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

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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. baumanni* strains, to study the co-resistance to other classes of antibiotics and to determine the prevalence of some antibiotic resistance determinants (bla $_{OXA\ 51}$ 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 bla $_{OXA\text{-}51\text{-}like}$ and Class I integrase genes. The highest rates of resistance were against ciprofloxacin (64.7%), amoxacillin clavulanic acid (58.8%), amikacin (58.8%), ceftriaxone (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 bla OXA-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.
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1. Introduction

Acinetobacter baumanni 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 dissimination of these genetic elements (Walsh *et al.,* 2005; Perez *et al.,* 2007).

Other carbapenemases include, carbapenem-hydrolysing 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 bla$_{OXA\text{-}58\text{-}like}$ genes (Poirel and Nordmann, 2006). In addition, the chromosomal bla$_{OXA\text{-}51}$-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. baumanni* strains, to study the co-resistance to other classes of antibiotics and to determine the prevalence 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. baumanii* 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. baumanii* 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 (BioMèrieux, 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 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 amoxacillin 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 $_{\text{oxa- 51 like}}$ gene and Class I intgrase gene by multiplex PCR:

The bla $_{\text{oxa- 51 like}}$ 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 baumanni* isolates had significantly higher resistance rates to amoxacillin 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. baumanni* 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 bla IMP, bla VIM or bla SIM 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* bla$_{\text{OXA-51-like}}$" gene by PCR. This agreed with the results of Turton *et al.* (2006) who reported the presence of the *bla*$_{\text{OXA-51gene}}$ -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 bla$_{\text{OXA-51-like}}$ 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 1 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

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 *et al.,* 2001).

In this study, we demonstrated that 81% (30/37) of integron positive *A. baumannii* showed resistance to five or more of the antibiotics tested. In concordance , Koeleman *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 *A. baumannii* and integron negative isolates regarding 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 *et al.,* 2001; Perez *et al.,* 2007; Peymani *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 *et al.,* 2007).

In conclusion, *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 imerging threat in Egypt. MBLs are detected in both imipenem sensitive and resistant *A. baumannii* isolates. CDT is a simple, easy, economic test for detection of MBLs that can be be incorporated into the routine testing of any busy microbiology laboratory. Class I integrase gene is predominantly found in *A. baumannii* strains isolated from the ICUs which confers resistance to many goups of antibiotics and suggest the epidemic potential. Infection control measures must be implemented to control the spread of such strains.

Table 1: Sequences of primers of *bla*OXA-51-like gene and Class 1 integrase gene .

Primer	Sequence	Target gene	Amplicon size (bp)[a]
OXA-51-likeF[b]	5_-TAA TGC TTT GAT CGG CCT TG-3_	$bla_{\text{OXA-51-like}}$	353 bp
OXA-51-likeR[b]	5_-TGG ATT GCA CTT CAT CTT GG-3_	$bla_{\text{OXA-51-like}}$	
Int1F[c]	5_-CAG TGG ACA TAA GCC TGT TC-3_	Class 1 integrase	160 bp
Int1R[c]	5_-CCC GAG GCA TAG ACT GTA-3	Class 1 integrase	

[a] bp : base pair
[b] Woodford *et al.,* 2006.
[c] Koeleman *et al.,* 2001.

Table 2: Resistance patterns of *A. baumannii* isolates to different Antibiotics.

	Strains from clinical samples N=24		Strains from environmental samples N=27		Total N= 51	
	Resistance		Resistance		Resistance	
Members	N	%	N	%	N	%
Amoxacillin-Clavulinic 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

* All imipenem resistant strains had MICs ranging from 16- 256 µg/ml by the IMP -E test.

Table 3: Detection of MBLs in imipenem susceptible and resistant strains by CDT.

A. baumannii strains	Imipenem susceptible			Imipenem resistant		
	No.	Positive CDT		No.	Positive CDT	
		No	%		No	%
Isolated from clinical samples (n= 24)	17	16	94	7	7	100
Isolated from environmental sample (n= 27)	18	16	89	9	9	100
Total (n=51)	35	32	91.4	16	16	100

Table 4: Detection of MBLs in imipenem susceptible and resistant strains by DDST.

A. baumannii strains	Imipenem susceptible			Imipenem resistant		
	No .	Positive DDST		No.	Positive DDST	
		No.	%		No.	%
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

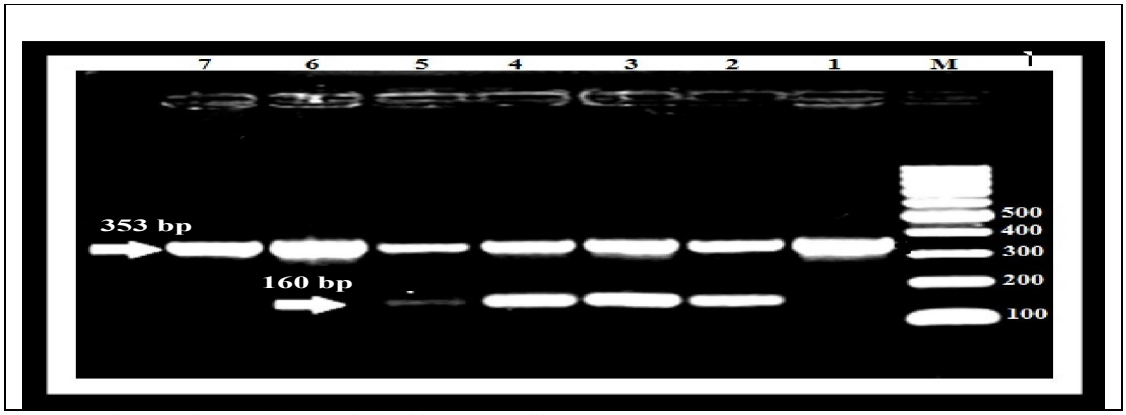
Table 5: Sensitivity & specificity of phenotypic tests

	Class I integrase gene positive isolates N= 37		Class I integrase gene negative isolates N=14		Sensitivity		Specificity	
	IPM-S n=25	IPM-R n=12	IPM-S n=9	IPM-R n=5	IPM-S	IPM-R	IPM-S	IPM-R
CDT (n= 48)	23	12	9	4	2%	00%	00%	100%
DDST (n=44)	21	12	8	3	86.2%	00%	00%	100%

Table 6: Detection of bla $_{\text{oxa- 51 like}}$ gene and Class I intgrase gene by PCR

Isolates from	bla $_{\text{oxa- 51 like}}$ gene positive isolates		Class I integrase gene positive isolates	
	N	%	N	%
Clinical samples (N= 24)	23	95.8	18	75
Enviornmental samples (N=27)	26	96.3	19	70.4
Total (N=51)	49	96.1	37	72.5

Figure 1: Multiplex PCR for detection of bla $_{\text{OXA-51-like}}$ 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

Antibiotic groups	Resistance pattern of *Acinetobacter* isolates				*P* value
	Class I integrase gene positive isolates N= 37		Class I integrase gene negative isolates N= 14		
	No	%	No	%	
Amoxacillin clavulanic acid	27	73	4	28.6%	0.004(S)
Ceftriaxonej	27	73	5	35.7	0.014 (S)
Amikacin	26	70.3	4	28.6	0.007 (S)
Tetracycline	9	24.3	4	28.6	0.7 (NS)
Ciprofoloxacin	20	54	13	92.9	0.01(S)
Chloramphenicol	18	48.6	10	71.4	0.14 (NS)
Imipenem	12	32.4	4	28.6	0.7(NS)
Overall Total main resistance	20	54.4	6	43	0.4 (NS)

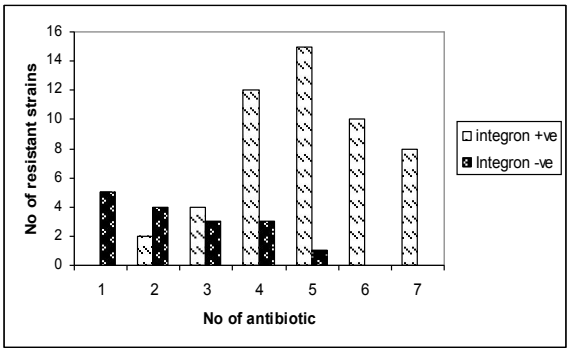


Figure 2: Comparison of resistance among integron-positive *A. baumannii* isolates and integron-negative strains in terms of the numbers of antibiotics to which isolates were resistant.

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