



Metallo-B-Lactamases Resistance and Bacteriological Profile in Ventilator Associated Pneumonia

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Abstract

Metallo- β -lactamases (MBLs) resistance spread easily via plasmids among bacteria and cause nosocomial infections lead to outbreaks in intensive care units (ICU). Ventilator-associated pneumonia (VAP) is an important nosocomial infection in mechanically ventilated patients at intensive care unit. VAP requires an early diagnosis and appropriate antibiotic treatment, to prevent mortality and morbidity. Hence, knowing the bacterial isolates and antibiotic resistance is essential to improve the clinical outcome of VAP. During the one-year study period, 392 patients received mechanical ventilation. Endotracheal aspirates were collected from 38 mechanically ventilated patients with suspected ventilator associated pneumonia. 31 organisms were isolated. *Klebsiella pneumoniae* (35%) was the most common organism followed by *Pseudomonas aeruginosa* (23%), and *Acinetobacter* spp (19%). 3 *Acinetobacter* spp and 2 *Pseudomonas aeruginosa* isolates were positive for metallo beta lactamase production. Out of 25-gram negative isolates 5 (20%) were positive for metallo beta lactamase production. Carbapenems are often considered as the last resort antibiotics in the treatment of Gram-negative isolates. Early detection of Metallo- β -lactamases (MBLs) resistance can reduce the spread of drug resistant pathogens. This in turn leads to reduce the morbidity and mortality from Ventilator-associated pneumonia.

Keywords: Metallo- β -lactamases (MBLs) resistance, Ventilator-associated pneumonia, (VAP).

INTRODUCTION

Ventilator associated pneumonia (VAP) is the most common hospital acquired infection in patients receiving mechanical ventilation, The VAP rate is measured as episodes per 1000 ventilator days. It occurs in 9-27% of mechanically ventilated patients. (1,2,3,4) Ventilator associated pneumonia (VAP) is a hospital acquired pneumonia that occurs 48 hours or more after tracheal intubation. It can be classified as early onset or late onset pneumonia. (1,2,3,4) Early onset pneumonia occurs within four days of intubation and late onset pneumonia develops after five days. In general, early VAP is caused by pathogens that are sensitive to antibiotics, whereas late onset VAP is caused by drug resistant pathogens (5,6). The common

causative agents of VAP includes *Klebsiella* spp, *Pseudomonas* spp., *Acinetobacter* spp and staphylococci (7).

Carbapenems are often considered as the last resort antibiotics in the treatment of infections due to multidrug-resistant Gram-negative isolates. However, during the last decade carbapenem resistance has been increasingly reported among Gram negative bacteria and is largely attributed to the production of Ambler class B metallo-beta lactamases (MBLs) (8,9,10). MBLs lead to resistance to antibiotics like penicillin, cephalosporins and carbapenems. There are a number of genes encoding MBLs such as imipenemase (IMP),

Verona integron-encoded metallo-beta-lactamases (VIM), Sno Paolo metallo (SPM), New-Delhi metallo-β-lactamase (NDM), German imipenemase (GIM), Kyorin University Hospital imipenemase (KHM), and Australian imipenemase (AIM). It has been suggested that different phenotypic tests identify MBLs based on metal-chelating ability such as EDTA inhibiting MBL activity. MBLs spread easily via plasmids and cause nosocomial infections lead to outbreaks⁽¹¹⁾. These nosocomial infections are more common in patients admitted to the intensive care units. Hence this study was conducted to detect MBL from the isolates of VAP for the prevention of drug resistant nosocomial infection.

Materials and methods

The prospective study was conducted over the period of one year after obtaining the institutional ethical committee clearance. During the study period 392 ventilated patients were observed. Endotracheal aspirates were collected from the patients on mechanical ventilation for more than 48 hours with new or progressive infiltrates, consolidation or cavitation on chest X-ray and one of the following: (a) New onset purulent bronchial secretions with leukopenia (white blood cell <1500/mm³) or leukocytosis (≥12,000/mm³), or core temperature ≥38.5 or ≤36°C without other cause.

Exclusion criteria: Patients with pneumonia prior to mechanical ventilation or within 48 hours of mechanical ventilation, patients with Adult Respiratory Distress Syndrome (ARDS), cavitary lung disease based on chest X-ray findings, primary lung cancer or another malignancy metastatic to the lungs and cystic fibrosis. Tuberculosis patients and patients with acquired, induced or congenital immunodeficiency, leukopenia <1000 cells/mm³, neutropenia <500 PN/mm³ were also excluded from the study.

The endotracheal aspirates were first subjected to Gram's staining and then cultures were performed. Samples were plated on blood agar, chocolate agar (CA), and MacConkey agar by wire loop. Culture plates were incubated overnight at 37°C and CA plates at 37°C in candle jar. Culture plates were checked for growth after 24 and 48 h of incubation. Significant growth was identified by biochemical tests. Susceptibility to antibiotics (concentration in µg) Ampicillin (10), Gentamycin (10), Amikacin(10),

Cefoxitin (30µg Cefotaxime(30), Ceftazidime (30), Ceftriaxone (30) Cotrimoxazole (25 µg) Ciprofloxacin) (5) Erythromycin(15µg), cefoperazone sulbactam and Imepenem (10 µg) were tested by Kirby Bauer's disk diffusion method and interpreted as Clinical Laboratory Standard (CLSI) recommendations

Combined Disk Test

The test was performed as described by Yong et al. An overnight culture of an Imepenem resistant bacterial suspension of the 0.5 McFarland standards was spread on Mueller-Hinton (MH) agar plate using cotton swab. Two Imepenem (IPM) disks were placed on the surface of the agar at a distance of 20mm from each other. 5 µL of 750 µg/mL EDTA solution was then added to one of the IPM disks. The inhibition zones displayed around the IPM and the IPM-EDTA disks were compared after 14 to 16 hrs of incubation at 37°C. The difference of ≥7 mm between the inhibition zone diameter of the IPM-EDTA disk and that of IPM only disk was considered to be a positive for the presence of MBLs^(8,11).

Result

During the study period, 392 patients were received mechanical ventilation at Intensive Medical Care Unit. Endo tracheal aspirates were collected from 38 mechanical ventilated patients with suspected ventilator associated pneumonia. 31 organisms were isolated from Endo tracheal aspirates

Klebsiella pneumoniae 11(35%) was the most common organism followed by *Pseudomonas aeruginosa* 7(23%), *Acinetobacter* spp 6(19%), *Staphylococcus aureus* (16%), *Citrobacter* spp1 (3%) and *Streptococci* sp 1(3%).

The antibiotic susceptibility of Gram negative organisms revealed, *Klebsiella pneumoniae* 17% (n=1), *Pseudomonas aeruginosa* 33% (n=2) and *Acinetobacter* spp 50% (n=3) resistance to Imepenem. Gram negative isolates (n=6) resistant to Imepenem were tested for Metallo beta lactamase production by Combined disk test.

3 *Acinetobacter* spp and 2 *Pseudomonas aeruginosa* isolates were positive for metallo beta lactamase production. Out of 25-gram negative isolates 5 (20%) were positive for metallo beta lactamase production.

Chart -1 Distribution of early and late VAP cases

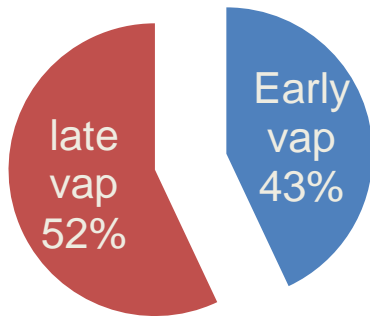


Chart 2: Organisms isolated from early and late ventilator associated pneumonia

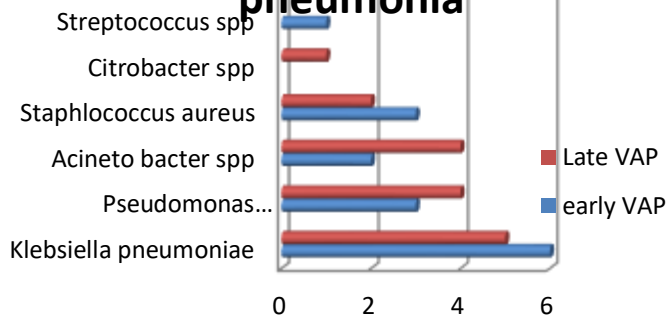
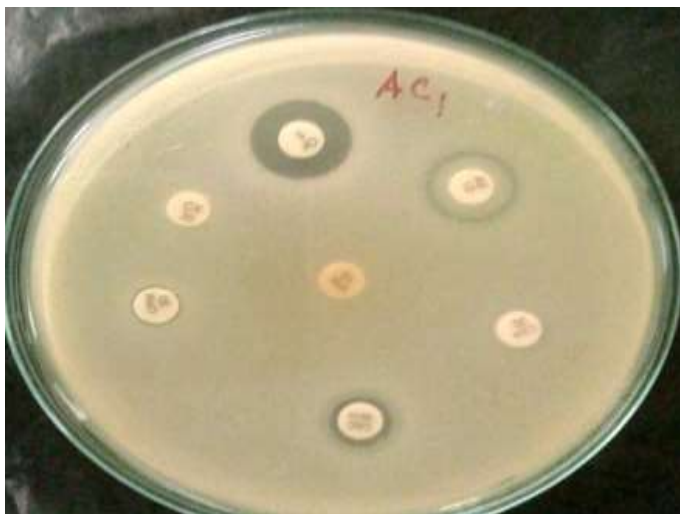
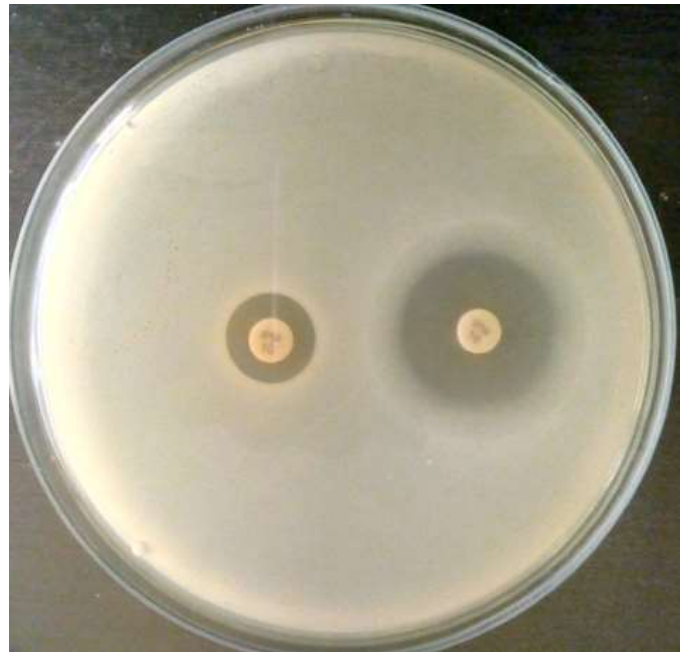


Fig 1. Screening for Metallo beta lactamase production



Resistance to Imepenem

Fig -2: Metallo beta lactamase Detection by Combined disk test



More than 7mm increase in zone size with addition of 0.5 M EDTA

Discussion

VAP requires a rapid diagnosis and initiation of appropriate antibiotic treatment, to prevent mortality and morbidity. Inappropriate and inadequate antibiotic treatment causes emergence of drug resistance in pathogens and poor prognosis in patients. (12,13,14,.)

Out of 31 VAP cases, 48% were categorized under early-onset VAP and 52% under late-onset VAP which was in concordance with studies conducted by Saroj Golia et al.

In our study Klebsiella pneumonia (35%) and Pseudomonas aeruginosa(23%) were the commonest isolates which is also reported by Ramakrishna et al. But Marcos et al reported that the predominant Gram-negative bacilli were Haemophilus influenzae, P. aeruginosa, and Klebsiella pneumoniae. In the present study S. aureus was more frequently isolated from early-onset VAP, when compared to late-onset VAP which is concordant with Marcos et al.

We observed that 20% of the isolates showed MBL production, which is concordant with a MBL prevalence identified by Chinjal et al (19.62%). But Gupta et al showed higher prevalence of MBL (50%)

Mansoor Khaledi et al reported that , the tracheal samples carried the most bacteria containing the MBLs. Franklin et al and Chinjal et al reported Combined Disk -0.1 M EDTA method is the most sensitive method in MBL phenotypic detection method. Detection of MBL by PCR is the most sensitive and specific method but the cost of this method limits the use for routine diagnostic microbiology laboratories.

To avoid the increase of nosocomial infections associated with MBL, strict infection control measures; including restrictive antibiotic use policies in the hospital and isolation of patients with MBL especially ICU patients should be followed.

Conclusion

Local epidemiological data like bacteriological profile and MBL resistance can help in guiding the initial empirical antibiotic therapy that lead to decrease mortality and morbidity and also help in preventing development of more resistant strains.

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