MOLECULAR GENOTYPING SURVEY FOR BLAIMP VIRULENCE GENE OF ACINETOBACTER BAUMANNII ISOLATES, IRAQ

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Abstract

The emergence of carbapenemase-producing Acinetobacter baumannii isolates have been worldwide reported and recognized, and A. baumannii isolates having blaIMP, blaCTX, blaSHV, and blaOXA-like genes are often resistant to various antimicrobial agents, also they are used as detecting genes for these isolates. Antimicrobial resistance can be especially strong for A. baumannii isolates.

The isolated A. baumannii from many clinical sources, were identified according to traditional microbiological, and molecular techniques (PCR for amplification of organism specific blaIMP gene).

In the present study (39) clinical isolates from different clinical sources including (Sputum, Wounds, Burns, UTI). (34) Samples were positively identified according to traditional biochemical tests. PCR was performed (34) samples, and the PCR results were (0) samples by using blaIMP gene detection.

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

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 β-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 β-lactams, aminoglycosides, fluoroquinolones (Wayne et al., 2016; Dent et al., 2010).

Metallo-β-lactamases have raised in the worldwide as a major source of acquired broad-spectrum β-lactam resistance. They hydrolyze practically all β-lactams classes (except monobactams), including carbapenems, which often consider the last chosen for the treatment of infections with MDR gram negative bacteria (Scotta C et al., 2011).

Molecular epidemiological case of A. baumannii isolated from clauses in the climates of hospital and the
existence of many different resistance determinants to infer their potential ability to cause human infections. There are two predominant kinds of transferable MβLs among clinical isolates, VIM and IMP. Most of the VIM- and IMP type MβL genes are found as gene cassettes inserted into integrons existing on plasmids or on the chromosome. These integrons may be related with transposon-like structures which may participate to their variable spread and position (Dent et al., 2010).

Carbapenems (meropenem, imipenem and ertapenem) have a wide phantom of antibiotic among all beta-lactams and are firstly used to treat infections with aerobic G-ve bacteria. Based on their dependency on divergent cations for activating the enzyme, carbapenemases able to split into a non metallo-carbapenemases (zincindependent classes A, C and D) and metallo-carbapenemases (zinc-dependent class B) (Jeon et al., 2014).

Class A carbapenemases, which have the KPC, SME, IMI and NMC-A families and some GES enzymes, have most considerably been discovered in isolates from Enterobacteriaceae and in other species like P. aeruginosa. Clavulanate inhibites these enzymes, excluding some KPC-type enzyme(s) like KPC-2, and hydrolyze cephalosporins or penicillins more efficiently than carbapenems. The IMP and VIM families as well as SPM-1 which involved in class B carbapenemases, have previously been detected in strains of P. aeruginosa, A. baumannii and members of the Enterobacteriaceae family (Thomson, 2010).

Multidrug-resistant (MDR) strains of Acinetobacter baumannii have emerged in recent decades. This opportunistic pathogen is responsible for severe infections, particularly hospital-acquired pneumonia and bloodstream, urinary tract, and wound infections, and has become of worldwide concern. As in other bacterial species, multidrug resistance can be achieved by two mechanisms: (i) horizontal transfer of genetic information and (ii) mutation of endogenous genes. Acquired resistance determinants that are carried by plasmids, transposons, and integrons have been described for Acinetobacter spp. Determination of the genomic sequence of several A. baumannii strains has improved our knowledge of the ways in which A. baumannii can develop antibiotic resistance. An 86-kb resistance island, AbaR1, found in strain AYE, contains as many as 25 antibiotic and 20 antiseptic and heavy metal resistance genes. Variants of this island are integrated at the same chromosomal locus in a significantly high proportion of MDR strains. In addition to these acquired resistance genetic elements, alterations in endogenous functions are involved in resistance, such as over expression of chromosomally encoded β-lactamases ADC and OXA-51-like; loss of porins CarO and Omp33–36 contributing to carbapenem resistance; mutation in the GyrA and ParC fluoroquinolone targets; and over expression of efflux systems (Se´bastien et al., 2010).

The option for this pathogen is carbapenems drugs, and carbapenem-resistant A. baumannii has been often hospital faced. Meropenem and imipenem are carbapenems that stay active against organisms carrying most Ambler classes of β-lactamases which included many Gram-negative bacilli, involving Acinetobacter spp. However, Acinetobacter isolates is increasingly faced carbapenem resistance in worldwide. One of the main carbapenem resistance mechanisms in this pathogen is the manufacturing of carbapenem hydrolyzing β-lactamases. These specific groups of β-lactamases are class B Metallo β-lactamases (MBLs) including IMP and VIM (M. Fattouh and A. N. El-din, 2014; Zuhair S. Alsehlawi et al., 2015).

Materials and Method

Phenotypic characterization

Sample collection

Samples (39) 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 microobial 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 following genes; bla IMP gene. PCR technique with a final volume of 20 μl was done. Table 1 indicates primers that have been used for PCR amplification, and table 2 involved PCR primers conditions. The PCR products were detected by agarose gel electrophoresis.

Results and Discussion

The recent studies gave an evidence that the A. baumannii diffused in a hospital environments and become threat of undetected reservoirs. However, the origin root of infection may comprise health care
Table 1: Sequences of the mentioned primers.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>PCR product size(bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>blaIMP</td>
<td>F ‘-GAAAGCGTATTAGTTCACTAC-3’</td>
<td>559</td>
<td>[11]</td>
</tr>
<tr>
<td>gene</td>
<td>R ‘-CTTCACCTGACTTGGAAC-3’</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: PCR primers conditions.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Initial denaturation</th>
<th>Time</th>
<th>Temperature (°C) / Time</th>
<th>Cycling Condition</th>
<th>Final extension</th>
<th>Number of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>blaIMP gene</td>
<td>95 °C 5 min</td>
<td></td>
<td>94 °C 15 sec 55 °C 30 sec 72 45 sec</td>
<td>Denaturation time Annealing time Extension Time</td>
<td>72 3 min</td>
<td>35</td>
</tr>
</tbody>
</table>

Fig. 2: PCR product 559 bp of blaIMP gene: all samples have negative result.

apparatus or the climate can related with transmit of microorganisms between patients and staff (Fattouh and El-din, 2014; Carla et al., 2018).

Identification of Acinetobacter baumannii isolates by ordinary phynotypic tests were done on the (39) samples, and only (34) sample were positive for Acinetobacter baumannii. Polymerase chain reaction (PCR) was performed on the (34) sample by using blaIMP gene detection. Fig. 1 showed PCR result of blaIMP detection gene (559pb).

Results of PCR for blaIMP gene for all bacterial isolates (68) were have negative results as shown in Fig. 2.

This result was agreed with Fattouh and El-din (2014) who revealed that the Acinetobacter baumannii isolates don’t have blaIMP gene in their genome. Also this result agreed with who reported that the present study showed no detectable bla IMP genes in CRAB isolates. This results is agreed with Rabeea and Eman (2017), who did not detect any bla IMP genes A. baumannii.

References


Multidrug resistant Acinetobacter baumannii: a descriptive study in a city hospital. BMC Infect Dis., 10:196.


