Original Research Article

Characterization of Carbapenem Resistant *Acinetobacter baumannii* causing Ventilator associated Pneumonia in ICUs of Zagazig University Hospitals, Egypt

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A B S T R A C T

Ventilator associated pneumonia (VAP) affects approximately 30% of intubated mechanically ventilated patients in intensive care units (ICUs) worldwide. *Acinetobacter baumannii* causing VAP is often resistant to a wide variety of antibiotics including carbapenems that have been considered the drug of choice for the treatment for this infection. To characterize *A. baumannii* as a cause of VAP in our ICUs and to study its incidence, antimicrobial resistant profile and to investigate the presence of carbapenem hydrolyzing class D β-lactamase genes as a cause of carbapenem resistance among the studied isolates. A total of 44 *A. baumannii* isolates were recovered from 135 endotracheal aspirate samples of VAP patients. A. *baumannii* were identified by Matrix-assisted laser desorption ionization–time of flight mass spectrometry and its antimicrobial susceptibility was investigated by VITEK 2 instrument and E test, class D β- lactamase genes were investigated by multiplex PCR technique. All *A. baumannii* isolates were multidrug resistant;75% were resistant to carbapenems while colistin remains the most active compound among the studied isolates with sensitivity rate of 93.2%. Multiplex PCR results showed that all *A. baumannii* isolates were positive for bla_{OXA-51}- like gene while 69.7% of carbapenems resistant isolates were positive for bla_{OXA-23} like gene,bla_{OXA-58} and bla_{OXA-24} like genes were not detected in any of the studied isolates. This study highlights the emergence of carbapenem resistant *A. baumannii* as a cause of VAP in our ICUs that was mostly due the presence of bla_{OXA-23} like gene.

Introduction

Ventilator-associated pneumonia (VAP) is a common serious healthcare-associated infection among patients in intensive care units (ICUs) who have endotracheal intubation or a tracheostomy for mechanical ventilation, affecting an estimated 10–30 % of ventilated patients worldwide (Bantar *et al*., 2008). *Acinetobacter baumannii (A. baumannii)*is an important opportunistic pathogen associated with ventilator-associated pneumonia and other variety of nosocomial infections in ICUs as central

line-associated bloodstream infections, urinary tract infections and wound infections(Peleg *et al*., 2008). Multidrug-resistant (MDR) *A. baumannii* isolates have been increasingly reported worldwide that are associated with an enhanced risk of mortality and prolonged durations of hospitalization (Antunes *et al*., 2014, Lemos *et al*., 2014).

Carbapenems (e.g., imipenem, meropenem) have historically retained the best antibacterial activity and are considered as the treatment of choice for this pathogen however nowadays clinicians are very concerned for the carbapenemase producer *A. baumannii*, the increasing rates of this resistance worldwide limit the range of therapeutic alternatives (Dai *et al*., 2013).

Carbapenem resistance in *A. baumannii*is most commonly caused by the production of carbapenemases; enzymes belonging to Ambler classes B, A and D (Bush and Jacoby, 2010). The most prevalent mechanism is carbapenem hydrolyzing class D β-lactamases (CHDLs) that can be divided into four main subgroups including OXA51, OXA 23, OXA24 and OXA 58, which are encoded by the intrinsic bla_{OXA-51}-like and the acquired carbapenemase genes including bla_{OXA-23}-like, bla_{OXA-24}-like and bla_{OXA-58}- like genes respectively (Poirel and Nordmann, 2006, Merkier and Centron, 2006).

The aim of this study was to characterize *A. baumannii* as a major cause of VAP in patients admitted to the ICUs of Zagazig University hospitals and to investigate its incidence, illustrate its antimicrobial resistance pattern and the genetic mechanisms of resistance involved in carbepenam resistant isolates by investigating the presence ofbla_{OXA-51}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-58} like gens.

Materials and Methods

A total of 44 *A. baumannii* isolates were recovered from 135 endotracheal aspirate samples of patients with VAP admitted to the ICUs of Zagazig University Hospitals over 18months period (September 2014 to February2016). Approval for this study was obtained from Research Administration and Research Ethics Committee of Faculty of Medicine, Zagazig University. The collected samples were transported to the microbiology laboratory and inoculated on Mac Conkey agar, blood agar and chocolate agar plates that incubated in aerobic, CO_2 condition at37°C for 24-48 hours.

Identification

All the organisms were identified by Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) using the VITEK MS system (Biomerieux. Inc, Durham, USA).*A. baumannii* isolates were further investigated by:

Antibiotic susceptibility testing

Antibiotic susceptibility testing was carried out using Vitek 2 System (card no 222) for Gram negative bacilli (Biomerieux. Inc, Durham, USA) in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2012), the following antibiotics were included: amikacin, aztreonam, cefepime, ceftazidime, ciprofloxacin, colistin, gentamicin, imipenem, meropenem, minocyclin, pefloxacin, piperacillin, piperacillin/ tazobactam, rifampicin, ticarcillin, ticarcillin/clavulanic acid, tobramycin, and sulfamethoxazole. Susceptibility of *A. baumannii* isolates to imipenem and meropenem was confirmed Etest strips (Biomerieux, France) that were performed according to the manufacturer's instructions.

PCR

All *A. baumannii* isolates were analyzed by multiplex PCR to detect bla_{OXA-51}-like, bla_{OXA-23}-like, bla_{OXA-24}-like and bla_{OXA-58}-like genes as follow:

DNA was extracted from isolated *A. baumannii* colonies by using QIAamp® DNA Mini kit (Qiagen GmbH, Germany), for DNA amplification Qiagen multiplex plus kit (Qiagen, Hilden, Germany) were used as described by the manufacture. PCR amplification conditions were as follows: initial denaturation at 94°C for 3 mins followed by 30 cycles of amplification. Each cycle consists of 25 s at 94°C, 40 s at 52°C and 50 s at72°C with a final extension of 5 min at 72°C. The amplified PCR products were visualized on 2 % agarose gel stained with ethidium bromide and examined under ultraviolet light; the molecular size marker is a 100-bp ladder (NEB, Frankfurt am Main, Germany). A single DNA band at 353 bp was recorded as positive for the bla_{OXA-51}-like gene and at 501 bp was recorded as positive for the bla_{OXA-23}-like gene, for bla_{OXA-58} like and bla_{OXA-24} like genes bands were expected to be at599 bp and 246 bp respectively. Primers used in this study are shown on table 1.

Statistical analysis

Data were analyzed using SPSS 20. Chi Square was used to compare categorical variables. P value of 0.05 was considered statistically significant

Results and Discussion

A. baumannii was the most common isolated organism from endotracheal aspirate samples of all VAP patients (44/135) 32.6%, followed by *Klebsiella* 23.7% (32/135), *Staphylococci* 22.2% (30/135),

Pseudomonas 14.8% (20/135), *E. coli* which represent 6.7%(9/135).

Hospitalization for more than 14 days, prior use of antibiotics were identified as significant risk factors for *A. baumannii* resistant to carbapenems (P0.016, P0.012 respectively), while age and sex were not significant risk factors (table 2)

Antimicrobial susceptibility

The resistant rate of carbapenem resistant *A. baumannii* (CRAB) to impenim was 75% (33) and to meropenim was72.7%. All *A. baumannii* showed 100% resistant to cefepime, ceftazidime, aztreonam, pipracillin and trimethoprim/ sulfemethoxazole. The resistance to antibiotics were as follow in a descending order: pipracillin/ tazobactam95.5% (42), ticarcillin 93.2% (41), ticarcillin/clavulanic acid 88.6%,(39), ciprofloxacin88%(39), pefloxacin 84.1% (37), gentamycin 75% (33), tobramycin 72.7 %(42), minocycline 70% (31), amikacin 57%(25), rifampicin 34.1% (15),colistin 6.8%(3). Percentages of antimicrobial resistance among the studied *A. baumanni* isolates are illustrated in (figure 1).

Colistin was the most active antibiotic against isolated *A. baumanni* (sensitivity was 93.2%). All *A. baumannii* isolates (100%) were multidrug resistant (MDR). These isolates sensitivity results to imipenem and meropemem were confirmed by E test and reported as resistant when MIC> 16 g/ml.

Molecular analysis of carbapenem resistance genes by PCR

All the 44 *A. baumannii* isolates were positive for the bla_{OXA-51}-like gene (100%), among 33 impenim resistant isolates there

were 23(69.7%) positive isolates for bla_{OXA-23}-like gene, while 10 (30.3%) were not carrying this gene. On the other hand 11 (25%) sensitive isolates to imipenem were also negative to bla_{OXA-23} gene. None of *A. baumannii* isolates were positive for bla_{OXA-24} or bla_{OXA-58} like genes.

Nowadays VAP is one of the most common infections in the intensive care units (ICUs), increasing the length of stay of patients in these units, the cost of the treatment, and the risk of death.(Baxter, 2005).

A. baumannii has probably been the most frequently isolated bacterium from patients with VAP around the world (Garnacho-Montero, 2005). This organism has emerged as one of the most problematic pathogens for healthcare institutions worldwide. Carbapenems remain the antibiotics of choice to treat *A. baumannii* due to both a wider spectrum of antibacterial activity and less frequent side effects. The development of resistance did not spare even this group of antimicrobial drugs that is largely due to the selective pressure from antimicrobials especially in ICUs, in addition to its incredible ability to acquire resistance (Fouad *et al*., 2013).Early detection of such resistance can help the clinician in managing infections, prevent the toxic adverse effect of different antimicrobial agents that may be caused by different trials of empirical treatment and also limit the spread of infections.

In this study, *A. baumannii* represented the predominant isolated organism from endotracheal aspiration samples of VAP patients (32.6%), this result was in agreement with many studies as that of Rit *et al*., (2014) and Chawla (2008)who reported that *A. baumannii* was the most common pathogen in VAP in Pakistan, India, Thailand and Malaysia however, other

organisms were often responsible for VAP in Korea and Taiwan Chawla (2008).The difference in incidences of isolated organisms may be due to different communities, different hospital wards and different strategies of infection control applications among countries.

In this study the risk factors that significantly associated with increase infection with carbapenem resistant *A. baumannii* (CRAB) in VAP patients were include; hospital stay > 14 days and prior use of antibiotics. This is similar to results of studies carried out by several investigators as Özgür *et al*. (2014),Marie *et al*. (2012), Robertino *et al*. (2010),they concluded that longer periods of hospitalization, and prior use of antibiotics are the recognized factors increasing the risk of VAP due to multidrug and impenim resistant *Acinetobacter* infection. On the other hand, there were no statistical significant differences regarding age and sex and presence of CRAB in VAP patients, the same finding concerning age of patients was reported by (Fattouh *et al*., 2014).

This study revealed that CRAB rates to impenim, meropenim were relatively high as the resistant rates to impenim was 75% and to meropenim was 72.7%, these results were in agreement with the results of other studies done in Egypt as that of AlHassan *et al*. (2013)who reported that *A. baumannii* isolates resistant rates were 73% to impenim and / or meropenem, (Al-Agamy *et al*., 2014) reported that the resistant rates were 70% to impenem, other authorsalso concluded that High resistance rates to carbapenems have been observed in Egypt, ranging from 75% to 100% for imipenem and from 61% to 77% for meropenem (Mohamed *et al*., 2011; Ahmed *et al*., 2011; Fouad *et al*., 2013). *A. baumannii* resistant rate to imipenem was found to be 65% in

Saudi Arabia, 95% in Turkey, 47.9% in Algeria, 45% in Tunisia, and 19.14% in Kuwait (Al agamy *et al*., 2013; Cicek *et al.,* 2013; Bakour *et al.,* 2013; Ben othman, 2007; Al Sweih *et al.,* 2012). The increased rates of resistant to carbepenems are most probably due uncontrolled extensive use of these drugs.

All *A. baumannii* isolates were multiple drug resistant (MDR) strains based on resistance to more than two antibiotic groups, these result is consistent with Cherkaoui *et al.,* 2015. Many authors also reported about MDR *A. baumannii* as Pleg *et al.,* 2008, Cicek *et al.,* 2014. None of the studied isolate was pan drug resistant strain. The same also reported by Cicek *et al.,* 2014.

The present study found 100% resistant isolates to cefepime, ceftazidime aztreonam, pipracillin and trimethoprim/ sulfemethoxazole, less resistant to pipracillin/ tazobactam, ticarcillin, ticarcillin/clavulanic acid, ciprofloxacin, pefloxacin, gentamycin, tobramycin, minocycline, amikacin, rifampicin and colistin. This results was consistent to some extent with previous studies carried out in Egypt byAl Agamy *et al*. 2014, Nasr and Attalla, 2012and Mohamed *et al.,* 2011andthey concluded that *A. baumannii* isolates in their studies were 100% resistant to third and fourth generation cephalosporins and variable results of resistance concerning other antibiotics.

We found that colistin was the most effective antibiotic against *A. baumannii* causing VAP infection as its sensitivity rate was 93.2% and it had the least resistant rate (6.8%) among 18 different antibiotic used for antimicrobial sensitivity test. This result is in agreement with a previous Egyptian study by Al-Agamy *et al*., 2014who reported

that the percentage of colistin susceptibility was 95%. Also our finding is consistent with the finding of other studies that reported *A. baumannii* isolates were100% sensitive to colistin such as Fouad *et al*., (2013), Josheghani *et al*., (2016), Cherkaoui *et al*., (2015), Cicek *et al*., (2013), Abdalhamid *et al*., (2014).There is tendency to increase using colistin in treatment of VAP caused by *A. baumannii* as it currently most effective drug (Aydemir *et al*., 2013; Jean & Hsueh, 2011). The colistin resistance rate is relatively low may be because its infrequent use (Al-Agamy *et al*., 2014).

On the other hand some studies reported lower rates of colistin sensitivity as one study in Egypt by Mohamed *et al*., (2011)) as they reported it was 82.2% and while in Saudia Arabia it was 70.9% (Al-Agamy *et al*., 2013).

The most prevalent resistant mechanism in CRAB isolates is carbapemem hydrolyzing class D β-lactamases (CHDLs) that can be divided into four main subgroups, and encoded by intrinsic bla_{OXA-51}-like gene and the acquired carbapenemase genes including bla_{OXA-23}-like, bla_{OXA-24}-like, bla_{OXA-58}- like genes (Adams-Haduch *et al*., 2006).

In this studybla_{OXA-23}gene was most common virulent oxacillinase gene detected among carbepenem resistant *A. baumannii*, this in agreement with many studies reported that bla_{OXA-23} was the most frequent type of carbapenamase identified (Al agamy *et al*., 2013; Cicek *et al*., 2013; Abbot *et al*., 2013; Al Hassan *et al*., 2013; Fouad *et al*., 2013). The percentage of bla_{OXA-23}-like gene detected in impenem resistant isolates were 69.7% (23/33), this result is in agreement with Zowawi *et al.*, 2015; Luo *et al.*, 2015, different percentages of presence of bla_{OXA-23}-like gene were reported in Egypt as 50% (Al Agamy *et al.,* 2014), 52.9% (Al Hassan

et al., 2013), 100% by Fouad et al. 2013, and others also reported all over the world as those reported by Cherkaoue et al., 2015 (51.8%), Val et al., 2015 (85%), 85.7% ElAbd et al., 2014and 100% Rolain et al., 2016.

None of the impenem resistant isolates were positive for bla_{OXA-24}-like or bla_{OXA-58}- like genes this result was in agreement with Rolain et al., (2016). However bla_{OXA-58}-like, and $bla_{OXA-24/40}$-like genes were detected in lower rate than ofbla_{OXA-23}-like genes in many studies in Egypt as that by Al Agamy et al., (2014)who reported that A. baumannii carryingbla_{OXA-58}-like gene were 5% and that carrying $bla_{OXA-24/40}$-like gene is

7.5% ,and another study by AlHassan et al.(2013) reported that the percentage of bla_{OXA-40}, and bla_{OXA-58} were 2.9%, and 14.7%, respectively.

However carbapenem resistance of A. baumanniihas mostly related to the production of bla_{OXA-58}in other countries as Italy and Turkey (Metan et al., 2013, Migliavacca et al., 2013).

Moreoverbla_{OXA-24}-like gene was detected with variable rates inmany countries as Saudi Arabia from 4-45% (Al agamy, 2014, Al Arfaj, 2011), Poland (Nowak et al., 2012), Spain (Villalon, 3013) and in United states (Qi et al., 2007).

Table.1 Primer used for detection of genes encoding oxacillinases in *A. baumannii* isolates

Primer	Nucleotide Sequence (5′—3′)	Amplicon size	
OXA-23F	GATCGGATTGGAGAACCAGA	501	
OXA-23R	ATTTCTGACCGCATTTCCAT		
OXA-51F	TAATGCTTTGATCGGCCTTG	353	
OXA-51R	TGGATTGCACTTCATCTTGG		
OXA24 F	GGTTAGTTGGCCCCCTTAAA	246	
OXA24 R	AGTTGAGCGAAAAGGGGATT		
OXA58F	AAGTATTGGGGCTTGTGCTG	599	
OXA58R	CCCCTCTGCGCTCTACATAC		

Table.2 Risk factors associated with isolation of imipenem sensitive and resistant *A. baumannii* among VAP patients

Risk factors		Imipenem resistant *A. baumannii*(33)		Imipenem sensitive *A. baumannii*(11)		X^2	P value
		No.	%	No.	%		
Age	≤ 40	14	42.4%	5	45.5%	0.32	0.86
	> 40	19	57.6%	6	54.5%		
Length of hospital stay:	<14days	1	3%	3	27.3.1%	5.73	0.016*
	> 14days	32	97%	8	72.7%		
Prior use of antibiotics	Yes	29	87.9%	5	45.5%	6.21	0.012*
	No	4	12.1%	6	54.5%		
Sex	Male	16	48.5%	5	45.5%	0.03	0.86
	Female	17	51.5%	6	54.5%		

* Significant

Fig.1 Percentages of antimicrobial resistance among *A. baumanni* isolates

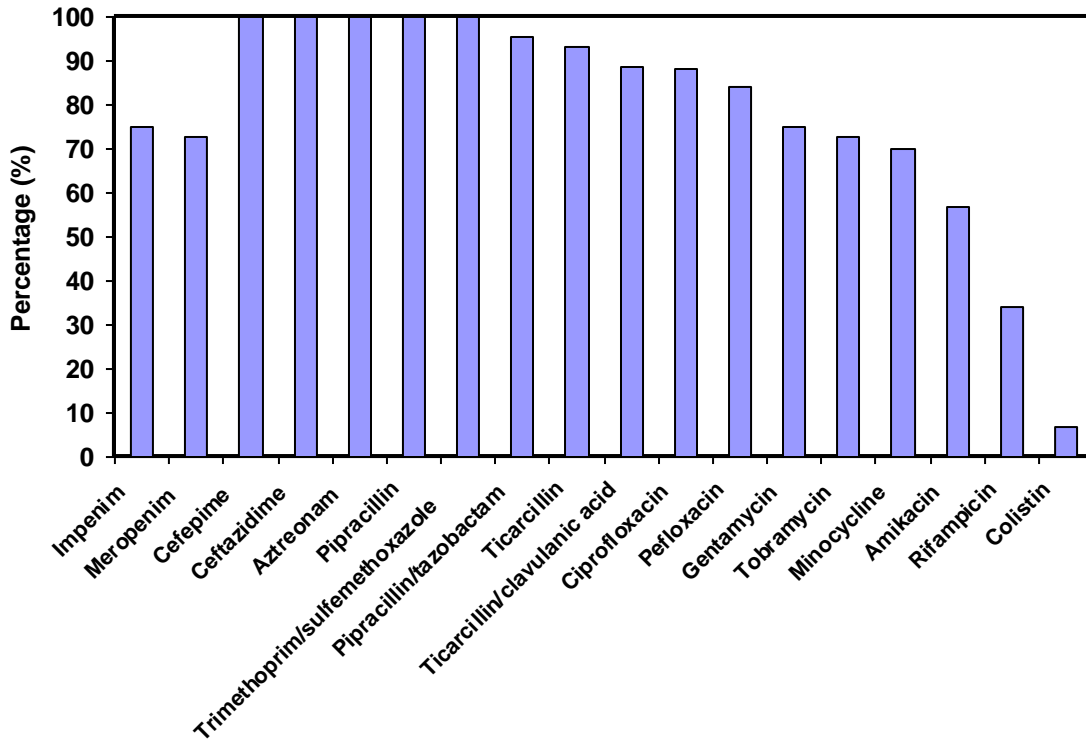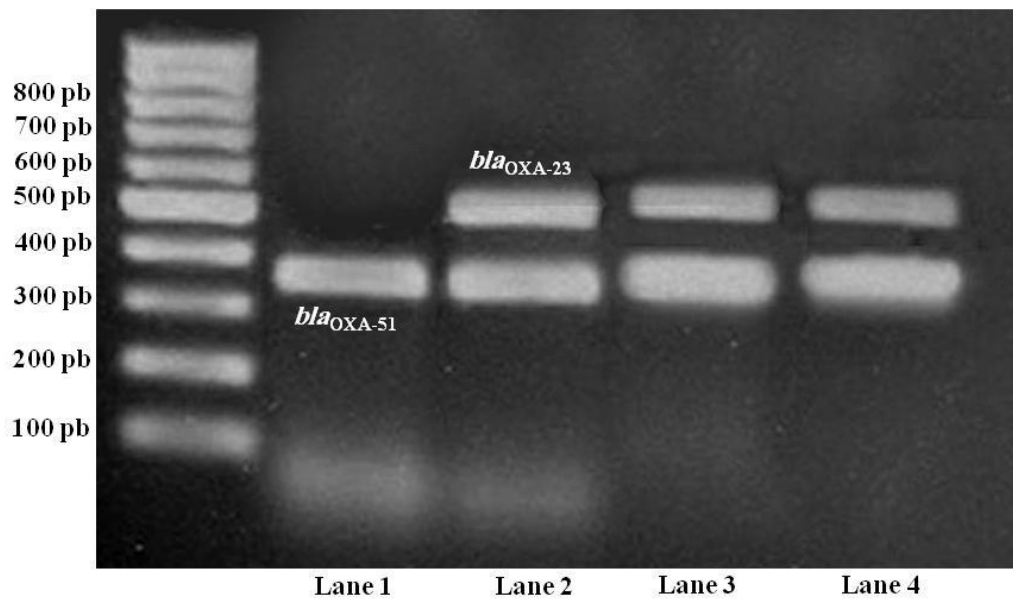**Fig.2** Multiplex PCR results of some *A. baumannii* isolates show intrinsic blaOXA-51-like gene at353 (lanes 1 to 4) and the acquired blaOXA-23-like gene at501 bp (lanes2 to 4).

These variable rates of detecting different types of oxacillinases are mostly due to different antibiotic treatment strategy that may affect the evolutionary direction of *A. baumannii*.

All of the studied isolates including carbepenem sensitive and resistant strains were carrying bla_{OXA-51}-like gene (100%), this in agreement with El Abd *et al.,* 2014 and many other author as Howard *et al.,* 2012; Lee *et al.*, 2012who concluded that this gene occur naturally in *A. baumannii* and it is chromosomally located and widely prevalent and it can be used as a supplementary tool to identify *A. baumannii* at species level.

In this study there were 33 carbapenem resistant strains 23 of them were carrying both bla_{OXA-51}-like gene andbla_{OXA-23}-like gene. There were11 resistant strains carrying only bla_{OXA-51}-like gene and no other CHDLs genes detected, this may be due to presence of other resistance mechanisms as acquisition of m*et al*lo β-lactamases.

This study had some limitation as a relatively small number of *A. baumannii* that was due to low numbers of VAP cases in comparison to other types of pneumonia admitted in our hospitals during the period of study. Other limitation of this study is being it is a single center study and our finding may not be applied to other hospitals in Egypt.

Multicenter molecular based epidemiological studies of *A. baumannii* with longer surveillance duration are recommended for better understanding of the prevalence and distribution of the carbapemase genes, that can help in prevention of the spread of carbapenem resistant *A. baumannii* in Egyptian hospitals and support the determination of priorities for local intervention actions. Also careful choose of empirical antimicrobial agent in VAP patients should be of great concern.

In conclusion, this study highlights multidrug resistant *A. baumannii* as the main cause VAP in our ICUs. Carbapenem resistance was significant in the studied isolates; the most common gene responsible for this resistance was bla_{OXA-23}-like gene. Colistin is the most effective antimicrobial agent against the studied isolates. The recovery of these resistant strains for the first time is emerging threat in our hospitals, as it will contribute in increasing patient hospital stay and associated costs; also it will lead to some difficulties in choice of empirical treatment in VAP patients. Monitoring the use of carbapenems and strict infection control measures are required for controlling the spread of the resistance and for optimizing the treatment of patients with *A. baumannii*.

Conflict of interest

The authors declare no conflict of interest This research did not receive any specific grant from funding agencies in the public, commercial or not-for- profit sector.

Abbreviations

VAP:Ventilator-associated pneumonia, MALDI-TOF MS: Matrix-assisted laser desorption ionization–time of flight mass spectrometry,MDR: multidrug-resistant, CRAB: carbapenem resistant *A. baumannii*, CHDLs:hydrolyzing class D β-lactamases

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