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Original article

Prevalence and antimicrobial resistance pattern of *Acinetobacter baumannii* isolates from intensive care units in Zagazig University Hospitals

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ABSTRACT

Background: Multidrug resistant (MDR) *Acinetobacter baumannii (A. baumannii)* has been recognized as a serious causative agent of health care associated infections (HAIs) in different countries of the world with increasing morbidity and mortality, therefore continuous monitoring and evaluation in health care settings are mandatory. We aimed to assess *A. baumannii* as a cause of HAIs in intensive care units (ICUs) of Zagazig University Hospitals and to demonstrate its antimicrobial resistance pattern. **Methods:** Different bacteriological specimens were collected from 71 patients with HAIs for isolation of *A. baumannii*, followed by *invitro* antibiotic susceptibility test. **Results:** Ten *A. baumannii* isolates were recovered (13.5%), of which eight isolates showed multidrug resistance pattern (80%). Prior use of antibiotics was a significant risk factor of development of multidrug resistance ($P < 0.05$). **Conclusion:** Predominance of MDR *A. baumannii* as an important agent of HAIs in ICUs of Zagazig University Hospitals. Inappropriate administration of antimicrobial agents appears to be the most important risk factor.

Introduction

Multidrug resistant (MDR) *A. baumannii* has become an important causative agent of healthcare associated infections (HAIs) including pneumonia, urinary tract infections (UTIs), septicemia, meningitis and wound infections [1,2]. In the past, *Acinetobacter* infections were simply managed with common antibiotics as β-lactams and sulfonamides, but emergence of drug resistance against different antibiotics has led to difficult treatment of *A. baumannii* infections with ability to cause extended outbreaks [3].

Multidrug-resistant *A. baumannii* is defined as resistant to at least three classes of the following antibiotics: cephalosporins, aminoglycosides, fluoroquinolones, carbapenems and beta-lactam/beta-lactamase inhibitors [3].

Infections with MDR *A. baumannii* have been reported in various spots of the world including Europe, America, Far East region, the United Arab Emirates, Bahrain, Saudi Arabia, Palestine and Lebanon [4]. It has also been reported in Egypt [5,6]. Such a condition needs continuous monitoring and evaluation to allow better understanding and control. This study aimed to assess *A. baumannii* as a cause of HAIs in intensive care units (ICUs) of Zagazig University Hospitals and to demonstrate its antimicrobial resistance pattern.

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Patients and Methods

Study design and setting

This cross -sectional study was conducted over nine months, from June 2019 to March 2020. It was carried out at the ICUs of Zagazig University Hospitals and Medical Microbiology & Immunology Department, Faculty of Medicine, Zagazig University, Egypt.

These ICUs are 29-bedded, closed, medical-surgical, tertiary facility units, with about 800-1000 admissions per year.

The beds are separated by curtains allowing proper movement of healthcare workers, each unit has alcohol rub dispenser, safety box and biological waste basket beside each bed. The consultant is in charge of all catheter devices for all patients.

Health care associated infections were suspected when infections had occurred after at least 48 hours of hospitalization and up to 30 days after an operation, with clinical symptoms as fever, cough and shortness of breathing, burning with urination or difficulty urinating, headache, nausea, vomiting and diarrhea, these varieties are according to type of infection and according to CDC's definitions [7].

The first isolation of *Acinetobacter* infections were the samples sent at least 48 h after admission to the ICU and these samples were considered the ICU-acquired infections. If a patient was diagnosed with an *Acinetobacter* infection within 48h of ICU admission, but the patient was admitted to the ICU from an in-patient ward or the operating room, then the infection is considered hospital acquired [8].

All antibiotic changes were made by the primary admitting clinician.

Ethical consideration

The study was approved by the institutional review board (IRB), Faculty of medicine, Zagazig University. This study was carried out in accordance with the revised Declaration of Helsinki. All subjects provided an informed written consent.

Study subjects

This study included 71 patients admitted to ICUs in Zagazig University hospitals (40 males and 31 females with ages ranged from 18 to 80 years). The study was conducted on patients who developed ICU-acquired HAIs. Exclusion criteria included admission with infections or development of infection within 48 hours of hospitalization, readmission 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 data (age, sex, social status and residence, etc.), clinical data (ICU admission and antibiotic administration) were collected from all patients. Medical history of comorbidities; diabetes, obesity, etc. and surgical history; length of the procedure, type of the wound, prosthesis and length of postoperative stay were reported for each patient.

Samples collection

A total of 71 clinical samples were collected from patients, including surgical site wound swabs, pus, sputum, endotracheal tube, urine samples and central venous catheter (CVC) tips as shown in **table (1)**. All samples were collected under strict aseptic precautions. Respiratory secretions were collected by blind endotracheal aspiration via suction catheters and collected in sterile, dry, wide-necked and leak-proof containers, while surgical site wound and pus samples were collected using sterile cotton swabs or syringes before an antiseptic dressing was applied with attention to avoid contamination from surrounding skin.

Urine samples in catheterized patients were collected by cleaning the sample port with a swab saturated with 70% isopropyl alcohol and allowed to dry. A sterile lock syringe was inserted into the port at 90° angle and turned half a turn clockwise, then a urine sample was slowly drawn. Samples were collected in sterile dry, wide-necked and leak-proof containers.

Bacterial isolation and identification

All samples were inoculated on MacConkey, nutrient and blood agar plates in addition to cystine lactose electrolyte deficient agar (CLED) in cases of urine samples then incubated at 37°C for 24 hours. All media were purchased from (Oxoid, England). Suspected colonies, (non-lactose fermenting isolates, Gram-negative cocco-bacilli) were identified as *A. baumannii* using VITEK® 2 compact system (Bio-Mérieux, France). After bacterial identification, all *A. baumannii* isolates were stored at -70ºC in glycerol and nutrient broth.

Antimicrobial susceptibility testing

The test was performed according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2020) using standard diffusion disk method on Mueller–Hinton agar (MHA) (Becton-Dickinson, USA). Plates were examined and diameters of the complete inhibition zones were measured in mm and interpreted according to (CLSI, 2020). The following antimicrobial disks (HImedia, India) were included:

Gentamycin (10 ug), ciprofloxacin (5 ug), imipenem (10 ug), piperacillin tazobactam (100/10 ug), ampicillin/sulbactam (10/10 ug), ceftazidime (30 ug), cefepime (30 ug) and tobramycin (10 ug), meropenem (10 ug), levofloxacin (5 ug), trimethoprim-sulphamethoxazole (1.25/23.75ug). Susceptibility of *A. baumannii* isolates to colistin was performed by microdilution method using Cation-adjusted Mueller Hinton broth, the only reliable method for colistin susceptibility according to (CLSI, 2020) instructions, as colistin is poorly diffused in agar media. *E. coli* ATCC 25922 and *Ps. aeruginosa* ATCC 27853 were used as quality control organisms to ensure accuracy of the antimicrobial susceptibility assay.

Table 1. Distribution of *A. baumannii* isolates according to type of infection and clinical specimens.

Type of infection	**Clinical specimens** (n=71)		***A. baumannii*** (n=10)	
	Type	**No**	**No**	**%**
Surgical wound infections	Surgical site infection (SSI)*	17	4	23.5
Chest infections	ET tube**	24	6	25
	Sputum	5	0.0	00
Urinary tract infections	Urine	13	0.0	00
Infected wounds	Pus	8	0.0	00
	CVC tip***	4	0.0	00

*SSI, surgical site wound ** E.T. tube, endotracheal tube ***CVC tip, central venous catheter

Statistical analysis

Data were analyzed using SPSS 20. Chi square test (X2) and Fisher exact test were used to compare categorical variables. P value of *0.05* was considered statistically significant.

Results

Among the collected 71 clinical specimens, ten samples (13.9%) were positive for *A. baumannii* as identified by VITEK 2 system. Most of *A. baumannii* isolates (60%) were recovered from respiratory samples followed by SSI specimens (40%).

The results of antimicrobial susceptibility testing showed that all *A. baumanni* isolates were resistant to ciprofloxacin, ceftazidime & cefepime. Moreover, the isolates showed high percentage of resistance to gentamycin, tobramycin, levofloxacin, imipenem, meropenem, ampicillin/sulbactam & piperacillin-tazobactam. Percentages of antimicrobial resistance among the studied *A. baumanni* isolates are shown in **figure (1)**. Also, all strains showed intermediate resistance to colistin ≤ 2 µg/ml according to (CLSI, 2020).

Table 2 shows that most of the isolates (80%) were MDR and were mainly recovered from respiratory secretions (62.5 %).

On investigating the possible risk factors associated with the isolation of MDR *A. baumannii*, prior use of antibiotics was a statistically significant association **(*P<0.05**)**, while age, sex and duration of hospitalization were non -significant risk factors as shown in **table (3)**.

Table 2. Distribution of MDR *A. baumanii* isolates according to the site of infection.

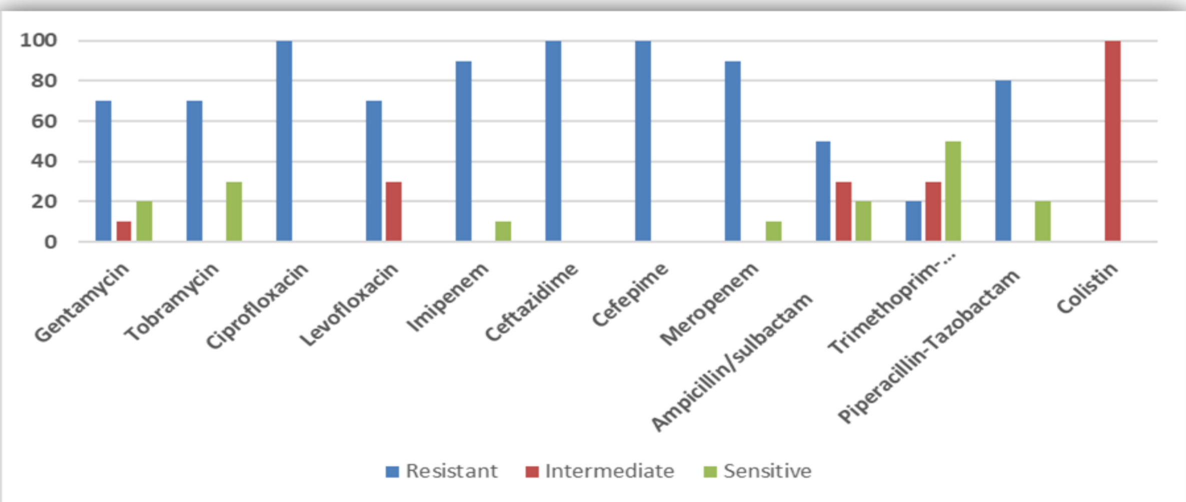
Sample	***A. baumanii*** **isolates (n=10)**	**MDR *A. baumanii* (n=8)**	
		No	**%**
• SSI	4	3	37.5
• ET tube	6	5	62.5

Table 3. Risk factors associated with MDR *A. baumanii* isolates.

Risk factors	Pattern of resistance				a Test	*P value*
	MDR (n=8)		Not MDR (n=2)			
	No	%	No	%		
Age (years)						
<45 years (n=7) ≥45 years(n=3)	5 3	71.4 100	2 0.0	28.6 00	Fisher	*0.301*
Sex						
Female (n=4) Male (n=6)	2 6	50 100	2 0.0	50 00	Fisher	*0.053*
Prior use of antibiotics						
Yes (n=9) No (n=1)	8 0.0	88.9 00	1 1	11.1 100	Fisher	***0.035****
Length of hospitalization						
< 7 days (n=0.0) ≥ 7 days(n=10)	0.0 8	00 80	0.0 2	00 20	-	-

[a] Fisher exact test

Figure 1. Percentages of antimicrobial resistance among the studied *A. baumanni* isolates.



Discussion

In the past few years, *A. baumannii* has become a recognized healthcare associated pathogen which is disseminated worldwide, resistant to many newly developed antimicrobial drugs, causing huge economical cost, increased morbidity and mortality rates and became a major threat to the infection control and treatment plans in clinical practices [9].

In this study, about (23.4%) of the identified Gram-negative bacilli of the samples were *Acinetobacter* isolates which represent its high prevalence in ICUs, our result is nearly the same conducted by **Nazeih et al.** [10] in ICUs in Zagazig University Hospitals. Higher rates of isolation were reported in Saudi Arabia (40.9%) [11] and Malaysia (32%) [9]. In Sudan, a fewer rate was reported (9.5%) [12].

In agreement with **El Maghraby et al.** and **Fatani et al.** [5,9], most of MDR Acinetobacters were recovered from respiratory samples. However, other investigators reported higher isolation rates from sources other than respiratory samples, as **El-Din** [13] who revealed that most of the *Acinetobacter* isolates (53.3%) were recovered from wound infections followed by respiratory tract infections, while **Behera et al.** [14] observed that most isolates were recovered from pus samples (45%).

The difference in isolation rates among different studies could be attributed to the difference in the hospital environment, clinical conditions of the patients and numbers of investigated specimens.

No *A. baumannii* was detected in urine specimens in this study. This finding is similar to a study conducted at Minia [3]. This may be caused by the small number of collected urine specimens.

In our study, the risk factor that is significantly associated with increased infection with MDR *A. baumannii* was prior use of antibiotics which supports the concept that *A. baumannii* is an ICU superbug. Several other studies reported the same finding as **Fattouh and El-din** [15]. On the other hand, there were no statistically significant differences regarding age, sex and prolonged hospitalization. The same finding concerning age of patients was reported by **Fattouh and El-din** [15]. In contrast, a different study observed that *A. baumannii* infection affected more male patients with a percentage of (55.8%) but the reason was not justified [16].

During the last decades, Emergence of drug resistance feature in *A. baumannii* against various antibiotics including carbapenems, aminoglycosides and fluoroquinolones has become a worldwide problem making treatment of *A. baumannii* difficult [3].

A big proportion of our *A. baumannii* isolates (80%) were MDR showing resistance to three or more classes of antibiotics. **Tolba et al.** [17] also found that MDR strains were 88.8% among all the isolates, **Farsiani et al.** [18] 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 [19, 20].

Carbapenem resistance rate (90%) was similar to other studies conducted in Egypt which showed resistance rate of 88.9% and 95% [17,21]. On the other hand, a report of strains isolated from Zagazig ICU showed only 46.4% were resistant to imipenem [10]. Inadequate infection control guidelines and inappropriate use of carbapenem may be the cause of this extreme resistance [21].

Regarding aminoglycoside, resistant rate was 70%, which coordinate with other studies as **Farsiani et al.** and **Amr and Abdel-Razek** [18, 22]. Higher resistance rate of 90% was reported in Egypt [21].

As regard resistance to quinolones, high rates of resistance were observed as the following, resistance rate for Levofloxacin was (70%R, 30%I) while for ciprofloxacin was (100%R). These results agreed with **El-Masry et al**. [23] and **Abdelwahab et al.** [24] 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 [25].

In accordance with the study carried out by **Fattouh et al.** [15], the isolates showed high frequency of resistance to β-lactams/β-lactamase inhibitors, this high resistance is mostly due to β-lactamases production [26]. Still high-dose ampicillin/sulbactam monotherapy was effective treatment for critically ill patients with MDR *A. baumannii* ventilator associated pneumonia [27].

Trimethoprim/Sulfamethoxazole (TMP-SMX) resistance pattern has been high in most studies, reaching to (92%) [26], even 100% in other studies [23]. These results don't agree with this study which represents half of strains, sensitive to sulfamethoxazole/trimethoprim, this is supported by [28].

Many results showed that the effect of TMP-SMX for Acinetobacters is inconstant and unpredictable and many resistant strains respond clinically well to TMP-SMX [29].

As regard to 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.** [30] 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) [31], moreover, the lack of a simple, easy-to-perform colistin susceptibility test has left most clinical microbiology laboratories in an unenviable position where they cannot provide clinicians with an accurate assessment of colistin susceptibility.

Conclusion

Our findings, together with previous studies confirm that MDR *A. baumannii* represent a real problem as an important agent of HAIs in ICUs. Its high resistance may be due to inappropriate administration of antimicrobial agents. Cephalosporins, quinolones and even colistin antibiotics are no longer recommended for the treatment of MDR *A. baumannii* infections.

Limitations

The sample size included in this study was relatively small. The outcomes of ICU patients with *Acinetobacter* infections were not assessed. Therefore, further studies with larger sample size and patient follow up are recommended. In addition, extensive investigation of more risk factors associated with MDR *A. baumanii* infection is needed.

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