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RESEARCH ARTICLE

ANTIMICROBIAL RESISTANCE OF BACTERIAL ISOLATES FROM RESPIRATORY SECRETIONS OF VENTILATOR ASSOCIATED PNEUMONIA IN THE INTENSIVE CARE UNITS OF A TERTIARY CARE HOSPITAL

***Dr. Ruchita Attal, Dr. Vijayshri Deotale and Ms. Anagha Potharkar**

Department of Microbiology, MGIMS, Sevagram

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ABSTRACT

Background: Ventilator-associated pneumonia (VAP) contributes to approximately half of all cases of hospital-acquired pneumonia. VAP is estimated to occur in 9–27 % of all mechanically ventilated patients. Due to increase in incidence of drug resistance among the VAP isolates, correct diagnosis is a challenge for an accurate management.

Aim: The aim of the study was to isolate the causative agents for VAP in patients on mechanical ventilation and determine their antibiotic susceptibility testing and also
Study the risk factors associated with VAP in critically ill patients admitted in ICUs.

Material & Method: This prospective observational study was conducted in the intensive care units (ICUs) from July to Dec 2015 undergoing mechanical ventilation for >48hrs. Endotracheal aspirates were collected from patients suspected VAP and quantitative cultures were performed .VAP was diagnosed on the basis of CPIS score.

Results: The incidence of VAP in our study was 34.61% and incidence rate of VAP was 46.65 per 1000 ventilation days.83.83% (83/99) isolates were from MICU and predominant VAP pathogens were *Acinetobacter spp*. (51.75%) . *Acinetobacter baumanii and Enterobacteriaceae* were the prevalent MDR isolates. This might be attributed to prolong hospital stay and improper cleaning of the tubes.

Conclusion: Increase in VAP cases with the potential drug resistant organisms is an emerging threat in our ICUs. Quantitative culture of endotracheal aspirate is a useful test for diagnosis of VAP and also help to determine the drug resistance in ICUs.

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INTRODUCTION

Ventilator-associated pneumonia (VAP) contributes to approximately half of all cases of hospital-acquired pneumonia. (American Thoracic Society, 2005; Vincent *et al*., 1995) VAP occurs frequently in critically ill patients and is associated with significant morbidity. As per the definition given by American Thoracic Society & Infectious Disease Society of America, VAP is defined as pneumonia that occurs 48–72 hours or thereafter following endotracheal intubation, characterized by the presence of a new or progressive infiltrate, signs of systemic infection (fever, altered white blood cell count), changes in sputum characteristics, and detection of a causative agent. (American Thoracic Society, 2005) VAP is usually classified as either early onset, occurring within the first four days of Mechanical ventilation (MV) or late onset, developing five or more days after initiation of MV. (Hunter, 2012) VAP is estimated to occur in 9–27 % of all mechanically ventilated patients, with the highest risk of being early in the course of hospitalization. (American Thoracic Society, 2005; Chastre and Fagon, 2002) The incidence of VAP varies among different studies, depending on the definition, the type of hospital or ICU, the population studied, and the level of antibiotic exposure. (Hunter, 2012; Niederman and Craven, 2005) Intubation and mechanical ventilation are associated with 6- to 21-fold increased risk of acquiring pneumonia in hospital settings. (Chastre and Fagon, 2002) Several risk factors may predispose patients to either colonization of the respiratory tract with pathogenic microorganisms or aspiration of contaminated secretions. The complex interplay between the endotracheal tube, presence of risk factors, virulence of the invading bacteria and host immunity largely determine the development of VAP. The presence of an endotracheal tube is by far the most important risk factor, resulting in a violation of natural defense mechanisms (the cough reflex of glottis and larynx) against micro-aspiration around the cuff of the tube. (Chastre and Fagon, 2002; Zolfaghari and Wyncoll, 2011) The type of

Corresponding author: **Dr. Ruchita Attal,**
Department of Microbiology, MGIMS, Sevagram

organism that causes VAP usually depends on the duration of mechanical ventilation. In general, early VAP is caused by pathogens that are sensitive to antibiotics, whereas late onset VAP is caused by multi-drug resistant and more difficult to treat bacteria. Due to increase incidence of VAP in patients requiring mechanical ventilation, a study has been conducted to know the predisposing factors and predominant causative agents for VAP.

Objectives

1. To isolate the causative agents for Ventilator Associated Pneumonia in patients on mechanical ventilation
2. To determine the antibiotic susceptibility pattern of isolates.
3. To identify the risk factors associated with VAP in critically ill patients admitted in ICUs

Methodology

This prospective observational study was conducted in the intensive care units (ICUs) of a tertiary care hospital in central India for duration of six months from July to Dec 2015. The study was approved by the Ethical committee of Institute, and informed consent was taken from each patient's attendant. Postoperative patients requiring ventilation were admitted to the Surgery ICU (SICU). Patients with medical conditions, who were on ventilators were admitted to the MICU. Any lower respiratory tract infection that developed after 48 h of mechanical ventilation and was judged not to have been incubating before mechanical ventilation was taken as VAP. VAP rate was defined as the number of VAPs/1,000 ventilator days. (Rodrigues *et al*., 2009) Patients who were already on mechanical ventilators before admission were excluded. During the study period, a total of 3,156 patients were admitted to ICUs and amongst which 537 were intubated and put on mechanical ventilators. Among them, those patients who were ventilated for more than 48 hours were eligible as per inclusion in the study .

Data Collection

Data was collected of all the patients who were enrolled in the study from the attending physicians and nurses as well as from the medical records, bedside flow sheets, radiographic reports. It includes demographic data at ICU admission: name, age, gender, hospital number, primary diagnosis, date of admission in hospital and ICU. Associated risk factors for the development of VAP was recorded. From the day three study patients were monitored for the development of VAP using clinical and microbiological criteria until either patients extubated or death. Clinical criteria include white blood cell count > 12,000 or < 4,000/mm³ or bands count > 10%; (3) axillary temperature > 38°C or < 36°C; and (4) worsening of PaO2/FiO2 ratio > 15 and microbiological criteria include purulent tracheal secretions and quantitative culture of endotracheal aspirates showing significant growth.

Criteria for diagnosing VAP

VAP was considered as a subjective clinical impression. The patients fulfilling both the clinical and microbiological criteria were diagnosed to be suffering from VAP. Clinical criteria included modified clinical pulmonary infection score (CPIS) > 6 **(Table 1) (**Pugin *et al*., 1991) and microbiological criteria included positive Gram stain (> 10 polymorph nuclear cells/low power field and ≥ 1 bacteria/oil immersion field with or without the presence of intracellular bacteria) and quantitative endotracheal aspirate culture showing ≥ 10^5 CFU/ ml. (Porzecanski and Bowton, 2006; Wu *et al*., 2002; Koenig and Truwit, 2006)

Identification of VAP pathogens

a.Processing of Sample: Quantitative culture of endotracheal aspirates (EA) was performed for identification of VAP pathogens. EA were serially diluted in sterile normal saline as 1/10, 1/100, 1/1,000, and 0.01 ml of 1/1,000 dilution then inoculated on 5% sheep blood agar. After incubation at 37^{o}C in a 5% CO_2 incubator for 24 hours, a colony count was done and expressed as number of colony forming units per ml (CFU/ml). (Baselski *et al*., 1992)

b.Identification of isolate: The microorganisms isolated at a concentration of more than 10^5 CFU/ ml was considered as VAP pathogens and identified based on standard bacteriological procedures including Gram's stain, colony morphology on blood agar and Mac Conkey agar, and biochemical reactions. (Mackie and McCartney, 1996)

c.Antibiotic susceptibility Test: Antibiotic susceptibility of the isolated microorganisms was performed by Kirby – Bauer Disk Diffusion method as per CLSI guidelines. (Clinical and Laboratory Standard Institute, 2006) In our study, multidrug resistance (MDR) definition for Gram-negative organisms was taken as non-susceptible to more than one agent in at least 3 antimicrobial categories. (Magiorakos *et al*., 2012) *Staphylococcus* was considered as MDR if (i) it was methicillin-resistant and (ii) non-susceptible to more than one agent in 3 antimicrobial categories. (Magiorakos *et al*., 2012)

d.Data was expressed as mean ± standard deviation and percentages. Quantitative variables are analyzed with Z test. Statistical significance was considered when $p < 0.05$.

RESULTS

During the study period, a total of 3,156 patients were admitted to different ICUs and amongst which 546 were intubated and mechanically ventilated. Among them, only 286 patients were ventilated for more than 48 hours and were eligible for inclusion in the study (Table 2). Maximum i.e. 361 (66.11%) were from MICU followed by PICU (15.01%). During the study period, 171 endotracheal aspirates were received and processed in the Microbiology laboratory. Samples were evaluated for Gram stain and quantitative culture. Gram stain showed positive findings in 131 (76.60%) i.e. (> 10 polymorphonuclear cells/low power field and ≥ 1 bacteria/oil immersion field) and quantitative culture results were satisfactory in 99 (57.9%) samples and had shown to have growth of either one or two organisms, 25 (14.6%) were sterile, while 47 (27.5%) showed growth of multiple organisms. Amongst 171 total endotracheal aspirates, 99 (57.9%) which were shown significant culture growth and positive Gram stain findings were further studied and were subjected for microbiological analysis and antibiotic susceptibility testing. Clinical Pulmonary Infection Score (CPIS) based on above six clinical assessments, were calculated for these 99 microbiologically confirmed VAP patients and was found to be > 6.

Table 1. Clinical Pulmonary Infection Score (CPIS Score) : Criteria for diagnosing VAP

S. No.	CPIS points	0	1	2
1.	Temperature (0C)	≥36.5 and ≤ 38.4	≥38.5 and ≤ 38.9	≥39 and ≤36
2.	Leucocytes count (per mm^3)	4,000 – 11,000	<4,000 or > 11,000	<4,000 or > 11,000 + band forms ≥ 500
3.	Tracheal secretions	Rare	Abundant	Abundant+ Purulent
4.	PaO_2/ FiO_2 mm of Hg	>240 or ARDS	-	≤240 and no ARDS
5.	Chest Radiograph	No Infiltrate	Diffuse Infiltrate	Localized Infiltrate
6.	Culture of Tracheal Aspirate	Light growth or No growth	Moderate or Heavy growth of pathogenic bacteria	Moderate or Heavy growth of pathogenic bacteria and presence of same bacteria in Gram stain

Table 2. Distribution of Mechanically ventilated patients in ICUs

ICUs	No. of Total Ventilated Patients	Duration of Ventilation	
		<48 hrs	>48 hrs
MICU	361	163	198
TraumaICU	17	05	12
Maternity ICU	4	02	02
NICU	40	15	25
PICU	82	51	31
SICU	42	24	18
Total	546	260	286

Table 3. Demographic profile of 99 patients enrolled in this study

Profile		N = 99	%
Age	< 20yrs	26	26.26
	20- 40 yrs	34	34.34
	41-60Yrs	27	27.27
	>60 yrs	12	12.12
Gender	Male	66	66.66
	Female	33	33.33
ICUs	Medicine ICU	83	83.83
	Paediatric ICU	7	07.07
	NICU	7	07.07
	TRAUMA ICU	1	01.01
Outcome	Expired	46	46.46
	Discharged	53	53.53

Table 4. Underlying Clinical condition in Ventilator Associated Pneumonia (VAP) cases

S.No.	Underlying Cause	No. (%)	%
1	Poisoning	31	31.31
2	CNS infections (Meningitis/Encephalitis)	8	8.08
3	Snake bite (Neuroparalytic/vasculotoxic)	7	7.07
4	ARDS	13	13.13
5	Sepsis with Shock	21	21.21
6	Stroke	5	5.05
8	Ischemic Heart Disease	5	5.05
9	Preterm with LBW with ARDS	5	5.05
10	Full term with meconium aspiration syndrome with sepsis	3	3.03
11	Microcephaly with DIC with sepsis	1	1.01

Table 5. Distribution of pathogens associated with VAP (N=114)

Antibiotics	Percentage resistance to Enterbacteriaceae n=	Percentage resistance to P.aeruginosa n=	Percentage resistance to Acinetobacter spp.n=
Ampicillin			
Ampicillin-sulbactam			
Amoxicillin-Clavulanic acid			
Ceftazidime			
Gentamicin			
Piperacillin			
Amikacin			
Aztreonam			
Ceftriaxone			
Cefotaxime			
Cefepime			
Ciprofloxacin	62.96	14.28	75.47
Imipenem	18.5	28.57	60.37
Meropenem	18.5	28.57	26.41
Piperacillin+Tazobactam	40.74	14.28	66.03
Co-trimoxazole	59.25	-	-
Tigecycline	0	-	-
Chloramphenicol	-	-	-
Polymyxin-B	0	0	0
Colistin	0	0	0

P.aeruginosa: Pseudomonas aeruginosa

Table 6. Antibiotic resistance of Gram-negative organisms from 99 patients enrolled in the study

Antibiotics	Percentage resistance *to Enterbacteriaceae* n= 27	Percentage resistance to *P.aeruginosa* 7	Percentage resistance to *Acinetobacter spp.* 53
Ampicillin	74.07	-	-
Ampicillin-sulbactam	37.03	-	35.84
Amoxicillin-Clavulanic acid	55.55	42.85	79.24
Ceftazidime	66.66	28.57	62.26
Gentamicin	48.14	14.28	66.03
Piperacillin	-	14.28	71.69
Amikacin	48.14	42.85	71.69
Aztreonam	-	14.28	-
Ceftriaxone	51.85	28.57	77.35
Cefotaxime	62.96	-	41.5
Cefepime	-	14.28	73.58

Table 7. Demographic data of ventilator-associated pneumonia patients in the intensive care Unit according to mortality status

Variables	Status at Discharge		(Z) Test result	P- value
	Died (n=46)	Alive (n=53)	-	-
Age (years)	33±23.3	35±20.3	-	-
CPIS score	6.65±1.02	6.56±0.77	0.1844	(P>0.05) not significant
ICU stay (Days)	11.56±9.50	16±12.1	2.0429	(P<0.05) significant
Male	32(69.56%)	34(64.15%)	-	-
Female	14(30.44%)	19(35.85%)	-	-

Growth of multiple organisms was not further studied as it suggests oropharyngeal contamination. (Henry D Isenberg 2nd edition)

Out of total 286 patients who were on mechanically ventilation for >48 hrs, 99 fulfilled the clinical and microbiological criteria for VAP. The incidence of VAP in our study was 34.61% and incidence rate of VAP was 46.65 per 1000 ventilation days. The demographic characteristics of the 99 patients who developed VAP are depicted in Table 3. Majority of the patients (34.34%) were between 20-40 yrs age whereas only 12.12% were more than 60 yrs. A total of 66(66.66%) male and 33(33.33%) female patients were enrolled in the study. The crude mortality rate of the patients was determined to be 46.46%. Most of the patients (83.83%) were from Medicine ICU. The most frequent cause of ICU admission was suicidal poisoning (31.31%) followed by sepsis with shock (21.21%). (Table 3 & 4)

Causative Agents

Most of the cases of VAP were caused by Gram negative bacteria, which accounted for 88.59% of causative organisms. *Acinetobacter spp*. was (51.75%) and *Enterobacteriaceae* (27.82%) were the common Gram negative bacteria associated with VAP and *Staphylococcus aureus* (6.14%) was the common Gram positive bacteria among patients with VAP. In our study we had also reported one isolate of *Streptococcus pneumoniae* and five (4.38%) *Candida spp.* (Table 5) Antibiotic susceptibility of all the bacterial isolates was performed by Kirby-bauer disk diffusion method. The antibiotic resistance among the *Enterobactericeae.* Highest resistance was observed against ß-lactam group of antibiotics (74.7% isolates were resistant to ampicillin and 55.55% isolates were resistant to amoxicillin-clavulanic acid); 66.66% isolates were resistant to third generation cephalosporins & 62.96% to ciprofloxacin. However they were relatively less resistant to carbapenems (18.5%), Amikacin (48.14%), Gentamicin (48.14%) and piperacillin-tazobactam (40.74%). Polymyxin B and tigecycline have been found to be sensitive in all the strains tested. Among the non-fermenting Gram negative bacilli, (Figure 1) 79.24% *Acinetobacter spp.* isolates were resistant to cefazoline and amoxycillin-clavulanic acid each, 77.35% to cepfaperazone and ceftriaxone, 75.47% to ciprofloxacin, 71.69% to amikacin & piperacillin each and 50.94% to doxycycline. However they were less resistant to ampicillin-sulbactam (35.84%), Meropenem (26.41%) and imipenem (60.37%). All the *Pseudomonas* isolates were found to be less resistant showing 14.28% resistance to piperacillin, gentamicin, ciprofloxacin, piperacillin-tazobactam, cefepime and aztreonam each. 42.85% isolates found to be resistant to amikacin and 28.57% to ceftazidime & carbapenem. All the nilfermenters were sensitive to polymyxin B. In *Staphylococcus aureus.*, 33.37% were methicillin-resistant. However no resistance was observed against co-trimoxazole, gentamicin and linezolid. (Figure 1)

In our study, we also compared the CPIS score, length of ICU stay and certain demographic variables like age, gender among the clinically and microbiologically confirmed VAP patients according to the mortality status. The mean age of VAP patients at dead was 33 years with standard deviation of 23.3 years. There was no statistically significant difference in the CPIS score of VAP patients died or alive at the time of discharge. The mean±SD ICU stay in died VAP patients compared to alive VAP patients was found to be significant (Table 7). Statistical significance is not considered for factor age due to large variation in standard deviation of dead and alive patients.

DISCUSSION

VAP is an important nosocomial infection among ICU patients, causing high morbidity and mortality. According to the National Nosocomial Infection Surveillance Program, the incidence of VAP is 7.6 cases per 1,000 patient ventilator days. (Edwards *et al.*, 2007) VAP occurs due to interplay of three factors - impaired host defense, access of pathogenic bacteria in sufficient numbers to the lower respiratory tract and the virulence of the organism. (Weber *et al.*, 1998) A total of 286 patients who were mechanically ventilated for > 48 hrs admitted in ICUs, 99(34.61%) patients fulfilled the clinical &

microbiological criteria for diagnosis of VAP. The overall incidence of VAP rate was 46.6 per 1000 ventilation days. The incidence of VAP reported in different studies conducted at various centers varies from 24% to 67%. (Fagon *et al*., 1988; Kerver *et al*., 1987; Torres *et al*., 1990; Mukhopadhyay *et al*., 2003; Kanafani, 2003; Rakshit *et al*., 2005; Ranjan *et al*., 2014; Dominic *et al*., 2012) This variation in the incidence of VAP as observed above is probably related to factors like differences in patient populations, hospital infection control and critical care practices and variability in data collection methods as well as variability in the definition of VAP. A study conducted in Pondicherry, India, showed a incidence rate of 22.94 per 1,000 ventilator days. (Joseph *et al*., 2009) In other Asian countries, the incidence rate is relatively less, ranging from 9 to 12 per 1,000 ventilator days. (Aly *et al*., 2008; Suka *et al*., 2007) The higher incidence of VAP in our study could be attributed to a lower number of cases and lack of adequate nursing staff which may have adversely affected the quality of care given to the patients. The health-seeking behavior of our patients is different compared with that in developed world. Patients seek medical help only when it is absolutely inevitable. By the time patient is referred to the tertiary-care centre, his underlying condition is well advanced and may be irreversible. This may necessitate longer duration of MV, which is directly proportional to development of VAP. The other most important factor in our set-up the number of cases of poisoning that predominated requiring prolonged ventilation, which is proved to be a risk factor.

Most common VAP pathogens are *P. aeruginosa, Acinetobacter spp., E. coli, K. pneumoniae* and *S. aureus* (American Thoracic Society, 2005; Chastre and Fagon, 2002) In our study 83.83% (83/99) isolates were from MICU and predominant VAP pathogens were *Acinetobacter spp*. (51.75%) followed by *Enterobactericeae* (27.82%). It is well correlated with Dey *et al*. (Arindam Dey and Indira Bairy, 2007) who had found 48.94% of *Acinetbacter sp.* The resistance pattern show that most of the VAP pathogens from ICUs have significantly increased in vitro resistance against most of the antibiotics tested according to CLSI guidelines and our ICU recommendation. In our study, mainly *Acinetobacter baumanii and Enterobacteriaceae* were the prevalent MDR isolates. This might be attributed to prolong hospital stay and improper cleaning of the tubes. Hence, steps must be taken to prevent the development and spread of the drug resistant strains. Alterations and rotation in antibiotic prescribing patterns might decline the development and acquisition of antibiotic resistance. Thus, the present study gives importance of knowing the pathogens and their antibacterial susceptibility pattern, common in the particular ICU, to initiate the empirical antibacterial therapy for patients on mechanical ventilation. Although Polymyxin B and tigecycline still effective against most resistant Gram-negative isolates and Linezolid and Gentamicin is still holding the fort against Gram-positive organisms, caution should be observed against rampant use of these drugs.

Conclusion

Ventilator associated pneumonia (VAP) continues to be a major challenge to the critical care physicians. It is the leading cause of morbidity and mortality in mechanically ventilated patients in the intensive care units. Most of the risk factors of VAP are preventable. Aspiration of colonized pathogenic microorganisms on the oropharynx and gastrointestinal tract is the main route for the development of VAP. On the other hand, the major risk factors for VAP are intubation and the duration of mechanical ventilation. Current guidelines for the management of VAP strongly recommend the use of early, appropriate empirical antibiotic therapy based on patient risk factors for multi-drug resistant pathogens. Vigilance is required for patients admitted in the ICU and on mechanical ventilation. The endotracheal aspirate of patients on mechanical ventilation should be sent for routine culture and sensitivity. This study showed that quantitative culture of endotracheal aspirate is a useful test for diagnosis of VAP and also help to determine the drug resistance in ICUs.

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