Isolation of Acinetobacter baumannii from Different Clinical Source and Study some Antibiotic Resistant and β-Lactamase Production

Mays Hadi Jabur
College of Physical Education, University of Babylon, Hilla, Iraq.

Received 12 January 2014 Accepted 24 February 2014

Abstract
A total of (10) isolates of Acinetobacter baumannii out of (75) samples were isolated from various clinical sources (urinary tract infections (UTIs), wounds, burn and sputum). From patients were attending to Hilla Teaching Hospital. Maximum isolates were obtained from urine samples 5(50%), wound samples 3(30%), burn and sputum samples 1(10%). All isolates were tested for their resistance to (10) different antibiotics and the results showed that all isolates were entirely resistant to Tetracycline (100%) but lesser to Ampicillin (70%), Ceftaxime (60%), Pipracillin, Polymyxine B and Norfloxacin (20%), Imipenem and Ciprofloxacin (30%), Amikacin and Gentamicin (40%). Besides, β-Lactamase production was detected in all samples of Acinetobacter baumannii and results of this test were positive for all tested isolates in this study.

Keywords: Acinetobacter baumannii, Multidrug-resistant, antibiotic, nosocomial infection, β-Lactamase.

Introduction
Acinetobacter baumannii is a Gram-negative rod that is aerobic, pleomorphic and non-motile. An opportunistic pathogen, Acinetobacter baumannii has emerged as an important and problematic human pathogen as it is the causative agent of several types of infections including pneumonia, meningitis, septicemia, and urinary tract infections. Several virulence determinants, such as biofilm formation, adherence and ability to invade host cells as well as iron acquisition and host cell death [1]. Acinetobacter baumannii readily adheres both to biological and abiotic
surfaces, on which it is able to form biofilms. This is an important pathogenic feature of many bacteria, facilitating colonisation of prosthetic material and contributing to drug resistance and evasion of the host immune system in vivo [2]. A. baumannii has a high incidence among immunocompromised individuals, largely associated with infected combat troops returning from conflict zones, coupled with a dramatic increase in the incidence of multidrug-resistant (MDR) strains, has significantly raised the profile of this emerging opportunistic pathogen [3]. Moreover, the resistance of A. baumannii to common disinfectants and ability to survive for long periods on dry surfaces make it difficult to eradicate from the hospital environment. Current multidrug resistance rates range from 48–85% of isolates, with the greatest burden in Asia and Eastern Europe [4]. Multidrug-resistant Acinetobacter baumannii infections represent a growing problem, especially in traumatic wounds and burns suffered by military personnel injured in Middle Eastern conflicts. Effective treatment with traditional antibiotics can be extremely difficult [5].

**Aims of Study**
The aims of the study were:
1- Isolation and identification of Acinetobacter baumannii from different clinical speciemens.
2- Study of antibiotics resistance detection.
3- Study of β-Lactamase production.

**Material and Methods**

**Specimen and isolates:**

**Patients:**
The study included (10) Acinetobacter baumannii isolates out of (75) samples from different clinical sources (urinary tract infections (UTIs), wounds, burn and sputum). From patients the period from October 2012 to December 2012 at Hilla teaching hospital.

**Specimen collection:**
(75) swab specimens were collected from infected patients with infections (UTIs), wounds, burn and sputum). Each swab taken carefully from the site of infection and placed in tubes containing readymade media to maintain the swab wet during transferring to laboratory. Each specimen was immediately inoculated on the blood agar plates, nutrient agar and MacConkey's plates. All plates were incubated at 37ºC for 24-48 hr.

Identification of Acinetobacter baumannii according to biochemical and physiological tests were studies.

1 - **Antibiotic susceptibility tests:**
The susceptibility of Acinetobacter baumannii isolates were determined by antibiotic disk diffusion method and compare with zones of inhibition determined by CLSI [6].and to decide the susceptibility of bacteria to antimicrobial agents, whether being resistant or sensitive.

2 – **Detection of β-Lactamase production**: The test was done according to procedure described in [7].

**Results and Discussion**
In this study a total of (75) samples were obtained from patients suffering from (urinary tract infections (UTIs), wounds, burn and sputum), Table(1)
Table 1 Distribution of samples and number of *Acinetobacter baumannii* isolates:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Number of samples</th>
<th>Number of <em>A. baumannii</em> isolates:</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>30</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>Wound</td>
<td>21</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>Burn</td>
<td>9</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Sputum</td>
<td>15</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>

In this study, the distribution of *Acinetobacter baumannii* isolates according to the site of infection was studied; it was found that the most infections of this bacterium occur in the urine (50%). It is commonly found in the presence of an indwelling urinary catheter, although it is occasionally associated with pyelonephritis, and urosepsis, it is rarely invasive and usually limited to the lower urinary tract [8,9]. This result was compared to the result obtained by [10,11] and [12] have isolated *Acinetobacter baumannii* from urine rate reaching to (16,10 and 11.5) respectively.

The results of bacterial isolation in Table (1) show that only 3 isolates (30%) of *Acinetobacter baumannii* were isolated from wound samples.

This result is identical with those obtained by [13] who has succeeded to isolate Acinetobacter baumannii from wound samples at a rate reaching (2%) but disagreement with those obtained by [14], who has isolated these bacteria from wound samples at rate of (23.6%).

The results obtained by the present study show that 1 isolate (10%) of *Acinetobacter baumannii* was isolated from burn. The results of this study are identical with those obtained by [15], who has isolated these bacteria from burn samples at rate of (17%) Burns are one of the most common and devastating forms of trauma and a major public health concern in all around the world. Burn patients are at high risk of developing nosocomial infection because of their destroyed skin barrier and suppressed immune system. The burn patients have unique predisposition to different infections which are linked to impaired resistance from disruption of the skin's mechanical integrity and generalized immune suppression. The skin barrier is replaced by a protein rich, avascular environment that provides a favourable niche for microbial colonization and proliferation. Additionally migration of immune cells is hampered, which contributes to septic process.

*Acinetobacter baumannii* infections in burn patients may lead to delays in wound healing, graft losses, and development of sepsis. Determining the risk factors for multidrug resistant *A. baumannii* (MDR-AB) infections is essential for infection control [15,16].

On the other hand, in this study 1 isolate (10%) of *Acinetobacter baumannii* was also isolated from sputum infection. *Acinetobacter* infections are uncommon but, when they occur, usually involve organ systems that have a high fluid content (e.g., respiratory tract, *A. baumannii* rarely cause community-acquired pneumonia and sepsis. These uncommon manifestations of *A. baumannii* infection are found among patients who reside in tropical climates and who have chronic obstructive pulmonary disease and/or who abuse alcohol [17].
This result agreement with those obtained by [18] and [13], who has isolated these bacteria from suptum samples at rate of (7%) and (15%) but disagreement with those obtained by [19], who has isolated these bacteria from suptum samples at rate of (31%).

The effects of different antibiotics on A. baumannii isolates were investigated such as Tetracycline, Ampicillin, Cefotaxime, PIPracillin, Polymyxin B, Imipenem, Amikacin, Gentamicin, Ciprofloxacin and Norfloxacine. All isolate of A. baumannii high resistant (100%) to Tetracycline, whereas some isolates showed resistance to Ampicillin (70%), Cefotaxime (60%), Pipracillin and Polymyxine B (20%), Imipenem (30%), Amikacin and Gentamicin (40%), Ciprofloxacin (30%), Norfloxacin (40%) for each antibiotic as shown in table (2).

Table 2 Effect of antibiotics on the growth of A. baumannii isolates

<table>
<thead>
<tr>
<th>Antibiotic agents</th>
<th>Total no. of resistant isolates</th>
<th>Resistance %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracyclin</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>7</td>
<td>70</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td>Pipracillin</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Imipenem</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>Amikacin</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>2</td>
<td>20</td>
</tr>
</tbody>
</table>

The results showed that (100%) of isolates are resistant to Tetracycline these results of resistance to these antibiotics were in agreement with the results obtained by [18] These results are comparable with the data reviewed by [20] have reported that the isolates of A. baumannii are susceptible to Tetracycline (90%). Two different mechanisms of resistance to Tetracyclines have been widely described in A. baumannii. TetA and TetB are specific transposon-mediated efflux pumps; TetB determines the efflux of both tetracycline and minocycline, whereas TetA drives only the efflux of tetracycline. The second mechanism is the ribosomal protection protein, which shields the ribosome from the action of tetracycline. The Tet(M) gene encodes this protein, which serves to protect the ribosome from tetracycline Tetracycline is bacteriostatic especially in large doses and it is short in acting. The small doses of oral antibiotic do not reduce the number of organisms but they affect their function. The antibiotic can also inhibit various enzyme activities such as lipase by interfering with protein synthesis combining with bacterial ribosome [21].

The results also showed that A. baumannii resistant to Ampicillin(70%). This result agrees with the result obtained by [22], he found that (75%) of isolates were resistant to Ampicillin. In present study all isolates also appeared to have resistance to Cefotaxime (60%), and the result in
this study was identical with the results obtained by [13] and [22] who have reported that most isolates of A. baumannii are considered to be resistant to Cefotaxime in rate reach to (80%) and (60%) respectively. but not identical with the results obtained by [23] he found that (12.2%) of isolates were resistant to Cefotaxime. Definitions of multidrug-resistant Acinetobacter species vary, referring to a wide array of genotypes and phenotypes. The mechanisms of resistance generally fall into 3 categories: (1) antimicrobial-inactivating enzymes, (2) reduced access to bacterial targets, or (3) mutations that change targets or cellular functions. For the first category, Acinetobacter species possess a wide array of β-lactamases that hydrolyze and confer resistance to penicillins, cephalosporins, and carbapenems. AmpC cephalosporinsases are chromosomally encoded and confer resistance to broad-spectrum cephalosporins [24]. The capacity of Acinetobacter species for extensive antimicrobial resistance may be due in part to the organism's relatively impermeable outer membrane and its environmental exposure to a large reservoir of resistance genes [25].

20% of A. baumannii was resistant to Pipracillin (the newer β-lactam). This result agrees with the result obtained by [18], he found that (29%) of isolates were resistant to Pipracillin.

Most A. baumannii isolates showed susceptibility to these antibiotics, due to found inhibitor and less of uses. Also (20%) of isolates have shown resistance to Polymyxin-B. These results are comparable with the data reviewed by [13] have reported that the isolates of A. baumannii are resistant to Polymyxin-B (3.07%) , while [26] have observed that the isolates of A. baumannii are sensitive to Polymyxin-B (100%).

[21], have mentioned that the Polymyxin-B is bactericidal against Acinetobacter species, and its effect is concentration dependent. Resistance to polymyxins has been reported, possibly as a result of outer cell membrane alterations or an efflux pump mechanism.

Observational studies have reported rates of cure or improvement for Polymyxin-B of 57%–77% among severely ill patients with multidrug-resistant Acinetobacter infections, including pneumonia, bacteremia, sepsis, intra-abdominal infection, and CNS infection. Although high-quality pharmacokinetic data are lacking, Polymyxin-B is reported to have relatively poor lung and CSF distribution, and clinical outcomes vary for different types of infections [27].

Also, the results showed that (30%) of isolates are resistant to Imipenem. These results are comparable with the data reviewed by [13] have reported that the isolates of A. baumannii are resistant to Imipenem (32.3%), while [28] have observed that the isolates of A. baumannii are resistant to Imipenem (85%).

The results also showed less resistance to Aminoglycoside (Amikacin and Gentamicin) as in table (2), this result agreement with (18), he found that (15%) of isolates were resistant to Amikacin and (31%) of isolates were resistant to Gentamicin. Nazmul et al.[22] have observed that the isolates of A. baumannii are resistance to (50%) Amikacin and(52.5%) Gentamicin.

The resistance of A. baumannii to Aminoglycoside is probably attributed to the fact that most clinical isolates of A. baumannii associated with Aminoglycoside-modifying enzymes or efflux pump mechanisms [29].
On the other hand, isolates (30%) and (40%) have shown resistance to Ciprofloxacin and Norfloxacine respectively. Shareek et al.[23] have observed that the isolates of A. baumannii are resistant to Ciprofloxacin (28%). While [18] he found that (34%) of isolates were resistant to Norfloxacine. This antibiotic (Quinolines) can inhibit bacterial DNA synthesis an event that is followed by rapid bacterial death.

Ciprofloxacin was bactericidal drug, they were affected against Gramnegative and Grampositive bacteria and the resistance to fluoroquinolones was through chromosomal mutation or alternation affecting the ability to fluoroquinolones to permeate to bacterial cell wall [30].

In this study β-Lactamase production was examined. The predominant mechanism of resistance to β-lactamantibiotics in Gram negative bacteria is by the synthesis of β-lactamases. Among the β-lactamases the production of ESBLs is the most common. Chromosome-mediated β-lactamases have been described in a wide variety of Gram negative bacilli, such as Pseudomonas aeruginosa and Enterobacterspp., Acinetobacter spp. and E. coli, Acinetobacter baumannii has an intrinsic resistance to Ampicillin and Cephalosporin due to extended spectrum β-lactamase (ESBLs) β-lactamases (ESBLs) are enzymes that compromise the efficacy of all β-lactams by hydrolysis of the β-lactam ring[31] and [32]. Results of this test were positive for all tested isolates in this study.

This method depends on the detection of penicilloic or cephalo spoic acid, resulted from breakdown of amide bond in β-lactam ring for each of Penicillins or Cephalosporins. Iodine reacts with starch to form dark blue complex, which stays without changes in the absence of β-lactamase enzymes. In the case of β-lactamase-producing bacteria, the resulting penicilloic or cephalosporic acid will reduce iodine into iodide; consequently, decolorization of starch-iodine complex occurs (changing the color directly to white) if an isolates a β-lactamase producer [33].

Acinetobacter baumannii, became a serious problem worldwide. Especially the increasing resistance to 3rd and 4th generation cephalosporins and carbapenems is of particular concern. Gram-negative bacteria pursue various molecular strategies for development of resistance to these antibiotics: (a) generation of extended-spectrum beta lactamases (ESBL) according to the original definition due to extension of the spectrum of already widely disseminated plasmid-encoded beta lactamases by amino acid substitution; (b) acquisition of genes encoding ESBL from environmental bacteria as, for instance the CTX-M-type beta lactamases (c) high-level expression of chromosome-encoded beta lactamase (bla) genes as bla(OXA) or bla(ampC) genes due to modifications in regulatory genes, mutations of the beta-lactamase promoter sequence as well as integration of insertion sequences containing an efficient promoter for intrinsic bla genes [34]. The result in this study was identical with the results obtained by [35] have reported (99%) of all isolates were Metallo-beta-lactamase (MβL) producing. Also this result was compared to [13] have shown β lactamase production was positive for (13.3%) strain.

Also, β-Lactam resistance was detected by [36] who found that most strains of Acinetobacter baumannii are increase of beta-lactam resistance mediated by the production of beta-lactamases in high ratio, mostly from intensive care, are among the most multiresistant nosocomial bacteria.
known and are often susceptible only to polymyxins.

References
13. Madanan, A., Yadav, D., Madhavan, R.(2009) Imipenem resistance and biofilm production in Acinetobacter. Department of Microbiology, SRM Medical College, Tamil Nadu, India
19. Kanokorn, M.D., Peninnah O.M. (2010) *Acinetobacter baumannii* Infection and Colonization among Pediatric Patients at Chiang Mai University Hospital. Ph.D. Division of Infectious Diseases, Department of Pediatric, Faculty of Medicine, Chiang Mai University, Thailand.


35. Shyam, K. M., Basista, P. R., Bharat, M. P.( 2013) Emerging threat of multidrug resistant bugs – Acinetobacter calcoaceticus baumannii complex and Methicillin resistant Staphylococcus aureus. Department of Microbiology, Institute of Medicine, Tribhuvan University, Kathmandu, Nepal, Vol 13, No 2; 6:98.