Multidrug resistant Egyptian isolates of *Acinetobacter baumannii*

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Abstract: The resistance of *Acinetobacter baumannii* to antimicrobial agents is mediated by all of the major resistance mechanisms, including modification of target sites, enzymatic inactivation and active efflux of drugs. Antibiotic susceptibility testing has been performed on fifty-two *A. baumannii* isolates. Twenty isolates have been recovered from patients suffering from wound and burn wound infections attending general surgery, plastic surgery and obstetrics and gynecology departments and thirty-two isolates have been recovered from the environment of these departments. Different mechanisms of antimicrobial resistance have been detected among resistant isolates. Broth dilution method have been used to investigate antimicrobial susceptibility pattern, iodometric method has been used to detect β-lactamase enzymes and polymerase chain reaction has been used to detect $bla_{oxa\text{-}51\text{-}like}$ genes, *aph (3')-VIa* genes and *adeB* gene. Tetracycline was the most effective antimicrobial agent against *A. baumannii*. It has showed high resistance to both of amikacin and meropenem (76.9%), cefipime (80.8%) and both of cephradine and imipenem (96.2%). An extreme resistance to the other antimicrobial agents has been shown by the same organism. β-lactamase enzyme has been detected in β-lactam resistant isolates, $bla_{oxa\text{-}51\text{-}like}$ carbapenemase genes have been detected in carbapenem resistant isolates, *aph (3')-VIa* genes have been detected in amikacin resistant isolates and *adeB* gene have been detected in some multidrug resistant strains. So, resistance to β-lactams, carbapenems and amikacin has been high in *A. baumannii* isolates which has caused appearance of multidrug resistant isolates with different resistance mechanisms like $bla_{oxa\text{-}51\text{-}like}$ genes, *aph (3')-VIa* genes and *adeB* gene.
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1. Introduction

The resistance of *A. baumannii* to antimicrobial agents is mediated by all of the major resistance mechanisms that are known to occur in bacteria. β-lactamases are the most diverse group of enzymes that are associated with resistance, and more than 50 different enzymes, have been identified so far in *A. baumannii*. OXA-51-like carbapenemases are class D β-lactamases which are intrinsic to *A. baumannii* and confer resistance to carbapenems **(1).** Aminoglycoside resistance has been attributed to at least nine distinct modifying enzymes. The emergence of APH (3') enzymes has effectively removed aminoglycosides such as kanamycin and neomycin from clinical use. Resistance to other aminoglycosides such as amikacin and lividomycin serve as the basis for classification into seven distinct classes (I-VII)**.** Another chromosomal system that is typical of *A. baumannii* is the AdeABC efflux system **(2).** Upregulation of AdeABC is so far the only mechanism that has been proven to decrease susceptibility to multiple antimicrobial classes in *A. baumannii*. This work aims to investigate antimicrobial susceptibility pattern among different *A. baumannii* isolates and detect possible mechanisms of resistance in multidrug resistant strains.

2. Material and Methods

Antimicrobial Susceptibility testing. Susceptibility testing was performed on fifty-two *A. baumannii* isolates by using broth dilution method **(3)** in accordance with the guidelines established by EUCAST standards. Twelve different antimicrobial agents were used as follows: Imipenem, meropenem, cephradin, cefipime, amoxycilline/clavulanic acid (Sigma), ciprofloxacin, nalidixic acid, ceftazidme, tetracycline, chloramphenicol, oxacillin (Himedia) and amikacin (Bristol-Myers Squibb).

Detection of β-lactamases . β-lactamase detection was performed using iodometric method **(4).**

Sample preparation for PCR. Isolation of bacterial DNA was performed using DNA extraction kits (Qiagen).

Amplification by PCR. The $bla_{oxa\text{-}51\text{-}like}$ primers (laboratories of The Midland Certified Reagent Company Inc. of Midland) used to amplify $bla_{oxa\text{-}51\text{-}like}$ genes as follows: (5'-TAA TGC TTT GAT CGG

CCT TG-3') as the forward primer and (5'-TGG ATT GCA CTT CAT CTT GG-3') as the reverse primer **(5).** PCRs were carried out using thermal cycler (Techne PROGENE) in 50 µl reaction volumes with 5 µl extracted DNA, 50 pmole of each primer, 25 µl of Go Taq® Green Master Mix (Promega). Conditions were the following: 94°C for 3 min, and then 35 cycles at 94°C for 45 s, at 60°C for 45 s and at 72°C for 1 min, followed by a final extension at 72°C for 5 min. The *aph (3')-VIa* primers (laboratories of The Midland Certified Reagent Company Inc. of Midland) used to amplify *aph (3')-VIa* genes as follows: (5'-ATACAGAGACCACCATAC AGT-3') as the forward primer and (5'-GGACAATCAATAATAGCAAT-3') as the reverse primer **(6).** PCRs were carried out in 50 µl reaction volumes with 5 µl extracted DNA, 50 pmole of each primer, 25 µl of Go Taq® Green Master Mix. Conditions were the following: 94°C for 3 min, and then 30 cycles at 94°C for 1 min, at 55°C for 1 min and at 72°C for 1 min, followed by a final extension at 72°C for 5 min. The *adeB* primers (laboratories of Eurofins MWG Operon) used to amplify *adeB* genes as follows: (5'-GTATGAATTGATGCTGC-3') as the forward primer and (5'-CACTCGTAGCCAATACC-3') as the reverse primer **(7).** PCRs were carried out in 50 µl reaction volumes with 5 µl extracted DNA, 50 pmole of each primer, 25 µl of (Taq PCR Master Mix (2X)) (USB Corporation). Conditions were the following: 94°C for 2 min, and then 35 cycles at 94°C for 30 s, at 55°C for 30 s and at 72°C for 2 min, followed by a final extension at 72°C for 2 min. After amplification, 10 µl of the PCR mixture was analyzed by agarose gel electrophoresis (2% agarose in Tris-borate-EDTA stained with ethidium bromide). The Gene Ruler 100 bp DNA ladder (Fermentas) was used as a DNA size marker.

3. Results

A- Antimicrobial susceptibility testing:

In Table 1, tetracycline was the most effective antimicrobial agent against *A. baumannii*. It showed high resistance to both of amikacin and meropenem (76.9%), cefipime (80.8%) and both of cephradine and imipenem (96.2%). An extreme resistance to the other antimicrobial agents was shown by the same organism.

B- Detection of β-lactamases:

Out of 52 *A. baumannii* strains resistant to different β-lactams tested, 51 strains were β-lactamase producers constituting 98% of the total tested strains (Figure 1).

C- Detection of *bla*$_{oxa\text{-}51\text{-}like}$ genes:

In Figure (2), a group of carbapenem resistant *A. baumannii* was tested for detection of *bla*$_{oxa\text{-}51\text{-}like}$ genes using PCR. It was found that, all tested isolates have been *bla*$_{oxa\text{-}51\text{-}like}$ enzyme producers giving amplicons of 353 bp. size.

D- Detection of *aph (3')-VIa* genes :

A group of amikacin resistant *A. baumannii* was tested for detection of *aph (3')-VIa* genes using PCR. It was found that, all resistant isolates were *aph (3')-VIa* enzyme producers giving amplicons of 234 bp. Size, (Figure 3).

E- Detection of *adeB* gene:

In Figure (4), a group of multidrug resistant *A. baumannii* isolates was tested for detection of *adeB* genes using PCR. It was found that some of the tested isolates were *adeB* gene positive giving amplicons of 979 bp. size and the other *A. baumannii* isolates were *adeB* gene negative.

Table 1: Incidence of antimicrobial resistance among *A. baumannii* isolates (52).

Antimicrobial agents	Number of resistant strains (%)*
Imipenem	50 (96.2%)
Meropenem	40 (76.9%)
Ciprofloxacin	52 (100%)
Nalidixic acid	52 (100%)
Chloramphenicol	52 (100%)
Tetracycline	0 (0%)
Amikacin	40 (76.9%)
Cefipime	42 (80.8%)
Oxacillin	52 (100%)
Amoxycilline/ clavulanic acid	52 (100%)
Cephradine	50 (96.2%)
Ceftazidime	52 (100%)

* Percentage was correlated to the no. of *A. baumannii* isolates.

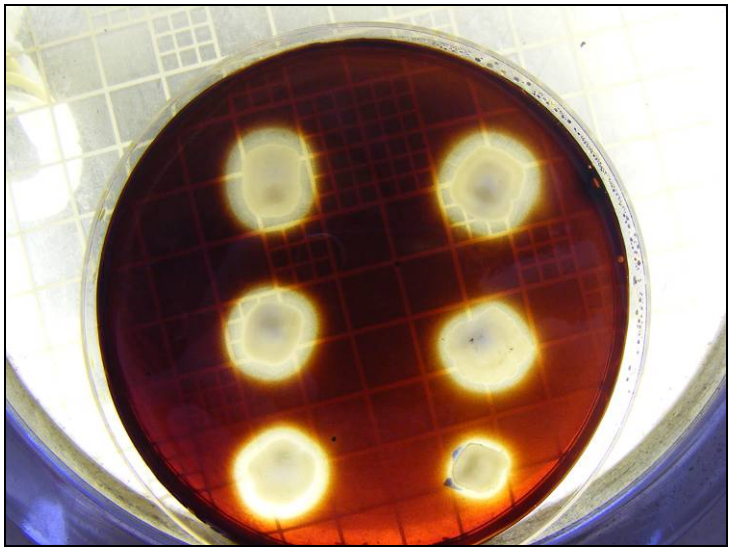

Figure (1): β-lactamase production by *A. baumannii isolates*.
The bottom right of the picture: control strain, The other strains: β-lactamase +ve strains

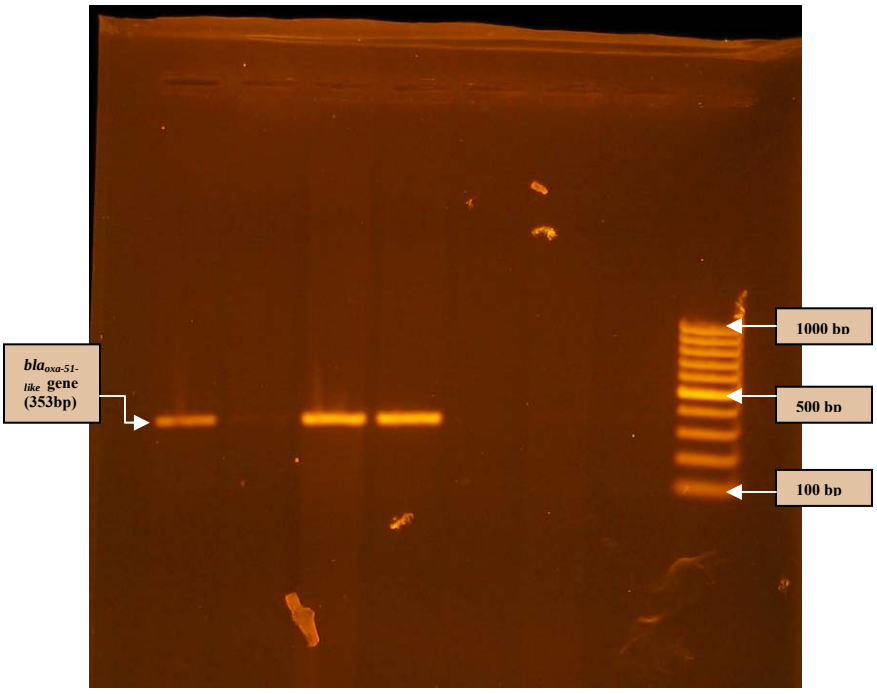

Figure (2): PCR results to amplify fragments of $bla_{oxa-51-like}$ genes in different carbapenem resistant *A. baumannii* isolates.
From right to left: Lane 1: DNA ladder, lanes 2 and 3: two control *E. coli* isolates, lane 4: control Klebsiella isolate, lanes 5, 6, 7 and 8: four carbapenem resistant *A. baumannii* isolates.

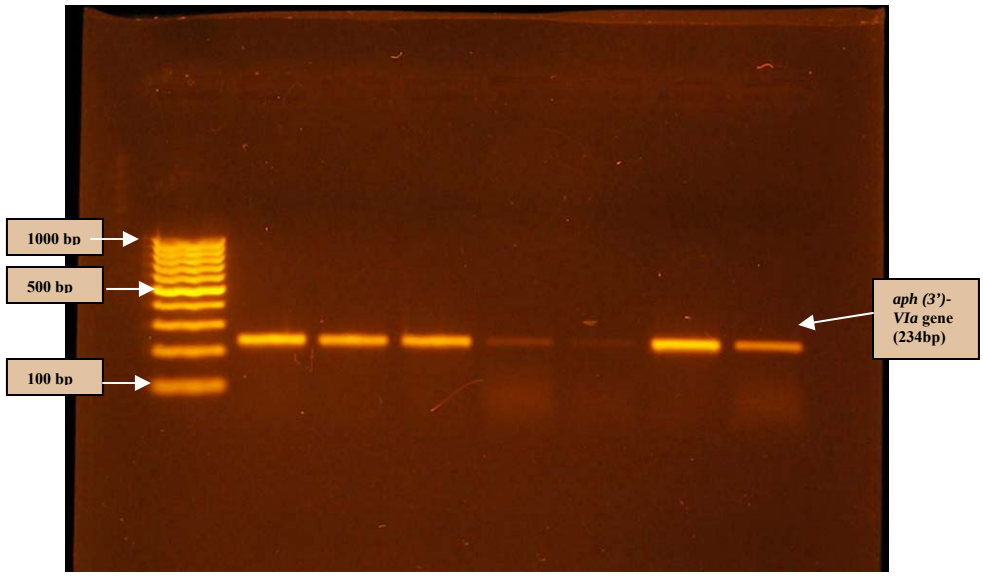

Figure (3): PCR results to amplify fragments of *aph (3')-VIa* genes in different amikacin resistant *A. baumannii* isolates.
From left to right: Lane 1: DNA ladder, lanes 2, 3, 4, 5, 6, 7 and 8: seven amikacin resistant *A. baumanni* isolates.

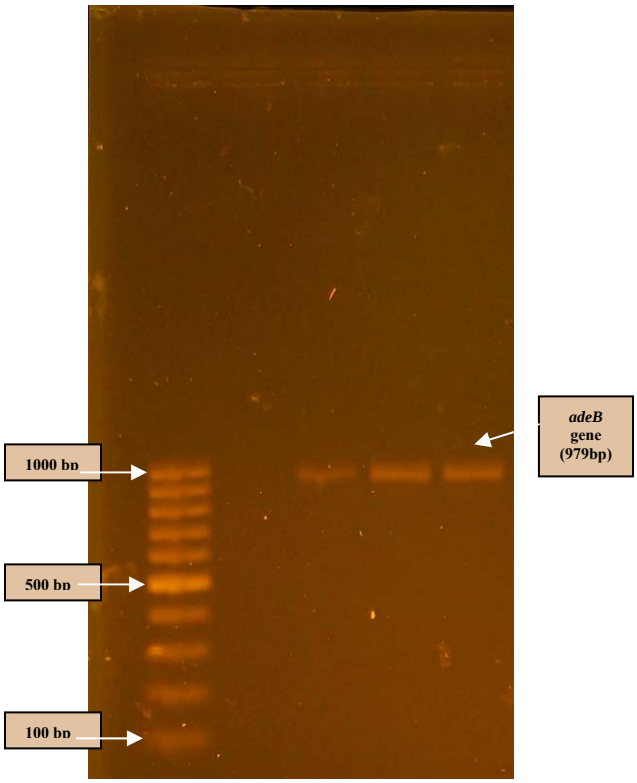

Figure (4): PCR results to amplify fragments of *adeB* genes in multidrug resistant *A. baumannii* isolates.
From left to right: Lane 1: DNA ladder, lane 2: *adeB* negative *A. baumannii* isolate, lanes (3-5): *adeB* positive *A. baumannii* isolates.

4. Discussion

Antimicrobial Susceptibility Testing:

A. baumannii isolates have showed high resistance to imipenem, meropenem, cefipime, ceftazidime, ciprofloxacin and β-lactam combination (100%), amikacin (98.8%) and no resistance to tigecycline. These results agree with the results obtained in our study for tetracycline derivative (tigecycline) (most effective), ceftazidime, ciprofloxacin and β-lactam combination, but it showed higher resistance with imipenem, meropenem, cefipime and amikacin **(8).**

A. baumannii have showed high resistance to ceftazidime (94.6%), tetracycline (81.2%), meropenem (80.4%), imipenem (78.6%) and ciprofloxacin (76.8%) and low resistance to cefipime (34.8%). These results are in agreement with the results obtained in the present study for ceftazidime, meropenem, imipenem and ciprofloxacin (high resistance) but it indicates higher incidence of resistance to tetracycline and lower incidence of resistance to cefipime **(9).**

It has been found that *A. baumannii* isolates have showed high resistance to cefipime (79.1%), meropenem and imipenem (70.5%), amikacin (61.2%) and low resistance to tigecycline (7.9%) **(10).** These results agree with the results obtained in our study.

It has been reported that *A. baumannii* strains had high resistance to ciprofloxacin (90.53%), cefipime (82.30%), imipenem (81.61%) and meropenem (75.31%) and low resistance to amikacin (33.33%). Our results showed the same behaviour with ciprofloxacin, cefipime, imipenem and meropenem (high resistance) but indicates higher resistance to amikacin **(11).**

It has been found that *A. baumannii* strains had high resistance to ciprofloxacin (98%), amikacin (96%), meropenem (95.5%) and imipenem (87.8%). These results are in agreement with the results obtained in the present study for ciprofloxacin, amikacin, meropenem and imipenem **(12).**

A. baumannii showed high resistance to ceftazidime (83.3%) and amikacin and ciprofloxacin (77.8%) and low resistance to meropenem (11.1%) and imipenem (9.1%). Our results showed the same behaviour with ceftazidime, amikacin and ciprofloxacin (high resistance) but indicated higher resistance to meropenem and imipenem **(13).**

Detection of β-lactamases and *bla*$_{oxa\text{-}51\text{-}like}$ genes:

Among imipenem-susceptible and resistant *A. baumannii* which were screened by PCR for different β-lactamases. The *bla*$_{oxa\text{-}51\text{-}like}$ gene was the only one detected, even in imipenem-susceptible strain **(14).** It has been reported that among *A. baumannii* isolates with *bla*$_{oxa\text{-}51\text{-}like}$ as sole carbapenemase gene, imipenem and/or meropenem resistance was associated only with isolates in which ISAba1 was upstream of *bla*$_{oxa\text{-}51\text{-}like}$, suggests that ISAba1 is providing the promoter for this gene **(1).** Oxa-51-like subgroup enzyme may be involved in the expression of carbapenem resistance under certain circumstances. Also, all imipenem-resistant *A. baumannii* isolates were positive for carbapenemase production and negative for metallo β-lactamase. They all possessed the encoding gene for an intrinsic oxa-51-like carbapenemase and an acquired oxa-23-like carbapenemase **(15).**

Detection of *aph (3')-VIa* genes:

All 16 clinical amikacin resistant *A. baumannii* isolates had positive PCRs with primers specific for the amplification of the *aph (3')-VIa* gene which confirms the contribution of the *aph (3')-VIa* gene to the incidence of amikacin resistance in *A. baumannii* **(6).** 97% of *A. baumannii* isolates that are amikacin resistant contained the phosphotransferase gene aphA6 (*aph (3')-VIa*) **(16).** Among 106 multidrug resistant clinical *A. baumannii* strains from hospitals in the Czech Republic and other European countries, aph A6 gene was predominant in 55 strains representing (52%) **(17).** It has been illustrated that among 49 clinical isolates of multidrug resistant *A. baumannii* identified at a tertiary medical center in Pennsylvania, the *aph (3')-VIa* gene was detected in 3 isolates representing (6.1%) **(18).**

Detection of *adeB* efflux pump gene:

All tested 39 multidrug resistant Acinetobacter strains had the *adeB* gene and disruption of the *adeB* gene has greater effect on resistance to meropenems than *adeA* gene in Acinetobacter spp. isolated from university Malaya medical centre **(19).** High distribution of *adeB* (91.8%) gene in multidrug resistant *A. baumannii* isolates from the three military hospitals in China has been observed **(12).** The majority of the *A. baumannii* isolates (75%) that generally display high-level multidrug resistance were positive for *adeSR-adeABC*, suggesting a potential linkage between these genes and multidrug resistance **(20).**

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