed, M. (2016).Presence of metallo-beta-lactamases (MBL), extended-spectrum betalactamase (ESBL) &AmpC positive non-fermenting Gram-negative bacilli among Intensive Care Unit patients with special reference tomolecular detection of blaCTX-M &blaAmpCgenes.Indian journal of Medical Research, 144(2): 271-275

Ibrahimagić, A.; Kamberović, F.; Uzunović, S.; Bedenić, B. and Idrizović, E. (2017). Molecular characteristics and antibiotic resistance of Acinetobacterbaumanniibeta-lactamase-producing isolates, a predominance of intrinsic blaOXA-51, and detection of TEM and CTX-M genes. Turk J Med Sci., 47(2): 715-720.

Jahangiri,S;Malekzadegen,Y;Motamedifar,M and Hadi,N .(2019).Virulene genes profile and biofilm formation abililty of Acinetobaterbaumanniiof a tertiary care hospital in Southwest of Iran . GeneReports.17:100481.

Jeon, J.H.; Lee, J.H.; Lee, J.J.; Park, K.S.; Karim, A.M.; Lee, C.R.; Jeong, B.C. and Lee, SH. (2015). Structural basis for carbapenem-hydrolyzing mechanisms of carbapenemases conferring antibiotic resistance. Int J Mol Sci., 16(5): 9654-9692.

Lee, C.R.; Lee, J.H.; Park, M.; Park, K.S.; Bae, I.K.; Kim, Y.B.; Cha, C.J.; Jeong, B.C. and Lee, S.H. (2017). Biology of Acinetobacterbaumannii: Pathogenesis, Antibiotic Resistance Mechanisms, and Prospective Treatment Options. Front Cell Infect Microbiol., 7: 55.

Lee, J.C.; Oh, J.Y.; Kim, K.S.; Jeong, Y.W. Park, J.C. and Cho, J.W. (2010). Apoptotic cell death induced by Acinetobacterbaumanniiin epithelial cells through caspase-3 activation. APMIS, 109(10): 679–684.

Momtaz, H., SeyedMortezaSeifati, M.S. and MarziyehTavakol, M. (2015). Determining the Prevalence and Detection of the Most Prevalent Virulence Genes in Acinetobacterbaumanniiisolated From Hospital Infections. Int. J. of Med. Lab., 2(2): 87-97.

Nahar, A.; Anwar, S.; Abu-Saleh, A. and Amin, M. (2012). Virulence factors sand Antibiotic Susceptibility Pattern of Acinetobacter Species In a tertiary care Hospital in Bangladesh. Ibrahim Medical College Journal, 6(1): 27-30.

Qureshi, Z.A.; Hittle, L.E.; O'Hara, J.A.; Rivera, J.I.; Syed, A.; Shields, R.K.; Pasculle, A.W.; Ernst, R.K. and Doi, Y. (2015). Colistin-resistant Acinetobacterbaumannii: beyond carbapenem resistance. Clin. Infect. Dis., 60(9): 1295-1303.

Tuwaij,N.S.S.(2016).Molecular Screening of Some β-Lactam Resistance Genes Producing Clinical Isolated of Acinetobacterbaumannii.Journal of Babylon University /Pure Applied Sciences, 24(7).

Vijayakumar, S.; Rajenderan, S.; Laishram, S.; Anandan, S.; Balaji, and Biswas, I. (2016). Biofilm formation and motility depend on the nature of the Acinetobacterbaumanniiclinical isolates Front. Public Health, 4:105.(B).

Koga, L.V, Tomazetto, G, Cyoia, S.P., Neves, S.M., Vidotto, C.M.,GersonNakazato, G. and Kobayashi, T.K.R. (2014). Molecular Screening of Virulence Genes inExtraintestinal Pathogenic Escherichia coli Isolated from Human Blood Culture in Brazil. Volume Article ID 465054, 9 pages.

Weiner, L.M.; Webb, A.K.; Limbago, B.; Dudeck, M.A.; Patel, J.; Kallen, A.J.; Edwards, J.R. and Sievert, D.M. (2016). Antimicrobial-Resistant Pathogens Associated With Healthcare-Associated Infections: Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011-2014. Infect. Control Hosp. Epidemiol., 37(11):1288-1301.

WHO (World Health Organization). (2003). Basic Laboratory procedures in clinical Bacteriology . 2nd ed .Geneva, Switzerland.

Zaki, M.E.S.; AbouElKheir, N.; Mofreh, M. (2018). Molecular Study of Quinolone Resistance Determining Regions of gyrA Gene and parC Genes in Clinical Isolates of Acintobacter Apr 30;12:116-122. doi: 10.2174/1874285801812010116. PubMed PMID: 29785218; PubMed Central PMCID: PMC5958293.

Kareem, MiasemHilan (2019). Inhibition the biosynthesis of Acinetobaterbaumannii that resistant to Imipenem. M.S. thesis. Univbersity of Diyala.

Al-Dulaimi, A.A.F; Al-Taai, H.R.R. and Al-Bajlany, S. M. M. (2017). Virulence Factors of Acinetobacterbaumannii isolated from different clinical specimens in Baquba. Diyala Journal For Pure Science, 13(issue1 part1): 13-26.