Isolation and Identification of Nosocomial Pathogen Acinetobacter baumannii From Al-Hussien Medical City in Karbala

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ABSTRACT

Objectives: Acinetobacter baumannii is among the most opportunistic pathogenic nosocomial associated with the hospital settings infections such as bacteremia, meningitis and UTIs. The growing emergence of these organisms’ multidrug resistance demonstrates the need to identify the spread of these bacteria and their resistance to the antibiotics which are used in Iraqi hospitals.

Methods: A total of 135 clinical samples from patients (urine, blood, CSF, swabs of wound and burns) and swabs of medical devices from ICU and burn units were collected in AL-Hussein Medical City in Karbala during the period from July to October 2018. Furthermore, Polymerase Chain Reaction Technique (PCR) was performed to identify the β-Lactamase enzyme encoding resistance genes include all of bla oxa-51 and bla ampc gene.

Results: 15 isolates (11.11%) of Acinetobacter baumannii have been described, with swabs of burns showing a high percentage of A. baumannii isolates (17.85%) Whereas other samples were variable in percentage. Susceptibility of 15 A. baumannii isolates to 16 different antimicrobials has been evaluated, the results showed that five isolates were multi drug resistance (MDR) and the others were extensive drug resistance (XDR). The PCR analysis showed that all isolates have bla oxa-51 gene while 11 isolates have bla ampc gene.

Conclusion: It was concluded that there is a high prevalence of Acinetobacter baumannii isolates in the burns unite and that all isolated bacteria characterized by their high antibiotic resistance, this resistance was confirmed by the detection of β-Lactamase enzymes which are encoded by the resistance genes bla oxa-51 gene and bla ampc gene.

INTRODUCTION

According to the World Health Organization (WHO), Acinetobacter baumannii at the top of the list of the most dangerous bacteria and antibiotic resistance 1 and the mortality rate caused by these bacteria range between 20 – 60 %. The bacterium can survive on solid and dry surfaces up to 5 months. There are several factors that are likely to be a significant cause of A. baumannii spreading inside the hospital settings such as the ability to develop...
in a broad variety of pH and temperature values, biofilm-forming capability on abiotic surfaces such as medical devices and catheters and the capacity to regulate innate and acquire antimicrobial resistance mechanisms 3. The treatment of infections caused by A. baumannii has lately started to be difficult because it has a flexible gene structure that has helped it to acquire many resistance genes for a large number of antibiotics through horizontal gene transfer, especially resistance to Carbapenemases, which are essential antibiotics in the treatment of these bacteria. The most common mechanism is to produce β-lactamases which are either acquired or intrinsic on the bacterial chromosome 4. The resistance to carbapenem is primarily mediated by the synthesis of class D β-lactamases (oxacillinases), particularly bla oxa-51, which are intrinsically located on the chromosome 5. Another important enzyme Class C β-lactamases (cephalosporinases) gives penicillin and cephalosporin resistance that is generally encoded on the chromosome (although the bla ampC gene has also been found in plasmids) 6. This study aimed to the isolation of Acinetobacter baumannii from clinical sources and investigation of antibiotic resistance genes in the isolated bacteria.

MATERIALS AND METHODS

Isolation and identification of A. baumannii

A total of 135 samples were collected from AL-Hussein Medical City in Karbala, these samples were cultivated on blood agar and MacConkey agar and incubation overnight at 37°C. Suspected bacterial isolates were identified by traditional techniques and API20NE system (bioMerieux, France).

Antimicrobial susceptibility Test

According to Kirby-Bauer method of disc-diffusion on Mueller-Hinton agar was dependent in antimicrobials susceptibility test 7. For 16 different antimicrobial agents which are included: Ticarcilline/Calvulate (TIM), Imipenem (IEM), Meropen (MER), Cefotaxime (CRO), Ceftazidine (CAZ), Cefepine (FEP), Amikazine (AK), Gentamicine (CN), Tobramycin (TOB), Ciprofloxacine (CIP), Levofloxacine (LEV), Tetracycline (TE), Sulfamethoxazole - Trimethoprim (SXT) (Bio-analyse, Turky), Tigecycline (TGC) and Colistin sulphate (CT) (MAST, UK).

DNA Extraction

DNA was extracted from bacterial isolates by using extraction kit (Favorgen Biotech Corporation, Taiwan).

Polymerase Chain Reaction (PCR) Techniques

Series of PCR reactions were conducted to detect the genes of two classes of hydrolysing carbapenem class D β-lactamases (CHDLs) (Oxacillinases) including bla oxa-51, and class C β-lactamases genes (Cephalosporinases) bla ampC, with specific primers for each gene as showed in Table 1. The program that used in the thermocycler PCR was carried out in which annealing at 55°C for 30sec for bla oxa51 and 57°C for 30sec for bla ampC. These primers were provided by (Bioneer-Company, Korea).

RESULTS

Identification of Acinetobacter baumannii

Fifteen isolates (15) of A. baumannii were described according to cultural characteristics, microscopic examination, and biochemical tests and confirmed the identification by using AP I20NE system from one hundred thirty five samples as showed in the Table 2.

Identifying A. baumannii isolates were performed in accordance with standard biochemical tests in the Bergey Manual of Determinative Bacteriology 9. Identification stage of A. baumannii began primarily by culturing the samples on MacConkey agar plates which appeared as lactose non-fermentative, while on blood agar they appeared white to grey and non-hemolytic, bacterial cell appeared microscopically as Gram-negative coco-bacilli, oxidase negative and catalase positive 10. As shown in the Table 3. At the species level, growth at 44°C was all positive. Isolates of A. baumannii which showed the ability to grow at this temperature. This test was used to distinguish A. baumannii which able to grow at this temperature degree from other Acinetobacter species which unable to grow at such temperature 11.

Table 1: Primers and their size

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer</th>
<th>Oligonucleotide sequence (5'-3')</th>
<th>Product size(bp)</th>
</tr>
</thead>
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<td>bla oxas1</td>
<td>OXA51-F</td>
<td>5'-TCGACCGAGTATGTACCTGC-3'</td>
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<tr>
<td>oxa51</td>
<td>OXA51-R</td>
<td>5'-TTGAGGCTGAACACCCATC-3'</td>
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<td>5'-ACTTACTCACTCAGCGACG-3'</td>
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<td>ampC</td>
<td>Ampc-R</td>
<td>5'-TAACACCCACATGTCCCG-3'</td>
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</table>

Table 2: Numbers and percentages of Acinetobacter baumannii isolates from different clinical sources.

<table>
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<th>Type of samples</th>
<th>Numbers of samples</th>
<th>Positive isolates</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burn swabs (BS)</td>
<td>28</td>
<td>5</td>
<td>17.85%</td>
</tr>
<tr>
<td>Medical devices swabs(ES)</td>
<td>25</td>
<td>3</td>
<td>12%</td>
</tr>
<tr>
<td>Urine samples (U)</td>
<td>30</td>
<td>3</td>
<td>10%</td>
</tr>
<tr>
<td>Blood samples (B)</td>
<td>20</td>
<td>2</td>
<td>10%</td>
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<tr>
<td>CNF samples (C)</td>
<td>10</td>
<td>1</td>
<td>10%</td>
</tr>
<tr>
<td>Wound swabs (W)</td>
<td>22</td>
<td>1</td>
<td>4.54%</td>
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<tr>
<td>Total number</td>
<td>135</td>
<td>15</td>
<td>11.11%</td>
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</table>

Identify A. baumannii isolates from clinical sources and investigation of antibiotic resistance genes in the isolated bacteria.
Antimicrobial Susceptibility tests:
Antibiotic susceptibility was determined by the procedure of disk diffusion test.
As shown in Figure 1 and Table 4 all isolates were resistance to cephalosporins in their third and fourth
generations in varying proportions. This resistance was 100% for Cefotaxime, (CTX), Ceftriaxone (CRO) and
80% for ceftazidime (CAZ), while it decreased to 60% for Cefepime (FEP).

Figure 1. Antibiotics susceptibility profile of A. baumannii isolates by disk diffusion method

Table 4. Antibiotics susceptibility profile of A. baumannii isolates by disk diffusion method (R-resistance, I-intermediate, S-sensitive).

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<th>BS2</th>
<th>BS3</th>
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<th>BS5</th>
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The results showed that the isolates of A. baumannii were 100% resistant to Tetracycline (TE). On the other hand, the bacteria under study were sensitive (93.3 and 73.3) % for both colistin (CO) and Tigecycline (TIG), respectively and had resistance rates to carbapenemes antibiotics Imepenem (IEM) and Meropenem (MEM) at 66.6% for each.

This study included the resistance of A. baumannii isolates toward aminoglycoside antibiotic, the resistance rates to Amikacine (AK), Tobramycine (TOB) and Gentamicine (CN) were (46.6, 66.6 and 66.6) %, respectively.

Molecular Detection of β-Lactamase Genes in A. baumannii isolates:
All isolates were investigated to determine the occurrence of β-Lactamase Genes. Detection of these genes (Class C and D of β-Lactamase) were performed with PCR. It was noticed that all of A. baumannii isolates had OXA β-lactamase (OXA-51-like) with chromosomal encoding, Figure 2.
Figure 2. OXA-51 gene result detection (amplified size 353 bp) using DNA template of Acinetobacter baumannii isolates by PCR (agarose gel electrophoresis, 1.5% agarose, 70 volt for 1-2 hrs.). Lane (M), DNA Ladder (1200 bp). All lane show positive results.

The current study also revealed that 11 (73.3%) of isolates harbored Ampc gens Figure 3.

Figure 3. Ampc Gene Output Identification (amplified size 663 bp) using DNA template of Acinetobacter baumannii isolates by PCR (agarose gel electrophoresis, 1.5% agarose, 70 volt for 1-2 hrs.). Lane (M), DNA Ladder (100 bp). 11 isolates of A. baumannii isolates show positive results. Only lanes 4 show negative results.

DISCUSSION

As shown in Table 2 the present study revealed that most bacterial species were isolated from burn samples 17.85%, this result in concordance with local study by Al-Hindawi & Jarallah 12 and Al-Harmoosh et al. 13. A high incidence of MDR A. baumannii was seen during outbreaks in burns unit. Isolates colonization were associated with increases skin grafting, antibiotic use and extended hospitalization procedures 14. The three main bacterial species that cause burn infections are A. baumannii, Pseudomonas aeruginosa and Staphylococcus aureus 15. Besides, burn patients are more susceptible to hospital-acquired infections 16.

Antimicrobial Susceptibility tests:
Antibiotic susceptibility was determined by the procedure of disk diffusion. A. baumannii isolates showed two types of antibiotic resistance, five isolates were Multi-Drug Resistance (MDR) and the others were Extensive-Drug Resistant( XRD) 17. Therefore, the five multidrug resistance isolates are likely to belong to hospital-endemic strains, while extensive drug resistant isolates are likely to belong to epidemic strains, as Jawad et al. 18 explained that epidemic strains are more resistant to antibiotics.

As shown in Figure 1 and Table 4 the resistance to cephalosporins in their third and fourth generations. Cephalosporins contain a β-Lactam ring and bacterial resistance to these antibiotics can be attributed to the possibility of hydrolysis of these antibiotics by β-Lactamase enzymes or by the modification of penicillin-binding proteins (PBP), or by reducing the permeability of the antibiotics into the bacterial cell 19.

The results of this study are in part consistent with several studies that included local studies conducted by Al-Hindawi & Jarallah 12, Tuwai 19 and a study in Egypt 20. In addition, a global study from Brazil 21 showed that bacterial resistance to Cefotaxime (CTX) and Ceftriaxone (CRO) was 100%. However, they did not agree with the results of another local study by AL – Harmoosh et al. 13 which bacterial resistance to Cefotaxime (CTX) and ceftazidime (CAZ) was 60% for each.

The isolates of A. baumannii were all resistant to Tetracycline (TE). The results obtained in this study are fully agreement with what found Jabur 22. All isolates of A. baumannii isolated from Babylon Governorate were 100% resistant to this antibiotic too. Resistance to this antibiotic can be achieved through three mechanisms : protection of bacterial ribosome, efflux pumps and antibiotic modification 23.

On the other hand, Polymixin E (colistin) is a promising antibiotic in the treatment of A. baumannii because it exhibits high inhibitory efficiency and safety 24. It is a positive polypeptide antibiotic used in the treatment of gram-negative bacteria as it interferes with the negatively charged lipopolysaccharide layer (LPS), especially lipid A causing disruption of the cytoplasmic membrane 25 resistance to colistin occurs through loss of lipid A by mutation 26.

The results of this study in agreement with those obtained in several studies such as in Qatar where the sensitivity of this antibiotic was 98.6% 24 as well as a study in Egypt reached to 95% 27. While they did not agree with a local study where it was only 80% of isolates were sensitive to colistin 28.

Tigecycline (TIG) is a modified Tetracycline belongs to the glycylcyclines group. Although its mechanism of action is similar to that of Tetracycline inhibition of protein synthesis by binding to tRNA at site A of the ribosome, it has the advantage of bypassing all of the resistance mechanisms which are used by bacteria to resist tetracycline antibiotic, which enhances its effectiveness for the treatment of A. baumannii 29. But in the long-term use of antimicrobial, the bacteria developed resistance to it 30. The results of this study in agreement with an Indian study 31. Where the sensitivity to this antibiotic was 80.5%.

Whereas susceptibility profile results from Fig.1 showed that the isolates had resistance to carbapenemes antibiotics Imipenem(IEM) and Meropenem(MEM). The high Carbapenem resistance levels observed in this study may be associated with the high frequency at which these antimicrobials were used in the health institutions.

While the resistance was increased to 100% in Brazil and Iran 21,33. The growing pattern of carbapenem resistance in Acinetobacter baumannii is a concern.
worldwide, because it restricts the range of therapeutic alternatives. The most common β-lactamases with activity at carbapenemase in A. baumannii like carbapenem-hydrolysing class D β-lactamases (CHDLs), most of which are unique to this genus, that may be plasmid- or chromosomal-encoded. Carbapenem resistance in A. baumannii including β-lactamases can also be the result of porins or protein modifications that bind penicillin. Many porins may be involved in carbapenem resistance, including the 33-kDa CarO protein 34.

**Molecular Detection of β-Lactamase Genes in A. baumannii isolates:**
All isolates were investigated to determine the occurrence of β-Lactamase Genes. Detection of these genes (Class C and D of β-Lactamase) were performed with PCR.

Although, bla oxa-51 in A. baumannii was regarded as a major factor in carbapenem resistance, it is intrinsic to A. baumannii, and used for species identification 35. Thus in our study bla oxa -51 has been used for identification of A. baumannii and their role in carbapenems resistance it encodes for Oxacillinase (carbapenemase), class D enzyme of β-lactamases that hydrolyzes the beta-lactam ring in carbapenem, including imipenem and meropenem. However, the expression of the gene in low levels but the resistance may increase especially when there is an insertion sequences in the upstream of the gene which causes over expression of the gene 35. This result is quite agreement with study in Egypt by AL-Agamy et al. 36 who detect bla oxa-51 in all A. baumannii isolates.

Ampe beta-lactamases are clinically significant cephalosporinides present on chromosomes in many of the Enterobacteriaceae as well as other species, where they mediate resistance to cephalothin, cefazoline, cefoxitin, most penicillins and beta-lactamase inhibitor-beta-lactam combinations. In many bacteria, Ampe enzymes are inducible, and can be released at high levels by mutation 37. Overexpression confers tolerance to cephalosporins of wide spectrum including cefotaxime, cefazidime and ceftriaxone 37.

In a local study at Al-Hilla city by Al-Hindawi& Jarallah 38 found Ampe genes in 87.5% of A. baumannii isolates and another study conducted in Egypt by Said et al. 39 who found 90% of A. baumannii isolates was harboring for Ampe genes. In spite of this gene intrinsic but some species do not possess it because of Insertional inactivation by an element of the transposase family IS5 39.

**CONCLUSIONS**
Acinetobacter baumannii has been identified as a significant cause of nosocomial infections and could even suddenly cause an outbreak of infections in burning unit and ICU and has exhibited a broad range of resistance to antimicrobials that are commonly used. To manage the spread of this organism, control interventions should be introduced in hospitals and work will concentrate on studying novel agents with less resistance.

**REFERENCES**


