Phenotypic characterization of *Acinetobacter baumannii* isolates from intensive care units at a tertiary-care hospital in Egypt

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التعرف على السمات المظهرية لمستفرَدات الراكدة البومانية من وحدات الرعاية المركزة في مستشفى للرعاية الثالثية في مصر

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الخلاصة: إن الذراري المقاومة لأدوية متعددة من جراثيم الراكدة البومانية هي الآن المسؤولة عن تزايد أعداد حالات العدوى الانتهازية في المستشفيات. وقد حددت هذه الدراسة معدلات انتشار الجراثيم المقاومة لأدوية متعددة من جنس الراكدة البومانية المستفردة من وحدات الرعاية المكثفة في أحد المستشفيات الكبيرة للرعاية الثالثية في الإسماعيلية، مصر، كما حددت الدراسة معدل وجود إنزيمات بيتالاكتاماز المختلفة في هذه المستفرَدات. وأعد الباحثون ملفاً يتضمن الأنماط البيولوجية والاستجابة لمضادات المكروبات للذراري المستفرَدة. فجمعوا عينات من الدم والقيح من الجروح والحروق ومن البول ومن الجهاز التنفسي لدى 350 مريضاً أدخلوا في مختلف الوحدات. واستفردوا 10 ذراري (2.9 ٪) من الراكدة البومانية، وتبين لهم أن جميع المستفردات لديها مقاومة لأكثر من 3 من أصناف المضادات الحيوية. وكان من بين المستفردات ستة جراثيم منتجة لإنزيم كاربابنيماز، وثلاثة جراثيم منتجة لإنزيم بيتا لاكتاماز AmpC، ولم يجدوا أي مستفردة منتجة لإنزيم ميتالو بيتا لاكتاماز. ورغم انخفاض معدل انتشار العدوى بجراثيم الراكدة البومانية في المستشفيات، فإن ملف مقاومتها للمضادات الحيوية يشير إلى ضرورة وأهمية الوقاية من العدوى المرتبطة بالرعاية الصحية بأنواع "الراكدة" المقاومة لأدوية متعددة.

ABSTRACT Multi-drug resistant (MDR) strains of *Acinetobacter baumannii* are responsible for an increasing number of opportunistic infections in hospitals. This study determined the prevalence of MDR *A. baumannii* isolates from intensive care units in a large tertiary-care hospital in Ismailia, Egypt, and the occurrence of different beta-lactamases in these isolates. Biotyping and antimicrobial susceptibility profile was done for isolated strains. Respiratory, urine, burn wound and blood specimens were collected from 350 patients admitted to different units; 10 strains (2.9%) of *A. baumannii* were isolated. All isolates showed resistance to more than 3 classes of antibiotics. Among the isolates, 6 isolates were carbapenemase producers, 2 were AmpC beta-lactamase producers and no isolates were metallo-beta-lactamase producers. Despite the low prevalence of *A. baumannii* infection in this hospital, the antibiotic resistance profile suggests that prevention of health-care-associated transmission of MDR *Acinetobacter* spp. infection is essential.

Caractérisation phénotypique des isolats d'*Acinetobacter baumannii* prélevés dans des unités de soins intensifs d'un hôpital de soins de santé tertiaires en Égypte

RÉSUMÉ Les souches d'A*cinetobacter baumannii* multirésistantes sont aujourd'hui responsables de l'augmentation du nombre d'infections opportunistes dans les hôpitaux. La présente étude a déterminé la prévalence des isolats d'*A. baumannii* multirésistants prélevés dans des unités de soins intensifs d'un grand hôpital de soins de santé tertiaires à Ismaïlia (Égypte), et la fréquence de différentes bêta-lactamases dans ces isolats. Le biotypage et le profil de sensibilité aux antimicrobiens ont été établis pour les souches isolées. Des échantillons des voies respiratoires, d'urine, de blessures par brûlure et de sang ont été prélevés sur 350 patients admis dans différentes unités ; 10 souches (2,9 %) d'*A. baumannii* ont été isolées. Tous les isolats présentaient une résistance à plus de trois classes d'antibiotiques. Parmi ces isolats, six produisaient des carbapénèmases, deux des bêta-lactamases AmpC mais aucun isolat ne produisait de métallo-bêta-lactamases. Malgré une faible prévalence de l'infection à *A. baumannii* dans cet hôpital, le profil de résistance aux antibiotiques laisse penser que la prévention de la transmission de l'infection à *Acinetobacter* spp. multirésistante associée aux soins de santé est essentielle.

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Introduction

Of the newer pathogens encountered in health-care settings, *Acinetobacter* spp. are now recognized to play a considerable role in the colonization and infection of patients admitted to hospitals. *Acinetobacter* spp. have been implicated in a range of nosocomial infections, including bacteraemia, urinary tract infection (UTI), and secondary meningitis, but their predominant role is as agents of nosocomial pneumonia, particularly ventilator-associated pneumonia in patients confined to hospital intensive care units (ICUs) [1]. Outbreaks have been increasingly reported and most of those outbreaks are caused by multidrug resistant (MDR) strains of this organism. MDR strains of *Acinetobacter* spp. are now observed worldwide [2,3]. Many mechanisms act together to contribute to the problem of MDR including reduced access to microbial targets through loss of porin channels, possession of efflux pumps that are capable of actively removing a broad range of antimicrobial agents from the bacterial cell, and possession of a wide group of beta-lactamases that hydrolyse and give resistance to penicillins, cephalosporins and carbapenems [4,5].

Regarding Egypt, there are no published data about the prevalence of *A. baumannii* in hospitals in our country. However, some reports are available from other developing countries. In the Islamic Republic of Iran, for example, a prevalence of 15% was reported [6], in India it was 9.5% [7], while in Kuwait it was 22.1% [8]. In one study carried out in Saudi Arabia, *A. baumannii* was the most common isolated organism among Gram-negative bacteria, with a prevalence of 31.7% [9]. The current study aimed to phenotypically characterize *A. baumannii* isolated from different ICUs in our hospital and to describe the resistance profile of isolated strains. One of the objectives of our study was also to screen for the occurrence of these beta-lactamases and carbapenemases in our hospital as this is of importance in deciding the most appropriate therapeutic regimen for treatment of these beta-lactamase-resistant non-fermenting bacilli.

Methods

Study setting

This study was conducted over a period of 8 months, from June 2011 to February 2012, at the Suez Canal University Hospital, Ismailia, Egypt. Suez Canal University hospital is a tertiary-care teaching hospital in which the ICUs comprise an internal medicine unit (10 beds), a coronary care unit (7 beds), a hepatology unit (7 beds), a burns unit (10 beds) and a neonatal ICU (12 beds).

Study sample

A total of 350 specimens were collected randomly from 350 patients with clinical nosocomial infections admitted to different ICUs of the hospital. Only infections which occurred 48 hours following a patient's admission to hospital were evaluated. These included septicaemia (15 specimens), UTIs (120 specimens), respiratory infections (170 specimens) and burn wound infections (45 specimen). Only 1 specimen per patient was included in the study.

Ethical approval to perform the study was obtained from the ethics committee in the Faculty of Medicine, Suez Canal University and the management board of the hospital.

Data collection

Data collected from each patient included age, sex, length of hospital stay, use of invasive medical devices, receipt of antibiotics and the general surgical or medical problem necessitating admission.

Samples included urine (from both catheterized and non-catheterized patients), respiratory specimens (from both intubated and non-intubated patients), blood specimens and wound swab specimens. These were plated onto blood agar and MacConkey agar using the streaking method and then incubated aerobically at 37° C for 24–48 hours. Colonies which that were morphologically consistent with *A. baumannii* (i.e. oxidase-negative, catalase-positive, indole-negative, non-motile, and glucose, lactose and sucrose non-fermenting when inoculated onto triple-sugar-iron agar) were then confirmed using an API™ 20NE kit (bioMérieux).

Susceptibility testing

Antibiotic susceptibility testing was determined using modified Kirby–Bauer method following the Clinical Laboratory Standards Institute guidelines [10]. *E. coli* ATCC 25922 was used as a quality control strain. The following antimicrobial agents were included in the panel: piperacillin (100 μg), piperacillin/tazobactam (100/10 μg), ampicillin/sulbactam (20/10 μg), imipenem (10 μg), meropenem (10 μg), ceftazidime (30 μg), cefotaxime (30 μg), ceftriaxone (30 μg), cefepime (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), gentamicin (10 μg), amikacin (30 μg), tobramycin (10 μg), tetracycline (30 μg) and trimethoprim/sulfamethoxazole (1.25/23.75 μg) (Oxoid).

With regards to tigecycline and colistin, there are considerable technical difficulties associated with susceptibility testing, as the disk diffusion method has been found to be inaccurate and not reproducible for colistin and tigecycline susceptibility testing of *A. baumannii* and this method is not recommended by the CLSI [11–13]. Agar dilution and broth microdilution are considered the gold standard susceptibility test methods for this organism. For determining susceptibility to polymyxins (polymyxin B and colistin) (Sigma-Aldrich), the minimum inhibitory concentration (MIC) was determined using the agar dilution method according to the European Committee on Antimicrobial

Susceptibility Testing (EUCAST) [12]. The tested strains were deemed sensitive or resistant according to EUCAST breakpoints. For tigecycline (Wyeth Pharmaceuticals), both the disk diffusion and the agar dilution method were tested against the isolated strains. It is noteworthy that neither the CLSI [14] nor EUCAST [15] have any published MIC breakpoints for *Acinetobacter* spp. susceptibility testing against tigecycline. The interpretation of the zone diameters of tigecycline was done by using the US Food and Drug Administration breakpoints for susceptibility [16]. This showed a zone diameter of ≥ 19 mm as susceptible, 15–18 mm as intermediate, ≤ 14 mm as resistant. Most published studies to date have used a provisional breakpoint of ≤ 2 mg/L for determination of tigecycline susceptibility and resistance and this was used in our interpretation [17–19]. *E. coli* ATCC 25922 was used as a quality control strain for the agar dilution method used in MIC determination.

Phenotypic detection of resistance mechanisms among *A. baumannii* isolates

MDR *A. baumannii* isolates were defined as those resistant to more than 3 classes of antibiotic [20]. Stocks of 10 distinct MDR *A. baumannii* isolates, recovered from ICUs, were tested for the presence of different beta-lactamases by the following methods.

Modified Hodge test

An overnight culture suspension of *E. coli* 25922 adjusted to 1:10 dilution of 0.5 McFarland standard was inoculated using a sterile cotton swab onto the surface of Muller–Hinton agar (MHA) (Hi-Media). After drying for 3–5 min, a 10 µg meropenem disk (Oxoid) was placed at the centre of the test area. The test organism was streaked in a straight line from the edge of the disk to the edge of the plate. After an overnight incubation at 37 °C, the presence of a cloverleaf shaped zone of inhibition due to carbapenemase production by the test strain was considered as positive [21].

EDTA disk synergy test

An overnight liquid culture of the test isolate was adjusted to a turbidity of 0.5 McFarland standard and spread on surface of MHA plate. A 10 µg meropenem disk or 30 µg ceftazidime disk was placed on the agar. A 0.5 M EDTA solution was prepared by dissolving 186.1 g of EDTA disodium salt (Reachem) in 1000 mL of distilled water. The pH was adjusted to 8.0 using NaOH (Hi-Media) and sterilized by autoclaving. A blank disk (6 mm in diameter, Whatmann filter paper no. 1) was kept on the inner surface of the lid of the MHA plate and 10 µL of 0.5 M EDTA was added to it. The EDTA disk was then transferred to the surface of the agar and kept 10 mm edge-to-edge apart from the meropenem or ceftazidime disk. After incubating overnight at 37° C, enhancement of zone of inhibition in the area between the meropenem and EDTA disk in comparison with the zone of inhibition on the far side of the drug was interpreted as positive for MBL production [22].

AmpC disk test

Tris-EDTA disks were prepared in-house by applying 20 µL of 1:1 mixture of saline and 100 × tris-EDTA to sterile filter paper disks. The tris-EDTA disk was inoculated with heavy inoculums of the test organism prior to placing it on the plate. This was done in an empty Petri dish, using the wooden end of a sterile cotton swab to apply the organism. It was then inverted before placing it on the agar. This ensures optimal diffusion of the beta-lactamase into the agar. The susceptibility plate was inoculated with lawn of 0.5 McFarland suspension of *E. coli* ATCC 25922. The cefoxitin disk was placed on the susceptibility plate. Then the TE disk with the test organism was placed close to (1 mm) but not touching the cefoxitin disk. After overnight incubation, the zone margin was examined for indentation or flattening which indicated evidence of AmpC beta-lactamase production [23].

Results

Background data of patients

This study included 350 patients with different nosocomial infections admitted to different ICUs of Suez Canal University Hospital during the period from June 2011 to February 2012. The study sample comprised 167 females (47.7%) and 183 males (52.2%). Their age ranged from 1 day to over 60 years, the mean age was 32.8 (standard deviation 21.7) years and most of them (37.7%) were aged 35–60 years old (Table 1).

Half of our collected samples came from patients admitted to the general medical and surgical ICUs, while 22.9% were from patients admitted to the neonatal ICU, 12.9% from the burns ICU, 8.9% from the coronary care unit and 5.4% from the hepatology ICU. Most of the collected specimens were respiratory specimens (including both expectorated sputum and endotracheal aspirates) (170/350) (48.6%),

Table 1 Distribution of the studied intensive care unit patients (*n* = 350) according to age

Age group	No.	%
Neonate (0–28 days)	55	15.7
Infant (1 month–2 years)	25	7.1
Child (2–18 years)	20	5.7
Young adult (18–35 years)	107	30.6
Middle age (35–60 years)	132	37.7
Elderly (> 60 years)	11	3.1

Table 2 Frequency of occurrence of bacterial species isolated from specimens from intensive care unit patients (*n* = 350)

Organism	No.	%
Klebsiella spp.	60	17.4
Escherichia coli	35	10.0
Pseudomonas spp.	125	35.7
Acinetobacter spp.	10	2.9
Proteus spp.	15	4.3
Other Gram –ve	21	6.0
Staphylococcus spp.	53	15.1
Other Gram +ve	31	8.9

followed by urine specimens (both catheterized and non-catheterized) (120/350) (34.3%), burn wound swabs (45/350) (12.9%) and blood specimens (15/350) (4.3%).

Organisms isolated

The most frequently isolated organism from patients' samples was *Pseudomonas* spp. (125/350) (35.7%), followed by *Klebsiella* spp. (60/350) (17.1%). The least frequently isolated among the diagnosed organisms was *Acinetobacter* spp. (10/350) (2.9%) (Table 2).

All 10 of the *Acinetobacter* spp. isolates in our study were confirmed to be *A. baumannii* by the API 20NE system. Isolation of *A. baumannii* was greatest from respiratory specimens (50%, 5/10), followed by blood specimens and burn wound swab specimens (both 20%, 2/10), while 1 isolate came from a urine specimen.

Most of the *A. baumannii* isolates came from the general ICU and the neonatal ICU (40% for each, 4/10). The remaining 2 isolates were from the burns unit. It was noted that 90% of patients with isolated *A. baumannii* (9/10) had received antibiotics before isolation of the organism. In half of cases (5/10) the patient stayed more than 15 days in hospital before the organism was isolated.

By the API 20NE system, 6 different biochemical profiles (biotypes) were identified in our wards; biotype 0001073 was the most common, accounting for 30% (3/10) of isolates, followed by biotypes 0041073 and 4041473, each accounting for 20% (2/10). Biotypes 0001473, 4001073, 5041073 were each found in 10% (1/10) of isolates.

Antibiotic resistance of isolates

Antibiotic susceptibility patterns of various isolates are shown in Table 3, and the MIC results of polymyxins are shown in Table 4. All the 10 *A. baumannii* isolates were MDR (defined as resistance to more than 3 classes of antibiotics). All isolates were resistant to piperacillin, piperacillin/tazobactam, ampicillin/sulbactam, ceftazidime, cefepime, cefotaxime, ceftriaxone, imipenem, amikacin, ciprofloxacin and trimethoprim/sulfamethoxazole. Susceptibility to meropenem, gentamicin, tobramycin, tetracycline and levofloxacin enabled the definition of 5 phenotypic resistance patterns (A–E) which were equally distributed (20% each). All isolates were sensitive to polymyxin B, although 2 isolates were resistant to polymyxin E (colistin), according to established CLSI breakpoints for MIC. When testing for tigecycline, the disk diffusion method and agar dilution for MIC showed variable results (Table 5) and according to the MIC, 2 isolates were tigecycline resistant.

Table 3 Biotypes and antibiogram of the 10 *Acinetobacter baumannii* isolates using disk diffusion method

Isolate	Biotype	Antibiogram group	Interpretation of disk diffusion test															
			PRL	TPZ	SAM	CAZ	FEP	CTX	CRO	IPM	MEM	CN	TOB	AK	TE	CIP	LEV	SXT
1	0001073 I	A	R	R	R	R	R	R	R	R	R	**S**	R	R	**I**	R	R	R
2	4041473 III	B	R	R	R	R	R	R	R	R	R	**S**	**I**	R	R	R	R	R
3	0041073 II	B	R	R	R	R	R	R	R	R	R	**S**	**I**	R	R	R	R	R
4	404147 III	C	R	R	R	R	R	R	R	R	**S**	R	R	R	**S**	R	**S**	R
5	0041073 II	C	R	R	R	R	R	R	R	R	**S**	R	R	R	**S**	R	**S**	R
6	0001073 I	D	R	R	R	R	R	R	R	R	R	R	R	R	**I**	R	R	R
7	5041073 VI	D	R	R	R	R	R	R	R	R	R	R	R	R	**I**	R	R	R
8	0001073 I	E	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
9	4001073 V	E	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
10	0001473 IV	A	R	R	R	R	R	R	R	R	R	**S**	R	R	**I**	R	R	R

R = resistant; I = intermediate; S = sensitive.
PRL = piperacillin; TPZ = piperacillin/tazobactam; SAM = ampicillin/sulbactam; CAZ = ceftazidime; FEP = cefepime; CTX = cefotaxime; CRO = ceftriaxone; IMP = imipenem; MEM = meropenem; CN = gentamicin; TOB = tobramycin; AK = amikacin; TE = tetracycline; CIP = ciprofloxacin; LEV = levofloxacin; SXT = trimethoprim/sulfamethoxazole.

Table 4 Minimum inhibitory concentration (MIC) of polymyxin B and of colistin (polymyxin E) using disk diffusion method for the 10 *Acinetobacter baumannii* isolates

Isolate	MIC by disk diffusion test	
	Polymyxin B (mg/L)	Colistin (mg/L)
1	0.5	64
2	0.5	0.5
3	0.25	0.5
4	0.25	128
5	0.25	0.5
6	0.25	0.5
7	0.125	0.5
8	0.125	0.25
9	0.125	0.5
10	0.125	0.25

For polymyxins, in Acinetobacter spp., MIC ≥ 4 mg/L is considered resistant, MIC ≤ 2 is considered sensitive (Clinical and Laboratory Standards Institute guidelines [10])

Among the 10 imipenem-resistant isolates of *A. baumannii*, 6 isolates were carbapenemase producers (60%) (positive by modified Hodge test), 2 isolates were beta-lactamase producers (20%) (positive by AmpC test) and no isolates were MBL producers (all negative by EDTA disk synergy test) (Table 6). Two isolates were positive for both carbapenemase and AmpC beta-lactamases and another 4 isolates were positive for carbapenemase by modified Hodge test but were negative for MBL and AmpC beta-lactamases by EDTA disk synergy test and AmpC disk test respectively. Three isolates were not producing any of the 3 tested beta-lactamases by the 3 phenotypic tests used in our study.

Discussion

A. baumannii has emerged as a leading nosocomial pathogen, particularly in ICUs, where several outbreaks have been described [1,24]. The epidemic potential and the clinical severity of *A. baumannii* infections are primarily related to the propensity of this organism to develop resistance to a variety of antimicrobial agents, including broad-spectrum beta-lactams, aminoglycosides, fluoroquinolones and carbapenems [25]. This renders studies of epidemiology and antibiotic resistance necessary for prevention of further infection with these organisms.

In the current study the prevalence of *A. baumannii* was investigated from different ICU wards in our hospital by making phenotypic characterization using biotyping and antibiogram profiles to investigate the problem of MDR among *A. baumannii* isolates from these critical sites. During the study period, 350 clinical specimens collected from 350 patients with different nosocomial infections from different ICUs in Suez Canal University Hospital. Ten *A. baumannii* isolates (2.9%) were identified. The results of the present study agree with those of Richards et al., who stated that *A. baumannii* represented 2.9% of all nosocomial infections [26]. Similarly, a low prevalence was reported by both Ruiz et al. and Lone et al., who found that *A. baumannii* represented 1.43%

Table 5 Antibiotic susceptibility patterns of tigecycline using both disk diffusion and agar dilution methods for the 10 *Acinetobacter baumannii* isolates

Isolate	MIC by disk diffusion test (mg/L)	Interpretation	Zone diameter (mm) by agar dilution test	Interpretation
1	0.125	S	17	I
2	32	R	8	R
3	32	R	6	R
4	0.25	S	19	S
5	1	S	11	R
6	0.5	S	12	R
7	0.5	S	16	I
8	0.5	S	17	I
9	0.5	S	13	R
10	0.5	S	20	S

Breakpoint ≤ 2 mg/L was considered sensitive according to most published studies [17–19]. Neither CLSI nor EUCAST has any published MIC breakpoints for Acinetobacter spp. susceptibility testing against tigecycline).
Zone diameter ≥ 19 mm = sensitive, 15–18 mm = intermediate, ≤ 14 mm = resistant (US FDA susceptible breakpoints [16]).
R = resistant, I = intermediate, S = sensitive.
MIC = minimum inhibitory concentration;

Table 6 Phenotypic enzyme detection results for the 10 *Acinetobacter baumannii* isolates by 3 different methods

Isolate	Modified Hodge test	EDTA disk synergy test	AmpC disk test
1	–ve	–ve	–ve
2	–ve	–ve	–ve
3	–ve	–ve	–ve
4	+ve	–ve	+ve
5	+ve	–ve	+ve
6	+ve	–ve	–ve
7	+ve	–ve	–ve
8	+ve	–ve	–ve
9	+ve	–ve	–ve
10	–ve	–ve	–ve

–ve = negative; +ve = positive.

and 4.8% of all nosocomial isolates respectively [27,28].

The prevalence of *A. baumannii* in our study was much lower than the rates reported by other authors [29–31]. Kessaris et al. reported that the overall incidence of MDR *A. baumannii* positive cultures was 14.2% [29]. Also Caricato et al. reported that *Acinetobacter* spp. accounted for 11% nosocomial infections [30]. Joshi et al. found that *A. baumannii* represented 9% of bacteriologically positive samples collected from a teaching hospital in India [31]. This can be explained by Siau et al. who showed a relative high frequency of *A. baumannii* infections in South-East Asian countries, and postulated that the hot and humid climates contributed to this high incidence [32].

In our study *Acinetobacter* spp. were isolated from respiratory, urine, blood and burn wound samples. *Acinetobacter* were most commonly isolated from specimens of patients with respiratory infections (5/10) and more rarely from urine samples (1/10). These results agree with those of another 2 studies, which found that most of the *Acinetobacter* spp. isolates (71.4% and 54% respectively) were from respiratory tract infection specimens [29,33]. In contrast, Villers et al. isolated *Acinetobacter* spp. most commonly from urine samples (31%), followed by respiratory tract and wound samples (26.7% and 17.8% respectively) [34]. These variations could be explained by the findings of Bergongne-Berezin, who stated that the predominant sites of *Acinetobacter* spp. nosocomial infection vary with time [35]. In early observations, UTIs predominated in ICUs. More recently, the incidence of UTI has decreased, possibly due to better care of urinary catheters, whereas the incidence of nosocomial pneumonia has increased significantly as reported by several recent surveys [35].

In our study, *A. baumannii* was the only *Acinetobacter* spp. encountered in clinical specimens and this supported the finding that infections by other *Acinetobacter* spp. are infrequent. Other studies found that among different *Acinetobacter* spp., *A. baumannii* was the most prevalent in clinical specimens and the one most often responsible for nosocomial infections [28,31,36]. As no single typing method has so far gained acceptance for typing *Acinetobacter* spp., and this area is still the subject of research, 2 approaches were used in our study: biotyping and antibiograms. By using those 2 phenotypic methods, our isolates were found to be distinct and non-duplicates [37].

Our isolates were shown to be 100% MDR (resistant to more than 3 groups of antibiotics) and 80% extensively drug resistant (XDR) (also resistant to meropenem). Evans et al. in Pakistan found that MDR were 82.4% of his isolates and XDR were 65.0% [38]. This differs from the results of Dent et al., who found that 72% of isolates were MDR and 58% were XDR [39]. This difference may be due to the much larger total number of *A. baumannii* isolates in the latter study (247 isolates) than in our study (10 isolates).

Using the disk diffusion method for testing susceptibility to tigecycline showed that 5/10 were resistant, 3/10 were intermediate and 2/10 were sensitive. However, these results cannot be relied upon, as testing susceptibility against tigecycline using the disk diffusion method has been found to be inaccurate and not reproducible [11] and for this reason the agar dilution method for MIC determination was used (which is now considered the golden standard for testing susceptibility to tigecycline [11]) and this showed that 2/10 were resistant and 8/10 were sensitive.

Beta-lactamase production is the most common mechanism of beta-lactam drug resistance in Gram-negative bacteria. Newer beta-lactamases that hydrolyse cephamycins, oxyimino and zwitterionic cephalosporins, monobactams, or carbapenems are of concern because they put a ceiling on the therapeutic options, lead to treatment failure and are increasing in prevalence [40,41].

Currently, there is no CLSI recommended guidelines to detect AmpC beta-lactamases. In this study, we used AmpC disk testing and found that 20% (2/10) isolates were positive for the enzyme. This agrees with the results of Rodríguez-Martínez et al., who reported 29% incidence of AmpC [42]. However, care is required in interpreting such results with isolates showing reduced carbapenem susceptibility since this

may be due to other currently rare beta-lactamases (carbapenemases) capable of hydrolysing cefoxitin used in the test.

Carbapenems are often used as the antibiotics of last resort for treating infections due to MDR Gram-negative bacilli, because they are stable even in response to extended-spectrum and AmpC beta-lactamases. However, Gram-negative bacilli producing acquired MBLs (integron-encoded MBL and Verona integron-encoded MBL) have been increasingly reported in Asia and Europe [43]. Lee et al. have reported that the Hodge test can be used to screen carbapenemase-producing Gram-negative bacilli and that the imipenem-EDTA double-disk synergy test can distinguish MBL-producing from MBL-non-producing Gram-negative bacilli [44].

In our study we used the modified Hodge test for carpabenemases detection. It showed 60% of strains to be carbapenemases producers. This agrees with Andriamanantena et al., who detected carbapenemases in 86.8% of carbapenems-resistant isolates using the modified Hodge test [45]. On the other hand, Noyal et al. showed that among 46 resistant strains, only 14.3% was positive for carbapenemases by modified Hodge test and this was attributed to the presence of other mechanisms of resistance among the studied strains [46]. In our study the EDTA disk synergy test showed that no single strain was an MBL producer. This corresponds with the results obtained by Andriamanantena et al., who reported that among 53 tested strains, no isolates produced MBLs [45]. It contrasts with Gupta et al., who found that 7.5% of 200 *Acinetobacter* isolates were MBL producers [47]. This shows that MBL production is not an important mechanism of resistance among *A. baumannii* strains and that our tested strains may have been positive for carpabenemases due to the production of OXA-type carbapenemases. However, this needs to be confirmed by molecular methods.

This study has shown that tigecycline had good *in vitro* activity against the clinical isolates of the MDR *A. baumannii*, and that it may be considered as a promising therapeutic option for treatment. Nevertheless, tigecycline resistance was found among 2/10 isolates, as shown by the MIC values using the agar dilution method, and it is concerning to note that those isolates had not previously been exposed to the drug. So before starting treatment, the *in vitro* susceptibility of the isolates to tigecycline, and its MIC, should be assessed to prevent the development and the dissemination of resistance against one of the last available promising and safe therapeutic options that are available to clinicians to fight these bacteria.

This study also raises the importance of screening high-risk patients for carpabenemase producers in order to make a proper decision about treatment regimens. We recommend that proper infection control measures should be initiated to avoid dissemination of resistant carbapenemase-producing strains.

This study had a number of limitations, including those related to the considerable technical difficulties associated with tigecycline and colistin susceptibility testing. The disk diffusion method has been found to be inaccurate and not reproducible for colistin and tigecycline susceptibility testing of *A. baumannii* [11]. Agar dilution and broth microdilution are considered the gold standard susceptibility test methods for this organism. Both are cumbersome to perform and impractical to implement as routine tests in clinical laboratories [11,14]. Another limitation was that tigecycline MICs determined on Mueller-Hinton agar containing manganese at concentrations higher than 8 mg/L may produce falsely elevated MICs, a problem that may occur if testing is performed on standard media [48].

Our study suggests that continuous evaluation of antibiotic policy in hospitals should be done on a routine basis, to avoid irrational prescribing of antibiotics and to treat different infections according to their antibiotic susceptibility profile. With increasing reports of resistance to *A. baumannii* by the new glycylcyclines and polymyxins, together with the side-effects associated with these agents used as monotherapy, further research is recommended to study the possible effect of novel combination therapies as our last resort to treat such infections.

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