

## Bacteriological Profile of Ventilator Associated Pneumonia in a Tertiary Care Hospital of South India with Special Reference to Multi Drug Resistant Pathogens

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### ABSTRACT

Ventilator associated pneumonia (VAP) occurs in approximately 9-24% of patients who are mechanically ventilated. Emergence of multidrug resistance (MDR) among the pathogens causing VAP is contributing to the increase in morbidity and mortality. The objective of our study was to isolate aerobic bacterial pathogens causing VAP, determine their antibiogram and detect the presence of drug resistance in the pathogens. Endotracheal aspirates from 120 patients undergoing mechanical ventilation for >48h were collected and processed by semi-quantitative method. Isolates were identified by standard methods and antibiotic susceptibility was done using Kirby Bauer disc diffusion method. Combination disk method, Modified Hodge test, EDTA disk synergy test and AmpC disk test were performed for detection of extended spectrum beta-lactamases (ESBL), carbapenemases, metallo-beta-lactamases (MBL) and AmpC β-lactamases respectively. Out of 120 cases 33 patients were diagnosed to have VAP using modified Clinical Pulmonary Infection Score (CPIS). The organisms isolated were *Acinetobacter* spp (37.83%), *Pseudomonas aeruginosa* (24.32%), *Klebsiella pneumoniae* (13.52%), *Escherichia coli* (10.81%), *Enterobacter* spp (5.41%), *Citrobacter koseri* (2.7%) and *Staphylococcus aureus* (5.41%). ESBL was produced by 50% of *E.coli*, 60% of *K.pneumoniae*, 100% of *Enterobacter* spp and *C.koseri*. MBL was produced by 44.44% of *P.aeruginosa* and 42.8% of *Acinetobacter* spp. AmpC β-lactamases were produced by 71.43% of *Acinetobacter* spp, 44.44% of *P.aeruginosa*. VAP is associated with MDR pathogens. Rational antibiotic therapy for treatment of VAP will be beneficial to combat the increase in VAP caused by MDR pathogens.

### Keywords

Ventilator associated pneumonia,  
*Acinetobacter* spp,  
*Pseudomonas aeruginosa*,  
Multidrug-resistance,  
ESBL,  
carbapenemases,  
MBL, AmpC β-lactamase.

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### Introduction

Ventilator-Associated Pneumonia (VAP), a subgroup of hospital-acquired pneumonia is a highly lethal condition contracted by patients on ventilators in hospitals and long-term nursing facilities. VAP is defined as pneumonia occurring more than 48 hours after endotracheal intubation and initiation of

mechanical ventilation including pneumonia developing even after extubation (Chastre, 2002). VAP occurs in 9-27% of all intubated patients (Chastre, 2002).

It is commonly classified as either early onset (occurring within 96 hours of start of

mechanical ventilation) or late onset (>96 hours after start of mechanical ventilation) (Joseph *et al.*, 2010).

VAP may be caused by a wide spectrum of bacterial pathogens. Common pathogens include *Pseudomonas* spp., *Acinetobacter* spp., *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*, with varying prevalence (Panwar *et al.*, 2005; Dey *et al.*, 2007).

*Pseudomonas* spp., *Acinetobacter* spp. and even Enterobacteriaceae are quite often multidrug-resistant (MDR) due to production of extended spectrum  $\beta$ -lactamases (ESBL), AmpC  $\beta$ -lactamases or metallo- $\beta$ -lactamases (MBL) (Bradford, 2001; Noyal *et al.*, 2009).

The present study was carried out to detect bacteria commonly associated in causation of VAP. Also to determine their antibiotic susceptibility patterns as well as to detect the presence of ESBL, AmpC  $\beta$ -lactamases, carbapenemases and MBL in these VAP pathogens.

### **Materials and Methods**

This was a cross-sectional study conducted in the Department of Microbiology, M S Ramaiah Medical College from January 2012 to December 2012. 120 patients admitted into the Multidisciplinary Intensive Care Unit (MICU), who satisfied the inclusion criteria, were enrolled for the study.

### **Inclusion criteria**

Patients admitted and put on mechanical ventilation for >48 hours in MICU's

### **Exclusion criteria**

Age <12 years.

Patients diagnosed to have lower respiratory

infections like pulmonary tuberculosis, chronic obstructive pulmonary disease, acute respiratory distress syndrome, bronchial asthma on admission.

Patients with pneumonia prior to mechanical ventilation or within 48 hours of mechanical ventilation.

Clinically suspected VAP cases were observed and relevant data were obtained. Endotracheal aspirates were collected aseptically from the patients and subjected to microbiological processing.

### **Microbiological processing**

All samples were first vortexed for one minute then, Gram stained preparations were performed and observed for the presence of epithelial cells, polymorphonuclear cells and to differentiate Gram positive and Gram negative bacteria. Following findings were considered significant (Rajasekhar *et al.*, 2006).

> 10 polymorphonuclear neutrophils / high power field

> 1 bacteria / oil immersion field and presence of intracellular bacteria.

Simultaneously, semi-quantitative cultures by the calibrated loop method using 4mm nichrome wire loop (Hi-media Mumbai, India) that holds 0.01ml of solution was performed on media such as nutrient agar (NA), 5% sheep blood agar (BA) and MacConkey's agar (MA) using standard techniques and incubated at 37° C under aerobic atmosphere. For diagnosis of VAP semi-quantitative culture threshold was considered as 10<sup>5</sup>cfu/ml. Any growth below the threshold was assumed to be due to colonization or contamination. The significant cultures were identified by studying the

colony morphology, Gram reaction and performing biochemical reactions according to standard methods (Collee *et al.*, 2006).

Antibiotic susceptibility testing was done by employing Kirby-Bauer standard disc diffusion method on Muller-Hinton agar and interpreted according to Clinical Laboratory Standards institute (CLSI) 2011 guidelines.

Clinical pulmonary infection score (CPIS) was considered for diagnosis of VAP. CPIS score >6 was considered as cut off value as described by Pugin *et al.*, (1991).

Isolates causing VAP screened for MDR were then tested for the enzyme production by confirmatory methods.

ESBL production was detected in Enterobacteriaceae family by Combination disk test using Cefotaxime (30µg) and Ceftazidime (30µg) alone and in combination with clavulanic acid (10µg) placed 50mm apart from centre to centre. An increase of 5mm in zone of inhibition in disk containing clavulanic acid compared to the drug alone is considered as ESBL producer (Thomson, 1992).

Modified Hodge Test was performed to detect carbapenemases as described by Lee *et al.*, (2001). The presence of distorted zone of inhibition or clover leaf type of indentation at the intersection of the test organism and *E. coli* within the zone of inhibition of the Imipenem susceptibility disc was interpreted as positive result.

MBL was detected by Imipenem-EDTA Double Disc Synergy Test (Lee *et al.*, 2001) using Imipenem (10µg) and 10 µL of 0.5 M EDTA (750 µg) disc placed 20 mm centre to centre. Enhancement of the zone of inhibition in the area between Imipenem and the EDTA disc in comparison with the far side of the

drug (Imipenem) was interpreted as a positive result.

AmpC Disk Test was done to detect AmpC beta lactamase enzyme. A sterile disks (6 mm) inoculated with test organism was placed beside a ceftoxitin disk (almost touching) on a lawn culture of *E. coli* ATCC 25922 on MHA plate. A positive test appeared as a flattening or indentation of the ceftoxitin inhibition zone in the vicinity of the test disk. A negative test had an undistorted zone (Singhal *et al.*, 2005).

## Results and Discussion

A total of 201 Patients were admitted to MICU and put on mechanical ventilation. 120 patients of them were enrolled for the study according to the inclusion criteria. 33 patients among 120 (27.5%) were detected with a CPIS of more than six and were diagnosed as VAP. Out of the 33 VAP cases, 42.42% (14/33) were categorized under the early-onset group and the remaining 57.58% (19/33) under the late-onset group (Table 1).

The most frequently isolated organisms in VAP patients were *Acinetobacter* spp (37.83%) followed by *P.aeruginosa* (24.32%), *K. pneumoniae* (13.52%), *E. coli* (10.81%), *Enterobacter* spp (5.41%), *Citrobacter koseri* (2.7%) and *S.aureus* (5.41%). The predominant organism in the early-onset as well as late-onset VAP group were *Acinetobacter* spp (Table 2).

Antimicrobial susceptibility pattern of the Gram negative and Gram positive isolates causing VAP is shown in table 3 and 4 respectively. Table 3 shows that, 55.5% of *P. aeruginosa*, 80% of *K. pneumoniae*, 100% of *C. koseri* and *Enterobacter* spp and 74% of *E. coli* were sensitive to carbapenems, making them a favourable choice for treatment. Amikacin was the other drug which showed a

good amount of sensitivity with 66.6% of *P. aeruginosa*, 60% of *K. pneumoniae*, 100% of *C. koseri*, 50% of *Enterobacter* spp and *E. coli* being sensitive. *Acinetobacter* spp showed resistance to most of the drugs like cephalosporins, fluoro-quinolones, aminoglycosides and even carbapenems with only 36% of the isolates being sensitive to either of the carbapenems tested. However all the isolates were sensitive to tigecycline. In table 4, both the isolates of *S. aureus* were methicillin and vancomycin sensitive.

MDR isolates when tested for production of various beta lactamases showed ESBL production in 66.6 % isolates of Enterobacteriaceae. ESBL was produced by 50% of *E. coli*, 60% of *K. pneumoniae*, 100% of *Enterobacter* spp and *C. koseri*. MBL was produced by 28.57 % of Gram negative isolates. 44.44% of *P. aeruginosa* and 42.8 % of *Acinetobacter* spp which were tested for MBL production were positive.

Also a single isolate of *K. pneumoniae* also produced MBL. AmpC  $\beta$ -lactamases were produced by 45.7% of the Gram negative isolates which included 33.3% and 52.17 % of

the members of Enterobacteriaceae and non-fermenters respectively. AmpC  $\beta$ -lactamases were produced by 71.43% of *Acinetobacter* spp, 44.44% of *P. aeruginosa*, 50% of *Enterobacter* spp, 25% of *E. coli*, 40% of *K. pneumoniae* (Table 5).

VAP is an important type of hospital acquired infection. VAP may be caused by a wide spectrum of bacterial pathogens (Chastre *et al.*, 2002). MDR pathogens causing VAP are a major concern in any kind of ICU set up.

In this study the VAP rate was found to be 27.5%, which is similar to other studies conducted by Reena *et al.*, (2011) and Mukhopadhyay *et al.*, (2010). Out of the 33 cases of VAP, 42.42% were early-onset and 57.58% were categorised as late-onset. Similar results were obtained by Mukhopadhyay *et al.*, (2010).

In the present study, 94.5% Gram negative bacilli and 5.5% Gram positive cocci were isolated from VAP cases. Chawla (2008) in his study also found that 87% of patients with VAP were infected with Gram negative bacilli.

**Table.1** Onset of VAP

Onset	Number (n=33)	Percentage
Early	14	42.42
Late	19	57.58

**Table.2** Organisms and onset of VAP

Organism	Early onset	Late onset	Total (n=37)
<i>Acinetobacter</i> spp	4	10	14 (37.83%)
<i>Pseudomonas aeruginosa</i>	3	6	9 (24.32%)
<i>Klebsiella pneumoniae</i>	2	3	5 (13.52%)
<i>Citrobacter koseri</i>	0	1	1 (2.7%)
<i>Enterobacter</i> spp	1	1	2 (5.41%)
<i>Escherichia coli</i>	2	2	4 (10.81%)
<i>Staphylococcus aureus</i>	2	0	2 (5.41%)

**Table.3** Antimicrobial susceptibility pattern of Gram negative isolates

Antibiotics	Susceptibility		<i>Acinetobacter</i> spp(14)		<i>P. aeruginosa</i> (9)		<i>K. pneumoniae</i> (5)		<i>C. koseri</i> (1)		<i>Enterobacter</i> spp (2)		<i>E. coli</i> (4)	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Amikacin	S	4	6	3	1	1	1	2	0	0	0	0	2	2
	R	10	3	2	0	1	2	0	0	0	0	0	2	2
Aztreonam	S	3	5	-	-	-	-	-	-	-	-	-	-	-
	R	11	4	-	-	-	-	-	-	-	-	-	-	-
Ceftriaxone	S	1	4	1	0	0	0	2	0	0	0	0	2	2
	R	13	5	4	1	2	2	0	1	2	2	2	2	2
Ceftazidime	S	1	5	1	0	0	0	2	0	0	0	0	2	2
	R	13	4	4	1	2	2	0	1	2	2	2	2	2
Cefipime	S	2	7	-	-	-	-	-	-	-	-	-	-	-
	R	12	2	-	-	-	-	-	-	-	-	-	-	-
Ciprofloxacin	S	1	2	1	0	0	0	1	0	0	0	0	1	1
	R	13	7	4	1	2	2	0	1	2	2	2	3	3
Gentamicin	S	3	3	4	0	0	0	2	0	0	0	0	2	2
	R	11	6	1	1	2	2	0	1	2	2	2	2	2
Imipenem	S	6	5	4	1	2	3	0	1	2	3	0	1	1
	R	8	4	1	0	0	0	1	0	0	0	1	1	1
Meropenem	S	5	5	4	1	2	4	0	1	2	4	0	0	0
	R	9	4	1	0	0	0	1	0	0	0	0	0	0
Piperacillin	S	4	6	-	-	-	-	-	-	-	-	-	-	-
	R	10	3	-	-	-	-	-	-	-	-	-	-	-
Tigecycline	S	14	-	-	-	-	-	-	-	-	-	-	-	-
	R	0	-	-	-	-	-	-	-	-	-	-	-	-

S: Sensitive; R: Resistant

**Table.4** Antimicrobial susceptibility pattern of Gram positive isolates

Isolate	Penicillin		Ceftriaxone		Ciprofloxacin		Erythromycin		Clindamycin		Doxycycline		Gentamicin		Vancomycin		
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	
<i>S. aureus</i> (2)	0	2	2	0	1	1	2	0	2	0	2	0	2	0	2	0	0

S: Sensitive; R: Resistant

**Table.5** Beta-lactamases production by isolates

Organism	<i>Acinetobacter</i> spp (14)	<i>P. aeruginosa</i> (9)	<i>E. coli</i> (4)	<i>K. pneumoniae</i> (5)	<i>C.koseri</i> (1)	<i>Enterobacter</i> spp (2)
Beta-lactamases						
ESBL	-	-	2 (50%)	3 (60%)	1 (100%)	2 (100%)
MBL	6 (42.8 %)	4 (44.4%)	Nil	1 (20%)	Nil	Nil
AmpC $\beta$ -lactamases	10(71.4%)	4 (44.4%)	1 (25%)	2 (40%)	Nil	1 (50%)

The present study showed that *Acinetobacter* spp. was the commonest isolate (37.83%) followed by *P. aeruginosa* (24.32%) in both early and late onset VAP. Similar findings were reported by Dey *et al.*, (2007).

MDR organisms are on the rise in intensive care settings. Antimicrobial susceptibility pattern of the isolates obtained in the present study showed that most of the Gram negative bacilli were MDR.

*Acinetobacter* species and *P. aeruginosa* were found to be resistant to most of the classes of antibiotics in current use, some including carbapenems. Enterobacteriaceae isolated showed a high level of resistance to beta-lactam antibiotics and were sensitive to carbapenems. Such a high level of drug resistance has also been documented in studies conducted by Joseph *et al.*, (2009) and Dey *et al.*, (2007).

*Acinetobacter* spp are generally less virulent than *P. aeruginosa*, these generally are susceptible to carbapenems but resistance is increasing due either to IMP-type metalloenzymes or carbapenemases of the OXA- type. In our study 44.44% of *Pseudomonas* and 42.8 % of *Acinetobacter* spp were plasmid-mediated MBL- producing strains as detected by EDTA disc synergy test

and Modified Hodge test similar to that of Gales *et al.*, (2001).

Emergence of ESBLs and AmpC beta-lactamases in a hospital set up are of increasing concern. ESBLs are most commonly produced by *Klebsiella* spp and *E. coli* but may also occur in other Gram-negative bacteria as documented by Joseph *et al.*, (2010). Among the isolates obtained in the present study, 60% of *K. pneumoniae*, 50% of *E. coli*, 100% of *Enterobacter* spp and *C. koseri* were found to be ESBL producers.

Although the current CLSI guidelines do not describe any method for detection of isolates producing AmpC beta lactamases, the present study incorporated the AmpC-disk method described by Singhal *et al.*, (2005) to detect AmpC beta lactamases and 71.43% of *Acinetobacter* spp, 44.44% of *P.aeruginosa*, 40% of *K.pneurnoniae*, 25% of *E. coli* and 50% of *Enterobacter* spp have shown production of AmpC beta lactamases, which is in concurrence with study by Philippon *et al.*,(2002). A study by Joseph NM *et al.*, (2009) showed methicillin sensitive *Staphylococcus aureus* (MSSA) to be a major Gram positive bacteria causing VAP. In our study 2 isolates of MSSA causing early-onset VAP were isolated.

The study population and number of isolates may not be representative of the scenario everywhere; further multi-centered studies are needed to strengthen the outcomes of the present study.

We conclude that the rate of VAP in mechanically ventilated patients is on the rise and it is increasingly associated with MDR pathogens. Knowledge of incidence of VAP, their causative microbial flora in a local setting along with information on the susceptibility patterns will help in selection of the appropriate antibiotic for therapeutic use and a better outcome. This will also prevent indiscriminate and irrational use of antibiotics which contribute to emergence of drug resistant strains.

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