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Prevalence of Efflux Pump Genes in Multidrug Resistant Acinetobacter Baumannii Isolates From Intensive Care Units in An Egyptian Hospital

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Abstract

Background and Objectives: Acinetobacter baumannii (A. baumannii) is a nightmare hospital acquired pathogen causing different infections that are problematic to treat due to multidrug resistance acquired by efflux pump mechanisms. One of the most important efflux pumps is AdeABC that is encoded by a group of genes, the prevalence of two of them (adeB and adeS genes) among hospital acquired A. baumannii infections were investigated in this study.

Materials and Methods: In this cross-sectional study, 50 isolates of A. baumannii were obtained from patients in ICUs of a tertiary hospital in Egypt. The sensitivity to different antibiotics was tested by the disc diffusion and broth dilution methods as stated by CLSI guidelines. AdeB and adeS genes were detected by the PCR method.

Results: The highest proportion of the isolates were resistant to Gentamycin, Amikacin, Tobramycin, Levofloxacin, Imipenem, Meropenem, Ampicillin/sulbactam & Piperacillin-Tazobactam, while all strains showed intermediate resistance to Colistin. All multidrug resistant isolates showed adeB gene while adeS gene was found in only 95% of the isolates.

Conclusion: AdeB and adeS genes are highly prevalent among hospital acquired multidrug resistant A. baumannii, therefore they are considered a mark of resistance.

Keywords: Acinetobacter baumannii, Multidrug resistant, ICU, Efflux pumps, adeB gene, adeS gene.

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Introduction

Multidrug resistance (MDR) has created a severe medical challenge causing high morbidity, mortality rates, and hospitalization costs; particularly, Gram-negative bacteria **(1).**

Acinetobacter baumannii is a Gram-negative bacterium causing a diversity of hospital acquired infections; mainly ventilator-associated pneumonia, skin, and soft-tissue infections, wound, urinary tract, and bloodstream infections **(2).** The ability to develop resistance to numerous antimicrobials combined with the ability to persist in hospital environments made *A. baumannii* represent a real issue in health care environments **(3).**

One important mechanism of MDR in *A. baumannii* is the use of efflux pumps as the bacteria can lead antibiotics towards the outside. Several studies demonstrated that efflux pump genes especially *adeB* and *ade*Sportray a main role in *A. baumannii*'s resistance to antibiotics**(4, 5).**Therefore, in our work, we sought to assess and confirm the frequency of some efflux pump genes among MDR *A. baumannii* isolates from hospital acquired infections in intensive care units (ICUs).

Patients and Methods

Study Design and Setting

This study was performed over ten months (June 2019 - March 2020) in Zagazig university hospital, a tertiary hospital in Egypt. Institutional Review Board (IRB) – Faculty of Medicine, Zagazig University approved this study. Informed consent was taken from the participants.

Patients

This study included 355 ICUs patients with suspected hospital acquired infections that developed after 48 hours of hospitalization without evidence of being incubated at the time of admission. Exclusion criteria included admission with infections or development of infection within 48 hours of hospitalization, re-admission from another hospital or other units, and previous hospitalization in the preceding three months. Immunocompromised patients and/or patients on immunosuppressive drugs and patients with chronic lung, liver or renal diseases were also excluded from the study. Demographic and clinical data were amassed from all patients as (age, sex, residence, devices, antibiotic administration, etc.). Also, medical and surgical histories were reported.

Samples collection, bacterial isolation, and identification

A sum of 50 *A. baumannii* isolates were recovered from 355 specimens that included tracheal aspirates, surgical wound swabs, urine specimens, central venous catheter (CVC) tips, and tracheostomy swabs.

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All specimens were acquired under the coverage of aseptic techniques.They were transported to the microbiology laboratory to be processed. Cultures were carried out on MacConkey, nutrient, and blood agar plates in addition to Cystine lactose electrolyte deficient agar (CLED) in cases of urine samples. After overnight incubation at 37°C, suspected *A. baumannii* colonies were diagnosed using the VITEK® 2 compact system (Biomérieux, France).

Antimicrobial susceptibility testing

Using the disc diffusion method, the test was executed using the following discs: piperacillin tazobactam (100/10 ug), ampicillin/sulbactam (10/10 ug), ceftazidime (30 ug), cefepime (30 ug), imipenem (10 ug), meropenem (10 ug), gentamycin (10 ug), amikacin (30 ug), tobramycin (10 ug), ciprofloxacin (5 ug), levofloxacin (5 ug) and trimethoprim- sulphamethoxazole (1.25/23.75ug) (HImedia, India). Susceptibility of *A. baumannii* isolates to colistin was done by microdilution method according to (CLSI, 2020) guidelines. Quality control strains: *Escherichia coli* ATCC® 25922™ and *Pseudomonas aeruginosa* ATCC® 27853™(American Type Culture Collection Global Bioresource Center, USA) were used for antimicrobial susceptibility testing.A recent study stated that MDR*A. baumannii* is resistant to at least three classes of the following antibiotics: aminoglycosides, cephalosporins, carbapenems, fluoroquinolones and beta-lactam/beta-lactamase inhibitors**(6)**.

Molecular detection of *adeB* and *adeS* genes:

Templates of DNA were prepared by suspending a loopful of each *A. baumannii* isolate in 300 µl sterile distilled water, followed by boiling for 10 min then centrifugation at (10.000 g) for 5 mins. Supernatant fluids were collected for PCR (7,8).

An initial denaturation stage at 94°C for 5 minutes was followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 52°C for 30 sec, extension at 72°C for 1 min, and a final extension at 72°C for 5 minutes in the PCR program for amplification of the *adeB*gene **(9, 10)**. The amplification conditions for the *adeS* gene were the same as (5), the PCR products were then separated on a (1.5 percent) agarose gel and visualized using ethidium bromide and a UV transilluminator documentation system.

Primers for *adeB* (11) and *adeS* (5) genes:

adeB Forward: 5′ TTAACGATAGCGTTGTAACC 3′

adeB Reverse: 5′ TGAGCAGACAATGGAATAGT 3′
adeS Forward: 5′TGC CGC CAA ATT CTT TAT TC 3′
adeS Reverse : 5′ TTA GTC ACG GCG ACC TCT CT 3′

Standard strains for PCR tests were not available; therefore, the first amplified PCR products of the expected size in the electrophoresis gel were sequenced and considered as positive controls **(8).**

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Statistical analysis

Data were analyzed using SPSS (Version 20.0. Armonk, NY: IBM Corp).

Results

In this study, 50 *A. baumannii* isolates were obtained representing 14% to the total number of isolates. The distribution of *A. baumannii* isolates in relation to the type of infection is shown in (Table 1).

Antibiogram results

For the 13 used antibiotics, all *Acinetobacter* isolates were resistant to Ciprofloxacin, Ceftazidime & Cefepime. Most of them were resistant to Gentamycin, Amikacin, Tobramycin, Levofloxacin, Imipenem, Meropenem, Ampicillin/sulbactam, & Piperacillin-Tazobactam, while 50% of them were sensitive to Trimethoprim-sulphamethoxazole (TMP-SMX). All strains showed intermediate susceptibility to Colistin ≤ 2 μg/ml according to (CLSI, 2020). The percentages of antimicrobial resistance among the *A. baumannii* isolates studied are described in the table below (Table 2).Forty of *A. baumannii* strains(80%) were MDR.

Results of genotypic tests

Table 3 demonstrates the abundance of *adeB* and *adeS* genes in MDR isolates, detected by the conventional PCR method.

Table 1. Frequency distribution of *A. baumannii* isolates according to the type of infection.

Type of infection	Clinical specimens (n=355)		*A. baumannii* (n=50)	
	Type	No	No	%
Surgical wound infections	SSI*	85	17	20
Chest infections	E.T. tube **	120	27	22.5
	Sputum	25	0.0	00
Urinary tract infections	Urine	65	4	6.1
Infected wounds	Pus	40	2	5
	CVC tip	20	0.0	00

*SSI, surgical site wound ** E.T. tube, endotracheal tube

Table 2. Antibiogram of antimicrobial resistance among the studied *A. baumannii* isolates

Antimicrobial agent	Resistant		Intermediate		Sensitive	
	No	%	No	%	No	%
Gentamycin	35	70	5	10	10	20
Tobramycin	35	70	0	0	15	30
Amikacin	30	60	5	10	15	30

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Ciprofloxacin	50	100	0	0	0	0
Levofloxacin	35	70	15	30	0	0
Imipenem	45	90	0	0	5	10
Ceftazidime	50	100	0	0	0	0
Cefepime	50	100	0	0	0	0
Meropenem	45	90	0	0	5	10
Ampicillin/sulbactam	25	50	15	30	10	20
Trimethoprim-sulphamethoxazole	10	20	15	30	25	50
Piperacillin-Tazobactam	40	80	0	0	10	20
Colistin	0	0	50	100	0	0

Table 3. Abundance of *adeB* and *adeS* genes among MDR *A. baumannii* isolates.

Efflux Pump Gene	MDR *A. baumannii* Isolates (n = 40)
adeB	40 (100%)
adeS	38 (95%)

Discussion

Acinetobacter baumannii infections have become a public health problem, contributing to high mortality, morbidity rates, and hospitalization cost in different countries. The attributable mortality rate of *A. baumannii* infections were reported to be 10-35%. The appearance of resistant strains of *A. baumannii* has initiated multiple and huge threats to the infection control strategy and treatment plans **(1, 12)**. As a result, *A. baumannii* has been designated as one of the "red alert" pathogens that threaten the efficacy of our antibacterial strategies by The Infectious Diseases Society of America **(13)**.

In our study, 50 (14%) samples were positive for *A. baumannii* out of 355 different clinical samples in ICUs during the study period. This was closely near the 15% isolation rate that was reported by a study conducted at ICUs in Zagazig University Hospitals, Egypt**(14)**.
In the current study, *Acinetobacter* isolates were retained from diverse clinical samples. Categorization of clinical *Acinetobacter* infection sources showed that they were mostlydivided from respiratory and surgical wound samples. This result agreed with those of **(15, 16, 17, 18)**.
Our results were similar to other studies where respiratory specimens have the largest proportion of *Acinetobacter* isolates, ranged from (21.8% - 32%) as that of **(19, 20, 21)**.
However, other investigators reported higher isolation ratios from sources other than respiratory samples. A study revealed that most of the *Acinetobacter* isolates (53.3%) were recovered from

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wound infection followed by respiratory tract infections (30%)**(22)**, while another study observed that most isolates were from pus samples (45%) followed by blood samples (18%)**(23)**.

The difference in isolation ratios from specimens between our study and other studies could be attributed to the difference in the hospital environment, clinical conditions of the patients, and numbers of clinical specimens investigated in different studies.

During the last decades, the resistance of *A. baumannii* against a variety FDA approved antibiotic including carbapenems, aminoglycosides and fluoroquinolones has become a worldwide problem, causing complexity in the treatment of *A. baumannii***(6)**. Therefore, the importance to search for new therapeutic strategies has been a major contest in the field of infectious diseases particularly MDR *A. baumannii* which is considered a main cause of severe HAIs. Eighty percent of our *A. baumannii* isolates were MDR, meaning they were resistant to three or more antibiotic groups. Tolba et al. also found that MDR strains were 88.8% among all the isolates, **(8)** as well.

In this study, *Acinetobacter* showed 100% resistance to third and fourth generation cephalosporins (ceftazidime and cefepime), which may point to the possibility of ESBLs production **(16, 25)**. The resistance rate to carbapenem (90%) was like studies operated in Egypt, which revealed resistance rates of 88.9% and 95%, respectively **(18, 24)**. On the other hand, a report of strains isolated from Zagazig ICU showed only 46.4% were resistant to imipenem **(14)**. Inadequate infection control guidelines and inappropriate use of carbapenem may be the cause of this extreme resistance **(18)**. Regarding aminoglycoside, the resistance rate was 70%, which coordinates with other studies as **(8,19)**. Higher resistance rate of 90% was reported in Egypt **(18)**.

As regard resistance to quinolones, high rates of resistance were observed as the following, resistance rate for Levofloxacin was (70% resistant, 30% intermediate) while for ciprofloxacin was (100% resistant). These results agreed with **(26, 27)** who reported (90.9%) and (100%) resistant rates for ciprofloxacin, also another study found that resistance rates for ciprofloxacin and levofloxacin were (97%) and (91%) respectively**(28)**.

Also, the isolates revealed a high prevalence of resistance to β-lactams/β-lactamase inhibitors as in **(29)**, this high resistance is mostly due to β-lactamases production **(30)**. Still high-dose ampicillin/sulbactam monotherapy was a powerful treatment for very sick patients with MDRAB ventilator-associated pneumonia **(31)**. Sulfamethoxazole/trimethoprim resistance pattern has been high in most studies, reaching (92%) **(30)**, even 100% in other studies **(26)**. These results don't agree with our study which represents half of the strains, sensitive to sulfamethoxazole/trimethoprim, this is supported by **(32)**. Many results showed that the effect of

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TMP-SMX for Acinetobacters is inconstant and unpredictable and many resistant strains respond clinically well to TMP-SMX **(33)**.

As regard colistin which is considered the last line of treatment for MDR *Acinetobacter* infections in the last few years, it was a surprise that all *A. baumannii* strains showed intermediate resistance to it. Al-Kadmy et al. had reported a high percentage of colistin resistance (76%) in Baghdad. Currently, efflux pumps particularly RND are considered the main cause of colistin resistance proved by suppression of this resistance with the use of efflux pump inhibitors (EPIs) **(35)**.
AdeB is an essential member of the RND superfamily, which is employed in the inner membrane of Gram-negative bacilli, *AdeB* arrests antibiotic from inside the phospholipid bilayer of the inner membrane or the cytoplasm, then transfer it outside via outer membrane proteins (*AdeC*) **(36)**.

In the current study, *AdeB* was found in 100% (40 MDR samples) of 50 *A. baumannii* isolates, this accounts for the high level of resistance in *A. baumannii*. These results agree with **(37)** and **(10)** who detected *AdeB* gene in 100% and 78.26% of the isolates respectively. Many studies state that all MDR *A. baumannii* strains have *adeB* gene expression. This indicates a strong correlation between *AdeB* gene and multidrug resistance. Also, a study has found that *AdeB* gene is not found in the environmental strains and appears to be related mostly with clinical isolates and helps in the reduction of susceptibility to disinfectants **(3)**.

In Baghdad, a recent study had estimated that 95.2 percent of *A. baumannii* isolates had the *adeS* gene**(37)**. This is similar to our analysis, but the prevalence of *adeS* gene was 81.66 percent which is lower than the frequency reported by **(5)**. Such differences between studies might be due to changes in the antibiotic policy, the number and kind of clinical sample and environmental factors.
Efflux pumps are the chief factors in the occurrence of antibiotic resistance in *Acinetobacter* infection **(38)**, so it is worth paying more attention to any changes in the performance of these pumps.

Conclusion

MDR A. *baumannii* is widespread in ICUs at Zagazig university hospitals.Efflux pumps genes especially (*adeB* and *adeS* genes) are an important contributor of resistance of Acinetobacters to multiple antimicrobial drugs among different geographical regions.

Limitations

Our limitation in this research project was the relatively small sample size. Therefore, further studies with a larger sample size are recommended. In addition, investigation of more risk factors associated with MDR *A. baumannii* infection is needed.

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