Original Article

# Metallo-Beta-Lactamase-Producing Multidrug-Resistant Acinetobacter Isolates in Patients with Ventilator-Associated Pneumonia

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## Abstract

**Background:** *Acinetobacter*, a nonfermenting Gram-negative coccobacilli, have emerged as significant pathogens causing multidrug-resistant (MDR) ventilator-associated pneumonia (VAP). Metallo-beta-lactamase (MBL)-producing *Acinetobacter* spp. have become an emerging therapeutic concern worldwide due to the MDR isolates. **Aim and Objectives:** Phenotypic detection of MBL producing MDR *Acinetobacter* isolates in patients with VAP and to study the antibiotic susceptibility pattern of MBL-producing isolates. **Materials and Methods:** This was a prospective observational and noninterventional study conducted on patients with VAP over a period of 2 years. This study was conducted at a tertiary care teaching hospital in the intensive care unit. A total of 164 MBL-producing MDR AB isolates were included in the study. MBL was detected by imipenem-EDTA double-disc synergy test (DDST), imipenem-EDTA combined disc synergy test (CDST-IPM), and MBL-E test. **Results:** A total of 188 samples were enrolled for the study, fulfilling the inclusion criteria of VAP. Total MDR *Acinetobacter* spp. isolates were 188 (76.42%) of them, 164 (87.23%) were MBL producing and 24 (12.76%) were nonMBL ($P < 0.002$). Total 11.17% and 88.83% MDR VAP due to *Acinetobacter* spp. were early-onset VAP and Late-onset VAP, respectively ($P < 0.001$). Late-onset VAP due to MDR *Acinetobacter* spp. was predominant in the present study caused by *Acinetobacter* spp. Of total 188 MDR *Acinetobacter* isolates, 156 (82.98%) were *Acinetobacter baumannii*, 15 (7.98%) were *Acinetobacter iwoffii*, 9 (4.79%) were *Acinetobacter calcoacetiucs*, 5 (2.66%) were *Acinetobacter hemotyticus,* and 3 (1.59%) were ABC complex, predominated by *A. baumannii* ($P < 0.001$). Of total 188 MDR *Acinetobacter* spp. 164 (87.23%) were putative MBL producing and 24 (12.67%) were nonMBL *Acinetobacter* spp. Of 164 MBL-producing isolates, 141 (85.98%) were detected by the DDST method, and 23 (14.02%) were DDST negative. Total 146 (89.02%) MDR *Acinetobacter* spp. were detected by a combined disc test-IMP test. A total of 152 (92%) MDR *Acinetobacter* spp. were detected by MBLe-Test. All MBL-producing MDR *Acinetobacter* spp. isolates (164) were resistant to piperacillin (PI), piperacillin + tazobactam (PIT), ciprofloxacin (CIP), ceftazidime (CAZ), cefepime (CPM), imipenem (IMP), and meropenem (MRP). The tigecycline (21.34%) resistance was significantly less compared to all other antibiotics. **Conclusions:** The present study highlighted the burden of MDR MBL producing *Acinetobacter* spp. in patients with VAP. About three-fourth of patients with VAP had MDR *Acinetobacter* spp. Eighty percent were MDR *Acinetobacter* spp. were MBL producers. MDR *Acinetobacter* isolates, including MBL producer, were significantly higher in late-onset VAP. The ability of phenotypic identification of *Acinetobacter* spp. for MBL producer among imipenem-EDTA double-disc synergy test (DDST), CDST-IPM and MBL-E Test were comparable. All MBL-producing MDR *Acinetobacter* spp. isolates were resistant to PI, Ciprofloxacin, CAZ, CPM, IMP, and MRP. The tigecycline resistance was significantly less ($1/5^{th}$). The study of antibiotic sensitivity patterns and screening for MBL production among *A. baumannii* isolates is essential for controlling *Acinetobacter* infections. The judicious use of antimicrobial therapy and combined approaches of rotational antibiotic therapy is strongly suggested.

**Keywords:** *Acinetobacter* species, metallo-beta-lactamase, multi-drug resistant, ventilator-associated pneumonia

## Introduction

Ventilator-associated pneumonia (VAP) is the most common nosocomial infection in intensive care units (ICU), which accounts for >25% of all ICU infections.

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*Acinetobacter* is a nonmotile, encapsulated, nonlactose fermenting Gram-negative coccobacillus. Documenting carbapenem-resistant *Acinetobacter* is very important as these strains may often cause outbreaks in the ICU setting and are responsible for the increased mortality and morbidity or limiting therapeutic options. Treatment of these pathogens has become a major challenge to clinicians worldwide, due to their increasing tendency to antibiotic resistance. MBL-producing *Acinetobacter* spp. have become an emerging therapeutic concern worldwide. *Acinetobacter baumannii* is a pleomorphic aerobe Gram-negative bacterium. Resistance to broad-spectrum beta-lactams, mediated by metallo-beta-lactamase (MBL) enzymes, is an increasing problem worldwide. *A. baumannii* is an emerging multi-drug resistant (MDR) opportunistic pathogen that causes a variety of nosocomial infections, including VAP. Metallo-β-lactamase (MBL)-producing isolates have a strong impact on diagnostic and therapeutic decisions. A high frequency of MBL-producing gram-negative bacilli has been reported worldwide. *A. baumannii* is an emerging MDR opportunistic pathogen that causes a variety of nosocomial infections. In recent years, carbapenem resistance (CR) in *A. baumannii* has increased due to Ambler class B Metallo β-lactamases or class D OXA Carbapenemases. The increased prevalence of carbapenem-resistant Gram-negative isolates caused by Metallo-β-lactamase (MBL) is worrisome in clinical settings worldwide. The mortality rate associated with infections caused by MBLs-producing organisms ranging from 18% to 67%.[1] MDR *A. baumannii* has emerged as an important nosocomial pathogen associated with VAP. Limited therapeutic options contribute to increased morbidity and mortality. *A. baumannii* can persist in the environment for prolonged periods. There is an increasing trend of CR and multi-drug resistance (MDR) in *A. baumannii* worldwide with limited therapeutic antibiotic therapy options. All isolates exhibited MDR phenotype.[2] There are scanty data available regarding Metallo-β-lactamase (MBL) producing *Acinetobacter* spp. causing VAP in Indian context. The present study was conducted to find the occurrence of MBL producing MDR *Acinetobacter* spp. and to study their antibiotic susceptibility pattern in patients with VAP.

## Materials and Methods

Aim and objectives: Phenotypic detection of MBL-producing MDR *Acinetobacter* species isolates in patients with VAP and to study the antibiotic susceptibility pattern of MBL-producing isolates. Study design: This was a prospective observational and noninterventional study conducted on a patient with VAP over a period of 2 years (January 2016-December 2017). Study Setting: This study was conducted at a tertiary care teaching hospital in ICU. Sample size: A total of 164 MBL-producing MDR AB isolates were included in the study. Ethical approval: This study obtained ethical approval from the Ethics committee Krishna Institute of Medical Sciences Karad Maharashtra, India (Reference No.: KIMSDU/IEC/4/2013). Criteria for the diagnosis of VAP: The diagnosis of VAP was based on clinical and microbiological criteria. The patients who had mechanical ventilation by endotracheal tube (ETT) for >48 h. A clinical suspicion of VAP was made in patients with a Modified Clinical Pulmonary Infection Score >6; the diagnosis was confirmed by performing a quantitative culture of the endotracheal aspirate (ETA) and observing $\geq 10^5$ cfu/ml. Fever/hypothermia or leukocytosis/leucopenia; purulent tracheal discharge; positive chest X-ray (chest X-ray shows consolidation or infiltration or pleural effusion) were included in the study.[2] Sampling technique: The ETA was collected by nonbronchoscopic method. The ETA was collected using a 22-inch Ramson's 12-F suction catheter with a mucus extractor, which was gently introduced through the ETT for a distance of approximately 25-26 cm. Gentle aspiration was then performed without instilling saline, and the catheter was withdrawn from the ETT. After the catheter was withdrawn, 2 mL of sterile 0.9% normal saline was injected into it with a sterile syringe to flush the exudates into a sterile container for collection and transported to the microbiology laboratory. ETA samples were immediately processed. The results of the Gram's stain were obtained within the 1st and quantitative cultures were performed immediately as proceeded by Rajashekar *et al*. Exclusion criteria: Patients who had severe hypoxemia (PaO2/FiO2 <100), immunocompromised, or neutropenic symptoms were excluded from the study [Figure 1]. Sample selection: All the samples were subjected to Gram's staining for microscopic examination and culture as per standard guidelines. The ETT secretions were cultured on blood agar and MacConkey agar. The culture plates were incubated at 370 C. [Figures 2 and 3]. The standard guidelines were used for the identification of the isolates.[3] Antibiotic sensitivity testing was done using the Kirby–Bauer disc diffusion test using Mueller–Hinton agar and commercially available antibiotic discs (HiMedia, Mumbai). Antibiotic susceptibility testing was performed using Kirby-Bauer Disc Diffusion method according to CLSI guidelines. The selection of antibiotics was based on CLSI guidelines. *P. aeruginosa* ATCC 27583 was used as quality control strain.[4,5] All the isolates were screened simultaneously for MBL detection by using imipenem (IMP) and meropenem (MRP) discs by Kirby–Bauer disc diffusion method as per Clinical and Laboratory Standard Institute guidelines. All IMP-resistance strains were confirmed for MBL production by Imipenem-EDTA double-disc synergy test (DDST), Imipenem-EDTA combined-disc synergy test (CDST-IPM) and E-test.

### Metallo-beta-lactamase detection methods [Figure 4]

#### *Imipenem-EDTA double-disc synergy test (DDST)*

Imipenem-EDTA double-disc synergy test (DDST) was performed as described by Lee *et al*. Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by CLSI. An IMP (10 μg) disc was placed 10 mm edge to edge from a blank disc containing 10 μL of 0.5 M EDTA (750 μg). Enhancement of the zone of inhibition in the area between IMP and EDTA disc in comparison with the zone of inhibition on the far side

of the drug was interpreted as a positive result for MBL production.[6]

### *Imipenem-EDTA combined disc synergy test*

The test isolates along with standard control strains (opacity adjusted to 0.5 McFarland opacity standard) were lawn cultured on Mueller-Hinton agar plate as recommended by CLSI. After drying, two 10 µg IMP discs were placed on the lawn culture with 20 mm distance from center to center of the discs. A volume of 10 µl of 0.5 M EDTA was added to one of the IMP discs and incubated overnight. Isolates showing ≥7 mm increase in the inhibition zone size of Imipenem-EDTA disc than the IMP disc alone were considered as MBL producers.[7]

### *MBL-E test*

The MBL E-test strip (HiMedia, Mumbai) containing a double sided of IMP (4–256 µg/ml) and IMP (1–64 µg/ml) in combination with a fixed concentration of EDTA was used for MBL detection. It was evaluated according to the instructions. A ratio of the MICs of the IMP (IP) to IP plus EDTA (IPI) of ≥8 or the presence of a phantom zone, i.e., an extra inhibition zone between the IP and IPI regions, or a deformation of the IP or IPI ellipses was interpreted as being positive for MBL production.[8]

### Statistical analysis

Data collected were entered in Microsoft Excel. The mean, percentage, standard deviation, and Chi-square test was calculated for quantitative data using Microsoft Excel spreadsheet. Appropriate statistical tests were applied using SPSS Software (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. IBM Corp.: Armonk, New York, NY, USA) was used to analyze the dependent variables and $P < 0.05$ was considered statistically significant.

## Results

A total of 246 isolates were of *Acinetobacter* spp. among patients with VAP. A total 188 samples were enrolled for the study fulfilling inclusion criteria of VAP. Of total 246 isolates with *Acinetobacter* spp. 188 (76.42%) were MDR *Acinetobacter* spp. of them, 164 (87.23%) were MBL-producing *Acinetobacter* spp. and 24 (12.76%) were nonMBL *Acinetobacter* spp. and was statistically significant ($P < 0.002$). Total 11.17% and 88.83% MDR VAP due to *Acinetobacter* spp. were in early-onset VAP and late-onset VAP, respectively ($P < 0.001$). Late-onset VAP due to MDR *Acinetobacter* spp. was predominant in the present study [Table 1].

Of the 188. *Acinetobacter* spp. isolates, 121 (64.36%) were male and 67 (35.64%) were female ($P < 0.05$). Total 112 (92.56%) of male and 52 (77.61%) female isolates were MBL positive. Of total 188 MDR *Acinetobacter* isolates, 156 (82.98%) were *A. baumannii*, 15 (7.98%) were *Acinetobacter iwoffii*, 9 (4.79%) were *Acinetobacter calcoacetiucs*, 5 (2.66%) were *Acinetobacter hemotyticus* and 3 (1.59%) were ABC complex, predominated by *A. baumannii* ($P < 0.001$) [Table 2].

Out of 188 *Acinetobacter* MDR isolates, 57 (30.32%) isolates were from early onset VAP, with 38 (31.40%) males and 19 (28.36%) femles. Amongst remaining 131 (69.68%) *Acinetobacter* MDR isolates were in late onset VAP, of them 83 (68.60%) were males and 48 (71.64%) were females [Table 3].

Total 624 patients fulfilled criteria of VAP according to CIPS ≥6, of them 246 (39.42%) were *Acinetobacter* spp. A total of 188 (76.42%) were MDR *Acinetobater* spp. Of total 188 MDR *Acinetobacter* spp. 164 (87.23%) were putative MBL producing and 24 (12.67%) were nonMBL *Acinetobacter* spp. Of 164 MBL-producing isolates, 141 (85.98%) were detected by DDST method and 23 (14.02%) were DDST negative. Total 146 (89.02%) MDR *Acinetobacter* spp. were deteted by combined disc test (CDT)-IMP test. Total 152 (92%) MDR *Acinetobacter* spp. were deteted by MBLe-Test [Table 4].

All MBL-producing MDR *Acinetobacter* spp. isolates (164) were resistant to piperacillin (PI), piperacillin + tazobactam (PIT), ciprofloxacin (CIP), ceftazidime (CAZ), cefepime (CPM), IMP, and MRP. A total of162 (98.78%) MBL isolates were resistant to ceftriaxone, whereas 152 (92.68%) were resistant to tetracycline. A total of 147 (89.63%) MBL were found to be resistant to doxyoycline, 143 (87.20%) resistant to gentamycin, 137 (83.54%) resistant to amikacin and 131 (79.88%) resistant to trimethoprim-sulfamethoxazole. The tigecycline (21.34%) resistance was significantly less compared to all other antibiotics. Of the nonMBL isolates, 24 (14.63%) were resistant of PI, CAZ, CPM, 21 (12.20%) were resistant to PIT, 20 (12.20%) resistant to ciprofloxacin and tetracyclin each. Ceftriaxone resistance was found in 19 (11.59%) nonMBL isolates, whereas 18 (10.98%) were resistant to doxycycline, 17 (10.37%) nonMBL isolates were resistant to gentamycin, 16 (9.76%) to amikacin, 15 (9.15%) to Trimethoprim-Sulfamethoxazolee and 4 (2.44%) were resistant to tigecycline [Table 5].

Comorbidites, H2 blockers, proton pump inhibitors, steroids, longer length of ICU stay, impaired consciousness, prior antibiotic

**Table 1: Total number of isolates included for the study among the ventilator associated pneumonia patients**

| | *Acinetobacter* spp. (*n*=246), *n* (%) | Early onset VAP, *n* (%) | Late onset VAP, *n* (%) |
|---|---|---|---|
| Total *Acinetobacter* spp. | 246 (39.42) | 56 (22.76) | 190 (77.24) |
| Total MDR *Acinetobacter* spp. | 188 (76.42) | 21 (11.17) | 167 (88.83) |
| MBL producing *Acinetobacter* spp. | 164 (87.23) | 14 (8.54) | 150 (91.46) |
| Non-MBL *Acinetobacter* spp. | 24 (14.63) | 11 (45.83) | 13 (54.17) |

VAP: Ventilator associated pneumonia, MDR: Multidrug resistant, MBL: Metallo-beta-lactamase

therapy, and high SOFA score were significantly associated with MBL *Acinetobacter* spp. associated VAP [Table 6].

## Discussion

The emergence and rapid spread of *bla*IMP and *bla*VIM MBL producing Gram-negative bacteria causing nosocomial infections are of concern worldwide due to limited treatment options. *A. baumannii* is a central cause of nosocomial infections that particularly increase the mortality and morbidity at the ICU of the hospitals.[9] Metallo-β-lactamase (MBL)-producing bacteria leads to resistance to carbapenem, an antibiotic that used as the last resort for the treatment of multidrug-resistant bacteria. The emergence of MBL-producing GNB is a challenge to microbiology laboratories because there are no standardized guidelines available to detect them.[10] *A. baumannii* is an important opportunistic pathogen due to its capabilities for developing mechanisms of resistance to a wide range of antimicrobial agents, including carbapenems. Dissemination of MDR *A. baumannii* is attributed to the extreme use of wide-spectrum antimicrobial drugs in hospitals, cross-infection between inpatients, invasive ICU procedures, and hospitalized patients with diabetes and cancer those are under frequent invasive diagnostic and therapeutic interventions. Although an increasing prevalence of colistin and tigecycline resistance has been reported in many hospitals, combinations of these agents with carbapenems or other antibiotics remain the best therapeutic choice and reasonably safe to treat patients with MDR *A. baumannii* infections. The wide distribution of carbapenem-resistant *A. baumannii* (CRAB) due to several mechanisms with diverse genetic determinants has been documented. The high rates of MDR *A. baumannii* indicate that extensive investigation into the molecular basis of MDR and developing new therapies of CRAB is needed. The development of a local antibiogram database coupled with nationwide antimicrobial stewardship and infection prevention program might help to improve our knowledge of the resistance patterns of *A. baumannii* and in developing a treatment protocol for decreasing the infection burden.[11] The worldwide proliferation of life-threatening metallo-β-lactamase (MBL)-producing Gram-negative bacteria is a serious concern to public health. MBLs are compromising the therapeutic efficacies of β-lactams, particularly carbapenems, which are

**Table 2: Different species of multidrug resistant *Acinetobacter* isolated**

| Species | *n* (%) | MBL (%) | Non-MBL (%) |
|---|---|---|---|
| *A. baumannii* | 156 (82.98) | 153 (93.29) | 3 (12.50) |
| *A. lwoffii* | 15 (07.98) | 6 (03.66) | 9 (37.50) |
| *A. calcoaceticus* | 9 (4.79) | 3 (01.83) | 6 (25.00) |
| *A. hemolyticus* | 5 (2.66) | 2 (01.22) | 3 (12.50) |
| ABC complex | 3 (1.59) | 0 | 3 (12.50) |
| Total | 188 (100.0) | 164 (100.0) | 24 (100.0) |

MBL: Metallo-beta-lactamase, *A. hemotyticus: Acinetobacter hemotyticus, A. calcoacetiucs: Acinetobacter calcoacetiucs, A. iwoffii: Acinetobacter iwoffii, A. baumannii: Acinetobacter baumannii*

**Table 3: *Acinetobacter* spp. multidrug resistant isolates**

| | Total, *n* (%) | Male (*n*=121), *n* (%) | Female (*n*=67), *n* (%) |
|---|---|---|---|
| Early onset VAP | 57 (30.32) | 38 (31.40) | 19 (28.36) |
| Late onset VAP | 131 (69.68) | 83 (68.60) | 48 (71.64) |

VAP: Ventilator associated pneumonia

**Table 4: Different methods for metallo-beta-lactamase detection**

| Results | DDST-IMP (%) | CDT-IMP (%) | MBLe-test (%) |
|---|---|---|---|
| Positive | 141 (85.98) | 146 (89.02) | 152 (92) |
| Negative | 23 (14.02) | 18 (10.98) | 12 (8) |

MBL: Metallo-beta-lactamase, DDST: Double disc synergy test, IMP: Imipenem, CDT: Combined disk test

**Table 5: *Acinetobacter* spp. antibiotics sensitivity profile/pattern**

| Antibiotic disc | MBL (*n*=164) resistant, *n* (%) | MBL sensitive, *n* (%) | Non-MBL (*n*=24) resistant, *n* (%) | Non-MBL sensitive, *n* (%) | *P* |
|---|---|---|---|---|---|
| Piperacillin | 164 (100.00) | 0 (0.00) | 24 (14.63) | 0 (0.00) | <0.01 |
| Piperacillin + tazobactam | 164 (100.00) | 0 (0.00) | 21 (12.80) | 3 (1.83) | <0.01 |
| Ciprofloxacin | 164 (100.00) | 0 (0.00) | 20 (12.20) | 4 (2.44) | <0.01 |
| Trimethoprim-sulfamethoxazole | 131 (79.88) | 33 (20.12) | 15 (9.15) | 9 (5.49) | 0.056 |
| Ceftazidime | 164 (100.00) | 0 (0.00) | 24 (14.63) | 0 (0.00) | <0.001 |
| Cefepime | 164 (100.00) | 0 (0.00) | 24 (14.63) | 0 (0.00) | <0.001 |
| Ceftriaxone | 162 (98.78) | 2 (1.22) | 19 (11.59) | 5 (3.05) | <0.001 |
| Imipenem | 164 (100.00) | 0 (0.00) | 0 (0.00) | 24 (14.63) | <0.001 |
| Meropenem | 164 (100.00) | 0 (0.00) | 0 (0.00) | 24 (14.63) | <0.001 |
| Gentamycin | 143 (87.20) | 21 (12.80) | 17 (10.37) | 7 (4.27) | 0.035 |
| Amikacin | 137 (83.54) | 27 (16.46) | 16 (9.76) | 8 (4.88) | 0.047 |
| Tetracycline | 152 (92.68) | 12 (7.32) | 20 (12.20) | 4 (2.44) | 0.125 |
| Doxycycline | 147 (89.63) | 17 (10.37) | 18 (10.98) | 6 (3.66) | 0.041 |
| Tigecycline | 35 (21.34) | 129 (78.66) | 4 (2.44) | 20 (12.20) | 0.59 |

PI: Piperacillin, PIT: Piperacillin + tazobactam, CIP: Ciprofloxacin, CAZ: Ceftazidime, CPM: Cefepime, CTR: Ceftriaxone, IMP: Imipenem, MRP: Meropenem, GEN: Gentamycin, AK: Amikacin, TE: Tetracycline, DO: Doxycycline, Tg: Tigecycline

**Table 6: Relation of *Acinetobacter* spp. isolates with clinical variables**

| Age | MBL *Acinetobacter* spp. (*n*=164) | Non-MBL *Acinetobacter* spp. (*n*=24) | *P* |
|---|---|---|---|
| Comorbidites | 57 | 6 | <0.02 |
| Prior antibiotic therapy | 69 | 7 | <0.01 |
| Impaired consciousness | 27 | 5 | <0.05 |
| Use of steroids | 55 | 6 | <0.05 |
| H2 blockers proton pump inhibitors | 155 | 7 | <0.02 |
| Length of ICU stay | 14±6 | 7±4 | <0.01 |
| SOFA score | 11±6 | 8±5 | <0.01 |

SOFA: Sequential organ failure assessment, ICU: Intensive care unit

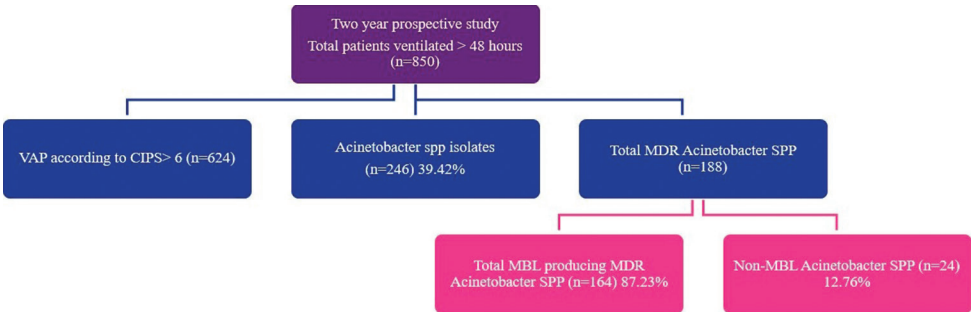



**Figure 1:** Flow chart of enrolling patients for study according to inclusion and exclusion criteria

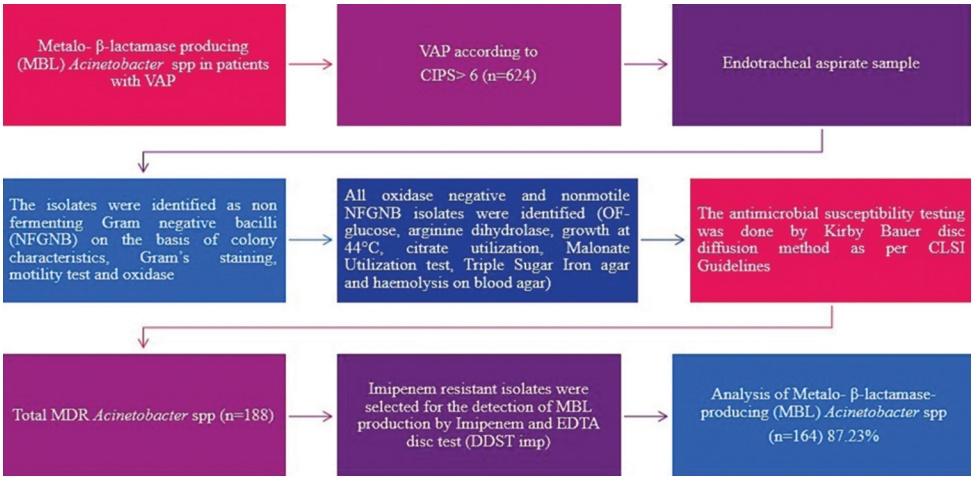



**Figure 2:** Processing of sampling

last-resort antibiotics indicated for various multidrug-resistant bacterial infections. Inhibition of enzymes mediating antibiotic resistance in bacteria is one of the major promising means for overcoming bacterial resistance. Compounds having potential MBL-inhibitory activity have been reported, but none are currently under clinical trials. The need for developing safe and efficient MBL inhibitors (MBLs) is obvious, particularly with the continuous spread of MBLs worldwide.[12] Nonfermenting Gram-negative bacteria such as *A. baumannii* are widespread in the environment and are increasingly associated with nosocomial infections, often associated with multidrug-resistance phenotypes. These organisms are well adapted to different environments and confirm the difficulty of therapeutic management of patients with infections associated with multidrug-resistant microorganisms, with a direct impact on mortality and epidemiological control of these strains in health centers.[13] Carbapenem hydrolyzing enzymes belong to classes A, B, and D according to molecular Ambler classification and are called carbapenemases. However, the carbapenemases in class B require one or two zinc ions for their full catalytic activity, and these enzymes are therefore called MBLs. MBLs are considered to be more crucial than other resistance mechanisms because they can almost hydrolyze all beta-lactam antibiotics. There are no clinically approved MBL inhibitors, making these enzymes a serious threat to human health. MBL encoding genes can be easily disseminated from one bacterium to another through the mechanism of horizontal gene transfer.[14] Metallo-β-lactamases (MBLs)-producing strains

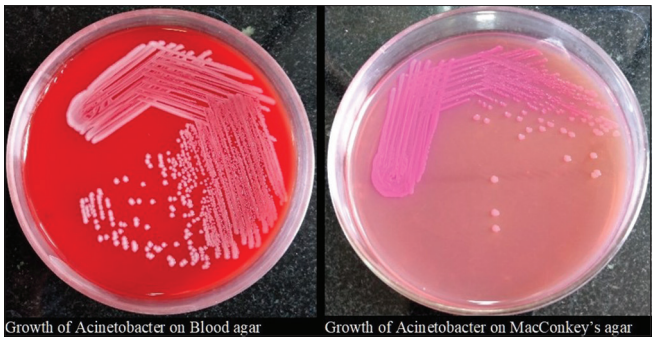



**Figure 3:** Growth of *Acinetobacter* on culture media

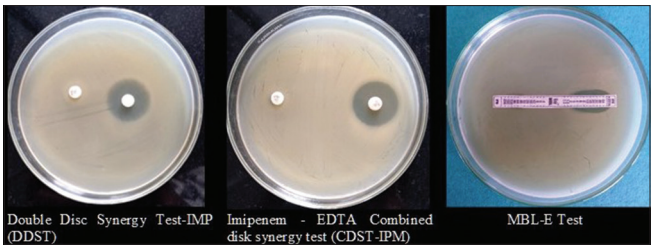
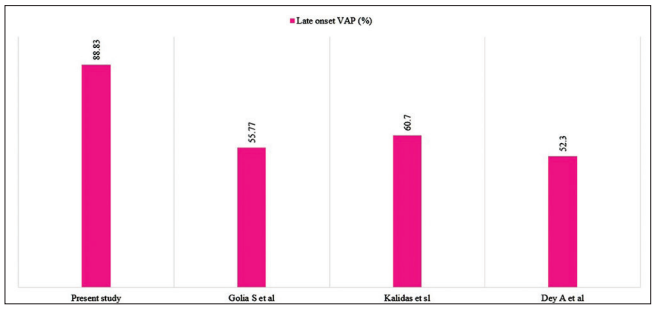


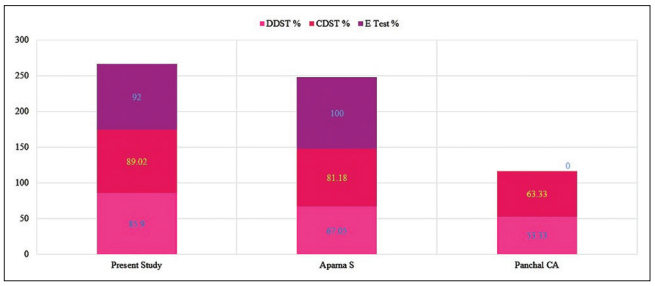

**Figure 4:** Metallo-beta-lactamase detection by various methods-DDST -IMP, CDST-IPM and metallo-beta-lactamase-E test

of *A. baumannii* are serious etiological agents of hospital infections worldwide. Among the β-lactams, carbapenems are the most effective antibiotics used against *A. baumannii*. However, resistance to these drugs among clinical strains of *A. baumannii* has been increasing in recent years.[15] The overall prevalence of multidrug-resistance among *A. baumannii,* causing VAP pooled from 114 studies, was 79.9%. Central America (100%) and Latin America and the Caribbean (100%) had the highest prevalence, whereas Eastern Asia had the lowest (64.6%).[16] The increasing trend of CR in *A. baumannii* worldwide is a concern since it limits drastically the range of therapeutic alternatives. MBL (VIM, IMP, SIM) have been reported worldwide, especially in Asia and Western Europe, and confer resistance to all beta-lactams except aztreonam. The most widespread beta-lactamases with carbapenemase activity in *A. baumannii* are carbapenem-hydrolyzing class D beta-lactamases that are mostly specific for this species.[17]

In the present study, total 11.17% and 88.83% MDR VAP due to *Acinetobacter* spp. were in early-onset VAP and late-onset VAP, respectively ($P < 0.001$). Late-onset VAP due to MDR *Acinetobacter* spp. was predominant in the present study. Total 246 (39.42%) VAP was caused by *Acinetobacter* spp. in the present study. Similarly, Golia *et al*. quoted incidence of VAP of 35.14%, out of which 44.23% had early-onset (<4 days MV) VAP and 55.77% had late-onset VAP. The most common organisms isolated in early-onset and late-onset VAP was A baumanii. The incidence of MDR *Acinetobacter* were 40%.[18] Rit *et al*. ($n = 140$) quoted 60.7% late-onset VAP due to *Acinetobacter* spp.[19] Dey and Bairy incidence of VAP was found to be 45.4%, of which 47.7% had early-onset (<5 days MV) VAP and 52.3% had late-onset (>5 days MV) VAP. Multiresistant bacteria, mainly *Acinetobacter* spp. (47.9%) was the most commonly isolated pathogens in both types of VAP[20] [Graph 1]. Total 82.98% were *A. baumannii*, 7.98% *A. iwoffii* 4.79% *A. calcoacetiucs,* 2.66% *A. hemotyticus* and 1.59% were ABC complex, predominated by *A. baumannii* ($P < 0.001$) in the present study. Similarly, the majority of isolates were *A. baumannii* quoted by Amudhan *et al*.[21] *A. baumannii* is an emerging MDR opportunistic pathogen that causes a variety of nosocomial infections, including VAP.[22] One leading factor responsible for resistance in *A. baumannii*, is the production of carbapenemases like metallo-β-lactamases (MBLs), which hydrolyze a variety of β-lactams including penicillin, cephalosporins, and carbapenems.[23] In the present study, total 188 MDR *Acinetobacter* spp. 164 (87.23%) were putative MBL producing and 24 (12.67%) were non-MBL *Acinetobacter* spp. Of 164 MBL producing isolates, 141 (85.98%) were detected by the DDST method and 23 (14.02%) were DDST negative. Total 146 (89.02%) MDR *Acinetobacter* spp. were detected by CDT-IMP test. Total 152 (92%) MDR *Acinetobacter* spp. were detected by MBLe-test. Similarly, Shivaprasad *et al*. studied 168 *A. baumannii* isolates and MBL screening was done by Imipenem-EDTA double-disc synergy test, Imipenem-EDTA combined disc test, Modified Hodge test, and MBL E-test. Out of 168 *A. baumannii* isolates, 85 (50.59%) were IMP resistant. Among these 85 isolates, 57 (67.05%) were MBL positive by DDST, 69 (81.18%) by CDT, 85 (100%) by MHT, and all these 85 isolates were confirmed to be MBL positive by MBL E-test method. Combined disc test, Modified Hodge test, and E-test are equally effective to detect MBL production. However, considering the cost constraints of the E-test, simple MHT and CDT can be used[22] [Graph 2]. In contrast with the present study Purohit *et al*. quoted less *A. baumannii* isolates, 4 (9.3%) were MBL producers by EIM, and 3 (6.97%) by eEDS.[23] Elbrolosy *et al*. in their study of 64 *Acinetobacter* isolates from late-onset VAP, 42 (65.6%) quoted sensitivity and specificity of MHT were 52.38% and 41.67%, while for CDT they were 92.86% and 83.33%, respectively. *Acinetobacter* isolates showed high susceptibility to colistin.[24] Nusrat *et al*. in their cross-sectional study ($n = 105$) quoted 48.42% IMP resistance, 65.22% were MBL producers by CDST.[25] Anwar *et al*. in their study of 112 *A. baumannii* isolates, 58.9% were resistant to both IMP and MRP and were 83.3% carbapenemase producers, 2/3rd isolates were positive by CDT and DDST. All MBL producing strains showed remarkable resistance to cephalosporins, fluoroquinolones, aminoglycosides, and piperacillin/tazobactam; these findings are comparable with present study in which significant resistance was observed against cephalosporins, fluoroquinolones, aminoglycosides, and piperacillin/tazobactam with 100% agaist IMP.[14] Similar to the present study Aghamiri *et al*. studied 169 IMP-resistant isolates by DDST phenotypic method and observed 165 strains were MBL positive.[15] Alkasaby *et al*. *A. baumannii* Phenotypic expression of MBLs resistance was demonstrated by CDT in 273 isolates (97.5%). MBLs genes were positive in 266 isolates (95%). They conclude that MDR *A. baumannii* with MBLs




**Graph 1:** Late onset ventilator associated pneumonia comparison

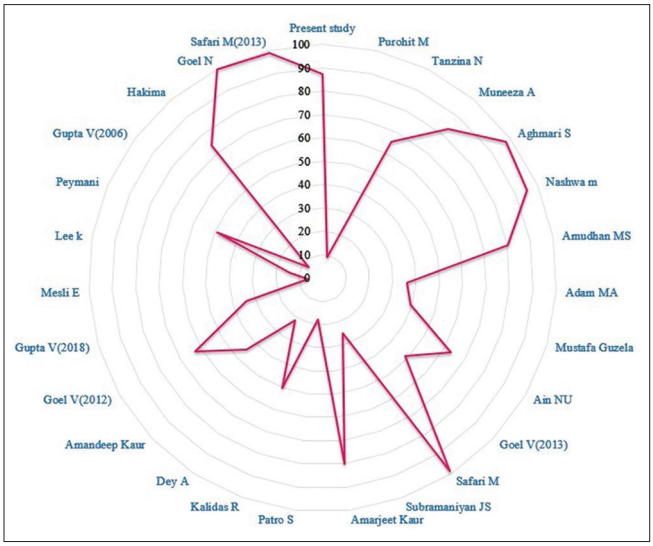



**Graph 2:** Metallo-beta-lactamase producer by various methods

activity was the most common isolate; these findings are comparable with the present study, in which 164 (87.23%) were MBL positive.[26] Similar to the present study Amudhan *et al*. quoted MBL screening with EDTA positive in 80.4%. CR in *A. baumannii* mediated by MBL production.[21] Panchal *et al*. in 107 clinical isolates, 70% isolates were MBL positive by CDST-0.1 M EDTA, 63.33% by CDST-0.5M EDTA, 56.67% by DDST-0.1 M EDTA, and 53.33% by DDST-0.5M EDTA. All MBL producer were resistant to ampicillin/sulbactam; these findings are comparable with the present study.[10]

Total 39.42% isolates were of *Acinetobacter* spp. present among patients with VAP in the present study. Total 76.42% *Acinetobacter* spp. were MDR of them 87.23% were MBL-producing *Acinetobacter* spp. and 12.76% were non-MBL *Acinetobacter* spp. and was statistically significant ($P < 0.002$). Adam and Elhag in their descriptive cross-sectional study quoted the prevalence of MBL genes 36.1%. MBL positive genes among carbapenems sensitive and resistant isolates were 27% and 45%, respectively.[1] Similarly, in the present study, all MBL positive *Acinetobacter* spp. were resistant to IMP and MRP, while all nonMBL isolates were sensitive to IMP and MRP. Guzela *et al*. quoted MBL-positive *A. baumannii* in 39.4%.[27] Ain *et al*. quoted incidence of MBLs of 63.38%–86.61%.[28] Similar to the present study, Goel *et al*. in their prospective study, reported 48.72% MBL-producing among *A. baumannii*.[29] Safari *et al*. in their cross-sectional study, observed 99% *A. baumannii* isolates were MBLs producing. In the present study 100% MBL positive *Acinetobacter* spp. were resistant to IMP and MRP and 87.23% were MBL producer.[30] Abd El-Baky *et al*. stated that the CRAB high prevalence of MBLs producing phenotypically and genotypically.[31] Subramaniyan and Sundaram quoted a relatively low prevalence of MBL producers *A. baumannii* (25%) compared to the present study.[32] Kaur *et al*. in their prospective study, reported all isolates of *Acinetobacter* with the high level of resistance to cephalosporins, cotrimoxazole and PI. *A. lwoffii* and *A. hemolyticus* showed lesser resistance to all antibiotics. IMP resistance was 40.3% and 80.3% of *A. baumannii* had MBL activity with higher resistance as compared to MBL negative isolates; these findings are comparable with the present study.[33] In contrast to the present study, Patro *et al*. reported MBL producer in 17.64% nonfermenters. Late-onset VAP is increasingly associated with MDR pathogens. Treatment with polymyxin B and tigecycline should be kept as last-line reserve drugs against the MDR *Acinetobacter* spp.[34] Rit *et al*. quoted *Acinetobacter* spp. were significantly associated with late-onset VAP and MBL was produced by 50%.[19] Dey and Bairy quoted MBLs that were produced by 21.74% of *Acinetobacter* spp. with 45.4% of VAP multidrug-resistant organisms.[20] Kaur *et al*. ($n = 116$) quoted MBL production in 44.8% Acinetobacter spp. isolates with very poor susceptibility to cephalosporins, aminoglycosides, fluoroquinolones, and even carbapenems. These findings are comparable with the present study.[35] Moghadam *et al*. In their cross-sectional study of 98 *A. baumannii* isolates quoted 98% carbapenem-resistant with half of the isolates were phenotypically positive for MBL with all MBL producer isolates were multidrug resistance.[36] Goel *et al*. ($n = 53$) reported 62.96% MBLs producer *A. baumannii* in their prospective study.[37] Gupta *et al*. in their prospective study ($n = 372$) reported MDR was high, with 34% of *Acinetobacter* being MBL producers.[38] Mesli *et al*. Among the 113 isolates of *Acinetobacter* spp, 80 (70.8%) were found to be resistant to IMP with metallo-β-lactamase in five isolates (6.2%).[39] Lee *et al*. Among the isolates nonsusceptible to IMP that were collected from 28 hospitals, 38 (14.2%) of 267 *Acinetobacter* spp. produced MBL and had alleles of blaVIM-2 or blaIMP-1. MBL-producing isolates were detected in 60.7% of the hospitals.[6] MBL-producing *A. baumannii* has become a growing therapeutic concern worldwide. Among 63 carbapenems (IMP and MRP) nonsusceptible isolates of *A. baumannii*, 31 (49%) were found to be MBL producers. Of 31 MBL-producing isolates, 19 (61%) carried the bla(IMP) gene, and 9 (29%) carried the bla(VIM) gene. All MBL-producing isolates were MDR.[40] Gupta *et al*. ($n = 200$) quoted 7.5% of *Acinetobacter* were MBL producers.[41] Similar to the present study Kabbaj *et al*. quoted 74% *A. baumannii* isolates MBL producers with the increasing prevalence of MBL producer strain (38% in 2005 vs. 75% in 2010).[42] Goel *et al*. quoted 100% MBL *A. baumannii* in their study.[43] Safari M (2013) in their cross-sectional study quoted that the by E-test 99% isolates were MBL producing [Graph 3].

Keskin *et al*. reported 94.5% MDR rate of *A. baumannii*.[9] Goel *et al*. in their prospective observational study quoted that, the *A. baumannii* isolates with high MDR (100%) and XDR 76 (86.33%).[43] Hasanin *et al*. quoted the prevalence




**Graph 3:** Metallo-beta-lactamase producer in various study

of XDR-AB was 63.8% (30 patients). Carbapenems showed poor activity against all isolates.[44] Royer *et al*. reported all carbapenem-resistant clinical and environmental isolates of *A. baumannii* were OXA-23 producers.[45] Safari *et al*. in their cross-sectional study of 100 *A. baumannii* isolates with significant resistance rate against MRP, IMP, amikacin, CIP, piperacillin/tazobactam, and cefotaxime.[46] All MBL-producing MDR *Acinetobacter* spp. isolates were resistant to PI, PIT, Ciprofloxacin, CAZ, CPM, IMP, and MRP in the present study. Total 162 (98.78%) MBL isolates were resistant to ceftriaxone, while 152 (92.68%) were resistant to tetracycline. Total 89.63% MBL were found to be resistant to doxyoycline, 87.20% resistant to gentamycin 83.54% resistant to amikacin and 79.88% resistant to trimethoprim-sulfamethoxazole. There was no resistance found for IMP and MRP amongst Non-MBL isolates. Kabbaj *et al*. cited all *A. baumannii* isolates were resistant to ticarcillin, ticarcilline/clavulanate, PI, piperacillin/tazobactam, gentamicin, tobramycin, and CIP. Amikacin and trimethoprim/sulfamethoxazole were, respectively, sensitive by 59.5% and 53% and 57,4% isolates were IMP nonsusceptible.[42] Salehi *et al*. reported *A. baumannii* strains were susceptible to colistin and 77% were nonsusceptible to tigecycline. A majority of the clinical and environmental isolates (97%) were considered as MDR strains.[47] Akter and Shamsuzzaman cited 92.1% resistant to IMP/MRP; these findings are comparable with the present study in which all MBL producer were resistant to IMP/MRP.[48] Hasanin *et al*. quoted the tigecycline showed good activity against half isolates. Colistin demonstrated potent *in vitro* activity against all isolates of *A. baumannii*. Similarly, tigecycline (21.34%) resistance was significantly less compared to all other antibiotics in the present study (one fifth).[44] Goel *et al*. quoted that, the 100% XDR resistant to cephalosporins, tetracycline, doxycycline, gentamycin, netilmicin, and ticarcillin/clavulinic acid. About 25 (32.8%) XDR strains were resistant to all the carbapenems.[43] Safari *et al*. cited no resistant isolate was observed against tigecycline with 99% were MBL producing with 85% resistance to IMP and MRP.[46] Mahdian *et al*. quoted all *A. baumannii* isolates were susceptible to colistin and polymyxin B. Eighty-one percent of the isolates was resistant to IMP or MRP; these findings are comparable with the present study.[49] Al-Agamy *et al*. reported 100% of *A. baumannii* isolates were resistant to amoxicillin-clavulanate, aztreonam, CPM, cefotaxime, and CAZ. Total 5% isolates were resistant to colistin, 45% to amikacin, 70% to IMP and 85% to CIP. These findings are similar to the present study.[50] Colistin appeared to be the most effective drug, followed by tetracycline and beta lactam/beta lactamase inhibitor combinations.[51] Keskin *et al*. 94% of the isolates were susceptible to colistin, followed by amikacin and SXT with a susceptibility rate of 32%.[9] Banerjee *et al*. reported significant resistance to IMP.[52] Colistin is still the most effective antibiotic for *A. baumannii* infections.[53] Various studies have quoted MBL producing *A. baumannii* in VAP ranging from 6.2% to 100% (mean:55.22%) [Table 7].

## Conclusions

The present study highlighted the burden of MDR MBL producing *Acinetobacter* spp. in patients with VAP. About three fourth of patients with VAP had MDR *Acinetobacter* spp. Eighty percent were MDR Acenetobacter spp. were MBL producer. MDR Acenetobacter isolates including MBL producer were significantly higher in late onset VAP (91.46%) compared to early onset VAP (8.54%) in present study. The ability of phenotypic identification of *Acinetobacter* spp. for MBL producer were comparable among Imipenem-EDTA double disc synergy test (DDST), Imipenem-EDTA combined disc synergy test (CDST-IPM) and MBL-E Test. All MBL producing MDR *Acinetobacter* spp. isolates were resistant to PI, Ciprofloxacin, CAZ, CPM, IMP and MRP. The Tigecycline (21.34%) resistance was significantly less compared to all other antibiotics. No resistance was found to IMP and MRP among Non-MBL isolates. Currently, considering limited availability of antimicrobial agent against MDR *Acinetobacter* spp, developing novel drugs and antibiotic combinations is the only therapeutic option available to combat antimicrobial resistance of MDR *Acinetobacter* spp. It is obvious that nosocomial infections associated with multidrug-resistant *Acinetobacter* spp. are on the rise. The increasing pattern of antimicrobial resistance, including Tigecycline is an alarming threat in VAP. The antimicrobial resistance of *Acinetobacter* spp. needs aggressive implementation of infection control measures as well as antibiotic stewardship at large. The determination of antibiotic sensitivity patterns and screening for MBL production among *A. baumannii* isolates is important for controlling clinical *Acinetobacter* infections. The judicious use of antimicrobial therapy, combined approaches of rotational antibiotic therapy and education programs might be

**Table 7: Comparison of various studies**

| Author | Type of study (*n*) | MDR-AB and MBL producer | Conclusion |
|---|---|---|---|
| Shivaprasad *et al*.[22] | Cross sectional study (*n*=168) | MBL positive by DDST: 67.05%<br>CDT: 81.18%, MHT: 100% | MHT and E-test were equally efficient to detect MBL production, followed by combined disc test |
| Safari *et al*.[30] | Cross sectional | 99% *A. baumannii* isolates were MBLs producing | *A. baumannii* isolates were drug resistant |
| Kabbaj *et al*.[42] | Cross sectional | 74% *A. baumannii* isolates MBL producers | Increasing prevalence of MBL producer strain (38% in 2005 vs. 75% in 2010) |
| Goel *et al*.[43] | Prospective observational | 100% XDR resistant to cephalosporins, tetracycline, doxycycline, gentamycin, netilmicin | 32.8% XDR strains were resistant to all the carbapenems |
| Hasanin *et al*.[44] | Prospective cohort (*n*=243) | Prevalence of XDR-AB: 63.8%. Carbapenems resistance against AB | Half of the *A. baumannii* strains resistant to tigecycline, colistin appears to be an appropriate first-line drug for Ab-VAP |
| Royer *et al*.[45] | Cohort study | Imipenem resistant: 25.8% and positive by the MHT: 75.0% | Monitoring of MDR in order to control the spread of these clones in the hospital environment |
| Safari *et al*.[46] | Cross sectional study of 100 *A. baumannii* isolates | 85% resistance to imipenem and meropenem | Significant resistance rate against meropenem, imipenem, amikacin, ciprofloxacin, piperacillin/tazobactam, and cefotaxime |
| Salehi *et al*.[47] | Cross sectional | *A. baumannii* strains were susceptible to colistin and 77% were nonsusceptible to tigecycline. | A majority of the clinical and environmental isolates (97%) were considered as MDR strains |
| Al-Agamy *et al*.[50] | Cross sectional study | 100% of *A. baumannii* isolates were resistant to amoxicillin-clavulanate, aztreonam | 100% of *A. baumannii* isolates were resistant to cefepime, cefotaxime, and ceftazidime |
| Banerjee *et al*.[52] | laboratory-based audit (*n*=993) | Significant resistance to imipenem ($P$<0.05) and 88.02% MDR and 61.97% XDR | All the 100 MDR isolates were imipenem resistant *A. baumannii*. Stringent measures to eradicate the reservoir of MDR *Acinetobacter* spp |
| Ziółkowski *et al*.[54] | Retrospective, *n*=187 | 76.5% Ab strains were extensively drug resistant and sensitive to colistin | Fluoroquinolones, amikacin, and trimethoprim/sulfamethoxazole: >90% *A. baumannii* resistant. Imipenem and meropenem: 95% resistant<br>Cephalosporins and tetracyclines: 100% resistant |
| Mohamed *et al*.[55] | Cross-sectional study (*n*=208) | Carbapenem-resistant: 35<br>MBL production: 20 | *A. baumannii* is the most common pathogen associated with VAP |
| Khelgi and Prathab[56] | Cross-sectional study (*n*=120) | MBL was produced by 42.8% of *Acinetobacter* spp. | Rational antibiotic therapy for treatment of VAP will be beneficial to combat the increase in VAP caused by MDR |
| Kumar *et al*.[57] | Prospective observational (*n*=308 isolates) | MBL producer *Acinetobacter* spp. (27.27%) | Tigecycline was found to be highly effective against MBL-producing *Acinetobacter* isolates |
| Joseph *et al*. 2010[58] | Prospective study (*n*=200) | 20% resistance with late-onset VAP | *Acinetobacter* spp. (32%): Late-onset VAP. *Acinetobacter* spp. causing early-onset VAP were colistin sensative |
| Werarak *et al*.[59] | Cross-sectional (*n*=146) | MDR: 92.3% | *A. baumannii* most common isolate |
| Present study | Prospective<br>A total 188 samples were enrolled for the study fulfilling inclusion criteria of VAP. | Total MDR *Acinetobacter* spp. isolates were 188 (76.42%) of them 164 (87.23%) were MBL producing and 24 (12.76%) were Non-MBL ($P$<0.002). All MBL producing MDR *Acinetobacter* spp. isolates (164) were resistant to PI, PIT, ciprofloxacin, ceftazidime, cefepime, imipenem and meropenem. The tigecycline (21.34%) resistance was significantly less compared to all other antibiotics | Of 164 MBL producing isolates, 141 (85.98%) were detected by DDST method and 23 (14.02%) were DDST negative. Total 146 (89.02%) MDR *Acinetobacter* spp. were deteted by CDT-IMP test. Total 152 (92%) MDR *Acinetobacter* spp. were detected by MBLe-Test. Total 11.17% and 88.83% MDR VAP due to *Acinetobacter* spp. were early onset VAP and Late onset VAP respectively ($P$<0.001). Late onset VAP due to MDR *Acinetobacter* spp. was predominant in present study caused by *Acinetobacter* spp. Of total 188 MDR *Acinetobacter* isolates, 156 (82.98%) were *A. baumannii*, 15 (7.98%) were *A. iwoffii,* 9 (4.79%) were *A. calcoaceticus*, 5 (2.66%) were *A. hemotyticus* and 3 (1.59%) were ABC complex, predominated by *A. baumannii* ($P$<0.001) |

MBL: Metallo-beta-lactamase, DDST: Double disc synergy test, MHT: Modified Hodge test, XDR: Extensively drug-resistant, CDT: Combined disc test, *A. hemotyticus: Acinetobacter hemotyticus, A. calcoacetiucs: Acinetobacter calcoacetiucs, A. iwoffii: Acinetobacter iwoffii*, *A. baumannii*: *Acinetobacter baumannii*

valuable to fight against these MDR *Acinetobacter* associated VAP. Carbapenems use should be restricted.

## Limitation

Non-MBL carbapenemases were not evaluated.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KIMS deemed to be university, Karad, Maharashtra.


## Conflicts of interest

There are no conflicts of interest.

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