Evaluation of different phenotypic assays for the detection of metallo-β-lactamase production in carbapenem susceptible and resistant Acinetobacter baumannii isolates

Enas A. Daef¹, Ismail S..Mohamed¹, Ahmed S. Ahmed¹, Nahla M Elsherbiny¹, Ibrahim M. Sayed¹.

¹Department of Medical Microbiology & Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt

nahlaelsherbiny@hotmail.com

Abstract: This study was conducted to evaluate the combined disc test and the double disc synergy test for MBL detection among imipenem sensitive and resistant A. baumannii strains, to study the co-resistance to other classes of antibiotics and to determine the prevalence of some antibiotic resistance determinants (blaOXA-51 like gene and class I integron) among these isolates. We isolated a total of 51 A. baumannii strains. The antibiotic sensitivity pattern was determined by Kirby Bauer disc diffusion method. For imipenem, the minimum inhibitory concentrations (MICs) were determined using the Epsilometer (E test). The isolates were tested for the presence of MBLs by the combined disc test (CDT) and the double disc synergy test (DDST). For all isolates, PCR was performed for the detection of the blaOXA-51 like and Class I integrase genes. The highest rates of resistance were against ciprofloxacin (64.7%), amoxicillin clavulanic acid (58.8%), amikacin (58.8%), ceftaxime (56.9%) and chloramphenicol (52.9%). Lower rates of resistance were to imipenem (31.4%) and tetracyclines (25.5%). MBLs were detected in both imipenem sensitive and resistant A. baumannii isolates. The CDT had a sensitivity ranging from 92% to 100%, while the DDST had a sensitivity ranging from 86.2% to 100%. The blaOXA-51 like gene was detected in 96.1% and Class I integrase gene was detected in (72.5%) of A. baumannii strains. The later conferred significantly higher resistance rates to various antibiotics.


Keywords: A. baumannii, Metallo beta lactamase, phenotypic methods.

1. Introduction

Acinetobacter baumannii has now been recognized as one of the most difficult health care associated infections to control and treat (Muthusamy and Boppe, 2012) because of its intrinsic and acquired resistance mechanisms (Navon-Venezia et al., 2005). Carbapenems are the drugs of choice for A. baumannii infections and are often used as a last resort. However, decreased susceptibility to carbapenems has been recently observed worldwide (Peleg et al., 2008; Valenza et al., 2010).

There are several carbapenem resistance mechanisms described in Acinetobacter species. (Peleg et al., 2008). Many carbapenem-hydrolyzing β-lactamases have been identified so far, amongst which are the metallo-β-lactamases (MBLs) (Ambler class B). Based on amino acid sequence homology, five MBL types have been recognized; IMP (imipenemase), GIM (German imipenemase) and SIM (Seoul imipenemase) types (Lee and Lee, 2006). 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).

Other carbapenemases include, carbapenem-hydrolyzing class D oxacillinase (CHDL) gene clusters that have been identified either in the chromosome or in plasmids of A. baumannii strains, represented by the blaOXA-23,-blaOXA-24/40,- and blaOXA-51 like genes (Poirel and Nordmann, 2006). In addition, the chromosomal blaOXA-51-like gene, intrinsic to A. baumannii species, has been demonstrated to confer carbapenem resistance (Turton et al., 2006).

The MBLs, as thought earlier, are just not restricted to the carbapenem resistant strains (Yan et al., 2004; Franklin et al., 2006). Identifying MBL-carrying isolates has been challenging due to the emergence of carbapenem-susceptible MBL-carrying organisms which may be missed in daily laboratory practice, compromising the sensitivity of detection methods. These carbapenem susceptible organisms with hidden MBL genes can spread unnoticed in hospitals if such isolates are reported as sensitive without screening for the presence of MBLs. The treatment of these organisms pose a serious therapeutic challenge as these strains are most often resistant to multiple drugs (Walsh et al., 2005).

The Clinical Laboratory Standard Institute (CLSI) has not yet included any standardized phenotypic detection method for screening MBL positive strains in the Acinetobacter calcoaceticus-baumannii complex, though it has included screening and confirmatory tests for suspected carbapenemase production in Enterobacteriaceae (CLSI, 2010). Polymerase chain reaction (PCR) is the gold standard method for the detection of MBL production, but it is not feasible in routine microbiology laboratory (Pandya et al., 2011). Several non molecular techniques have been
studied, all taking advantage of the enzyme's zinc dependence by using chelating agents, such as EDTA to inhibit its activity (Franklin et al., 2006). Various phenotypic methods for MBL detection include combined disc test (CDT), double disc synergy test (DDST) and MBL E-test (Monoharan et al., 2010).

This study aimed to evaluate the combined disc test and the double disc synergy test for MBL detection among imipenem sensitive and resistant A. baumannii strains, to study the co-resistance to other classes of antibiotics and to determine of some antibiotic resistance determinants (bla _OXA_ 51 like gene and class I integron) among these isolates.

2. Material and Methods

**Bacterial strains:** A total of 51 consecutive, non duplicate, _A. baumannii_ strains were isolated from various clinical and environmental specimens from the ICUs of Assiut University Hospitals, Egypt during period of October 2009 to February 2011. Regarding the clinical specimens, _A. baumannii_ strains (24 strains) were isolated from urine (n= 5), sputum (n= 8), swabs from endotracheal tubes (n= 6), blood cultures (n=1), throat swabs (n=3) and wound swabs (n=1) that were submitted for bacteriological testing from patients admitted to the ICUs.

A total of 27 isolates were obtained from environmental swabs from the ICUs. Swabs were taken from call bells, bedrails, and bedside tables, bedside equipments, carts, commodes, doorknobs and faucet handles.

All the isolates were characterized up to the species level by using standard microbiological techniques (Washington et al., 2006) which included morphological and cultural properties as well as biochemical characteristics which were determined by the commercial test system API 20NE Index system (BioMérieux, France). Species identification was confirmed by detection of _bla OXA-51_ like gene as described previously (Turton et al., 2006).

**Antimicrobial susceptibility testing.** Antibiotic susceptibility testing was done for all isolates using commercially available discs (HiMedia, Mumbai, India) by Kirby Bauer disk diffusion method and interpreted as recommended by Clinical Laboratory Standards Institute (CLSI, 2010). The antibiotics which were tested were imipenem 10 μg (IPM), amoxicillin clavulanic acid 30 μg (AMC), tetracycline 30 μg (Te), ceftriaxone 30 μg (CRO), amikacin 30 μg (AK), ciprofloxacin 5 μg (CIP) and chloramphenicol 30 μg (C). _A baumannii_ ATCC 19606 was used as control. MIC for imipenem was identified using the E-test strip (AB Biodisk, Solna, Sweden). The organisms were considered resistant to imipenem if the MIC was ≥16 μg/ml and susceptible if the MIC was ≤ 4 μg/ml (CLSI, 2006).

The phenotypic MBL detection methods. All the isolates were screened for the presence of MBLs by the combined-disk test (CDT) and the double-disk synergy test (DDST). All the MBL-positive isolates were repeatedly checked for reproducibility.

**The combined-disk test (CDT).** It was performed according to Yong et al. (2002) with some modifications. Two 10 μg imipenem discs (HiMedia, Mumbai, India) were placed on a plate 25 mm apart (center to center), inoculated with the test organism, and 10 μL of 0.1 M (292 mg) anhydrous Ethylenediaminetetraacetic acid (EDTA) (Sigma Chemicals, St. Louis, MO) solution was added to one disc. The inhibition zones of the imipenem and imipenem+EDTA discs were compared after 18 hours of incubation in air at 35°C. According to Franklin et al. (2006) an increase in zone diameter of >4 mm around the IPM-EDTA disk compared to that of the IPM disk alone was considered positive for an MBL enzyme.

**The double-disk synergy test (DDST).** It was performed according to Lee et al. (2003) with slight modifications. An IPM (10 μg) disk was placed 20 mm (center to center) from a blank filter paper disc (6 mm in diameter, Whatman filter paper no. 2) containing 10 μL of 0.1 M (292 μg) EDTA. After overnight incubation, enhancement of the zone of inhibition in the area between the two disks was considered positive for an MBL.

**Multiplex PCR for detection of _bla oxa-51_ like gene & Class I integrase gene** (Turton et al, 2005)

**DNA extraction.** The boiling method was used to extract the DNA from the bacteria (Vaneechoutte et al., 1995). Briefly, one colony of a pure culture was suspended in 50 μl of sterile water and heated at 100°C for 15 min. After centrifugation in a microcentrifuge (6,000 x g for 3 min), the supernatant was stored at -20°C for further use.

**PCR amplification and detection.** This was carried out in 25 μl reaction volumes with 3 μl of extracted DNA, 12.5 pmol of each primer as shown in table (1) and 1.5 U of _Taq_ DNA polymerase in 1X PCR buffer containing 1.5 mM MgCl2 (QIAGEN) and 200 μM of each deoxynucleoside triphosphate. Conditions for the multiplex PCR were as following: 94°C for 3 min, and then 35 cycles at 94°C for 45 s, at 57°C for 45 s, and at 72°C for 1 min, followed by a final extension at 72°C for 5 min. Amplified products from the isolates were analysed by electrophoresis on 1.2% (w/v) agarose gels, stained with ethidium bromide.

**Statistical analysis**

All data were analyzed using the computerized statistical analysis (SPSS, version 16). Descriptive statistics was used. The p value <0.05 was considered statistically significant. Sensitivity and specificity of the phenotypic MBL detection
methods was evaluated using PCR as the gold standard (Listrup, 1990)

3. Results
A total of 51 strains of A.baumannii were isolated from clinical (24 strains) and environmental (26 strains) samples.

Antimicrobial susceptibility testing and MIC determination
By Kirby Bauer disc diffusion method, the antimicrobial susceptibility pattern of 51 A. baumannii isolates was determined and is shown in Table (2). The highest rates of resistance were against amoxicillin clavulanic acid, ceftriaxone, amikacin, cephalosporins, aminoglycosides, chloramphenicol and ciprofloxacin.

Phenotypic tests for the detection of MBLs:
The combined-disk test (CDT)
A total percentage of 94.1% (48/51) of Acinetobacter isolates showed a positive CDT as shown in table (3). All imipenem resistant Acinetobacter isolates showed positive results for detection of MBLs (expressed phenotypically) , while 91.4% (32/35) of Imipenem susceptible Acinetobacter isolates showed positive results.

Double Disk Synergy test (DDST)
Out of the 51 Acinetobacter isolates, 44 strains showed synergistic zones of inhibition between Imipenem and EDTA discs (representing 86.3%) as presented in table (4). All the Imipenem resistant Acinetobacter isolates showed positive results for detection of MBL, while 80% (28/35) of Imipenem susceptible Acinetobacter isolates were positive by DDST.

On comparing the phenotypic tests, the sensitivity of CDT was higher (92%-100%) than that of the DDST (86.2%-100%) as shown in table (5).

Detection of bla\_oxa-51 like gene and Class I integrase gene by multiplex PCR:
The bla\_oxa-51 like gene was detected in 96.1% of all A. baumannii isolates and Class I integrase gene was detected in 72.5% of the isolates as shown in table (6) and in figure (1).

Antibiotic resistance pattern of integron-positive and integron-negative A. baumannii isolates
Integron positive A baumannii isolates had significantly higher resistance rates to amoxicillin clavulanic acid, ceftriaxone, amikacin and ciprofloxacin compared to the integron negative isolates as presented in table (7). Integron positive isolates also showed resistance to a significantly larger number of different antibiotics compared to integron negative isolates (Figure 2).

4. Discussion
A. baumannii infections present a global medical challenge. The interest in this organism has been growing rapidly because of the emergence of multi-drug resistant strains (MDR), some of which are pan resistant to antimicrobial agents (Muthusamy and Boppe, 2012).

In the present study the majority of A. baumannii isolates were MDR showing resistance to three or more classes of antibiotics. There has been a lot of debate concerning the definition of multidrug resistance (MDR). Renu et al. (2010) defined MDR as resistance to 4 or more classes of antimicrobials. MDR A.baumannii was also defined as an isolate with intermediate or complete resistance to at least 3 of the following classes of antibiotics: betalactam, aminoglycoside, carbapenem and fluoroquinolone (Zapantis et al., 2007). Others defined MDR as resistance to two or more drugs or drug classes of therapeutic relevance (Navon-Venezia et al., 2005).

Resistance against carbapenems is, in itself, considered sufficient to define an isolate of A. baumannii as highly resistant (Poirel and Nordmann, 2006).

We found that 31.4% of all A. baumannii isolates were imipenem resistant. For other antibiotics, we recorded high rates of resistance to ciprofloxacin (64.7%), amoxicillin clavulanic acid (58.8%), amikacin (58.8%) and ceftriaxone (56.9%). The least rates of resistance was against tetracycline (25.5%). Our results were lower than that reported in another Egyptian study where resistance rates approached nearly 100% against many antibiotics among carbapenem resistant Acinetobacter isolates. In that study all imipenem resistant isolates showed very high resistance to amikacin (100%), 3rd and 4th generation cephalosporins (100%), ampicillin/ sulbactam (100%). Lower rates of resistance were reported against tetracycline (73.9%) and ciprofloxacin (69.6%) (Mohamed and Raafat, 2011). The difference in the resistance rates is attributed to the isolates that were tested. In our study we included all A. baumannii strains that were resistant and sensitive to imipenem while the previous study was restricted to imipenem resistant strains only. In addition, our results were also lower than that of Ahmed et al. (2011) who reported 77% resistance to meropenem among 52 A baumannii isolated from hospitals of Upper Egypt. In the Middle East, the occurrence of imipenem resistant A. baumannii is alarmingly recognized. In Saudi Arabia, the imipenem- resistance rate of A. baumannii isolated from a tertiary care hospital was reported to be as high as 90% (Al-Johani et al., 2010). In Bahrain, 58% of A. baumannii isolates from a tertiary care hospital showed resistance to imipenem (Mugnier et al., 2009). Very high prevalence rates were reported in other studies. In an Indian study, Muthusamy and Boppe (2012) found 100% resistance to imipenem, meropenem and cefipime, 99% resistance to ceftazidime, 95% resistance to ciprofloxacin, 73% resistance to doxycycline, 83% resistance to ampicillin- sulbactum, and 55%...
resistance to tobramycin. The extensive use of carbapenem has created a selective antibiotic pressure which in turn has resulted in an increased prevalence of carbapenem resistant A. baumannii. (Mohamed and Rafaat, 2011).

Since there are no standard guidelines for detection of MBL, different studies have reported the use of different methods. In this study we compared the phenotypic methods for detection of MBL with the presence of class I integrase gene. As most of the MBL-encoding genes including the blalmp, blavim or blasim genes are embedded in class-1 integron structures (Walsh, 2005; Peleg et al., 2008; Poirel and Nordmann, 2006).

In this study, MBL was detected in both imipenem susceptible and imipenem resistant A. baumannii isolates. By the CDT, we found MBLs in 94.1% of all A. baumannii isolates. We detected MBL by this method in all imipenem resistant isolates and in 91.4% of imipenem sensitive strains. Our results were very high compared to those of another Egyptian study where only 48.7% of A. baumannii IMP-resistant isolates were MBL producer by CDT (Mohamed and Rafaat, 2011). To our knowledge, data on the prevalence of MBLs in A. baumannii is lacking in Egypt. In another study, MBL production was reported in 76% of imipenem resistant gram negative isolates (Renu et al., 2010). Recently, it was found that among all carbapenem resistant isolates, 77% of the isolates were found to be MBL producers (Omair et al., 2012).

The detection of MBLs among imipenem sensitive isolates was reported in many studies with varying percentages. It was reported that 20% of the MBL carrying isolates were found to be susceptible to IMP (Renu et al., 2010). Rate varying from 30%-88% were reported by other workers (Yan et al., 2004; Franklin et al., 2006).

In the current study, we found that the CDT was more sensitive than the DDST. This is in agreement with many previous studies (Franklin et al., 2006; Muthusay and Bobbe, 2012; Omair et al., 2012). In our study the sensitivity and specificity of the CDT for detection of MBLs in imipenem sensitive A. baumannii isolates, were 92% and 100% and for imipenem resistant isolates the sensitivity and specificity were 100%. Our results agreed with the results of Pandya et al. (2011) who reported that CDT-IPM was found to be a more sensitive method (96.30%) compared to DDST-IPM (81.48%). Our also results also agreed with Franklin et al. (2006) who found that the sensitivity of the CDT was 100% and the specificity was 98%. On the other hand, we disagreed with the findings of Kumar et al. (2011) who reported that the sensitivity of the combined disc test in the detection of carbapenemase was only 21%. The findings of the present study confirm and extend the results of previous studies regarding the feasibility, accuracy, of the combined disc test, in the detection of MBL in clinical lab.

In this study, 86.3% of A. baumannii isolates produced MBLs by the DDST. The percentages varied in many studies from 14% to 70.9% (Lee et al., 2003; Uma et al., 2009; Anwar, Amin, 2011). The studies differed in the cut off chosen for MBL detection. Our cut off value was 4 mm according to Franklin et al. (2006) while it was 7mm for others (Renu et al., 2010). We reported the sensitivity of the DDST to be 86.2% and 100% for IMP sensitive and resistant strains respectively. Our results were somewhat higher than those of Franklin et al.(2006) who reported a sensitivity of 79% and a specificity of 98%. Other studies documented a higher sensitivity of DDST (100%) as was reported by Yan et al. (2004). Lee et al. (2003) reported it to be 33.3%. The variation in different studies may be due to the different gold standard taken to which the phenotypic test is compared to in each study. DDST results are more subjective as it depends upon the technician’s expertise to discriminate true synergism from the intersection of inhibition zones (Pandya et al., 2011).

There was mounting evidence that A. baumannii has a naturally occurring carbapenemase gene intrinsic to this species (Turton et al., 2006). In this study, 96% or 49/51 of the isolated Acinetobacter strains had the band of blaOXA-51-like gene by PCR. This agreed with the results of Turton et al. (2006) who reported the presence of the blaOXA-51gene band in all isolates of A. baumannii but they mentioned the possibility of non detection of some variants. These results provided evidence that detection of blaOXA-51-like gene can be used as a simple and reliable way of identifying A. baumannii.

We reported on the presence of class I integron only as it was shown to be the most prevalent class among Acinetobacter isolates (Sirichot et al., 2009). We found that class I integron was widely distributed among A. baumannii isolates in the ICUs of our hospital (72.5%). This finding agreed with Koeleman, et al. (2001) and Lin et al. (2009) who detected that in 74% and in 75% of Acinetobacter isolates in their study. But, our percentage (72.5%) is considerably higher than the rates found in other geographical regions including Thailand (52%) (Sirichot et al., 2009), United Kingdom (60%) (Turton et al., 2005). Other areas reported much higher rates. In an Iranian study, 92.5% of A. baumannii strains had class I integron (Peymani et al., 2012).

In this study, integron positive strains were associated with increase resistance to antibiotics compared with integron negative isolates. This finding is in agreement with many previous studies (Koeleman et al., 2001; Peymani et al., 2012). This is not surprising, since many antibiotic resistance mechanism...
gene cassettes encoding resistance to a wide range of antibiotics have been reported. It was previously reported that the presence of Class I integrons correlates with the epidemic behavior of the strains (Koeleman \textit{et al.}, 2001).

In this study, we demonstrated that 81\% (30/37) of integron positive \textit{A. baumannii} showed resistance to five or more of the antibiotics tested. In concordance, Koeleman \textit{et al.} (2001) found that 95.8\% of integron positive strains (23 of 24) showed resistance to five or more of the antibiotics tested. We also reported a significant difference between resistance of integron positive \textit{A. baumannii} and integron negative isolates regardless amoxicillin clavulanic acid, cefotrixone, ciprofloxacin and amikacin, while there was no significant difference between them regarding the resistance to imipenem, tetracyclines and chloramphenicol. Our results agreed to a great extent with many previous studies (Koeleman \textit{et al.}, 2001; Perez \textit{et al.}, 2007; Peymani \textit{et al.}, 2012).

As many studies reported, we also found that many integron-negative isolates were MDR and showed the same antibiogram patterns of integron-positive strains. The antibiotic resistance genes of these isolates could be acquired by plasmid or other mobile elements (Perez \textit{et al.}, 2007).

In conclusion, \textit{A. baumannii} is a cause of concern due to multidrug resistance. The high incidence of isolates possessing MBL activity in the present study represents an emerging threat in Egypt. MBLs are detected in both imipenem sensitive and resistant \textit{A. baumannii} isolates. CDT is a simple, easy, economic test for detection of MBLs that can be incorporated into the routine testing of any busy microbiology laboratory. Class I integrase gene is predominantly found in \textit{A. baumannii} strains isolated from the ICUs which suggests resistance to many groups of antibiotics and suggests the epidemic potential. Infection control measures must be implemented to control the spread of such strains.

\begin{table}[h]
\centering
\caption{Sequences of primers of blaOXA-51-like gene and Class 1 integrase gene.}
\begin{tabular}{|c|c|c|c|}
\hline
Primer & Sequence & Target gene & Amplicon size (bp) \\
\hline
OXA-51-likeF\textsuperscript{a} & 5' -TAA TGC TTT GAT CCG CCT TG-3' & \textit{blaOXA-51-like} & 353 bp \\
OXA-51-likeR\textsuperscript{a} & 5'TGG ATT GCA CTT CAT CTT GG-3' & Class 1 integrase & 160 bp \\
Int1F\textsuperscript{b} & 5' -CAG TGG ACA TAA GCC TGT TC-3' & Class 1 integrase & \\
Int1R\textsuperscript{b} & 5' -CCC GAG GCA TAG ACT GTA-3' & & \\
\hline
\end{tabular}
\textsuperscript{a}bp : base pair
\textsuperscript{b}Woodford \textit{et al.}, 2006.
\textsuperscript{c}Koeleman \textit{et al.}, 2001.
\end{table}

\begin{table}[h]
\centering
\caption{Resistance patterns of \textit{A. baumannii} isolates to different Antibiotics.}
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
& \multicolumn{2}{|c|}{Strains from clinical samples N=24} & \multicolumn{2}{|c|}{Strains from environmental samples N=27} & \multicolumn{2}{|c|}{Total N= 51} \\
\hline
& Resistance & N & % & Resistance & N & % & N & % \\
\hline
Amoxicillin-Clavulanic acid & 16 & 66.7 & 14 & 51.9 & 30 & 58.8 & \\
Ceftriaxone & 15 & 62.5 & 14 & 51.9 & 29 & 56.9 & \\
Amikacin & 16 & 66.7 & 14 & 51.9 & 30 & 58.8 & \\
Tetracycline & 5 & 20.8 & 8 & 29.6 & 13 & 25.5 & \\
Imipenem & 7* & 29.2 & 9* & 33.3 & 16 & 31.4 & \\
Chloramphenicol & 13 & 54.2 & 14 & 51.9 & 27 & 52.9 & \\
Ciprofloxacin & 16 & 66.7 & 17 & 63 & 33 & 64.7 & \\
\hline
\end{tabular}
\textsuperscript{*}All imipenem resistant strains had MIC’s ranging from 16-256 \(\mu\text{g/ml}\) by the IMP - E test.
\end{table}

\begin{table}[h]
\centering
\caption{Detection of MBLs in imipenem susceptible and resistant strains by CDT.}
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
\textit{A. baumannii} strains & Imipenem susceptible & Imipenem resistant & \\
& No. & Positive CDT & No. & Positive CDT & \\
\hline
Isolated from clinical samples (n= 24) & 17 & 16 & 94 & 7 & 9 & 100 \\
Isolated from environmental sample (n= 27) & 18 & 16 & 89 & 9 & 9 & 100 \\
Total (n=51) & 35 & 32 & 91.4 & 16 & 16 & 100 \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Detection of MBLs in imipenem susceptible and resistant strains by DDST.}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textit{A. baumannii} strains & Imipenem susceptible & Imipenem resistant & \\
& No. & Positive DDST & No. & Positive DDST & \\
\hline
Isolated from clinical samples (n=24) & 17 & 15 & 88 & 7 & 7 & 100 \\
Isolated from environmental sample (n= 27) & 18 & 13 & 72 & 9 & 9 & 100 \\
Total (n=51) & 35 & 28 & 80 & 16 & 16 & 100 \\
\hline
\end{tabular}
\end{table}
Table 5: Sensitivity & Specificity of phenotypic tests

<table>
<thead>
<tr>
<th>Phenotypic test</th>
<th>Class I integrase gene positive isolates N= 37</th>
<th>Class I integrase gene negative isolates N= 14</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IPM-S</td>
<td>IPM-R</td>
<td>IPM-S</td>
<td>IPM-R</td>
</tr>
<tr>
<td>CDT (n=48)</td>
<td>23</td>
<td>12</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>DDST (n=44)</td>
<td>21</td>
<td>12</td>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 6: Detection of bla_{oxa-51-like} Gene and Class I integrase gene by PCR

<table>
<thead>
<tr>
<th>Isolates from</th>
<th>bla_{oxa-51-like} gene positive isolates</th>
<th>Class I integrase gene positive isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Clinical samples (N=24)</td>
<td>23</td>
<td>95.8</td>
</tr>
<tr>
<td>Environmental samples (N=27)</td>
<td>26</td>
<td>96.3</td>
</tr>
<tr>
<td>Total (N=51)</td>
<td>49</td>
<td>96.1</td>
</tr>
</tbody>
</table>

Figure 1: Multiplex PCR for detection of bla_{OXA-51-like} gene and Class I integrase gene
M: DNA marker (100bp)

Lane 1 to lane 7: show positive results for bla-OXA 51-like gene
Lane 2 to lane 5: Show positive results for class I integrase gene (160 bp).

Table 7: Antibiotic resistance pattern of integron-positive and integron-negative A. baumannii isolates

<table>
<thead>
<tr>
<th>Antibiotic groups</th>
<th>Resistance pattern of Acinetobacter isolates</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Class I integrase gene positive isolates N=37</td>
<td>Class I integrase gene negative isolates N=14</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Amoxicillin clavulanic acid</td>
<td>27</td>
<td>73</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>27</td>
<td>73</td>
</tr>
<tr>
<td>Amikacin</td>
<td>26</td>
<td>70.3</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>9</td>
<td>24.3</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>20</td>
<td>54</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>18</td>
<td>48.6</td>
</tr>
<tr>
<td>Imipenem</td>
<td>12</td>
<td>32.4</td>
</tr>
<tr>
<td>Overall Total main resistance</td>
<td>20</td>
<td>54.4</td>
</tr>
</tbody>
</table>

Figure 2: Comparison of resistance among integron-positive A. baumannii isolates and integron-negative strains in terms of the numbers of antibiotics to which isolates were resistant.
References


4. CLSI (2010). Performance standards for antimicrobial susceptibility testing. Twentieth informational supplement, document M100-S20, Clinical and Laboratory Standards Institute, Wayne, PA, USA.

5. CLSI (2006). Performance standards for antimicrobial susceptibility testing. 16th informational supplements. CLSI document, M2 Clinical and Laboratory Standards Institute, Wayne, PA, USA.


