

Original Research Article

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## Detection of Extended Spectrum $\beta$ Lactamase and Amp C $\beta$ Lactamase Resistance in the Gram Negative Bacterial Isolates of Ventilator Associated Pneumonia

David Agatha and B. Subitha\*

Department of Microbiology, Thanjavur Medical College, Thanjavur, India

\*Corresponding author

### ABSTRACT

Ventilator-associated pneumonia (VAP) is an important nosocomial infection in mechanically ventilated patients at intensive care unit (ICU). The administration of accurate and timely initial empirical antibiotic therapy is essential to reduce the morbidity and mortality from Ventilator-associated pneumonia. Initial empiric antimicrobial therapy for VAP greatly depends on the type of causative pathogen and its resistance pattern. During the six months study period, 196 patients received mechanical ventilation. Endo tracheal aspirates were collected from 22 mechanically ventilated patients with suspected ventilator associated pneumonia. 19 organisms were isolated. All Cefoxitin resistant isolates were studied for the presence of plasmid mediated AmpC beta-lactamase enzyme by Inhibitor disk based method and inducible AmpC beta-lactamase production by Ceftazidime-imipenem antagonism test (CIAT).ESBL production in the gram negative isolates was detected by Phenotypic Confirmatory Test. Incidence rate of VAP was 9.7%. *Klebsiella pneumoniae* (26%) was the most common organism followed by *Pseudomonas aeruginosa* (21%) and *Acinetobacter* spp (16%). (53%) of Gram negative isolates were positive for ESBL production. (6%) was positive for plasmid mediated Amp C beta lactamase production and (6%) was positive for inducible Amp C beta lactamase production. Due to the increasing incidence of drug-resistant organisms, VAP requires an early diagnosis and appropriate antibiotic treatment, to prevent mortality and morbidity. Hence, knowing the bacterial isolates and their antibiotic resistance pattern is essential to improve the clinical outcome of VAP.

#### Keywords

$\beta$  Lactamase, Gram negative bacteria, Ventilator, Pneumonia

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### Introduction

Ventilator associated pneumonia is the most common nosocomial infection in patients receiving mechanical ventilation, It occurs in 9-27% of mechanically ventilated patients (Arindam Dey *et al.*, 2007, Gupta *et al.*, 2011). Ventilator associated pneumonia (VAP) is a hospital acquired pneumonia that

occurs 48 hours or more after tracheal intubation. It is classified as early onset or late onset pneumonia (Hanan H *et al.*, 2014, Xiaofang Cai *et al.*, 2011). 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 such as various beta-lactamases (AmpC  $\beta$  lactamase (AmpC), extended spectrum  $\beta$ -lactamases (ESBL) and metallo- $\beta$ -lactamases (MBL) producing gram negative isolates and methicillin-resistant *Staphylococcus aureus* (MRSA) (Marcos *et al.*, 2013, Ramakrishna *et al.*, 2012).

The most common mechanism of resistance in Gram negative bacteria is by the production of  $\beta$  lactamases which inactivate  $\beta$  lactam antibiotics. Among the  $\beta$  lactamases, Extended Spectrum  $\beta$  lactamases (ESBL) and Amp C  $\beta$ -lactamases are most commonly produced. Organisms producing ESBL are resistant to all penicillins, first, second and third generation cephalosporins and monobactam, however remain sensitive to carbapenems and cephamycins. AmpC beta-lactamases have broad substrate specificity and are classified as class C according to Ambler and group 1 by Bush-Jacoby-Medeiros. These enzymes are both chromosomal and plasmid mediated and confers resistance to narrow, broad spectrum cephalosporins, and  $\beta$  lactam  $\beta$  lactamase inhibitor combinations (Varsha Gupta *et al.*, 2007; Gupta *et al.*, 2013)

Initial empiric antimicrobial therapy for VAP greatly depends on the type of causative pathogen and its resistance pattern. Increasing drug resistance rates among the pathogens that frequently cause VAP may compromise treatment and result in prolongation of hospital stay and increase in mortality. There is a wide geographic and temporal variability of antibiotic resistance among the bacterial isolates of VAP (Chittawatanarat *et al.*, 2014, Jean-Louis Trouillet *et al.*, 1998). Hence this prospective study was conducted to evaluate the bacteriological profile, antibiotic resistance pattern, ESBL and AmpC  $\beta$  lactamase (AmpC) production in gram negative isolates of ventilator associated pneumonia.

## Materials and Methods

This prospective study was conducted in a Tertiary care hospital over a period of 6 months. In that period 196 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  $<1500/\text{mm}^3$ ) or leukocytosis ( $\geq 12,000/\text{mm}^3$ ), or core temperature  $\geq 38.5$  or  $\leq 36^\circ\text{C}$  without other cause.

The endotracheal aspirates were sent to the lab and processed immediately. The samples were first subjected to Gram's staining and then quantitative cultures were performed.

Samples were mechanically liquefied and homogenized by vortexing for 1 min. Then 0.01 mL of sample solution was inoculated on sheep blood agar, chocolate agar (CA), and MacConkey agar by using 4 mm Nichrome wire loop. All plates were incubated overnight at  $37^\circ\text{C}$  and CA plates at  $37^\circ\text{C}$  in candle jar. All plates were checked for growth after 24 and 48 hrs of incubation. A detailed biochemical tests were performed to identify the significant growth of organism, and antibiotic sensitivity testing were performed on Mueller–Hinton agar plates by Kirby-Bauer disc diffusion method. Zone diameter was measured and interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines

Gram negative isolates resistant to 3rd Generation Cephalosporins were tested for ESBL production.

Phenotypic detection of ESBL production in the gram negative isolates by Phenotypic Confirmatory Test.

Ceftazidime (30 µg) disk and a Ceftazidime plus Clavulanic acid (Ca 30 µg + Caz 10 µg) disks were placed at a distance of 20 mm apart on a lawn of culture of the suspected ESBL producing clinical isolates on MHA.

The plates were incubated at 37°C overnight. The test organism was considered to produce ESBL if the zone size around the Ceftazidime plus Clavulanic acid disk increased >5 mm in comparison to the third generation Ceftazidime disk alone.

All Cefoxitin resistant Gram negative isolates were tested for AmpC beta-lactamase enzyme production.

Detection of plasmid mediated AmpC beta-lactamase production by Inhibitor disk based method.

The test culture was swabbed on Mueller-Hinton agar plates. Cefoxitin (30 µg) disk and Cefoxitin /Boronic Acid (BA) disk were placed at a distance of 20 mm from center to center.

An increase of >.5 mm around Cefoxitin /BA compared to Cefoxitin alone was considered positive for the presence of AmpC production (Philip *et al.*, 2005).

Detection of inducible AmpC beta-lactamase production by Ceftazidime-imipenem antagonism test (CIAT)

The test culture was swabbed on Mueller-Hinton agar plates. Imipenem disk (10 µg) and Cefoxitin disk were placed 20 mm apart (edge-to-edge) from a Ceftazidime disk (30 µg). Antagonism was indicated by a visible reduction in the inhibition zone around the Ceftazidime disk adjacent to the Imipenem or Cefoxitin disks. This was regarded as positive for inducible AmpC beta-lactamase production (Vladimir *et al.*, 2007).

## Results and Discussion

During the study period, a total of 196 patients were on mechanical ventilation at Intensive Medical Care Unit, Neonatal Intensive Care Unit and Pediatric Intensive Care Unit in a Tertiary care Hospital. Endo tracheal aspirates were collected from 22 mechanically ventilated patients with suspected ventilator associated pneumonia. 19 organisms were isolated and 3 Endo tracheal aspirates were reported as no growth. Incidence rate was 9.7%. *Klebsiella pneumoniae* (26%) was the most common organism followed by *Pseudomonas aeruginosa* (21%), *Acinetobacter* spp (16%), *Staphylococcus aureus* (16%), *Klebsiella oxytoca* (11%), *Citrobacter* spp (5%) and *Streptococcus* sp (5%).

The antibiotic susceptibility testing for Gram positive organisms revealed 100% sensitivity to Vancomycin and all the three *Staphylococcus aureus* isolates were resistant to cefoxitin (Fig. 1 and 2).

Gram negative organisms except *Klebsiella pneumoniae* and *Acinetobacter* spp were 100% sensitive to Imipenem.

Gram negative isolates (n=11) resistant to 3rd Generation Cephalosporins were tested for ESBL production. 4 *Klebsiella pneumoniae* isolates, 2 *Klebsiella oxytoca* isolates, 1 *Acinetobacter* spp isolate and 1 *Pseudomonas aeruginosa* were positive for ESBL production

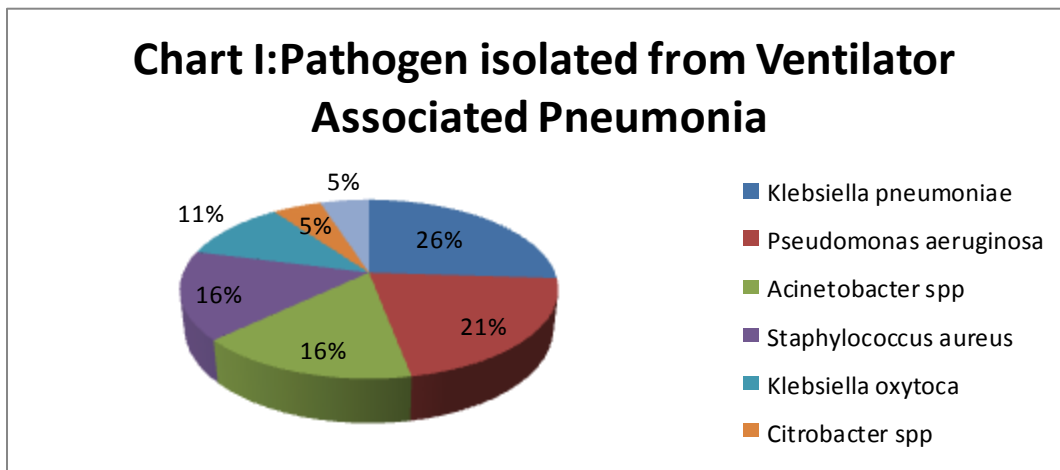
Two *Klebsiella pneumoniae*, two *Acinetobacter* spp one *Pseudomonas aeruginosa* resistant to Cefoxitin were tested for Amp C β lactamase production. One *Acinetobacter* spp was positive for plasmid mediated Amp C beta lactamase production. One *Pseudomonas aeruginosa* was positive for inducible Amp C beta lactamase

production. Out of 15 gram negative isolates, 8 (53%) were positive for ESBL production, 1 (6%) was positive for plasmid mediated Amp

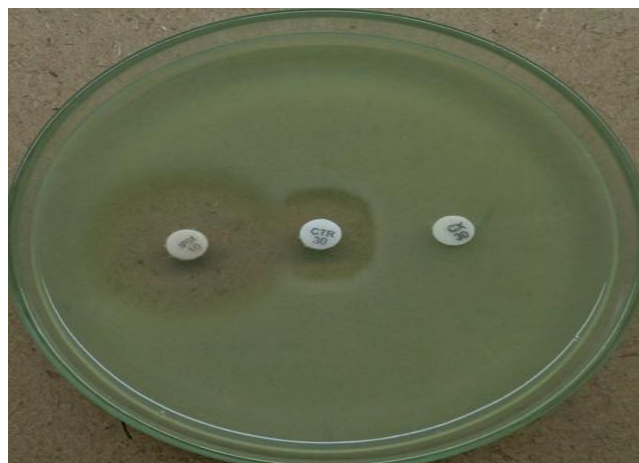
C beta lactamase production and 1(6%) was positive for inducible Amp C beta lactamase production (Table 1).

**Table.1** Antibiotic sensitivity pattern of Gram negative organisms isolated from ventilator associated pneumonia

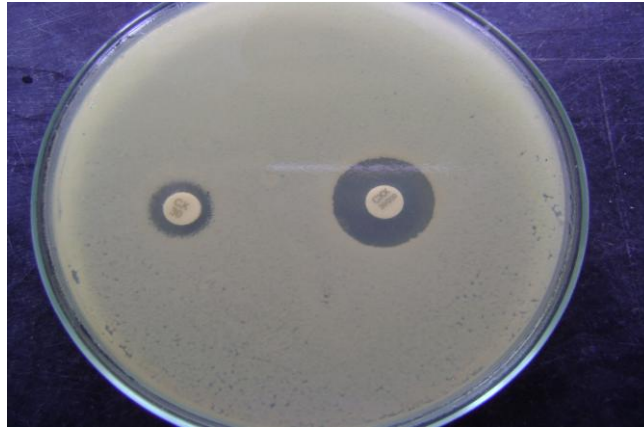
Drugs	<i>Klebsiella pneumoniae</i>	<i>Klebsiella oxytoca</i>	<i>Pseudo monas aeruginosa</i>	<i>Acineto bacter spp</i>	<i>Citrobacter spp</i>
Amikacin	80	50	50	67	100
Gentamycin	40	50	50	33	100
Ciprofloxacin	20	0	75	0	100
Ceftazidime	20	0	50	33	100
Piperazilin tazobactum	80	100	75	33	100
Imipenem	80	100	100	33	100
Cefoxitin	60	100	75	33	100



**Fig.1** Inducible Amp C beta lactamase detection by Ceftazidime-imipenem antagonism test



**Fig.2** Plasmid mediated Amp C beta lactamase detection by Inhibitor disk based method



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 (Steven *et al.*, 2006, Ali Amanati *et al.*, 2017, Su Young Chi *et al.*, 2012)

The incidence of VAP in our study was 9.7%, which was lower than studies done by Alok Gupta *et al.*, (28.04%), SarojGolia *et al.*, (35.78%), Gadani *et al.*, (37%) and Dey *et al.*, (45.4%) Rajashekar *et al.*, reported a very high incidence rate of 73%.The lower incidence rate may be due to death of most of the patients on the day of mechanical ventilation itself.

Out of 19 VAP cases, 43% were categorized under early-onset VAP and 57% under late-onset VAP which was in concordance with study conducted by Dey *et al.*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were the commonest isolates obtained in both early and late onset VAP cases, which were also reported as the commonest isolates by study conducted by Ramakrishna *et al.*, (2012).

In our study 53 % of Gram negative isolates were ESBL producers. Saroj Golia *et al.*, and

Dey *et al.*, also observed a high prevalence of ESBL producers in their study. Chromosomal Amp C  $\beta$  Lactamase resistance was seen in 6% of our isolates and plasmid mediated Amp C beta lactamase production was seen in (6%) which was similar to Gupta *et al.*, observation (11%). Cefoxitin resistance in non-Amp C producing *Klebsiella pneumoniae* is often due to porin deficient mutants. The interruption of a porin gene by insertion sequences is a common type of mutation that causes the loss of porin expression and increased Cefoxitin resistance in *Klebsiella pneumoniae*.

Our results suggest no difference in the rate of drug resistant pathogens between early-onset and late-onset VAP. Many studies have shown a higher association between resistant pathogens and late-onset VAP. This association is due to previous antibiotic therapy, time on mechanical ventilation, and local factors, which are institution specific. Ibrahim and colleagues have reported resistant pathogens to be common in both early-onset and late-onset. The overall picture suggests that number of drug-resistant strains of various organisms is rising and is an important cause of VAP in our setting.

In conclusion, this study suggests that most cases of VAP in our setting are caused by highly resistant strains. Local epidemiological



data like this should be collected at all centers, as such information can help in guiding the initial empirical antibiotic therapy, which would be more rationale and help in decreasing mortality and morbidity. This would also help in preventing development of more resistant strains.

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