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ORIGINAL ARTICLE

Multi- and Extensive-Drug Resistant *Acinetobacter baumannii* in ICUs: Risk Factors, Antimicrobial Resistance Profiles and co-harboring of *gyrA* and *parC* mutations

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ABSTRACT

Background: *Acinetobacter baumannii is a gram-negative organism that is implicated in hospital acquired infections. It confers high resistance to many classes of antibiotics.* ***Objectives:*** *To assess the prevalence of multi and extensive drug-resistant (MDR & XDR) Acinetobacter baumannii, their risk factors, antimicrobial resistance patterns and the presence of gyrA and parC gene mutations of quinolone resistance.* ***Methodology:*** *The study included 106 ICU patients (56 males & 50 females), samples were collected according to sites of infections, Acinetobacter baumannii was identified by morphology, biochemical reactions &API 20NE. Antimicrobial susceptibility testing was performed by disc diffusion method. The E-test was used to detect MIC of Ciprofloxacin & Levofloxacin, then a polymerase chain reaction- restriction fragment length polymorphism was performed to detect the occurence of gyrA and parC gene mutations of Quinolone resistance.* ***Results:*** *Thirty isolates were identified as Acinetobacter baumannii, most of which from respiratory infections (P=0.005) prolonged hospitalization, antibiotic use, urinary catheters & ventilator supports were found to be risk factors of infections. Acinetobacter baumannii isolates showed high resistance to most of the tested antibiotics (29 MDR & 28 XDR). All isolates were resistant to Ciprofloxacin & Levofloxacin with the co-presence of gyrA and parC mutations in all isolates (P<0.001).* ***Conclusions:*** *There is an increased prevalence of MDR & XDR Acinetobacter baumannii among ICU infections. The co-occurrence of gyrA and parC mutations is associated with high resistance to Quinolones.*

INTRODUCTION

Acinetobacter baumannii (A. baumannii) is a gram-negative bacterium that is found ubiquitously in nature and is considered part of the normal flora with a high carriage rate in healthy and hospitalized subjects. It has the ability to persist on dry surfaces and medical equipment as well as surviving exposure to the commonly used disinfectants enabling its spread in the hospital environment [1].

Acinetobacter baumannii is implicated in different hospital acquired and intensive care unit (ICU) infections as ventilator associated pneumonia (VAP), urinary tract (UTI), blood stream (BSI) and wound infections. The ICU patients are not only critically ill, but are also subjects to major surgeries, invasive medical devices and broad-spectrum antibiotic therapy. Moreover, its isolation from hospitalized patients indicates severe illness and increased mortality [2].

Multi-drug resistance (MDR) is one of the assets of *A. baumannii* and is now a global health problem as about half the strains worldwide are resistant to multiple antimicrobials, it is one of the "ESKAPE" group of bacteria with high antimicrobial resistance, including; *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa & Enterobacter* [3]. The antimicrobial resistance is due to the co-existence of numerous mechanisms including the intrinsic β-lactamase genes, extended spectrum β-lactamases (ESBLs), metallo β-lactamase (MBLs), weak permeability, active efflux systems and various acquired mechanisms[4]. *Acinetobacter baumannii* exhibits resistance to fluoroquinolones via mutations in the DNA gyrase (*gyrA* & *gyrB*) and topoisomerase IV (*parC* & *parE*) genes, involving mainly the Quinolone Resistance Determining Regions (QRDRs) of *gyrA* and *parC* genes [5].

Our objectives were to investigate the prevalence of MDR and XDR (extensive drug resistant) *A. baumannii* among Zagazig University hospitals' ICU infections and the risk factors associated with these infections. In addition, analysis of the antimicrobial resistance profiles of isolates and detection of the co-presence of *gyrA* (Ser83Leu) and *parC* (Ser80Leu) gene mutations in relation to quinolone resistance.

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METHODOLOGY

Study design and subjects:

The current cross-sectional study was conducted in the Microbiology & Immunology Department and the ICUs of Zagazig University Hospitals over the period from June 2016 to June 2017. Samples were obtained from 106 patients (56 males & 50 females), who were admitted to different ICUs including; Emergency, Surgery, Internal medicine and Pediatric ICUs, their ages ranged from 18 to 80 years.

Inclusion and exclusion criteria

The study enrolled ICU patients with hospital acquired infections that appeared after 48-72 hours and were not incubating at their admissions. While, patients with community acquired infections (present or incubating at their admissions), patients diagnosed with an organism other than *A. baumannii*, or patients who refused to participate in the study were excluded.

Ethical considerations

An informed consent was taken from each participant after explaining the nature as well as the purpose of this work. The study was approved by the Institutional Reviewer Board (IRB) of Faculty of Medicine, Zagazig University.

Samples collection:

Clinical samples were collected according to sites of infections including; endotracheal aspirates, urinary catheter samples, sputum, pus and conjunctival swabs. Collected samples were transported to the Microbiology laboratory as soon as possible, recovered on MacConkey's and blood agar plates and incubated aerobically at 37ºC for 24 hours [6,7].

Presumptive identification of *A. baumannii*:

Isolates were presumptively identified based on colonial morphology (small, opaque, creamy, raised colonies on blood agar and pale lactose non-fermenters on MacConkey's agar) and on Gram-staining characters (Gram negative bacilli or coccobacilli). Colonies were then sub-cultured on nutrient agar plates at 37ºC for 24 hours to perform biochemical reactions [6].

Biochemical characteristics were based on catalase, oxidase (Oxoid, UK) and motility tests [6,8]. All catalase positive, oxidase negative, non-motile isolates were incubated aerobically on triple sugar iron (TSI) agar at 37ºC for 24 hours. *Acinetobacters* showed negative sugar fermentation and failed to grow anaerobically in the butt of tubes [6]. *Acinetobacter baumannii* was distinguished from other *Acinetobacter* species by the ability to grow at 44ºC on brain heart infusion broth (Himedia, India) after 24 and 48 hours [9]. Strips of API 20 NE (Bio-Merieux, France) were inoculated with *A. baumannii* and incubated according to the manufacturer's instructions, then the reactions were read and interpreted using the Analytical Profile Index provided by the manufacturer.

Antibiotic susceptibility testing:

Disc diffusion method

All isolates of *A. baumannii* were subjected to antibiotic susceptibility testing using the standard Kirby-Bauer disc diffusion method [10]. Susceptibility testing was performed on Mueller-Hinton agar (Becton-Dickinson, USA) using the antimicrobial discs; gentamycin 10µg, amikacin 30 µg, ciprofloxacin 5 µg, levofloxacin 5 µg, tigecycline 15 µg, trimethoprim-sulphamethoxazole 1.25/23.75 µg, imipenem 10 µg, aztreonam 30 µg, piperacillin-tazobactam 100/10 µg, amoxicillin/clavulinic acid 20/10 µg, ceftazidime 30 µg and cefepime 30 µg (Oxoid, UK). Testing results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) [11]. The *Acinetobacter* species were categorized as MDR if they were not susceptible to at least one agent in three or more of nine antimicrobial categories or to three classes of; all penicillins and cephalosporins (including inhibitor combinations), aminoglycosides and fluroquinolones [12]. The MDR *Acinetobacters* which conferred additional resistance to carbapenems were termed XDR, while XDR strains showing resistance to polymyxins and tigecycline were named pan-drug resistant (PDR) *Acinetobacter* species [13].

Epsillometer (E) test

The E-test strips (Oxoid, UK) were used to detect the antimicrobial susceptibility and the minimal inhibitory concentrations (MIC) of ciprofloxacin (CIP 32µg/ml-0.002µg/ml) and levofloxacin (LEV 32µg/ml-0.002µg/ml) according to the CLSI guidelines [11].

Detection of *gyrA* (Ser83Leu) and *parC* (Ser80Leu) gene mutations by polymerase chain reactions-restriction fragment length polymorphism (PCR-RFLP) techniques:

DNA extraction

Bacterial DNA was extracted physically by transferring several colonies from a nutrient agar subculture to 0.5 ml Tris EDTA buffer in Eppendorf tubes. Samples were boiled at 100ºC in a water bath for 10 minutes then centrifuged at 14000 rpm at 22ºC for 5 minutes. The supernatant containing the DNA was then transferred to new tubes and stored at -20ºC until further use. The optical density of each sample was measured at 260nm and 280 nm wavelengths and DNA concentrations were calculated [14].

DNA amplification by PCR and detection of gyrA (Ser83Leu) & parC (Ser80Leu) gene mutations

To detect the *gyrA* (Ser83Leu) and *parC* (Ser80Leu) gene mutations, we used the primers; forward 5′AAATCTGCCCGTGTCGTTGGT3′ and reverse 5′GCCATACCTACGGCGATACC3′ for *gyrA* gene and the primers; forward: 5′AAACCTGTTCAGCGCCGCATT 3′ and reverse 5′AAAGTTGTCTTGCCATTCACT3′ for *parC* gene (Bioron, Germany) to give 343 and 327 bp bands; respectively. The PCR reactions were performed in 20

µl reaction mixtures using PCR-GOLD master mix beads (Bioron, Germany) containing: 1 µl of each of the forward and the reverse primers, 5 µl of template DNA and 13 µl of distilled water. Reactions were carried on using Biometra T gradient thermal cycler (Germany) programmed for initial template denaturation at 95°C for 1 min; followed by 36 cycles of denaturation at 95°C for 30 sec, annealing at 53°C for 30 sec, extension at 72°C for 2 min; and final extension at 72°C for 10 min; for *gyrA* and an initial denaturation at 95°C for 2 min; then 36 cycles of denaturation at 95°C for 1 min , annealing at 60°C for 1 min, extension at 72°C for 2 min; and final extension at 72°C for 10 min; for *parC* genes [15,16]. The *HinfI* restriction enzyme (Promega) was used to digest the PCR products by incubation at 37°C for 2.5 hours. The digests were visualized under UV light after electrophoresis using 1.5% agarose gel, the *gyrA* (Ser83Leu) and *parC* (Ser80Leu) mutations resulted in 291bp & 52bp bands; respectively [17].

Statistical analysis

Data were checked, entered and analyzed using SPSS version 19.0 for Windows. Quantitative variables were expressed as means ± standard deviation (SD). Student's "t" test was used to ascertain the significance of differences between the mean values of two continuous variables. Qualitative data were compared by the chi-squared-test (X^2). P values < 0.05 indicated significant results.

RESULTS

The study enrolled clinical samples from 106 hospitalized patients (56 males & 50 females) whose ages ranged from 18-80 years (mean±SD = 41.8±18.2). The API 20 NE identified 30 (28.3%) *A. baumannii* isolates out of the 106 samples. Respiratory infections were the most common infections among our ICU patients (45.2%), followed by urinary tract (26.4%) then wound and eye infections (14.2%, each). The highest percentage of *A. baumannii* isolates was recovered from endotracheal aspirates of respiratory infections (12/30, 40%), followed by urine samples (8/30, 26.67%), pus from infected wounds (5/30, 16.67%), sputum (4/30, 13.33%), and the least percentage came from conjunctival swabs (1/30, 3.33%), with a statistically significant difference (P=0.005) (table 1).

Table 1: *A. baumannii* isolates recovered from different clinical samples

Infection N=106;%	Clinical specimen N=106;%	*A. baumannii* N=30,	%	χ^2	P value
Eye infections (15;14.2)	Conjunctival swabs (15;14.2)	1	3.33		
Infected wounds (15;14.2)	Pus (15;14.2)	5	16.67		
Urinary tract (28;26.4)	Urine (28;26.4)	8	26.67		
Respiratory tract (48;45.2)	Endotracheal aspirates (30;28.3)	12	40.0		
	Sputum (18;16.9)	4	13.33	14.58	0.005*

**Statistically significant*

Studying the prevalence of *A. baumannii* among different ICUs (28.3%; 30/106 of all infections) revealed that it represented 31.6% (12/38) of isolates recovered from the surgery ICU, 26.7% (4/15) of both the emergency and the pediatric ICU and 26.3% (10/38) of the internal medicine ICU isolates, with no statistically significant differences between them (P=0.95).

Assessment of the risk factors associated with the prevalence of *A. baumannii* among different ICUs showed that patients' hospitalization more than 7 days (OR:5.26, 95%CI:1.67-16.54, P<0.001) and the prior use of antibiotics (OR:2.85, 95%CI:0.98-8.23, P=0.046) significantly increased the risk of *A. baumannii* infections. In addition, the presence of a urinary catheter (OR:10.50, 95%CI:1.07-102.48, P=0.02) and/or a ventilator support (OR:2.33, 95%CI:0.61-8.82, P=0.01) significantly contributed to the occurrence of *A. baumannii* infections. On the other hand, neither age nor gender of patients, were associated with increased *A. baumannii* infection risk (table 2).

Table 2: Risk factors associated with *A. baumannii* infections

Risk factor	Total No. of samples	*A. baumannii* isolates	%	Odds Ratio (95%CI)	P value
Age group					
– < 45	54	13	24.0%	0.65	
– ≥ 45	52	17	32.7%	(0. 27-1.52)	0.32
Sex					
– Male	56	16	28.6%	1.02	
– Female	50	14	28.0%	(0.44 -2.39)	0.94
Length of hospitalization					
– < 7 days	38	4	10.5%	5.26	
– ≥ 7 days	68	26	38.2%	(1.67 -16.54)	< 0.001*
Prior use of antibiotics					
– Yes	66	22	33.3%	2.85	
– No	50	8	16.0%	(0.98-8.23)	0.046*
Urinary catheter					
– Present	15	7	46.7%	10.50	
– Absent	13	1	7.7%	(1.07-102.48)	0.02*
Ventilator support					
– Present	30	12	40.0%	2.33	
– Absent	18	4	22.2%	(0.61-8.82)	0.01*

*Statistically significant

The antimicrobial susceptibility profiles of *A. baumannii* isolates were examined using the disc diffusion method. All isolates were resistant to ciprofloxacin, levofloxacin & aztreonam, 29 (96.7%) of them were resistant to amoxicillin-clavulanic, ceftazidime & cefepime, 28 (93.3%) were resistant to imipenem and 26 (86.7%) were resistant to piperacillin-tazobactam. While, the highest detected susceptibility was to tigecycline (23, 76.76%) (table 3).

Table 3: Antimicrobial susceptibility profiles of *A. baumannii* isolates.

Antibiotics	Antimicrobial susceptibility pattern of *A. baumannii* (N=30)					
	Sensitive		Intermediate		Resistant	
	N	%	N	%	N	%
Ciprofloxacin	0	0.0	0	0.0	30	100.0
Levofloxacin	0	0.0	0	0.0	30	100.0
Gentamycin	6	20.0	2	6.7	22	73.3
Amikacin	4	13.33	5	16.7	21	70.0
Tigecycline	23	76.76	2	6.7	5	16.7
Trimethoprim-sulphamethoxazole	9	30.0	0	0.0	21	70.0
Imipenem	2	6.7	0	0.0	28	93.33
Aztreonam	0	0.0	0	0.0	30	100.0
Piperacillin-tazobactam	4	13.3	0	0.0	26	86.7
Amoxicillin-clavulanic	0	0.0	1	3.3	29	96.7
Ceftazidime	1	3.3	0	0.0	29	96.7
Cefepime	1	3.3	0	0.0	29	96.7

We classified the *A. baumannii* isolates according to their susceptibility patterns into 7 groups (I to VII), where group I was sensitive only to tigecycline and group VII was resistant to all the tested antimicrobials. Our data demonstrated that 29 (96.7%) *A. baumannii* isolates, all except group VI, were MDR (resistant to penicillins, cephalosporins & fluoroquinolones), 28 (93.3%) isolates were XDR with additional resistance to carbapenems (groups; I,II,III,V &VII) and 7 isolates (group VII) were PDR showing resistance to all the used antibiotics, with a statistically significant value (P=0.001) (table 4).

Table 4: Grouping of *A. baumannii* isolates according to their resistance patterns.

Antibiotics	I N (10)	II N (7)	III N (3)	IV N (1)	V N (1)	V1 N (1)	VII N (7)	Total	
								S	R
Ciprofloxacin	R	R	R	R	R	R	R	0 (0.0)	30 (100.0)
Levofloxacin	R	R	R	R	R	R	R	0 (0.0)	30 (100.0)
Gentamycin	R	R	S	S	S	S	R	6 (20.0)	24 (80.0)
Amikacin	R	R	R	S	S	S	R	3 (10.0)	27 (90.0)
Tigecycline	S	S	S	S	S	S	R	23 (76.7)	7 (23.3)
Trimethoprim-sulpha	R	S	R	R	S	S	R	9 (30.0)	21 (70.0)
Imipenem	R	R	R	S	R	S	R	2 (6.7)	28 (93.3)
Aztereonam	R	R	R	R	R	R	R	0 (0.0)	30 (100.0)
Piperacillin-tazobactam	R	R	R	R	R	S	R	1 (3.3)	29 (96.7)
Amox-clav	R	R	R	R	R	R	R	0 (0.0)	30 (100.0)
Ceftazidime	R	R	R	R	R	S	R	1 (3.3)	29 (96.7)
Cefepime	R	R	R	R	R	S	R	1 (3.3)	29 (96.7)

The E-test performed to assess the MICs of ciprofloxacin and levofloxacin showed that all the studied *A. baumannii* isolates were resistant to both agents (MIC ≥32µg/ml, for both of them). In addition, a PCR-RFLP technique was carried out to detect the presence of the quinolone resistance gene mutations; *gyrA* (Ser83Leu) and *parC* (Ser80Leu) among the studied isolates. The analysis revealed the simultaneous occurrence of *gyrA* and *parC* gene mutations in all isolates. There was an agreement between the presence of the resistance genes and results of disc diffusion antimicrobial susceptibility testing (P<0.001) as well as the results of the E-test for ciprofloxacin and levofloxacin (P<0.001& <0.001).

DISCUSSION

Acinetobacter baumannii is considered one of the common causes of ICU hospital acquired infections. It often possesses resistance to numerous antibiotics making its treatment difficult. *Acinetobacter baumannii* is regarded as an opportunistic pathogen that causes various infections in immunocompromised or critically ill hospitalized patients[18]. This work tries to assess the prevalence of MDR & XDR *A. baumannii* in ICU infections, their risk factors and antibiotic resistance patterns. In addition to exploring the co-harboring of *gyrA* and *parC* gene mutations in relation to quinolone resistance.

Respiratory tract infections were the most common among our ICU patients followed by urinary infections, then infected wounds and eye infections. This agrees with the study of Patel et al.[19], who found pneumonia the most frequent infection in his ICU patients followed by blood stream and urinary infections and Sharma et al.[20], who reported similar data about urinary infections in his patients. The current study found *A. baumannii* to be recovered significantly from endotracheal aspirates, followed by urine, pus, sputum and conjunctival samples. Samples from respiratory infections and VAP (endotracheal aspirates & sputum) represented the commonest source of *A. baumannii* followed by urinary tract infections. This is consistent with Khilnani et al.[21] who found *A. baumannii* the most frequent organism causing VAP and Sharma et al.[20], who isolated *A. baumannii* from UTI in a comparable percentage. While, Zheng et al.[22], found *A. baumannii* the most common Gram-negative organism causing blood stream infection. *A. baumannii* is a leading cause of pneumonia in ICU patients and it is also a main cause of urinary, blood stream and wound infections, which makes it a serious threat[23]. *A. baumannii* was recovered from surgery, emergency, pediatrics and medicine ICUs, with no statistically significant differences between them which agreed with the findings of Mamishi et al.[24].

Hospitalized, particularly ICU, patients are critically ill and subjected to numerous risk factors that increase morbidity and mortality. We studied the risk factors which contributed to *A. baumannii* infections in our patients, and we found the presence of ventilation support or urinary catheters, beside to the use of antibiotics and prolonged hospital stay to affect the occurrence of infections significantly. While, age and gender of patients were not considered risk factors in this study. Similarly, other researchers agreed that the presence of mechanical ventilation represented a principle risk factor for the occurrence of VAP in ICU patients[19,22,25]. While, Patel et al.[19] and Zheng et al.[22] could not relate urinary infections to the presence of urinary catheterization. Russotto et al.[26], found the previous antibiotic therapy to be a risk for hospital infections, but Zheng et al.[22], could not prove this relation. Prolonged hospitalization was a significant risk of infection in the studies of Ren et al.[25], but not Patel et al.[19]. Moreover, according to Zheng et al.[22], age and sex were not related to hospital infections. It is well known that ICU patients are vulnerable to infections, these patients may be immunosuppressed by medications they

take or by their underlying medical conditions. In addition, their immunological barriers are weakened by ventilators, urinary or blood catheterizations. Together with their exposure to invasive procedures, prolonged stay in ICUs and antibiotics, all put them in a significant risk of hospital acquired infections caused by *A. baumannii* which is a leading pathogen of invasive infections in ICU patients[26,27].

Antibiotic resistance is considered one of the most important problems threatening health, where *A. baumannii* and *Pseudomanas aeruginosa* are recognized as the principle and most serious resistant bacteria. Hospital acquired infections with MDR organisms are markedly increased especially with emerging opportunistic pathogens as *A. baumannii*[1,3]. Hereby, we tested the antibiotic susceptibility of *A. baumannii* isolates against various antibiotics using the Kirby-Bauer disc diffusion method. Isolates were completely resistant to fluoroquinolones (ciprofloxacin & levofloxacin) and aztreonam, they were highly resistant to amoxicillin-clavulinic (96.7%) ceftazidime (96.7%), cefepime (96.7%) & imipenem (93.3%), while they were least resistant to tigecycline. In line with our findings, studies by Ranjbari & Farahani[28] and Boral et al.[29] found similar patterns of *A. baumannii* resistance regarding fluoroquinolones, imipenem, ceftazidime, cefepime and piperacillin-tazobactam. While, they recorded higher resistance to amikacin and lower resistance to tigecycline. Studies of Tohamy et al.[30] and Bitew[31] recorded lower resistance to fluoroquinolones, gentamicin, piperacillin-tazobactam and aztreonam than ours.

Our study revealed a high prevalence of MDR and XDR *A. baumannii* recovered from ICU infections. We found 96.7% MDR, 93.3% XDR and 23.3% PDR *A. baumannii* isolates. This is comparable to the study of Bitew[31] who found 92.9% MDR &25% PDR, but only 35.7% XDR isolates. Additionally, Ranjbari & Farahani[28] found 93% of their isolates to be resistant to six antimicrobial groups and Ren et al.[25] who detected a significant infection with PDR *A. baumannii* in their patients. However, our rates were higher than other Egyptian studies, where Tohamy et al.[30], El-Mahalawy et al.[32]and Hassanin et al.[33], reported 68.6%, 69% & 86% MDR isolates; respectively. The MDR *A. baumannii* causes infections in seriously ill patients with underlying conditions and invasive procedures receiving broad-spectrum antibiotics as ICU patients[2]. *Acinetobacter baumannii* confers natural resistance to antimicrobials and its treatment with different agents contribute to the prevalence of PDR strains[25]. There was also a high rate of biofilm formation associated with MDR *A. baumannii*[28,34]. Variability of antibiotic resistance patterns of *A. baumannii* could be attributed to the use of different antimicrobial panels, antibiotic abuse or even in the definitions of MDR among countries [31].

Acinetobacter baumannii exhibits different mechanisms of resistance to many classes of antibiotics. Resistance to quinolones is mediated mainly by mutations in the quinolone resistance determining regions *gyrA* and *parC* of the target enzymes; DNA gyrase and topoisomerase IV. This lowers the affinity for binding of quinolones to these bacterial enzymes, another mechanism involves an efflux system decreasing drug levels in the bacterial cells[5].

This work focused on the genetic characterization of the simultaneous occurrence of *gyrA* (Ser83Leu) and *parC* (Ser80Leu) gene mutations of quinolone resistance among our *A. baumannii* isolates. We found all the recovered isolates to harbor both mutations by PCR-RFLP techniques. This also agreed with the results of the disc diffusion antibiotic testing and the MIC detected by the E-test against both ciprofloxacin and levofloxacin ($P<0.001$). Our findings are consistent with Zaki et al.[35] and Tawfick et al.[36], who recorded high resistance of *A. baumannii* to ciprofloxacin and levofloxacin where all the resistant isolates harbored the *gyrA* and/or *parC* mutations. It is known that mutations of *gyrA* together with *parC* are associated with high resistance to quinolones[16]. Results of the disc diffusion testing matched the MIC results of the E-test, which is agreed by Zaki et al.[35], where, the disc diffusion method is described as a good and rapid screening test for antibiotic resistance[37].

The emerging MDR, XDR and PDR *A. baumannii* leave very limited choices of antibiotics for treatment of its serious infections. In our study, the highest sensitivity was achieved by tigecycline, a bacteriostatic agent, that has a limited use in immunocompromised patient. Other studies recommended the use of colistin to which *A. baumannii* strains were mostly susceptible, however, it has toxic effects [28,29,38].

CONCLUSIONS

The wide irrational use of broad-spectrum antibiotics and disinfectants together with the natural resistance of *A. baumannii* and risk factors associated with ICU patients contributed to the increasing problem of antimicrobial resistance. Implementation of infection control measures including antibiotic stewardship, standard & contact precautions, personnel training and surveillance should be the effective management ways of this problem.

Recommendations:

Further multi center studies on the genetic basis of other antibiotic resistance mechanisms and prevalence of MDR *A. baumannii* should provide better understanding of the problem in Egypt.

Conflicts of interest:
The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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