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MOLECULAR GENOTYPING SURVEY FOR *BLA* TEM VIRULENCE GENE OF *ACINETOBACTER BAUMANNII* ISOLATES, IRAQ

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Abstract

ESBLs have altered enzymatic activity from their progenitors. Furthermore, they have the ability to hydrolyze penicillins, first and second generation of cephalosporins. Adding that, they can also hydrolyze the third generation of cephalosporins and monobactams. The most prominent members of this group are TEM and SHV enzymes. The main challenge in *A. baumannii* pathogenesis is unity, connection, expression and regulation of genetic elements that contribute to the MDR phenotype. For example, the presence of OXA and ESBLs genes such as TEM and SHV lead to resistance to almost all β-lactams antibiotics, including carbapenems. The isolated *A. baumannii* from many clinical sources, were identified according to traditional microbiological, and molecular techniques (PCR for amplification of organism specific *bla*TEM gene). In the present study (44) clinical isolates from different clinical sources including (Sputum, Wounds, Burns, UTI and blood). (39) Samples were positively identified according to traditional biochemical tests. PCR was performed (39) samples, and the PCR results were (25) samples by using *bla*TEM gene detection. This study dealing with the molecular genotyping for virulence gene (*bla*TEM) in Iraqi *A. baumannii* isolates.

Keywords: Molecular genotyping survey, Blatem virulence gene, *Acinetobacter baumanni*

Introduction

The *Acinetobacter baumannii*, which considers as newly giving rise opportunistic pathogen, which is in charge of a significant ratio of nosocomial infections inclusive endocarditis, urinary tract infections, septicemia, meningitis, asurgical site infections, and ventilator related to pneumonia among intensive care unit patients in hospitals. *Acinetobacter baumannii* is implicated in a variety of opportunistic nosocomial infections, including bacteremia, epidemic pneumonia, secondary meningitis, and urinary tract infections. Treatments of infections caused by epidemic strains of *A. baumannii* are often extremely difficult because of the widespread resistance of strains to diverse antimicrobial agents. Resistance to various antimicrobial agents by these bacteria has usually resulted from intrinsic factors or acquisition of genes encoding antimicrobial resistance determinants. The antimicrobial resistance mechanisms include production of beta-lactamases, production of aminoglycoside-modifying enzymes, decreased expression of outer membrane proteins, mutations in topoisomerase or gyrase, and over expression of efflux pumps (Ji *et al.,* 2016).

This organism raised multidrug resistance and can pull out in environments of hospital for a very long time, so this has enabled it to protrude as a succeeded opportunistic nosocomial pathogenic organism. *A. baumannii* is commonly diffused in clinical climates, pulling out as a commensal on the skin or hair of hospital crew and patients and able to colonize on a many of body surfaces. Currently, a lot of nosocomial *A. baumannii* isolates have antibiotics resistance against most or all major antibiotics classes inclusive beta-lactams, aminoglycosides, fluoroquinolones (Wayne *et al*., 2016; Dent *et al.,* 2010).

Since β-lactam antibiotics were used many years ago, thus β-lactamases have evolved along with them Acinetobacter species has a wide range of β-lactamases, hydrolyzing the penicillin, cephalosporins and carbapenem. Today more than 300 types of β- lactamase have been identified that are divided into groups based on the structure and substrate they affect. ESBLs belong to the 2be group according to the Bush-Jacoby-Medeiros classification which are produced by the initial mutation and amino acid substitutions of early β-lactamases. ESBLs have altered enzymatic activity from their progenitors. Furthermore they have the ability to hydrolyze penicillins, first and second generation of cephalosporins. Adding that, they can also hydrolyze the third generation of cephalosporins and monobactams. The most prominent members of this group are TEM and SHV enzymes. The main challenge in *A. baumannii* pathogenesis is unity, connection, expression and regulation of genetic elements that contribute to the MDR phenotype [4]. For example, the presence of OXA and ESBLs genes such as TEM and SHV lead to resistance to almost all β-lactams antibiotics, including carbapenems (Melika Sharif, *et. al*., 2014)

Inactivation of b-lactams by b-lactamases is a major antibiotic resistance mechanism in *A. baumannii*. Based on sequence homology, b-lactamases are grouped into molecular classes**,** A, B, C, and D (Jeon *et al*., 2015). All four classes of b**-** lactamases were identified in *A. baumannii*. Recent studies have shown that *A. baumannii* has natural competence to incorporate exogenous DNA and its genome has foreign DNA at high frequencies, implying frequent horizontal gene transfer in this pathogen (Ramirez *et al*., 2010; Touchon *et al*., 2014; Traglia *et al*., 2014). Additionally, albumin, a main protein in blood**,** enhances natural competence of *A. baumannii* (Traglia *et a*l 2016). Therefore, natural competence of *A. baumannii* may contribute to identification

of a large number of b-lactamases in this threatening human pathogen.

Class A b-lactamases inhibited by clavulanate hydrolyze penicillins and cephalosporins more efficiently than carbapenems, except for some KPC type enzymes (Jeon *et al*., 2015). A number of class A b-lactamases, including TEM**,** SHV, GES, CTX-M, SCO, PER, VEB, KPC, and CARB, have been identified in *A. baumannii* . Some of these enzymes**,** such as TEM-1, CARB-4, and SCO-1, are narrow-spectrum b-lactamases, whereas other enzymes (e.g., PER-1, TEM-92**,** CARB-10, SHV-5, PER-2, CTX-M-2, CTX-M-15, VEB-1**,** GES-14, and PER-7) are ESBLs. Some carbapenemases, such as GES-14 and KPC-2, have been detected in *A. baumannii* (Moubareck *et al*., 2009; Bogaerts *et al*., 2010).

Materials and Method

Phenotypic characterization

Sample collection

Samples (44) were collected from patients hospitalized in AL-Hillah Teaching Hospital and Marjan Medical City, at Babylon province, and the Medical city, at Baghdad. Clinical specimens comprising; wounds, burns, sputum and urine were collected with regarding experimental guidelines.

Identification of *Acinetobacter baumannii* isolates

Microscopical examination (Gram stain)

All the bacterial isolates were examined for gram stain ability, shape and color of the cells. All microbial tests were carried out according to Forbes *et al.* (2007) Oxidase test, Catalase test, Kligler iron agar (KIA), Indole production test, Motility test, Urease production test, Citrate utilization test, Lactose fermentation test, Hemolysin production, Growth at 44ºC.

Molecular examination and characterization

All bacterial isolates were subject to PCR in order to detect the bla_{TEM} gene. The forward and reverse primers in the table (1) and condition as shown in table (2).The PCR product is 800 bp fragments of bla_{TEM} gene. Using 20µL of PCR reaction, 2.5 µl DNA template (100 ng/µl) was amplified using 12.5 µl of Go *Taq*® green master mix 2X (Intrin, South Korea) and 1 µl of each primer (10 pmol/µL) for each. The PCR products were detected by agarose gel electrophoresis.

Table 1 : Listed the Sequences of the Primers Used for Conventional PCR to Detect *Acinetobacter baumannii*

Gene	Sequence	PCR product size(bp)
blaTEM	GAGTATTCAACATTTCCGTGTC	800bp
	TAATCAGTGAGGCACCTATCTC	

Table 2 : PCR Thermo-Cycling Conditions for *blaTEM* Genes

Loop's steps	Temperature	Time	Number of cycles
Initial denaturation	94 ºC	3 min	1
Denaturation	94 ºC	30 sec	30
Annealing	45 ºC	1min	
Extension	72 ºC	1min	
Final extension	72 ºC	10 min	1

Results and Discussion

The recent studies gave an evidence that the *A. baumannii* diffused in a hospital environment and become threat of undetected reservoirs. However, the origin root of infection may comprise health care apparatus or the climate can relate with transmit of microorganisms between patients and staff (Fattouh and El-din, 2014; Carla *et al.,* 2018). Identification of *Acinetobacter baumannii* isolates by ordinary phenotypic tests were done on the (44) samples, and only (39) sample were positive for *Acinetobacter baumannii* as shown in figure (1)

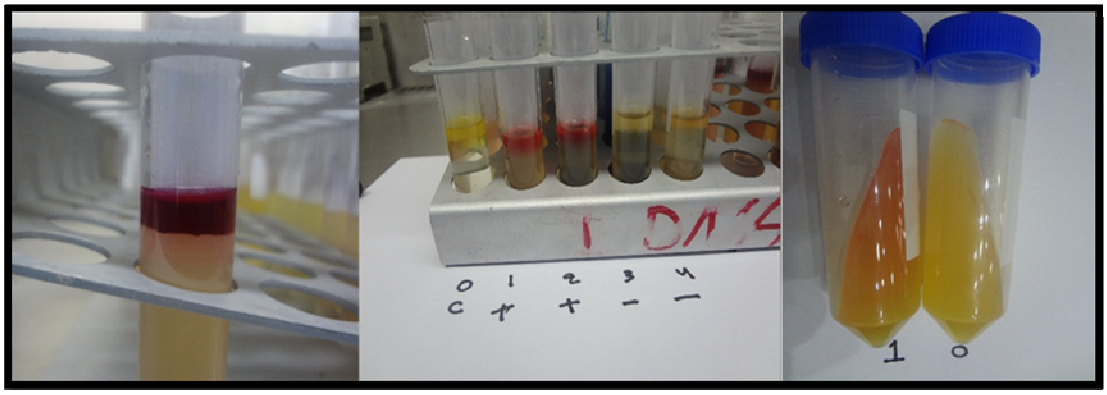

Fig. 1 : Identification of *Acinetobacter baumannii* by using biochemical test

Polymerase chain reaction (PCR) was performed on the (39) sample by using *bla*TEM gene detection Results of PCR for *bla*TEM gene for bacterial isolates were only (25) have positive results as shown in figure (2).

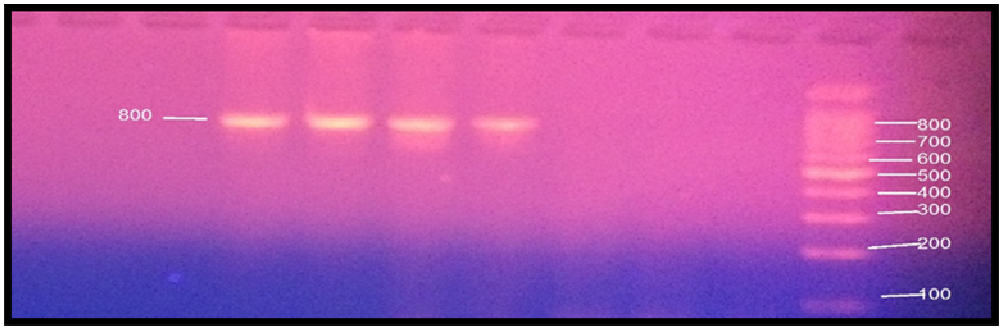

Figure (2): 2% Agarose Gel Electrophoresis of PCR Amplified Products for *bla*TEM *Gene*. Lane (M): l00bp ladder, Lane (4-7): Positive Result with Positive Bands of 800 bp *A. baumannii*, Lane (1-3): Negative Result. (5 v/cm)

The results of this study is widely accepted ton that of Mohsen Aziz, *et. al.* 2017, that indicated the spread of *bla*TEM gene in *Acinetobacter baumannii*. isolates. Also the results obtained in this study was agreed with Melika Sharif, *et al*., 2014.

References

Bogaerts, P.; Naas, T.; El-Garch, F.; Cuzon, G.; Deplano, A. and Delaire, T. (2010). GES extended-spectrum b-lactamases in *Acinetobacter baumannii* isolates in Belgium. Antimicrob. Agents Chemother. 54, 4872–4878.

Dias, C.; Borges, A.; Oliveira, D.; Martinez-Murcia, A.; Saavedra, M.J. and Simões, M. (2018). Biofilms and antibiotic susceptibility of multidrug-resistant bacteria from wild animals. PeerJ, DOI 10.7717/peerj.4974; Published June 12, 2018 PubMed 29910986.

Dent, L.L.; Marshall, D.R.; Pratap, S. and Hulette, R.B. (2010). Multidrug resistant *Acinetobacter baumannii*: a descriptive study in a city hospital.

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

Ji Youn Sung, Sun Hoe Koo, Semi Kim, and Gye Cheol Kwon, (2016). Persistence of Multidrug-Resistant *Acinetobacter baumannii* Isolates Harboring blaOXA-23 and bap for 5 Years. J. Microbiol. Biotechnol., **26(8):** 1481-1489.

Melika, S.; Reza, M. and Noor, A. (2014). Molecular identification of TEM and SHV extended spectrum β-lactamase in clinical isolates of *Acinetobacter baumannii* from Tehran hospitals. Volume 2014, Year 2014 Article ID jgmi-00020.

Mohsen, A.; Seyed, H.M.; Misagh, E.; Simin, G.; Siavash, V.; Amirhooshang, A.; Mahsa, K. and Kamal, A. (2017). Prevalence of Extended-Spectrum β-Lactamases and Antibiotic Resistance Patterns in *Acinetobacter baumannii* Isolated from Clinical Samples in Kermanshah, Iran. Jundishapur Journal of Microbiology: 10 (12): e61522.

Moubareck, C.; Bremont, S.; Conroy, M.C.; Courvalin, P. and Lambert, T. (2009). GES-11, a novel integron-associated GES variant in *Acinetobacter baumannii*. Antimicrob. Agents Chemother, 53: 3579–3581.

Ramirez, M.S.; Don, M.; Merkier, A.K.; Bistue, A.J.; Zorreguieta, A. and Centron, D. (2010). Naturally competent *Acinetobacter baumannii* clinical isolate as a convenient model for genetic studies. J. Clin. Microbiol., 48: 1488–1490.

Touchon, M.; Cury, J.; Yoon, E.J.; Krizova, L.; Cerqueira, G.C. and Murphy, C. (2014). The genomic diversification of the whole Acinetobacter genus: origins, mechanisms, and consequences. Genome Biol. Evol., 6: 2866–2882.

Traglia, G.M.; Chua, K.; Centron, D.; Tolmasky, M.E. and Ramirez, M.S. (2014). Whole-genome sequence analysis of the naturally competent *Acinetobacter baumannii* clinical isolate A118. Genome Biol. Evol., 6: 2235–2239.

Traglia, G.M.; Quinn, B.; Schramm, S.T.; Soler-Bistue, A. and Ramirez, M.S. (2016). Serum Albumin and Ca^{2+} Are Natural Competence Inducers in the Human Pathogen *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 60: 4920–4929.

Wayne, A.; Warner, S.N.; Kuan, R. Hernandez, M.C. Chong, P.J. Ewing, J. Fleischer, J. Meng1, S. Chu, D. Terashita, L'Tanya English; Chen, W. and Xu, H.H. (2016). Molecular characterization and antimicrobial susceptibility of *Acinetobacter baumannii* isolates obtained from two hospital outbreaks in Los Angeles County, California, USA.BMC Infectious Diseases, 16: 194.