MOLECULAR GENOTYPING SURVEY FOR BLA TEM VIRULENCE GENE OF
ACINETOBACTER BAUMANNII ISOLATES, IRAQ
Taif Majid Abdulhussein¹, Haider Turky Mousa Al-Mousawi², Jassim Mohamed Abdallah³,
Alyaa Saad Abed⁴ and Rand Jabbar Sattar⁴
¹Medical Laboratory Technology, College of Medical and Health Technology, Gilgamesh Ahliya University (Gau),
Baghdad, Iraq.
²Genetic engineering department, Biotechnologies College, University of Al-Qasim green, Iraq.
³Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq
*Corresponding author: Taifgpl@gmail.com

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 blaTEM 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 blaTEM gene detection. This study dealing with the molecular genotyping for virulence gene (blaTEM) in Iraqi A. baumannii isolates.

Keywords: Molecular genotyping survey, Blatem virulence gene, Acinetobacter baumannii

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 wide range 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 substance 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 al 2016). Therefore, natural competence of A. baumannii may contribute to identification...
of a large number of β-lactamases in this threatening human pathogen.

Class A β-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 β-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 44°C.

**Molecular examination and characterization**

All bacterial isolates were subject to PCR in order to detect the blaTEM 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 blaTEM 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**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
<th>PCR product size(bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>blaTEM</td>
<td>GAGTATCAACATTTCCGTGTC</td>
<td>800bp</td>
</tr>
<tr>
<td></td>
<td>TAATCAGTGAGGCACCTATCTC</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Loop’s steps</th>
<th>Temperature</th>
<th>Time</th>
<th>Number of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>94 °C</td>
<td>3 min</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94 °C</td>
<td>30 sec</td>
<td>30</td>
</tr>
<tr>
<td>Annealing</td>
<td>45 °C</td>
<td>1 min</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72 °C</td>
<td>1 min</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72 °C</td>
<td>10 min</td>
<td>1</td>
</tr>
</tbody>
</table>

**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).

![Identification of Acinetobacter baumannii by using biochemical test](image1.jpg)

Polymerase chain reaction (PCR) was performed on the (39) sample by using blaTEM gene detection Results of PCR for blaTEM gene for bacterial isolates were only (25) have positive results as shown in figure (2).
The results of this study is widely accepted on that of Mohsen Aziz, et. al. 2017, that indicated the spread of blaTEM gene in Acinetobacter baumannii isolates. Also the results obtained in this study was agreed with Melika Sharif, et al., 2014.

References


