Phenotypic and genotypic Detection of Some Antimicrobial Resistance Mechanisms among Multidrug-Resistant Acinetobacter baumannii Isolated from Immunocompromised Patients in Egypt

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ABSTRACT

Acinetobacter spp. are Gram-negative bacteria that have become one of the most difficult pathogens to treat. The species A. baumannii, largely unknown 30 years ago, has risen to prominence particularly because of its ability to cause infections in immunocompromised patients. It is now a predominant pathogen in many hospitals as it has acquired resistance genes to virtually all antibiotics capable of treating Gram-negative bacteria, including the fluoroquinolones and the cephalosporins. The aims of this study were to (i) evaluate the antimicrobial susceptibility of Acinetobacter baumannii isolated from immunocompromised host (ii) phenotypic assessment of the prevalent mechanism of resistance among multidrug resistant Acinetobacter spp. (iii) evaluate the prevalence of metallo-β-lactamases (MBLs) phenotypically and genotypically among multidrug resistant A. baumannii. Seventy-two non-replicated A. baumannii isolates were recovered from 631 clinical specimens referred for bacteriological cultures from immunocompromised patients of all age groups and both sexes admitted in El-Demerdash Hospital and National Cancer Institute (NCI) Cairo-Egypt. Isolates were identified conventionally using standard biochemical tests and also using Microscan (Dade Behring, West Sacramento, USA). Both manual (following the CLSI protocol) and automated methods (using Microscan system) were used to detect antimicrobial susceptibility pattern of Acinetobacter isolates. Different potential resistance mechanisms were investigated in 45 carbapenem resistant A. baumannii phenotypically. Genotypic detection of MBLs was carried out using PCR. Acinetobacter baumannii represents the predominant Acinetobacter isolates (83.3%). The mean age group of patient with Acinetobacter infection was more than 55 years old (36.1%). A. baumannii exhibited high resistance rate to the majority of commercially available drugs including imipenem (66.6%), meropenem (73.3%) and cefazolin & cephalothin (100%). Isolates show moderate susceptibility to tetracycline (40% of the isolates were susceptible) and gentamicin (33.3%). MDR A. baumannii represent 75% of the Acinetobacter isolates (45/72). Using phenotypic tests; none of the carbapenem-resistant A. baumannii were carbapenemase producers and 44.4% were AmpC β-lactamase positive. MBLs were detected in 55.6% using phenotypic tests and in 44.4% of isolates using PCR. Moreover Efflux pump was detected in 77.8% of isolates.

Conclusions: Multidrug resistant A. baumanniiis a problematic organism in immunosuppressed patients since it became resistant to the majority of commercially available antimicrobials with different resistance mechanisms. Metallo-β-lactamase production is an important mechanism of carbapenem resistance and AmpC - β-lactamase could be a contributory factor for meropenem resistance among MDRAB isolates. This underlies the importance of their accurate identification and reporting to prevent the emergence of absolute resistance to the useful drugs against Acinetobacter spp. in Egypt.

Key words: Acinetobacter, carbapenemase, metallo-β-lactamase, Efflux Pump

INTRODUCTION

Acinetobacter baumannii (A. baumannii) is an opportunistic pathogen with increasing clinical significance, particularly in immunocompromised patients, causing nosocomial infections of the lungs, urinary tract and surgical wounds[1].

Because of frequent resistance to commonly used antibiotics, carbapenems have become important for managing Acinetobacter infections. However, their effectiveness is being increasingly compromised due to enzymatic modification of antibiotic molecules especially by carbapenemases and expression of efflux pumps. Acquired carbapenemases can be either metallo-β-lactamases (MBLs) such as VIM and IMP, or non-MBL. MBL genes are mostly detected in class integrons’ structures and these...
integrons are detected in a high proportion of Acinetobacter isolates\(^3\).

Efflux pumps are transport proteins involved in the extrusion of toxic substrates (including virtually all classes of clinically relevant antibiotics) from within cells into the external environment. In Gram-negative efflux pump can result in both intrinsic and acquired multidrug resistance\(^9\).

To this end the present study was conducted to (i) evaluate the antimicrobial susceptibility of *Acinetobacter baumannii* isolated from immunocompromised patients (ii) To assess the prevalent mechanisms of resistance among *Acinetobacter* spp. (iii) To determine the prevalence of MBLs phenotypically and genotypically among multidrug resistant *Acinetobacter baumannii*.

**MATERIAL AND METHODS**

**Specimens**

Clinical specimens referred for bacteriological cultures from immunocompromised patients of all age groups and both sexes admitted in El-Demerdash Hospital and National Cancer Institute (NCI) Cairo-Egypt over the period of January 2011 to March 2012 were included in the study. The specimens comprised urine, blood, sputum, pus, drain fluid, swabs (pus, wound, throat and nasal) and chest tube.

**Microbial identification**

Bacterial isolates were identified conventionally using standard biochemical tests according to *Konemanet al.*\(^4\) and *Constantiniuet al.*\(^5\). *A. baumannii* showing resistance to more than two of the following five antimicrobial classes: antipseudomonal cephalosporins (cefazidime or cefepime), antipseudomonal carbapenems (imipenem or meropenem), ampicillin/sulbactam, fluoroquinolones (ciprofloxacin or levofloxacin), and aminoglycosides (gentamicin, tobramycin, or amikacin) were considered MDR\(^8\).

**Detection of multi drug resistant strains (MDR)**

Interpretation of both manual and automated antimicrobial susceptibility test results were evaluated according to Clinical and Laboratory Standards Institute guidelines\(^6\). *A. baumannii* isolates were subjected to modified Hodge test for detection of metallo-β-lactamases.

**1- Phenotypic Detection of Various Beta Lactamases:**

**A- Detection of carbapenemases
Modified Hodge Test**

The meropenem resistant strains were subjected to modified Hodge test for detection of carbapenemases. An overnight culture suspension of *E.coli* ATCC 25922 adjusted to 0.5 McFarland standard was inoculated using a sterile cotton swab on the surface of a Mueller-Hinton agar. After drying, 10µg meropenem containing disc was placed at the center of the plate and the test strain was streaked from the edge of the disc to the periphery of the plate in four different directions. The plate was incubated overnight at 37°C. The absence of a ‘cloverleaf shaped’ zone of inhibition due to carbapenemase production was considered as negative result\(^8\).

**B- Detection of the Metallo- β- lactamases (MBLs)**

**EDTA disc synergy test (EDS)**

Ethylene diamine tetra acetic acid (EDTA) disc synergy test was done using cefazidime and meropenem disc for detection of metallo-β-lactamases in meropenem and cephalosporins resistant isolates. A suspension of overnight culture of the test isolate was adjusted to match a turbidity of 0.5 McFarland standard and was spread on the surface of a Muller- Hinton agar.
plates. A 10 µg meropenem containing disk or 30 µg ceftazidime containing disk was placed on the agar surface. A blank disk (6 mm in diameter, Whatmann filter paper no. 1) was kept on the inner surface of the lid of the Muller- Hinton agar plate and 10 µl of 0.5 M EDTA is added to it. EDTA disk was then transferred to the surface of the agar and was kept 10 mm edge to edge apart from the meropenem or ceftazidime disk. After incubating overnight at 37°C, the presence of an expanded growth inhibition zone between the two discs was interpreted as positive for MBL production.

C- Detection of the AmpC β-lactamases

Amp C disc test

AmpC disc test was used for detection AmpC β-lactamases in meropenem and cephalosporin resistant strain. On a Muller-Hinton agar plate, lawn culture of *E. coli* ATCC 25922 was prepared from an overnight culture suspension adjusted to 0.5 McFarland standards. A 30 µg cefoxitin containing disc was placed on the surface of the agar. A blank disc (6 mm in diameter, Whatmann filter paper No. 1) was moistened with sterile saline and inoculated with a few colonies of the test strain. The inoculated disc was then placed beside the cefoxitin disc almost touching it. The plate was incubated overnight at 37°C. A flattening or indentation of cefoxitin inhibition zone in the vicinity of the disc with the test strain was interpreted as positive for the production of Amp C β-lactamase. An undistorted inhibition zone was considered as negative.

2- Phenotypic Detection of Efflux Pump

Resistance to ciprofloxacin by efflux pump (EP) was detected using ciprofloxacin and reserpine as an efflux pump inhibitor (EPI) according to Andrews. A minimum inhibition concentration (MIC) of ciprofloxacin was determined by microtiter broth dilution method (with and without reserpine) according to Andrews.

3- Genotypic detection of MBLs

In all carbapenem resistant isolates, conventional PCR was used for detection of *blaIMP* and *blaVIM* genes.

DNA extraction

Genomic DNA of Acinetobacter isolates was extracted using QIAamp® DNA Mini kit on QIAcube (QIAGEN, Germany) according to the manufacturer protocol. QIAcube is novel and complete automation of established nucleic acid extraction processes using spin columns. The innovative QIAcube uses advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample preparation.

PCR specific for *blaIMP* & *blaVIM*

*blaIMP* and *blaVIM* genes were amplified using the primers listed in table (1), the primers were synthesized by Invitrogen, USA. Taq PCR Master Mix Kit (QIAGEN, Germany) was used. PCR reaction mixture for both was carried out in final volume 25 µl consisting of 50 ng (1µl) of extracted DNA, 12.5µl of master mix (QIAGEN), 1µl of forward and 1µl reverse primers (20 pmol from each primer), then 9.5µl of distilled water was added in order to complete 25µl of total volume.

The PCR amplification was carried out initial denaturation for 5 min at 95°C followed by 35 cycles at 95 °C for 20 sec, 50 °C for 40 sec, and 72 °C for 30 sec, and a final extension for 5 min at 72 °C.

Detection of amplified PCR products

Agarose gel electrophoresis was used to detect PCR products. Ten micro liter volumes of PCR products separated through 1.5% agarose horizontal gel by electrophoresis at 84 volts. Gel was stained with ethidium bromide and was photographed.

### Table (1): Oligonucleotides used as primers for detection of MBLs genes.

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer Sequence (5’→3’)</th>
<th>Product size bp</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>blaIMP</em> F</td>
<td>CAT GGT TTG GTG GTT CTT GT</td>
<td>488</td>
<td>Sung <em>et al.</em>, (13)</td>
</tr>
<tr>
<td><em>blaIMP</em> R</td>
<td>ATA ATT TGG CGG ACT TTG GC</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>blaVIM</em> F</td>
<td>ATT GGT CTA TTT GAC CCG GTC</td>
<td>780</td>
<td></td>
</tr>
<tr>
<td><em>blaVIM</em> R</td>
<td>TGC TAC TCA ACG ACT GAG CG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F: forward          R: reverse.

Statistical analysis

SPSS V.13 was used for statistical analysis. P value < 0.05 was considered significant.
RESULTS

During the study period (January 2010 to March 2012), a total of 631 samples were received, from which 72 *Acinetobacter* spp. were isolated (non-repetitive). *Acinetobacter baumannii* was the predominant species (83.3%), while *Acinetobacter Iwoffii* has lower frequency (16.7%). With regard to the clinical origin, the *A. baumannii* were isolated from blood (n = 30) sputum (n = 14) pus (n = 13), wound swabs (n = 6), chest tube (n = 4), nasal swab (n = 2), throat swabs (n = 2) and urine (n = 1).

There is a statistically significant relation (P-value = 0.0135) between *Acinetobacter* infection and age group. The mean age group of patient with *Acinetobacter* infection was more than 55 years old (36.1%).

Antimicrobial susceptibility of *Acinetobacter* isolates

Studying the antibiogram of *A. baumannii* revealed that the isolates showed moderate susceptibility to tetracycline in which 40% of the isolates were susceptible to it, followed by gentamicin (33.3%), tobramycin (30%), and imipenem (28.3%).

Resistance to penicillins were high, ampicillin shows the lowest susceptibility (1.7%) followed by piperacillin (5%). Also β lactam-β-lactamase inhibitor combinations show high resistance pattern (ranged from 96.7-100%).

Regarding cephalosporins, all isolates were completely resistant to cefazolin & cephalothin (100%). Also cefoxitin show high resistance (98.3%) followed by cefotetan (95%). Also show highest resistance to chloramphenicol and trimethoprim/sulfamethoxazole (90% & 91.7% respectively).

Resistance pattern of *Acinetobacter baumannii* revealed that 75% were resistant to third-generation cephalosporins, aminoglycosides and quinolones, indicating high prevalence of MDR (Table, 2). MDR pattern were higher among isolates recovered from blood and sputum specimens (Table, 3).

A total of 45 meropenem-resistant *Acinetobacter baumannii* were detected on disc diffusion method and all were MDR. MIC for meropenem for these isolates ranged between 8 and 64 µg/ml.

EDST detected MBL production in 25/45 (55.5%) *Acinetobacter baumannii* (enhancement of the zone of inhibition in the area between MEM and EDTA discs or CAZ and EDTA discs; Figure 1: A and B) whereas 20 isolates were negative (Figure 2). Among the 25 MBL producing isolates, 23 were detected by simultaneous use of both meropenem and ceftazidime in EDS and 2 were detected using only EDTA-meropenem combination (100% sensitivity).

MHT did not pick even single carbapenemase producer in this study (no cloverleaf shaped zone of inhibition; Figure 3).

AmpC β-lactamase was found in 20 (44.4%) *Acinetobacter baumannii* (showed by flattening or indentation of cefoxitin inhibition zone in the vicinity of the disc and test strain; Figure 4, whereas negative result shown in figure 5). In eight isolates it was associated with a positive EDS.

On the other hand, *blaIMP* gene was detected in twenty Acinetobacter isolates and none of these were positive *blaVIM* (Figure 6: A, B & C). All PCR positive isolates were EDST positive for MBL.

The Frequency of efflux pump among *A. baumannii* was 77.8% (35/45), where MIC of ciprofloxacin in presence of reserpine was decreased one fold in 20% (9/45) and decreased four folds in 57.8% (26/45). On the other hand MIC of ciprofloxacin in presence of reserpine didn't change in the rest of isolates.
### Table (2): Frequency of antimicrobial susceptibility of Acinetobacter baumannii.

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>S</th>
<th>I</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>10</td>
<td>4</td>
<td>46</td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Ampicillin/sulbactam</td>
<td>2</td>
<td>0</td>
<td>58</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1</td>
<td>7</td>
<td>52</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Cefepime</td>
<td>5</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>1</td>
<td>5</td>
<td>54</td>
</tr>
<tr>
<td>Cefotetan</td>
<td>1</td>
<td>2</td>
<td>57</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>1</td>
<td>0</td>
<td>59</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>5</td>
<td>2</td>
<td>53</td>
</tr>
<tr>
<td>Ceftiraxone</td>
<td>5</td>
<td>2</td>
<td>53</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>1</td>
<td>1</td>
<td>58</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>5</td>
<td>1</td>
<td>54</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>15</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>20</td>
<td>6</td>
<td>34</td>
</tr>
<tr>
<td>Imipenem</td>
<td>17</td>
<td>3</td>
<td>40</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>13</td>
<td>4</td>
<td>43</td>
</tr>
<tr>
<td>Meropenem</td>
<td>15</td>
<td>1</td>
<td>44</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0</td>
<td>2</td>
<td>58</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>0</td>
<td>5</td>
<td>55</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>3</td>
<td>1</td>
<td>56</td>
</tr>
<tr>
<td>Tetracyclin</td>
<td>24</td>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>Ticarcillin/clavulanic acid</td>
<td>7</td>
<td>1</td>
<td>52</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>18</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>Trimethoprim / sulfamethoxazole</td>
<td>5</td>
<td>0</td>
<td>55</td>
</tr>
</tbody>
</table>

*percentage was correlated to total number of Acinetobacter baumannii.  
S: Sensitive, I: Intermediate, R: Resistant

### Table (3): Distribution of Acinetobacter baumannii isolates among different clinical specimens.

<table>
<thead>
<tr>
<th>Type of specimens</th>
<th>A. baumannii N=60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDR</td>
</tr>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Blood</td>
<td>18</td>
</tr>
<tr>
<td>Sputum</td>
<td>14</td>
</tr>
<tr>
<td>Throat</td>
<td>2</td>
</tr>
<tr>
<td>Pus Swab</td>
<td>11</td>
</tr>
<tr>
<td>Drain</td>
<td>2</td>
</tr>
<tr>
<td>Wound Swab</td>
<td>6</td>
</tr>
<tr>
<td>Chest Tube</td>
<td>4</td>
</tr>
<tr>
<td>Nasal Swab</td>
<td>2</td>
</tr>
<tr>
<td>Urine</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
</tr>
</tbody>
</table>

P-Value: 0.0097** 0.26***

*percentage was correlated to total number of Acinetobacter baumannii.  
** Significant., *** Non- significant.
Figure (1): Positive EDTA disk synergy test.
(A) Positive EDTA disk synergy test using meropenem.
(B) Positive EDTA disk synergy test using ceftazidime.

Figure (2): Negative EDTA disk synergy test.

Figure (3): Negative modified Hodge test.

Figure (4): Positive AmpC test.

Figure (5): Negative AmpC test.
Figure (6): Agarose gel electrophoresis of PCR products of \( \text{bla}_{\text{IMP}} \) and \( \text{bla}_{\text{VIM}} \).

A- Lanes 3-6 and 8-9: *Acinetobacter baumannii* with positive \( \text{bla}_{\text{IMP}} \) gene, showed bands at 488, lanes 4, 5, 7 & 10 *Acinetobacter baumannii* with negative \( \text{bla}_{\text{IMP}} \) gene.

B- Lanes 1-6 and 8 *Acinetobacter baumannii* with positive \( \text{bla}_{\text{IMP}} \) gene, showed bands at 488, lanes 7, 9 & 10 *Acinetobacter baumannii* with negative \( \text{bla}_{\text{IMP}} \) gene.

C- Lanes 1-10 negative \( \text{bla}_{\text{VIM}} \) showed no bands at 780.

Table (4): Distribution *A. baumannii* carrying MBLs \( \text{bla}_{\text{IMP}} \) among different specimens

<table>
<thead>
<tr>
<th>Type of specimens</th>
<th>MBL positive isolates (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{bla}_{\text{IMP}} )</td>
</tr>
<tr>
<td>Blood</td>
<td>9</td>
</tr>
<tr>
<td>Sputum</td>
<td>5</td>
</tr>
<tr>
<td>Throat swab</td>
<td>1</td>
</tr>
<tr>
<td>Pus</td>
<td>4</td>
</tr>
<tr>
<td>Drain</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
</tr>
</tbody>
</table>

*Percentage was correlated to the number of isolates carrying MBLs gene

**DISCUSSION**

*Acinetobacter* species has emerged as an important pathogen causing life-threatening infections both in community and hospital. Rapid emergence of multidrug-resistant *Acinetobacter* has further made the situation critical.\(^{(14)}\)

In the present study *A. baumannii* was frequently isolated from blood, (30%), sputum (23.33%) and pus (18.33%). This result is consistent to the results reported by Bazargani and Hashemizadeh\(^{(15)}\). The increased blood stream infection frequency was attributed to immunosuppression (include organ transplantation, corticosteroids and immunosuppressive therapy) which is considered as an important risk factor for developing Acinetobacter bacteremia. On the other hand Al Masoudi et al.\(^{(16)}\) reported that wound and
resistant to multiple antibiotics has always been an organism which was inherently an emerging phenomenon, as A. baumannii has always been an organism which was inherently resistant to multiple antibiotics. An alarming rise in antibiotic resistance rate has now become a serious and an increasingly common public health concern.

In the present study 86.7%, 93.3%, 96.7%, 100%, and 100% of A. baumannii were resistance to ticarcillin/clavulanic, piperacillin, ampicillin/sulbactam, acid, aztreonam and amoxicillin/clavulanic acid respectively. Similar resistant pattern was reported by Peymaniet al. (17). There were high resistant to different generation of cephalosporins in the present study where 88.3% of A. baumannii were resistant to ceftazidime and ceftriaxone, 90% to cefotaxime, 91.7% to cefepime, 95% to cefoteten, 96.6% to cefoxhydro and all isolates were resistant to cefazolin and cephalothin. These results were consistent with those reported by Feizabadi et al. (10), Peymaniet al. (17), Muthusamy and Boppe (8) and Erfaniet al. (19).

The interest in A. baumannii has been growing rapidly because of the emergence of multi-drug resistant strains, some of which are pan resistant to antimicrobial agents. Multi-drug resistant (MDR) A. baumannii is not a new or emerging phenomenon, as A. baumannii has always been an organism which was inherently resistant to multiple antibiotics (89). An alarming rise in antibiotic resistance rate has now become a serious and an increasingly common public health concern.

In the present study 86.7%, 93.3%, 96.7%, 100%, and 100% of A. baumannii were resistance to ticarcillin/clavulanic, piperacillin, ampicillin/sulbactam, acid, aztreonam and amoxicillin/clavulanic acid respectively. Similar resistant pattern was reported by Peymaniet al. (17).

There were high resistant to different generation of cephalosporins in the present study where 88.3% of A. baumannii were resistant to ceftazidime and ceftriaxone, 90% to cefotaxime, 91.7% to cefepime, 95% to cefoteten, 96.6% to cefoxhydro and all isolates were resistant to cefazolin and cephalothin. These results were consistent with those reported by Feizabadi et al. (10), Peymaniet al. (17), Muthusamy and Boppe (8) and Erfaniet al. (19) reported absolute resistance toceftazidime among A. baumannii isolates. This may be indicative that resistance to cephalosporins is due to continuing misuse of antibiotics.

The isolates in the present study show higher resistance to aminoglycoside; gentamicin, tobramycin and amikacin showed high resistance in which (56.7%, 58.3% and 76.7% respectively). These findings indicates that resistance of A. baumannii to aminoglycosides was increased than those previously reported in Egypt by Hassan et al. (20), in which 56.7%, 58.3% and 76.7% respectively. These findings indicates that resistance of A. baumannii to aminoglycosides was increased than those previously reported in Egypt by Hassan et al. (20), where lower resistant pattern detected (28% and 56% resistant to tobramycin and amikacin respectively).

Moreover A. baumannii isolates showed high resistance to quinolones in which 75% were resistant to ciprofloxacin and 71.7% to levofloxacin. This finding was consistent with the pattern of quinolone susceptibility reported by Elmanama (21). Feizabadi et al. (18) Idmiret et al. (22) Peymaniet al. (17) and Muthusamy and Boppe (8). Where higher resistance was reported (63-87%). Also previous study by Ahmed et al. (23) reported complete insusceptibility to ciprofloxacin (100%).

On the other hand, although tetracycline susceptibility pattern were found with high discrepancy. Ahmed et al. (25) reported that A. baumannii have showed no resistance to tetracycline. On the other hand Hassan et al. (20) and Japoni-Nejader et al. (24) reported higher tetracycline resistance rate (80% and 90% respectively) than those encountered in the present study.

90% of A. baumannii isolated in the study were resistant to chloramphenicol which indicates the rapidly growing resistance among A. baumannii where a previous report (25) detects only 10% resistant isolates to chloramphenicol. A. baumannii isolates exhibited a higher resistance rate (91.7%) totrimethoprim/sulfamethoxazole than that previously reported (89%) by Peymaniet al. (17).

The explanation of increasing paradigm of resistant bacteria is that the organism has a never increasing list of resistance determinants that can rapidly nullify most of the therapeutic armamentarium (26). Both acquired and intrinsic resistance mechanisms can contribute this multi resistance pattern. The ability to acquire such resistance for multiple drugs may be due to either the acquisition of genetic elements carrying multiple resistant determinants or mutations affecting the expression of porins and/or efflux pump, which can minimize the activity of unrelated antimicrobial agents (27). It is also indicated that the outer membrane of Acinetobacter spp. acts as a substantial barrier against the penetration of these antibiotics.

Many of the acquired resistance determinants found in A. baumannii may be difficult to detect in a routine laboratory. Lack of detection of these resistance mechanisms may further enhance their spread. Multidrug resistance seems to result from both the accumulation of multiple mutations and the acquisition of resistance genes from other bacterial genera (27).

Multidrug-resistant Acinetobacter baumannii has been reported worldwide and it has now been recognized as one of the most difficult health care associated infections to control and treat Dheepa et al. (28). In the present study, 75% of A. baumannii were MDR (MDRAB). This is of grave concern because treatment options become limited. This increased frequency of MDRAB was also reported by Dheepael et al. (28), Shin et al. (29) and Muthusamy and Boppe (8).

Carbapenem, the broadest-spectrum β-lactams, were generally considered as the last resort for the treatment of serious infection cause by A. baumannii, since they are not affected by most β-lactamases, including Extended-spectrum β-
β-lactamases (ESBLs). However, decreased susceptibility to carbapenem among A. baumannii strains has been observed worldwide\(^{38}\).

A. baumannii in the present work showed high level of resistance to carbapenem in which 66.7% and 73.3% of isolates were imipenem and meropenem resistant respectively. This finding is not expected as different reports on carbapenemase-producing Actinobacter isolates are on rise globally due to increased carbapenem usage and selection pressure Ahmed et al.\(^{23}\), Peymani et al.\(^{17}\) and Karmostaji et al.\(^{31}\).

A variety of β-lactamases which include ESBLs, AmpC β-lactamases and metallo-β-lactamases, have emerged as the most worrisome mechanism of resistance among the Gram negative bacteria, which pose a therapeutic challenge to the health care settings\(^{32}\). The present study was undertaken to detect the role of MBL, Amp C, carbapenemase and efflux pump system as a potential resistance mechanisms of A. baumannii isolates to β-lactams, carbapenems and quinolones. The present study investigated some rapid and simple phenotypic tests to identify the potential resistance mechanisms among MDRAB to carbapenem and other β-lactams.

Among MDRAB isolated in the study carbapenemase was not produced by any of the isolates (0/45) as all isolates yield negative results in modified Hodge test. This result were also reported by Noyal et al.\(^{9}\) whereas Muthusamy and Boppe\(^{36}\) detected 20% carbapenemase production rate among MDRAB.

Metallo-β-lactamases (MBL) has increasing frequency over the past few years, and infection with MBL producing organisms is associated with higher rates of mortality, morbidity and health care costs\(^{33,34,35}\). In the present study Metallo-β-lactamase production was detected in 25/45 (55.6%) of MDRAB isolates by EDTA disc synergy test. Two MBL producing isolates were detected only by EDTA-meropenem combination, but not by EDTA-ceftazidime combination. A synergistic zone of inhibition which must have been normally present between EDTA and ceftazidime indicating MBL production was not observed with these two isolates. Interestingly these two isolates were Amp C β-lactamase producers, therefore it is conclude that the synergistic zone of inhibition was masked by the resistance to ceftazidime that conferred by the Amp C β-lactamase production (which is independent of zinc ions for its action). These findings were agreed with those previously observed by Noyal et al.\(^{9}\) and Pandya et al.\(^{35}\).

Based on the present study and the previously results reported by Noyal et al.\(^{9}\) and Pandya et al.\(^{35}\), it is clear that both EDTA-meropenem and EDTA-ceftazidime combination must be used simultaneously to detect all the MBL producers, which may otherwise be missed by using either of this combination alone.

The present study supposes that EDTA disc synergy test seems to be a better method than MHT for detection of carbapenemase production since the 25 isolates of MDRAB that have carbapenemase activity were detected by EDTA disc synergy test but not by MHT. This finding is similar to that reported by Noyal et al.\(^{9}\) and Jesudason et al.\(^{36}\) as they reported that both EDTA-meropenem and EDTA-ceftazidime combination must be used simultaneously to detect all MBL producers, which may otherwise be missed by using either of this combination alone. The previous result indicates that EDTA-meropenem was more sensitive (100% sensitivity) than EDTA-ceftazidime Pandya et al.\(^{35}\).

The present study revealed that meropenem resistance was not detected by MHT but detected by EDTA disc synergy test, indicating that the type of carbapenemase produced by such strains was related to class B not class A or class D β-lactamases, or that resistance mechanisms may be employed in resistance of such strains to meropenem.

Unlike carbapenemase, resistance due to production of MBLs has a potential for rapid dissemination, since it is often plasmid-mediated. Consequently, the rapid detection of MBL production is essential to initiate an effective infection control policy to prevent their uncontrolled spread in clinical settings\(^{30}\).

The present study revealed that 44.4% (20/45) of isolates harbored bla\(_{VIM}\) and none of them were carrier of bla\(_{IMP}\). These findings are consistent with those previously reported by Poirel and Nordmann\(^{37}\) and Sung et al.\(^{43}\). Poirel and Nordmann\(^{37}\) stated that VIM enzymes have been identified very rarely in A. baumannii. On the other hand, Sung et al.\(^{43}\) reported similar prevalence of bla\(_{IMP}\) whereas Peymani et al.\(^{17}\) reported higher frequency of isolates carrying bla\(_{IMP}\) (61%).

In the present study five MBL producing isolates were only detected by phenotypic tests and were negative bla\(_{IMP}\) by PCR, this could be attributed to different variants genes, or other mechanisms (such as outer-membrane permeability and efflux pump). Amp C β-lactamases represent a new threat since they confer resistance to cephalosporins and are not affected by β-lactamase inhibitors and can provide resistance to carbapenems. This
resistance mechanism has been reported around the world, therefore it was evaluated in the present study among MDRAB.

Among the studied isolates, 44.4% of MDRAB (20/45) were Amp C β-lactamase producers. Other reports by Mohanudha et al. and Singh et al. detect higher rate of Amp C β-lactamase production among Acinetobacter isolates (46.1% and 66.6% respectively). Therefore, AmpC β-lactamase could be an important contributory factor for meropenem resistance among A. baumannii, this result illustrate the reason for finding 20 meropenem resistant isolates, despite they did not show production of MBL or other carbapenemases.

Another mechanism of resistance in bacteria is the efflux pumps that contribute to intrinsic resistance to a wide range of antibiotics and often have a broad substrate range. The over expression of multidrug efflux pumps can lead to low-level multidrug resistance, which poses a clinical problem. To overcome this problem an efflux pump inhibitor such as reserpine had been used.

Our study revealed that efflux pump system plays a role with/without other mechanisms in ciprofloxacin resistance among MDRAB, in which the MIC value of ciprofloxacin for MDRAB isolates were determined in presence and absence of reserpine. When reserpine was added the MIC values decrease in 77.8% (35/45) of isolates, on the other hand 22.2% (10/45) of isolates did not exhibit any change in MIC values after addition of reserpine which indicating that these isolates having efflux pump system.

These results are in agreement with Wei-Feng et al. who reported that there is increasing evidence that the efflux pump mechanism plays an important role in high level resistance to antibiotics.

CONCLUSION

Multidrug resistant A. baumannii became a problematic organism in immunosuppressed patients since it became resistant to the majority of commercially available antimicrobial agents (including aminoglycosides, cephalosporins, quinolones and imipenem). Infections caused by carbapenem-resistant Acinetobacter baumannii are therapeutically challenging because treatment options are limited. Resistance among A. baumannii attributed to different resistance mechanisms, of which Metallo-β-lactamase production is an important mechanism. EDTA disk synergy test is a relatively simple and sensitive method for MBL detection and seems to be a better method for detection of carbapenemase than MHT. Meropenem resistance that detected by EDTA disc synergy test but not by MHT indicating that the carbapenemase produced by such strains was related to class B not class A or D β-lactamases. Ceftazidime appears to be the better substrate for EDS compared to meropenem, but both meropenem and ceftazidime must be used simultaneously to detect all the MBL producers. AmpC β-lactamase could be a contributory factor for meropenem resistance among MRAB isolates. Efflux pump system plays a role in ciprofloxacin resistance among some A. baumannii isolates. This study underlies the importance of accurate identification and reporting of Acinetobacter spp. to prevent the emergence of epidemic of multiresistant A. baumannii circulating in hospitals.

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الكشف المذهري والنمط الجيني لبعض آليات المقاومة لمضادات الميكروبات بين الاستينتوباكتر
بوماني متعددة المقاومة للأدبيات المعزولة من مرضى نقص المناعة في مصر

فقطة الزهرا، م.، جمعة، و.، و.، وكول، و.، و.، والعزارة، م.، جامعة الأزهر، القاهرة، مصر

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خلفية: الاستينتوباكتر بوماني هو ميكروب يسبب تلك الجائحة التي انتشرت في العالم وكان ينتشر في المستشفيات. يعاني من نسبة المقاومة عالية للعلاجات، وينبغي تحديد هذه الظاهرة لتعزيز نوع الأمراض. وتتطلب الشفاء تقييم المقاومة معدل للاستينتوباكتر بوماني متعددة المقاومة للأدبيات (MBSLs) بفيومات-الاختيارات (Microscan).

المواضيع والأهداف: تشمل تقييم وأهداف الاستينتوباكتر بوماني متعددة المقاومة للأدبيات دراسة

النتائج: قام الأستاذ م.ج.، و.، وكول، و.، وكول، و.، بالبحث عن نمط المقاومة بين الاستينتوباكتر بوماني متعددة المقاومة للأدبيات (MBSLs) في السرطان كارباميكتين طاويا. وانتقدت النتائج الجيدة من الاستينتوباكتر بوماني متعددة المقاومة للأدبيات (MBSLs) على البكتيريا المتعددة المقاومة (MDR). وتتأثر شفاء المرضى ببعض آليات الاستئصال. وتتطلب الشفاء تقييم الاستئصال لدفعة المقاومة.

الاستنتاج: الاستئصال الاستئصال موحد في التحليلات المتعددة لانتشار الميكروبات المتعددة للمقاومة. وتساعد الاستئصال في تقييم الاستئصال على الفعالية في تعزيز النتائج الجيدة من الاستئصال. وتستلزم الاستئصال تعزيز الفعالية في تعزيز النتائج الجيدة من الاستئصال. وتستلزم الاستئصال تعزيز الفعالية في تعزيز النتائج الجيدة من الاستئصال. وتستلزم الاستئصال تعزيز الفعالية في تعزيز النتائج الجيدة من الاستئصال. وتستلزم الاستئصال تعزيز الفعالية في تعزيز النتائج الجيدة من الاستئصال.