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Detection of *bla* $_{VIM}$ and *bla* $_{IMP}$ metallo-β-lactamase genes by Multiplex PCR among carbapenem resistant *Acinetobacter baumannii* strains isolated from ventilator associated pneumonia in ICU patients

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ABSTRACT

This study was undertaken to examine the microbial causes of ventilator associated pneumonia (VAP) among adult intensive care unit (ICU) patients of Al-ansar Hospital, Medina, KSA and to detect the prevalence of metallo-β-lactamase (MBL) in carbapenem-resistant *A. baumannii*. Microorganisms obtained from different respiratory specimens in patients diagnosed with VAP between January 2011 to January 2014 were included in the current analysis. Standard laboratory methods were used to identify microorganisms, and a standardized susceptibility test was performed. Vitek 2 compact identification system (BioMerieux) using GP-67 and GN-291 cards were also used to confirm the identification. Screening for the detection of MBL in *A. baumannii* strains was done by disc potention test with EDTA impregnated- impenem and meropenem discs and confirmed by multiplex PCR. A total of 208 clinical isolates of bacteria including Gram negative bacteria constituted 150 (72.1%) of VAP, while Gram positive bacteria constituted 58 (27.9%) of isolates. The most common pathogen associated with VAP was *Acinetobacter* spp. (26.9%). Patients with primary VAP associated with *Acinetobacter* spp. were more likely to have nasogastric intubation, sedation and they were on mechanical ventilator for more than 15 days. Out of the 56 strains of *A. baumannii* isolated form VAP patients, 35 were carbapenem-resistant; of these, 20 were positive for MBL production by potentiated disk test, 17 were positive for *bla* $_{VIM}$ by multiplex PCR but none of our strains were positive for *bla* $_{IMP}$. In conclusion, gram-negative pathogens are responsible for most of VAP cases in our patients. *A. baumannii* is the most common pathogen associated with VAP, especially recurrent cases. Carbapenem resistance in *A. baumannii* is mainly mediated by MBL production. The common MBL gene is *bla* $_{VIM}$.

Keywords: Recurrent ventilator-associated pneumonia, *Acinetobacter baumannii, bla* $_{VIM}$, *bla* $_{IMP}$, multiplex PCR.

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INTRODUCTION

Ventilator-associated pneumonia (VAP) continues to complicate the course of 8 to 28% of patients receiving mechanical ventilation and accounts for 15 to 25% of all types of intensive care unit (ICU) acquired infections (Klevens et al., 2007). Hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) are important causes of morbidity and mortality, with mortality rates approaching 62% (Rosenthal et al., 2010). Increasing drug resistance rates among Gram negative pathogens that frequently cause VAP may compromise treatment and result in prolongation of hospital stays, inflation of health care costs and further increase in hospital mortality (Amin, 2009).

Acinetobacter spp. have become major pathogens in hospital-associated infections, especially in critical care settings such as ICUs. They can survive in the hospital environment for long periods and have a remarkable propensity to develop resistance to multiple classes of

antimicrobial agents.

Acinetobacter baumannii is the most clinical important species of the *Acinetobacter* genus, as it is responsible for over 90% of infections and it is often involved in outbreaks (Murray and Hospenthal, 2008; Ecker et al., 2006).

The broad-spectrum β-lactam antibiotics, carbapenems were introduced by 1985 and have been for years the most important agents for the treatment of infections caused by multidrug resistant *A. baumannii* (MDRAB). However, today carbapenem resistance is more frequently reported worldwide, attributable mainly to Ambler class D carbapenemases and class B metallo-ß-lactamases (MBLs) (Poirel and Nordmann, 2006).

Carbapenem-resistant isolates of *A. baumannii* are usually resistant to all classes of antimicrobials, and show susceptibility to tigecycline and colistin (Ecker et al., 2006; Poirel and Nordmann, 2006). The resistance of *A. baumannii* to carbapenems can be mediated by one of the resistance mechanisms that are known to occur in bacteria, including enzymatic inactivation, active efflux of drugs, and modification of target sites. The production of carbapenem-hydrolyzing ß-lactamases is the most common mechanism responsible for carbapenem resistance in *A. baumannii* (Poirel and Nordmann, 2006). Among them, bla_{IMP} and bla_{VIM} are the most common types of MBLs with worldwide distribution. VIM (Veronese Imipenemase) enzymes have been grouped into three main clusters designated VIM-1, VIM-2, and VIM-7. To date, VIM-2 is more widely spread among *P. aeruginosa* isolates, whereas VIM-1 is normally confined to *Enterobacteriaceae* isolates. The IMP gene has at least 27 unique variants differing by up to 22% amino acid sequence divergence (between IMP-9 and IMP-19) that exhibit important structural and functional differences from each other or from enzymes of other genes. IMP-type MBL determinants are mostly found in *P. aeruginosa*, *A. baumannii* and *Enterobacteriaceae* isolates (Kouda et al., 2009; Walsh et al., 2005).

This study was undertaken to examine the microbial causes of VAP among adult ICU patients of Al-ansar Hospital, Medina, KSA and to detect the prevalence of MBLs in carbapenem-resistant *A. baumannii*, which is one of the common organism associated with VAP.

MATERIALS AND METHODS

Setting

The current study was conducted at adult ICU of Al-ansar Hospital, Medina, KSA. Al-ansar Hospital an approximately 200-bed tertiary care facility that provides health care services to Saudi patients, Hajj and Omera patients. The ICU at Al-ansar Hospital is a 20-beds covered 24 h/7 days a week and admits approximately 950 patients /year. The nurse-patient ratio is 1:1.

VAP definition

Diagnosis of Ventilator Associated Pneumonia (VAP) was based on pneumonia that occurred 48 h after endotracheal intubation, with the following diagnostic criteria: new or progressive infiltrates, consolidation, or cavitation on chest X-ray, with one of the following: (a) new onset purulent bronchial secretions with leukopenia (white blood cells $<4 \times 10^9$/l) or leukocytosis ($>12 \times 10^9$/l), or core temperature >38.5 without other cause, (b) significant positive culture from endotracheal aspirate or bronchoalveolar lavage (BAL). Both primary (first) and recurrent (second or third) episodes of VAP during the same hospitalization were included in the analysis. Recurrent VAP was diagnosed using the same criteria as primary VAP only after an evidence of clearance of the previous VAP.

VAP surveillance

On a daily basis, data were collected prospectively from all the patients admitted in the ICUs. Data were gathered according to the CDC-NNIS and CDC-NHSN definitions for DA-HAI (Horan et al., 2008) and the methodology followed was as proposed by INICC (Rosenthal et al., 2008).

Microorganisms

Microorganisms obtained from different respiratory specimens from patients diagnosed with VAP between January 2011 and January 2014, were included in the current analysis. Specimens examined included endotracheal aspirate and BAL. Specimens were processed in a quantitative manner with a cut-off of 10^4 CFU/ml. The isolates were identified by conventional methods (Clinical Laboratory Standards Institute, 2010). All isolates were non-duplicate. *Escherichia coli* ATCC 25922 was used as quality control strain.

Susceptibility testing

Susceptibility testing was performed using amikacin, gentamicin, tobramicin impenem, meropenem, azteronam, ceftazidime, piperacillin, piperacillin/tazobactam, cefepime, ticarcillin, tigacycline, tetracycline, and ciprofloxacin (BBL chemical, USA) by disk diffusion method according to NCCLS guidelines (Clinical Laboratory Standards Institute, 2010). Vitek compact identification system (bioMe´rieux) cards was also used to confirm the identification using GP-67, and GN-291 cards and for antimicrobial susceptibility testing using AST –P580 and AST -N232 cards.

MBL production

Gram negative bacilli isolates were subjected to antibiotic susceptibility testing by Kirby Bauer disk diffusion method as per the CLSI guidelines (Hemalatha et al., 2005). Acinetobacter isolates resistant to imipenem and meropenam were subjected to screening for detection of MBL that was done by disc potention test with EDTA impregnated impenem discs and EDTA impregnated meropenem discs and confirmed by multiplex PCR (Hemalatha et al., 2005).

Disc potentiation test method

Acinetobacter spp. with resistant to imipenem, meropenam were inoculated into Mueller Hinton plates (opacity adjusted to 0.5 MacFarland). The plates were inoculated using cotton-tipped applicators. A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA. $2H_2O$ in 1000 ml of distilled water and

Table 1. PCR: genes, primer sequences, and amplicons size.

Genes	Primer sequence (5'-3')	Amplicon size (bp)	Reference
VIM	F : 5´-GAT GGT GTT TGG TCG CAT A-3´ R: 5´- CGA ATG CGC AGC ACC AG-3´	390	14
IMP	F : 5′-GGA ATA GAG TGG CTT AAY TCT C-3′ R : 5′- CCA AAC YAC TAS GTT ATC T-3′	188	14

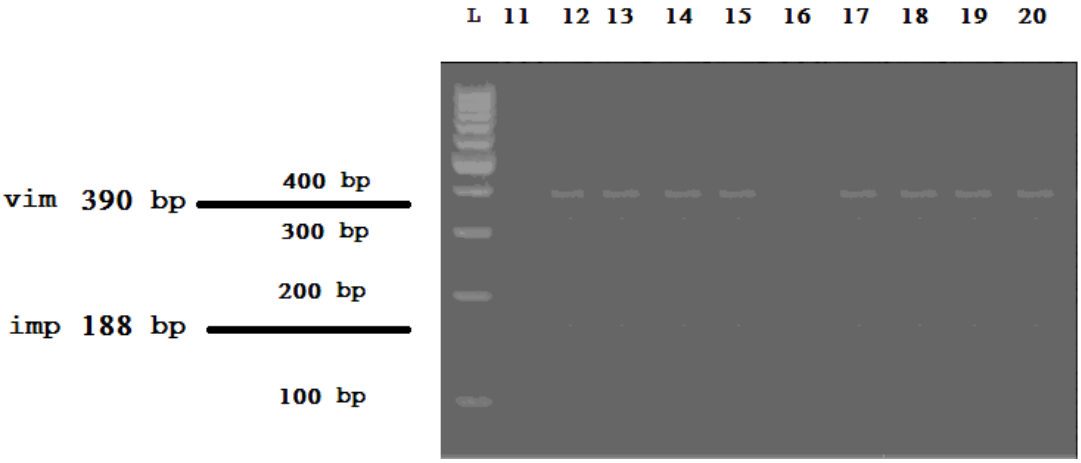

Figure 1. Multiplex PCR assay for *bla* $_{VIM}$ and *bla* $_{IMP}$ genes . Agarose gel electrophoresis showing: Lane L 100 to 1000 base pair (bp) ladder. Lane 11 to 20 are all positive for *bla* $_{VIM}$ gene (390 bp) except lane 11,16, None of the samples were positive for *bla* $_{IMP}$ gene (188 bp).

its pH was adjusted to 8.0 by using NaOH. The mixture was sterilized by autoclaving. Two 10 µg imipenem discs and meropenem discs (ETP; Becton Dickinson, Sparks, MD) were placed to the plate, 5µl of EDTA solution was added to one of them to obtain a concentration of 750 µg. The inhibition zones of the imipenem and imipenem EDTA discs were compared after 18 h of incubation at 35°C. An increase of the zone size to ≥7 mm around imipenem-EDTA and meropenem-EDTA discs was recorded as MBL-positive strain (Lee et al., 2003).

PCR

MBL-positive *A. baumannii* strains were tested for the presence of *bla* $_{VIM}$ and *bla* $_{IMP}$ by Multiplex PCR which was carried out using primers as described by Mendes et al. (2009) (Table 1) (Mendes et al., 2009). A total of 4 to 5 identical colonies of *A. baumannii* were re-suspended in 500 µl of sterile saline in 1.5 ml Eppendorf tubes. This was centrifuged at 8000 rpm for 5 min and the supernatant containing DNA was used for further processing. The PCR mixture used was as follow: 1 ul DNA template in a 50 µl mixture containing 10 mM Tris/ HCl (pH 8.8), 50 mM KCl, 4 mM $MgCl_2$, 0.2 mM dNTPs (Roche Diagnostics, Mannheim, Germany), and 1 µl of each of the forward and reverse primers (Promega, Madison, WI, USA) and one Unit of Taq Polymerase (Promega, Madison, WI, USA). The PCR conditions consisted of an initial denaturation step at 94°C for 5 min, followed by 33 cycles of denaturation at 94°C for 30 s, annealing at 56°C, for 30 s, elongation at 72°C for 45 s, plus a final extension step at 72°C for 7 min. The PCR products of 188 bp for *bla* $_{IMP}$ and 390 for *bla* $_{VIM}$ were visualized by 1.5% agarose gel electrophoresis containing ethidium bromide, 0.5 µg/ml (Bangalore Genei) (Mendes et al., 2009). (Figure 1)

Statistical analysis

The data were analyzed using Package for Social Sciences 19.0 for Windows" (SPSS-19) software (SPSS Inc., Chicago, IL, USA). Data were expressed as frequencies, percents. Chi-square and Fishers exact test used for comparisons of categorical data. A p value < 0.05 was considered statistically significant.

RESULTS

During the study period, a total of 2650 cases were admitted to the ICU; of these, 1720 cases were on ventilator and 239 cases were diagnosed as VAP. About 15% of the VAP cases were diagnosed mainly based on clinical signs and symptoms with no pathogen identified. A total of 208 pathogens were identified during the study; 170 (92.1%) were associated with primary VAP and 38 (7.9%) were associated with recurrent VAP. Gram negative and Gram positive bacteria constituted 150 (72.1%) and 58 (27.9%), respectively, of 208 clinical bacterial isolates recovered from patients diagnosed with VAP. The isolates were obtained from tracheal aspirates (90%) and BAL (10%) as all our patients are intubated (Table 2).

Table 2. Distribution of bacteria isolated from VAP among primary and recurrent cases.

Isolated organism	Primary VAP (n = 170)	Recurrent VAP (n = 38)	Overall (n =208)	P-value	Sig
GPC	49 (28.8%)	9 (23.7%)	58 (27.9%)		
S. aureus	30 (17.6%)	5 (13.1%)	35 (16.8 %)	0.66	NS
MRSA	15 (8.8%)	3 (7.8%)	18 (8.6%)	0.90	NS
S. pneumoniae	4 (2.3%)	1(2.6%)	5 (2.4%)	0.63	NS
GNB	121 (71.2%)	29(78.3%)	150 (72.1%)		
A. baumannii	40 (23.5%)	16 (42.1%)	56 (26.9 %)	0.03	S
P. aeruginosa	38 (22.3%)	6 (15.7)	44 (21.1%)	0.49	NS
Klebsiella spp.	21 (12.3%)	4(10.5%)	23 (11.0%)	0.97	NS
Proteus spp.	11 (6.4%)	2 (5.2%)	13 (6.2%)	0.92	NS
Escherichia coli	5 (2.9%)	1 (2.6%)	6 (2.9%)	0.66	NS
S. maltophilia	4 (2.3%)	0 (0.0%)	4 (1.9%)	0.77	NS
Serratia spp.	2 (1.1%)	0 (0.0%)	2 (0.96%)	0.76	NS

Table 3. Comparison of demographic and clinical characteristics of patients with primary VAP by type of pathogen.

Studied parameter	Acinetobacter spp. (n = 40)	Other pathogens (n = 130)	Total (n = 170)	P- value
Age				
20-49	7 (17.5%)	23 (17.7%)	30 (17.6%)	0.83
50-80	8 (20%)	28 (21.5%)	36 (21.2%)	0.98
>80	25 (62.5%)	79 (60.8%)	104 (61.2%)	0.99
Gender				
Male	29 (72.5%)	88 (67.7%)	117 (68.8%)	0.7
Female	11 (27.5%)	42 (32.3%)	53 (31.2%)	0.7
Interventional factors	32 (80%)	78 (60%)	110 (64.7%)	0.03
Nasogastric intubation sedation	24 (60%)	52 (40%)	76 (44.7%)	0.03
Prolonged antibiotic therapy	10 (25%)	28 (21.5%)	38 (22.03%)	0.98
Vevtilator days				
>15day	24 (60%)	51(39.2%)	75 (44.1%)	0.03
≤15day	16 (40%)	79(60.8%)	95 (55.9%)	0.03
ICU Length of stay	27.3 ± 15.2	21.6 ± 20.9	22.5 ± 18.4	0.11

The most common pathogen associated with VAP was *Acinetobacter* spp. (26.9%), followed by *Pseudomonas aeruginosa* (21.1%), *Staphylococcus aureus* (16.8%), *Klebsiella* spp. (11%), *MRSA* (8.6%), *Proteus* spp. (6.1%), *E. coli* (2.9%), *Streptococcus pneumoniae* (2.4%), *Stenotrophomonas maltophilia* (1.9 %), and lastly *Serratia* spp. (0.96%). Gram negative pathogens were slightly more encountered in recurrent VAP (78.3 %) than in primary VAP (71.2%), but the difference was not statistically significant (Table 2). *A. baumannii* was the only pathogen significantly associated with recurrent VAP compared to primary VAP (42.1% vs. 23.5%, p = 0.03). Patients with primary VAP associated with *A. baumannii* were more likely to have nasogastric intubation (p = 0.03), sedation (p = 0.03), and to be mechanically ventilated more than 15 days (Table 3). Fifty six strain of *A. baumannii* were isolated form VAP patients. Out of them, 35 were carbapenem-resistant; of these, 20 were positive for MBL production by potentiated disk test. MBL-positive *A. baumannii* strains were 100% resistant to ceftazidime, aztreonam, piperacillin, piperacillin/ tazobactam, cefepime, tigecycline, tetracycline, and ciprofloxacin, while the resistance rates were 95% for amikacin, impenem and meropenem and 65% for both tobramicin and levofloxacin (Table 3).

Detection of *bla* $_{VIM}$ and *bla* $_{IMP}$ genes by PCR

Among 20 *A. baumannii* isolates, *bla* $_{VIM}$ gene was

Table 4. Antimicrobial resistance profiles and resistant genes detected by Multiplex PCR for 20 carbapenum resistant *A. baumannii* isolates.

	Antibiotic sensitivity profile															Resistant genes detected by PCR	
	AK	**GN**	**TN**	**IMP**	**MER**	**AZT**	**CAZ**	**PIP**	**PIP/ TZ**	**CPM**	**TIC**	**TIG**	**CIP**	**Lev**	**TE**	**VIM**	**IMP**
1	R	S	I	R	R	R	R	R	R	R	R	R	R	R	R	+	-
2	R	S	S	R	R	R	R	R	R	R	R	R	R	I	R	+	-
3	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	+	-
4	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	+	-
5	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	+	-
6	R	S	S	R	R	R	R	R	R	R	R	R	R	I	R	-	-
7	R	S	S	R	R	R	R	R	R	R	R	R	R	I	R	+	-
8	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	+	-
9	R	S	S	R	R	R	R	R	R	R	R	R	R	I	R	+	-
10	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	+	-
11	S	S	R	R	R	R	R	R	R	R	R	R	R	I	R	-	-
12	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	+	-
13	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	+	-
14	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	+	-
15	R	S	S	R	R	R	R	R	R	R	R	R	R	I	R	+	-
16	R	S	S	S	S	R	R	R	R	R	R	R	R	I	R	-	-
17	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	+	-
18	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	+	-
19	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	+	-
20	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	+	-

AK: Amikacin, GN: Gentamicin, TN: Tobramicin, IMP: Impenem, MER: Meropenem, AZT: Azteronam, CAZ: Ceftazidime, PIP: Piperacillin, PIP/TAZ:Piperacillin/Tazobactam, CPM: Cefepime, TIC: Ticarcillin, TIG: Tigacycline, CIP: Ciprofloxacin, LEV: Levofloxacin, and TE: Tetracycline.

detected by multiplex PCR in 17 isolates (61.5%) as shown in Figure 1. However, *bla* $_{IMP}$ gene was not detected in any of the isolated *A. baumannii* strains (Table 4).

DISCUSSION

The incidence of nosocomial pneumonia in ventilated patients is high, ranging from 7% to more than 40%. Such HAIs prolong hospital stay and contribute to ICU patient mortality (Jones, 2010; Trouillet et al., 1998). *Acinetobacter* spp. are aerobic, Gram negative coccobacilli that can survive in moist and dry conditions for a prolonged period, so it often leads to nosocomial outbreaks (Manikal et al., 2003; Paterson, 2006).

Our study reports the bacterial causes of VAP among patients of a general adult ICU over a period of 3 years and. *A. baumannii* was the most common VAP pathogen (26.9%), followed by *P. aeruginosa* (21.1%) and *S. aureus* (16.8%). The 3 pathogens together are associated with almost two-thirds of all VAP cases. The current findings are in accordance with previous studies as *Acinetobacter* spp. was the most common isolated pathogen (21 to 36%) in six studies (Arabi et al., 2008). Meanwhile, different data were reported from western and several developing countries. Data from 55 ICUs between 2002 and 2005 in eight developing countries showed that *P. aeruginosa* and *Enterobacteriaceae* were the most common isolated pathogens (26% each), followed by *Acinetobacter* spp. (20%) (Rello et al., 2002). On the other hand, reports from the USA and Europe showed that *S. aureus* (23 to 32%)

and *P. aeruginosa* (16 to 22%) were the leading causes of VAP, while *Acinetobacter* spp. was responsible for only 4 to 8% of VAP cases (Jones, 2010; Hidron et al., 2008). The high association of *Acinetobacter s*pp. to VAP in our study can be explained by that our VAP patients were exposed to more prolonged use of ventilator (44.1% more than 15 days) than western patients (Rello et al., 2002) and lack of strict application of standard infection control precautions for surfaces and equipment in our ICU, that are insufficient to control *Acinetobacter* spp., which is particularly known for its resistance to disinfectants and long survival on dry surfaces (Barchitta et al., 2009; Weber et al., 2010).

The rate of VAP recurrence in our study (23.5%) was nearly the same as that reported in a meta-analysis done by Siempos et al. (2008) (26.8%) (Siempos et al., 2008). *A. baumannii* was the only pathogen significantly associated with recurrent VAP in our patients. Supporting our finding, Chastre and Fagon (2002) report, which showed that a high rate of VAP relapse was associated with primary infection by *Acinetobacter* spp. (Chastre and Fagon, 2002).

In our investigation, the majority of *A. baumannii* occurred among patients with age more than 50 (82.5%). Age more than 55 was reported by El-Ageery and Al-Hazmi (2014) as a co-factor for the acquisition of *A. baumannii* (El-ageery and Al-hazmi, 2014).

In our study the nasogastric intubation, use of sedation and prolonged use of ventilator were significantly associated with *A. baumannii* infection in VAP cases. This finding was also reported by Aiman et al. (2013) and Balkhy et al. (2014) both studies were carried in Saudi Arabia. As the commonly encountered organism with VAP (especially recurrent type) in this study was carbapenem-resistant *A. baumannii*. The prevalence of MBL (bla_{VIM} and bla_{IMP}) were investigated by phenotypic and molecular methods.

In the present study, the majority of the isolates (95%) were carbapenem resistant. High resistance rates to carbapenems have been observed in previous studies, ranging from 75 to 100% for imipenem and from 61 to 77% for meropenem (Bonnin et al., 2013; Mohamed and Raafat, 2011; Nasr and Attalah, 2013). The resistance to imipenem reflects a problem that might be described as countrywide. In the Middle East and North Africa, the occurrence of imipenem-resistant *A. baumannii* is recognized with alarm. The emergence of *A. baumannii* strains with increased carbapenem resistance in this area of the world may be due to the extensive misuse of carbapenems (Al-Agamy et al., 2014).

The most prevalent mechanism of carbapenem resistance in *A. baumannii* is the enzymatic degradation by carbapenem-hydrolyzing b-lactamases, MBL, mostly VIM and IMP, has been reported sporadically in some parts of the world (Alm El-Din et al., 2014). Nevertheless, in the present study, none of the *A. baumannii* isolates harbored bla_{IMP} but bla_{VIM} was discovered in 85% of *A. baumannii* isolates. Lower rate of detection was reported by other researchers, 15.38%, 16.28 and 46.55% were reported in a study conducted by Alam El Din et al. (2014), Karthika et al. (2009) and Amudhan et al. (2011), respectively.

The absence of bla_{IMP} and bla_{VIM} in 3 out of our 20 tested MBL positive *A. baumannii* strains could be either due to presence of unidentified MBL genes, limitation of the primer set used either with regards to picking up the variant IMP/VIM gene or because of presence of MBL genes other than IMP/VIM, presence of other enzymes (OXA like Ambler class D carbepenemase Amp C β-lactamases) or other mechanism of carbepenem resistance, namely loss of prions, increase in efflux pump activity alteration in penicillin binding proteins (PBPs) (Singh et al., 2009).

In conclusion, Gram negative pathogens are responsible for most of VAP cases in our patients. *A. baumannii* is the most common pathogen associated with VAP, especially recurrent cases. Carbapenem resistance in *A. baumannii* is mainly mediated by MBL production. The common MBL gene is bla_{VIM}.

RECOMMENDATIONS

1. Our ICU should continue to actively screen for *Acinetobacter* spp. in all admitted patients, shorten the duration of ventilation, minimize sedation, encourage oral gastric rather than nasogastric intubation, and improve currently implemented infection control measures, including environmental disinfection.
2. The development of simple and inexpensive screening methods to detect MBL production in microbiology laboratories is crucial for optimal treatment of patients, particularly critically ill and hospitalized patients, and to control the spread of resistance.

Limitation of the study

In this study, only *A.baumannii* strains were subjected to multiplex PCR for detection of the common MBL resistance genes (bla_{VIM} and bla_{IMP}), because they are the most common organisms isolated from VAP patients. Other commonly isolated organisms, such as *P. aeruginosa*, were not included due to limited resources.

Ethical approval

Adherence to Saudi Arabia regulations concerning the welfare of human subjects was maintained throughout the study. The participant's rights, privacy, health, and well-being were safeguarded through informed consent forms that they asked to read and sign if they agreed to participate in the study.

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