
# Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 6 (10): 750-760, 2011
ISSN 1816-4935 / DOI: 10.3923/jm.2011.750.760


# Phenotypic and Genotypic Detection of Metallo-beta-lactamases in Imipenem-resistant *Acinetobacter baumannii* Isolated from a Tertiary Hospital in Alexandria, Egypt

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## ABSTRACT

The wide use of carbapenems for empirical therapy of nosocomial infections caused by *Acinetobacter baumannii* has resulted in emergence of an additional type of resistance; the Metallo-β-lactamases (MBLs). This study analyzed the prevalence of MBL production in imipenem-resistant *A. baumannii* isolated from the Main University Hospital based in Alexandria, Egypt. The Main University Hospital is a 1500-bed tertiary teaching hospital with a heavy patient turnover and extensive antibiotic use. The majority of isolates (95.7%) were Multi-Drug Resistant (MDR), showing complete resistance to extended-spectrum cephalosporins, ureidopenicillin, β-lactam combinations and amikacin. All the isolates displayed elevated values for Minimum Inhibitory Concentration (MIC) of imipenem ($MIC_{50}$ and $MIC_{90}$ were 64 and 512 μg $mL^{-1}$, respectively). Phenotypic detection of MBLs by Combined Disc Test (CDT) and Modified Three-Dimensional Test (MTDT) identified a proportion of 70% as MBL producers. The presence of MBL-encoding genes ($bla_{IMP}$, $bla_{VIM}$, $bla_{SPM-1}$, $bla_{SIM-1}$ and $bla_{GIM-1}$) was checked by Polymerase Chain Reaction (PCR) and was confirmed in 52% of isolates where amplification of $bla_{VIM}$ and $bla_{SPM-1}$ genes was observed in 27 and 30%, respectively. A single isolate harbored a $bla_{GIM-1}$ gene while none of the isolates showed amplification of either $bla_{SIM-1}$ or $bla_{IPM}$ genes. Further investigation on phenotypically-positive/PCR-negative isolates by spectrophotometric assay revealed MBL activity in all isolates, suggesting the involvement of other genes. These results point out that MBL-producing *A. baumannii* isolates are now being detected in Egypt. This underlies the importance of their accurate identification and reporting to prevent the emergence of complete resistance to the useful drugs against *Acinetobacter* spp. in this country.

**Key words:** *Acinetobacter baumannii*, imipenem-resistance, metallo-beta-lactamases, phenotypic detection, genotypic detection

## INTRODUCTION

*Acinetobacter baumannii* is one of the most frequently isolated nosocomial pathogens in intensive care settings (Jazani *et al*., 2011). This organism is considered to be the cause of various infections such as ventilator-associated pneumonia, bloodstream and urinary tract infections, meningitis as well as wound infections (Jazani *et al*., 2007). Carbapenems, the broadest-spectrum β-lactams, were generally considered as the last resort for the treatment of these serious infections,

since they are not affected by most β-lactamases, including Extended-Spectrum β-lactamases (ESBLs) (Gupta, 2008). However, decreased susceptibility to carbapenems among *A. baumannii* strains has been recently observed worldwide (Peleg *et al*., 2008; Valenza *et al*., 2010). Resistance to carbapenems in *Acinetobacter* spp. might be associated with development of efflux pumps, alteration in membrane permeability, structural changes within penicillin-binding proteins or the production of carbapenem-hydrolyzing enzymes (Perez *et al*., 2007). Two types of carbapenemases are recognized: serine β-lactamases (having serine at their active site) and the Metallo-β-lactamases (MBLs), containing a metal ion that works as a cofactor for the enzyme's activity. Based on amino acid sequence homology, five MBL types have been recognized; IMP (imipenemase), VIM (Verona integron-encoded metallo-β-lactamase), SPM (Sao Paulo metallo-β-lactamase), GIM (German imipenemase) and SIM (Seoul imipenemase) types (Lee and Lee, 2006). The problem is aggravated by the fact that most of the MBL-encoding genes reside on integrons and plasmids which in turn allows for the widespread dissemination of these genetic elements (Walsh *et al*., 2005; Perez *et al*., 2007). Poor therapeutic outcomes and increased mortality rates have been associated with infections of such organisms (Peleg *et al*., 2008). Therefore, early detection of MBL-producing organisms is of crucial importance to permit rapid initiation of strict infection control procedures, allow timely institution of effective therapy and prevent nosocomial spread (Qu *et al*., 2009).

This study was undertaken to assess both phenotypically and genotypically the prevalence of MBL-producing strains among imipenem-resistant *A. baumannii* isolated over a period of four months from the Main University Hospital in Alexandria, Egypt.

## MATERIALS AND METHODS

**Test organisms:** Twenty-three non-replicated imipenem-resistant *A. baumannii* isolates were obtained from clinical specimens submitted for bacteriological testing from hospitalized inpatients admitted to the Main University Hospital (Alexandria University, Alexandria, Egypt) over the period of January to April 2010. With regard to the clinical origin, the *A. baumannii* were isolated from bronchoalveolar lavages (n = 11), urine (n = 5), blood (n = 4) and wound swabs (n = 3). *Escherichia coli* NCTC 10418 and *A. baumannii* ATCC 19606 were included in the study as quality control strains.

**Bacterial identification:** The test organisms were isolated as described in the classical literature. Culture identification was carried out by means of conventional methods and included morphological and cultural properties, as well as biochemical characteristics which were estimated by the commercial test system API 20 NE (BioMérieux, France). A growth temperature of 44°C was used to confirm the identity of these isolates. The identified strains were stored as glycerol cultures (20% [vol/vol]) at -70°C; working cultures were additionally kept at 4°C on appropriate nutrient agar plates which were subcultured fortnightly from the respective glycerol cultures at 37°C.

***In vitro* antimicrobial susceptibility testing:** Susceptibility of the isolates to the following antibacterial agents was tested by the Kirby-Bauer disk diffusion method (Bauer *et al*., 1966) using disks (Oxoid) on Mueller Hinton agar (Oxoid) and interpreted as recommended by Clinical Laboratory Standards Institute (CLSI, 2010) guidelines: imipenem (IPM, 10 μg), meropenem (MEM, 10 μg), ceftazidime (CAZ, 30 μg), cefotaxime (CTX, 30 μg), cefepime (FEP, 30 μg),

ampicillin/sulbactam (SAM, 10/10 μg), piperacillin (PRL, 100 μg), piperacillin/tazobactam (TPZ, 100/10 μg), ticarcillin (TIC, 75 μg), tetracycline (TE, 30 μg), doxycycline (DO, 30 μg), amikacin (AK, 30 μg), tobramycin (TOB, 10 μg), ciprofloxacin (CIP, 5 μg), sulfamethoxazole-trimethoprim (SXT, 1.25/23.75 μg), polymyxin B (PB, 300 units) and colistin (CT, 25 μg).

**Determination of the Minimum Inhibitory Concentration (MIC) of imipenem by the agar dilution technique:** The MIC of imipenem (Merck, Sharp and Dohme, Egypt) was determined by the agar dilution method as recommended by Clinical and Laboratory Standard Institute (CLSI, 2010). A control plate was included in which the antibiotic solution was replaced by sterile water. Results were read after incubation at 37°C for 24 h and the MIC was recorded as the lowest concentration of the carbapenem at which ≤5 colonies grew. The organisms were considered susceptible to the tested carbapenems if the MIC was ≤4 μg $mL^{-1}$ and resistant if the MIC was ≥16 μg $mL^{-1}$ (CLSI, 2010).

**Preparation of crude cell lysates:** Cell lysates were prepared from overnight cultures in Luria-Bertani (LB) broth (1% tryptone, 1% NaCl and 0.5% yeast extract in distilled water) by centrifuging each culture for 10 min at 4,000 rpm, discarding the supernatant and resuspending the cells in 1 mL of 0.05 M sodium phosphate buffer (pH 7). The cells were then lysed by seven repeated freeze-thaw cycles between -20°C and Room Temperature (RT). The crude cell lysate was separated from solid cell debris and unlysed cells by centrifugation (10 min, RT, 3,200×g) (Livermore and Williams, 1996). The protein content of the extract was determined using total protein assay kit (Biosystems) according to the accompanied protocol by measuring the absorbance of both provided bovine serum albumin standard and sample under test at 545 nm against blank.

**Phenotypic analysis of MBL production:** All imipenem-resistant isolates were investigated for MBL production by two phenotypic tests: the CDT (combined disc test with MBL inhibitor, EDTA) developed by Yong *et al.* (2002) and the MTDT (modified three-dimensional test) described by Shahid *et al.* (2004). After overnight incubation at 37°C, the plates were examined for developed inhibition zones. In the CDT, an increase in zone diameter by ≥6 mm around the IPM-EDTA disc compared to that of the IPM alone was considered positive for MBL. In MTDT, a distorted inhibition zone around the cup with the test crude enzyme extract was translated as a positive result. All the MBL-positive isolates were repeatedly checked for reproducibility of results.

**Genotypic analysis of MBL determinants:** Polymerase Chain Reaction (PCR) testing of all isolates for MBL genes was done employing each of the five primer pairs described in Table 1, specific for each family of acquired MBLs ($bla_{IMP}$, $bla_{VIM}$, $bla_{SPM-1}$, $bla_{GIM-1}$ and $bla_{SIM-1}$), respectively. Two microliters of genomic DNA extracted using the bacterial DNA isolation kit (Bio Basic Inc., Canada) were added to the PCR reaction mixture containing PCR master Mix (DreamTaq™) and specific primers (10 μM) prior to thermal cycling in a Perkin Elmer thermocycler. The cycling protocol involved an initial DNA release and denaturation step at 94°C for 5 min, followed by 36 cycles at 94°C for 30 sec, 52°C for 40 sec and 72°C for 50 sec, with a subsequent single elongation step at 72°C for 5 min. The PCR products were then detected by agarose gel electrophoresis using 1% agarose.

Table 1: Forward (F) and Reverse (R) primer pairs used for PCR detection of MBL genes as described by Ellington *et al.* (2007)

| Target gene | Primer pair (5' to 3') | Expected PCR product (bp*) |
|---|---|---|
| $bla_{IMP}$ | Imp-F GGA ATA GAG TGG CTT AAY TCT C | 188 |
| | Imp-R -CCA AAC YAC TAS GTT ATC T | |
| $bla_{VIM}$ | Vim-F GAT GGT GTT TGG TCG CAT A | 390 |
| | Vim-R CGA ATG CGC AGC ACC AG | |
| $bla_{SPM-1}$ | Spm-F AAA ATC TGG GTA CGC AAA CG | 271 |
| | Spm-R ACA TTA TCC GCT GGA ACA GG | |
| $bla_{GIM-1}$ | Gim-F TCG ACA CAC CTT GGT CTG AA | 477 |
| | Gim-R AAC TTC CAA CTT TGC CAT GC | |
| $bla_{SIM-1}$ | Sim-F TAC AAG GGA TTC GGC ATC G | 570 |
| | Sim-R TAA TGG CCT GTT CCC ATG TG | |

*bp (basepairs)

**Determination of MBL activity by spectrophotometric analysis:** Isolates which were positive for MBL production by the CDT and/or MTDT but were found to be PCR-negative were further investigated for enzyme activity using a double-beam spectrophotometer (Thermo Spectronic, Helios alpha, England) at 299 nm as previously described by Toleman *et al.* (2003). The amount of residual IPM was calculated using the regression equation of a prepared standard curve.

## RESULTS

**Antibiotic susceptibility testing:** The susceptibility of 23 imipenem-resistant *A. baumannii* isolates against different antibiotics showed that 95.7% (22/23) of them were Multi-Drug Resistant (MDR). Multi-drug resistance is defined as the resistance to three or more classes of antibiotics, including aminoglycosides, antipseudomonal penicillins, carbapenems, cephalosporins and quinolones (Adeniyi *et al.*, 2006). While all the isolates were resistant to imipenem, 60.9% of the isolates were resistant to meropenem. A 100% resistance was noticed to the tested third and fourth generation cephalosporins. High resistance to aminoglycosides was detected with 100% of isolates being resistant to amikacin and 82.6% to tobramycin. Some of the isolates remained susceptible to ciprofloxacin (30.4%). Among the tetracyclines, doxycycline displayed a lower resistance rate (21.7%), than tetracycline (73.9%). Polymixins (colistin and polymixin B) retained their activity against most of the tested isolates with a percentage of susceptibility of 82.6 and 91.3%, respectively (Fig. 1).

**The MIC of imipenem; phenotypic and genotypic detection of MBLs and spectrophotometric analysis:** The MIC of IPM was determined by the agar dilution technique for all clinical isolates (Table 2). According to CLSI standards, all of the tested strains were found to be resistant to imipenem, with MIC values ranging from 32 to 1024 μg $mL^{-1}$. The $MIC_{50}$ and $MIC_{90}$ were 64 and 512 μg $mL^{-1}$, respectively. Based on the significantly elevated imipenem MIC values obtained, all the isolates were subjected to two phenotypic screening tests for MBL production. Out of the 23 imipenem-resistant isolates included in this study, 11 isolates (47.8%) showed an increase in the zone diameter around the IPM+EDTA disc of ≥6 mm and were identified as MBL producers by the CDT (Fig. 2a, Table 2). In the MTDT, 16 isolates (69.6%) displayed a distorted inhibition zone around the cup with the test crude enzyme extract indicating a positive result (Fig. 2b, Table 2). Seven isolates (30.4%) were phenotypically found to be MBL non-

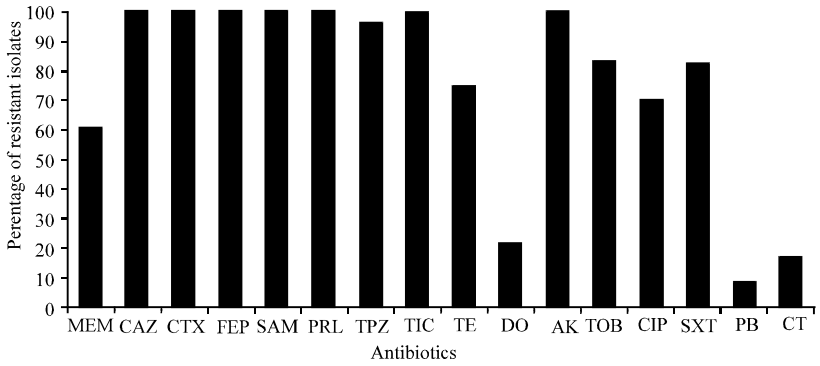


Fig. 1: Percentage of *A. baumannii* isolates resistant to the tested antibiotics. The percentage was calculated based on the number of resistant isolates to the respective antibiotic relative to the total number of tested isolates (n = 23). Abbreviations: MEM (meropenem); CAZ (ceftazidime); CTX (cefotaxime); FEF (cefipime); SAM (ampicillin/sulbactam); PRL (piperacillin); TPZ (piperacillin/tazobactam); TIC (ticarcillin); TE (tetracycline); DO (doxycycline); AK (amikacin); TOB (tobramycin); CIP (ciprofloxacin); SXT (sulfamethoxazole-trimethoprim); PB (polymixin B) and CT (colistin)

Table 2: MIC of imipenem, phenotypic, genotypic detection of MBLs and spectrophotometric analysis for *A. baumannii* isolates (n = 23)

| Strain ID | MIC of imipenem (μg mL$^{-1}$)* | Phenotypic tests | | Genotypic analysis (PCR) | | | | | Imipenem hydrolysis-spectrophotometric assay[c] |
|---|---|---|---|---|---|---|---|---|---|
| | | CDT[a] | MTDT[b] | *bla*$_{IPM}$ | *bla*$_{VIM}$ | *bla*$_{GIM-1}$ | *bla*$_{SPM-1}$ | *bla*$_{SIM-1}$ | |
| A1 | 128 | - | + | - | - | - | - | - | 45.4 |
| A2 | 128 | - | + | - | + | - | + | - | - |
| A3 | 128 | - | - | - | - | + | - | - | - |
| A4 | 32 | - | - | - | - | - | - | - | 2.6[§] |
| A5 | 128 | + | + | - | + | - | - | - | - |
| A6 | 128 | - | + | - | - | - | - | - | 56.5 |
| A7 | 1024 | + | + | - | - | - | + | - | - |
| A8 | 512 | + | + | - | - | - | + | - | - |
| A9 | 32 | - | - | - | - | - | - | - | 11.3[§] |
| A10 | 32 | - | - | - | - | - | - | - | - |
| A11 | 512 | + | + | - | + | - | - | - | - |
| A12 | 128 | + | + | - | + | - | - | - | - |
| A13 | 64 | + | + | - | - | - | - | - | 58.3 |
| A14 | 128 | - | - | - | + | - | - | - | - |
| A15 | 32 | + | + | - | - | - | - | - | 46.2 |
| A16 | 32 | - | - | - | - | - | - | - | - |
| A17 | 128 | + | + | - | + | - | + | - | - |
| A18 | 32 | - | + | - | - | - | + | - | - |
| A19 | 32 | - | + | - | - | - | + | - | - |
| A20 | 32 | + | + | - | - | - | + | - | - |
| A21 | 32 | - | - | - | - | - | - | - | - |
| A22 | 32 | + | + | - | - | - | - | - | 48.6 |
| A23 | 32 | + | + | - | - | - | - | - | 50.0 |

*CLSI breakpoint for imipenem: 16 μg mL$^{-1}$. [a]CDT: Combined disc test; breakpoint for positive result: ≥6 mm; (+): indicates a positive result while (-): indicates a negative result. [b] MTDT: Modified-three-dimensional test; positive result indicates the presence of a distorted inhibition zone around the cup with the crude enzyme extract. [c]Nanomoles of imipenem hydrolyzed per minute and mg protein in the crude enzyme extract at 37°C and pH 7.0. [§]Negative control

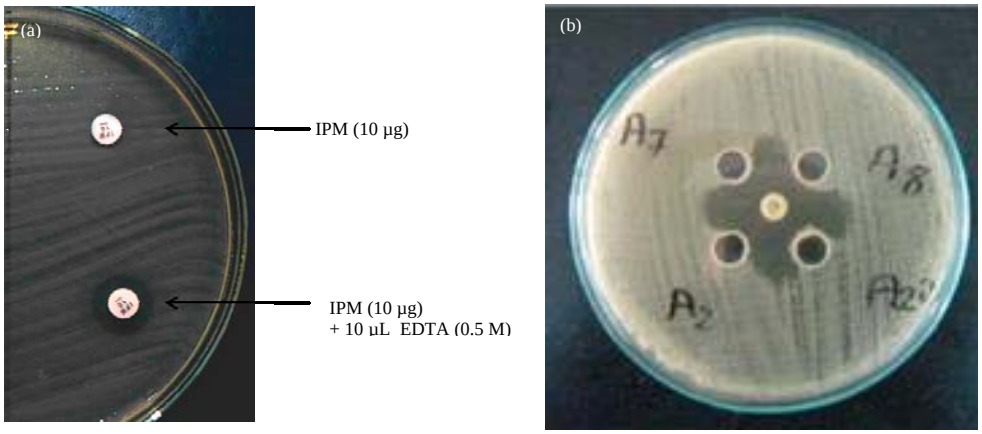


Fig. 2: Phenotypic detection of MBL-producing isolates by: (a) the Combined Disc Test (CDT) showing a positive result upon the increase in the inhibition zone diameter around the imipenem (IPM)+EDTA disc by 6 mm as compared to the imipenem disc alone (IPM); and (b) the Modified Three-Dimensional test (MTDT) showing positive results with a distorted inhibition zone of *E. coli* NCTC 10418 around cups containing the crude enzyme lysates of *A. baumannii* isolates A2, A7, A8 and A20, placed around a central IPM disc (10 µg)

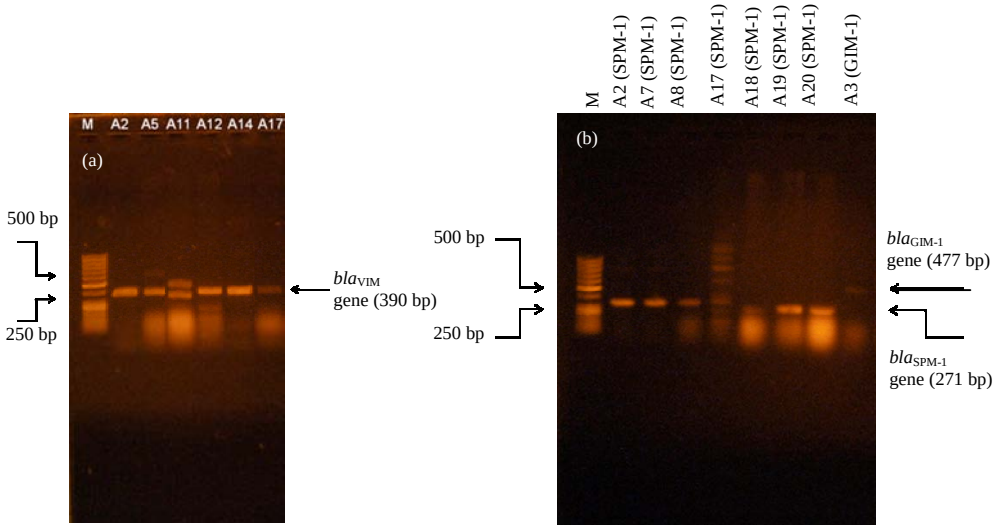


Fig. 3: Genotypic detection of MBL genes in *A. baumannii* isolates. PCR results of the amplification of $bla_{VIM}$ (a), $bla_{SPM-1}$ and $bla_{GIM-1}$ genes (b) in different *A. baumannii* isolates are shown. To the far left is the DNA marker (M), with bands of the following sizes: 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900 and 1000 basepairs (bp). The remaining lanes represent six (a) and eight (b) *A. baumannii* isolates, respectively. The respective gene tested in case of each isolate in (b) is mentioned in brackets. The location of the respective bands of all three genes $bla_{VIM}$ (a), $bla_{SPM-1}$ and $bla_{GIM-1}$ (b) are marked on the figures

producers. Genotypic analysis was positive for 52.2% (12/23) of the isolates. PCR revealed amplification of a 390 bp fragment corresponding to the $bla_{VIM}$ gene in 26.1% of isolates (6/23) (Fig. 3a) and a 271 bp fragment corresponding to the $bla_{SPM-1}$ gene in 7 isolates (30.4%). Isolate A3 gave an amplicon of 477 bp size corresponding to $bla_{GIM-1}$ (Fig. 3b). None of the isolates harbored $bla_{IMP}$ (188 bp) or $bla_{SIM-1}$ (570 bp) genes. It should be noted that the phenotypic tests identified 6 isolates as MBL-producers which in turn did not show amplification of any of the five tested MBL genes. These isolates were further investigated for MBL activity by a spectrophotometric assay. The freeze-thawed cell lysates were tested for their ability to hydrolyze imipenem by UV spectrophotometry at 299 nm after incubation for 1 h at 37°C. The test was positive for the six isolates as their crude enzyme extracts exhibited high hydrolytic efficiency on imipenem. Specific activity ranged from 45.4 to 58.3 nmol imipenem hydrolysed $min^{-1}$ (mg protein)$^{-1}$, compared to 2.6-11.3 nmol imipenem hydrolysed $min^{-1}$ (mg protein)$^{-1}$ by the two MBL-negative isolates used as negative controls (Table 2).

## DISCUSSION

Resistance to carbapenems in *A. Baumannii* has been noted in recent years in Egyptian hospitals. A recent study conducted on fifty-two *A. baumannii* isolated from hospitals of the Upper Egypt sector showed 77% resistance to meropenem (Ahmed *et al*., 2011). In the present study, twenty-three *A. baumannii* isolates, obtained from a tertiary teaching hospital in the city of Alexandria located on the Mediterranean coast in North Egypt, were all resistant to imipenem, reflecting a problem that might be described as countrywide. In addition, these twenty-three isolates displayed unusually high-level of imipenem resistance with 52.2% of the isolates showing MIC values ≥64 μg $mL^{-1}$ (Table 2). In the Middle East, the occurrence of imipenem-resistant *A. baumannii* is alarmingly recognized. In Saudi Arabia, the susceptibility rate of *A. baumannii* isolated from a tertiary care hospital to imipenem was reported to be as low as 10% (Al-Johani *et al*., 2010). In Bahrain, 58% of 454 *A. baumannii* isolated from a 1000-bed tertiary care centre were resistant to imipenem (Mugnier *et al*., 2009). In United Arab Emirates and Qatar, 100% resistance to imipenem was observed in the tested *A. baumannii* isolates (Mugnier *et al*., 2008; El-Shafie *et al*., 2004). The extensive use of carbapenems in this part of the world has likely created a selective antibiotic pressure which in turn has resulted in an increased prevalence of carbapenem-resistant *A. baumannii*.

Carbapenem-resistant *A. baumannii* often exhibit resistance to additional classes of antibiotics and behave as Multi-drug Resistant (MDR) bacteria (Lee *et al*., 2005; Bratu and Quale, 2006). The antibiogram of the twenty-three imipenem-resistant *A. baumannii* isolates investigated in this study showed that the majority of these isolates (96%) were MDR, being resistant to three or more classes of antibiotics (Fig. 1). While all the isolates were resistant to extended-spectrum cephalosporins, ureidopenicillin, β-lactam combination and amikacin; 61, 74, 83 and 70% of isolates were resistant to meropenem, tetracycline, tobramycin and ciprofloxacin, respectively. Unfortunately, these antibiotics are frequently prescribed in the Main University Teaching Hospital. In this disturbing situation, a strict policy for the use of antibiotics in the face of aggressive marketing by pharmaceutical companies is urgently needed. Implementation of effective surveillance programmes and antibiotic cycling succeeded in controlling drug resistance in some countries (Merz *et al*., 2004). On the other hand, few antimicrobials that were not used frequently in the hospital, such as polymixin B, colistin and doxycycline continued to show low resistance against the tested isolates, with a percentage resistance as low as 9, 17 and 22%, respectively

(Fig. 1). Such observations have also been noted by other authors wherein susceptibility was attributed to the decreased prescription of these antimicrobials in hospital settings (Manikal *et al*., 2000).

Unlike carbapenem resistance due to several other mechanisms, the resistance due to the production of Metallo-β-lactamases (MBLs) has a potential for rapid dissemination, since it is often plasmid-mediated (Walsh *et al*., 2005). Consequently, the rapid detection of MBL production is necessary to initiate effective infection control measures to prevent their uncontrolled spread in clinical settings. Molecular and non-molecular methods have been used for screening bacterial isolates for MBL production. In this study, the significantly elevated imipenem MIC values of the twenty-three *A. baumannii* isolates, ranging from 32 to 1024 μg $mL^{-1}$, rendered all the isolates eligible for further testing for MBL production by means of two phenotypic tests. The CDT was positive for 48% of isolates while the MTDT identified 70% of these isolates as MBL-producers (Table 2). Data on the prevalence of MBLs in *A. baumannii* in Egypt are lacking but the proportion of isolates producing MBLs is in accordance with that reported in India (Uma Karthika *et al*., 2009). Hence, the production of MBLs might be considered as an important contributory factor for imipenem resistance among *Acinetobacter* species in both countries.

The most common transferable MBL-encoding genes include the VIM-, IMP-, GIM-, SPM- and SIM-type enzymes which have been detected primarily in *Pseudomonas aeruginosa* but were also found in *A. baumannii*. Two new subgroups of MBLs, designated NDM-1 and DIM-1, are currently being identified in India, Pakistan, the United Kingdom and in the Netherlands (Valenza *et al*., 2010). Recently, an *A. baumannii* isolate recovered from a patient transferred to Germany from an Egyptian hospital revealed a new variant of NDM-1 that was designated NDM-2 (Kaase *et al*., 2011). The presence of MBL genes was confirmed in twelve cases (52%) by PCR amplification where six isolates harbored a $bla_{VIM}$ gene, seven isolates a $bla_{SPM-1}$ gene and one isolate a $bla_{GIM-1}$ gene. However, no isolates showed the presence of $bla_{IPM}$ when tested for MBL genes, a phenomenon which is also reported by others (Rossolini *et al*., 2008; Valenza *et al*., 2010).

Ten isolates were positive for MBLs by both the phenotypic detection methods (CDT and/or MTDT) as well as the PCR amplification. There were two isolates (A3 and A14) that were PCR-positive but showed negative results in the phenotypic tests. Since, MBL-encoding gene cassettes have been associated with integrons, it is possible that MBL gene alleles could be carried by *Acinetobacter* strains but do not phenotypically express the MBLs (Uma Karthika *et al*., 2009). Besides, the phenotypic detection tests showed six isolates (A1, A6, A13, A15, A22 and A23) positive for MBLs which did not show PCR amplification for MBL genes. These isolates were further assayed spectrophotometrically by monitoring the hydrolysis of imipenem at 299 nm to detect MBL activity which was positive for the six isolates wherein cell extracts caused breakdown of imipenem with a specific activity of >40 nmol imipenem hydrolysed $min^{-1}$ (mg protein)$^{-1}$ as compared to 2.6-11.3 nmol imipenem hydrolysed $min^{-1}$ (mg protein)$^{-1}$ by the two negative controls (Table 2). These results suggest that these six strains might harbor different variants of the studied genes. The imipenem-resistant strains with no phenotypic or genotypic sign of MBL production may possess other enzymes mediating carbapenem resistance, such as OXA-type lactamases (class D) or AmpC β-lactamases and/or other mechanisms such as outer-membrane permeability and efflux mechanisms that were not evaluated in this study.

Carbapenem resistance in *A. baumannii* isolates is now being detected in many countries, obviously not excluding Egypt. Among the several mechanisms responsible for resistance to

carbapenems, the production of MBLs remains one of the most clinically significant mechanisms. The rapid ability of MBL-producing *A. baumannii* to disseminate within any hospital setting poses a tremendous challenge to physicians and infection control practitioners alike, minimizing the available therapeutic options for this pathogen. To confront this challenge, effective infection control strategies such as restriction of the use of antibiotics, especially those with broad-spectrum activity and those identified as antibiotics of last resort and the accurate identification and reporting of MBL-producing bacteria, need to be implemented and maintained thoroughly to prevent the emergence of complete resistance to the useful drugs against *Acinetobacter* spp. in Egypt.

**ACKNOWLEDGMENTS**

We would like to thank the staff of the microbiology laboratory affiliated with the Main University Hospital in Alexandria for providing isolates used in this study as part of our collaborative efforts.

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