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Antibiotic Susceptibility Pattern of Bacteria Isolated from Adult Patients with Ventilator Associated Pneumonia (VAP) in Intensive Care Units in a Tertiary Care Hospital

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Abstract
Ventilator-associated pneumonia (VAP) refers to pneumonia developing in a patient on mechanical ventilator >48 hours after intubation or tracheostomy. VAP is an important cause of morbidity and mortality in mechanically ventilated patients. The aim of the study was to assess the clinical and bacteriological profile of VAP, risk factors, prevalence of multidrug-resistant pathogens in VAP cases in ICU setting and to correlate Endotracheal aspirate (ETA) with blood culture in those cases. The study included 130 adult patients of both sexes who were admitted to ICUs and fulfilling the criteria of VAP. Endotracheal aspirates (ETA) and blood samples were collected from the suspected VAP cases and subjected to culture and antimicrobial susceptibility testing as per standard protocol. Incidence of VAP was found to be 40.8%, out of which 81.13% had late-onset VAP. Diabetes mellitus, advancing age (>60 years) and Chronic Obstructive Pulmonary Disease were the important risk factors associated with VAP. The most frequently isolated microorganism was Acinetobacter species (70.37%), followed by Pseudomonas aeruginosa (14.81%) and Klebsiella pneumoniae (5.56%). All Acinetobacter species and 75% of Pseudomonas aeruginosa isolates were multidrug resistant. Overall carbapenem resistance was 46%. Blood culture results were positive for 47.16% cases of VAP of which 76% showed bacteraemia of pulmonary origin. Acinetobacter species (33.96%) was the most common isolate from blood. Mortality in VAP cases was 50.94%. Due to the increasing incidence of multidrug-resistant organisms in ICUs, early and correct diagnosis of VAP is an urgent challenge for an optimal antibiotic treatment and cure. Hence, knowledge of the local microbial flora causing VAP and effective infection control practices are essential to improve clinical outcome.

Keywords: *Ventilator-associated pneumonia, Endotracheal aspirate, Acinetobacter species, Multidrug resistant.*

Introduction

Ventilator Associated Pneumonia (VAP) is defined as pneumonia occurring more than 48 hours of mechanical ventilation and not incubating at the time of intubation.VAP is the most frequent Intensive Care Unit (ICU) acquired infection occurring in 9–24% of patients intubated for longer than 48 hours.[1] Diagnostic testing in VAP is necessary as it allows one to define whether a patient has pneumonia or any other related diseases. Diagnosing VAP requires a high clinical suspicion combined with bedside examination, radiographic examination and microbiological analysis of respiratory secretions. Due to increasing incidence of Multi Drug Resistant (MDR) organisms in ICUs, early and correct diagnosis of VAP is an urgent challenge for optimal antibiotic treatment.[2] The present study aimed to find out the incidence of VAP in ICUs in this tertiary care hospital, major pathogens responsible for VAP, the antimicrobial susceptibility pattern of the organisms isolated from cases of VAP and to correlate Endotracheal aspirate (ETA) with blood culture in cases of VAP. This will help in initiating appropriate antibiotic treatment, thus reducing the morbidity, mortality, length of hospital stay, cost of treatment and the adverse effects of inadequate antibiotic treatment on patient prognosis.

Materials and Methods

This was a prospective study, conducted over a period of one year and seven months (1st April 2013 to 31st October 2014) after taking permission from the Institutional Ethics Committee. The study included 130 adult patients (> 14 years of age), of both sexes who were admitted to ICUs and fulfilling the criteria of VAP.[3] Patients with respiratory malignancies, HIV positive patients and those not giving consent were excluded from this study.

Endotracheal aspiration was performed under aseptic precautions using a sterile 30 cm long 12F suction catheter. Culture of endotracheal aspirates (ETA) of all suspected cases of VAP was done. ETA was inoculated on blood agar, chocolate agar and MacConkey agar and incubated over night at 37°C. Any growth observed was identified using standard biochemical tests.[4] Antibiotic susceptibility testing was performed on Mueller Hinton agar, according to CLSI guidelines.[5] Blood culture was also performed in these 130 cases, for which 10 ml blood was inoculated in 50 ml of tryptic soy broth and incubated at 37°C. Subcultures were done on MacConkey agar and Blood agar on 2nd day, 4th day and 7th day. Growth seen any subculture was identified by standard biochemical tests.[4]

Results

In this prospective study, 130 patients were enrolled as per inclusion criteria. VAP was found to be more common in males (60%), than in females (40%). Maximum patients belonged to the age group of 21-40 years (42.3%), with mean age of 37.42±16.95 years. Eighty percent of the cases were from Medical Intensive Care Unit (MICU) and 20% from Intensive Respiratory Care Unit (IRCU). The predominant clinical conditions were poisoning (17.7%), followed by meningoencephalitis and Acute Febrile illness with Acute Respiratory Distress Syndrome (12.3% each). Diabetes mellitus, advancing age (> 60 years) and Chronic Obstructive Pulmonary Disease were the important risk factors associated with VAP.

As per Clinical Pulmonary Infection Score (CPIS),[3] 53 cases (40.8%) turned out to be VAP and the remaining 59.2% were Non-VAP cases. Mechanical ventilation days, leukocyte count, PaO_2 and CPIS was highly significant in patients with VAP as compared to Non VAP cases (Table 1). Late-onset VAP was more common (81.13%), than early-onset VAP. Sensitivity and specificity of qualitative culture was 100% and 22.08%, respectively while the positive and negative predictive values were 46.9% and 100%, respectively.

Overall gram-negative bacilli predominated (96.3%) over gram-positive cocci. Most of the endotracheal aspirates showed monomicrobial pathogens. Only 7.5% were polymicrobial. The most frequently isolated microorganism was *Acinetobacter species* (70.37%), followed by *Pseudomonas aeruginosa* (14.81%), *Klebsiella pneumoniae* (5.56%), *Enterobacter species* (3.7%), Methicillin Resistant *Staphylococcus aureus* (3.7%) and *Escherichia coli* (1.86%) [Figure 1].

With first line antibiotics, all gram-negative bacilli showed 100% resistance to amoxicillin-clavulanic acid and high degree of resistance to amikacin, piperacillin and ciprofloxacin. All gram-negative

bacilli showed resistance to cephalosporins (cefotaxime and cefepime), except one *Enterobacter species* which was sensitive to cefepime. Overall carbapenem resistance was 46% [Figure 2]. For *Acinetobacter species*, susceptibility to colistin and tigecycline was 100% and 94.74%, respectively. Two *Acinetobacter species* showed ESBL production, while one showed MBL production. All *Acinetobacter species* and 75% of *Pseudomonas aeruginosa* isolates were MDR.

All *Pseudomonas aeruginosa* were susceptible to colistin (100%) and 75% were susceptible to tigecycline. Two of the *Pseudomonas aeruginosa* species showed MBL production [Figure 3]. Two MRSAs isolated from late onset VAP cases were susceptible to vancomycin, linezolid and netilmycin. Blood culture results were positive for 47.16% cases of VAP, of which 76% showed bacteraemia of pulmonary origin [Figure 4]. *Acinetobacter species* (33.96%) was the most common isolate from blood, followed by MRSA (5.67%), and *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (3.8% each). Mortality in VAP cases was 50.94%.

Table 1: Parameters of VAP and Non-VAP cases with significance

Parameters	VAP (n=53)	NON-VAP (n=77)	P value	Significance
Mechanical ventilation days	22.68 ± 13.462	7.43 ± 3.266	<0.0001	Significant
Leucocytes (cells/µl)	20,024.53 ± 3417.652	16,361.04 ± 3359.91	<0.0001	Significant
CPIS score	7 ± 2	5 ± 2	<0.0001	Significant
PaO2	76.11 ± 13.098	128.75 ± 37.63	<0.0001	Significant
PaCO2	38.87 ± 2.354	39.56 ± 3.91	0.261	Non-significant
Growth in quantitative culture (>10^5 CFU/ml)	50	09	<0.0001	Significant
Duration of hospital stay	27.77 ± 16.595	11.21 ± 5.320	0.311	Non-significant

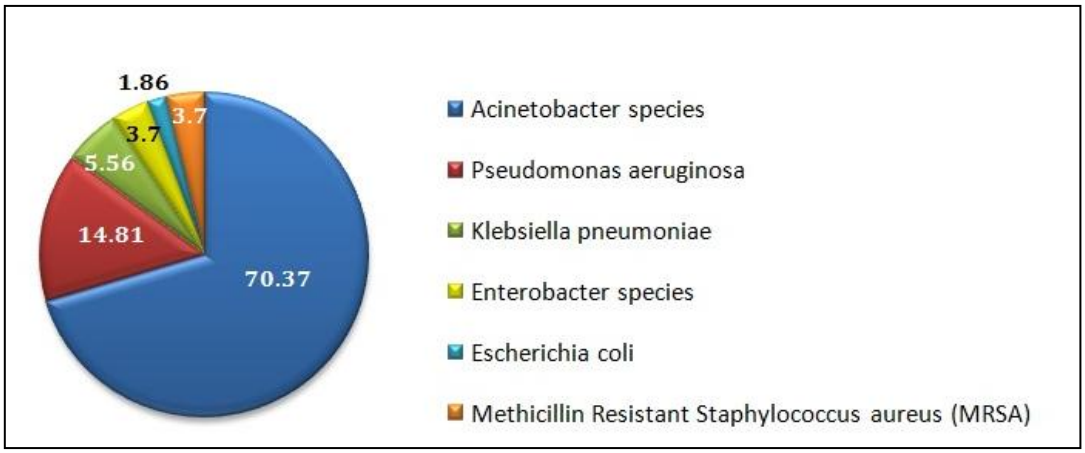

Figure 1: Organisms responsible for VAP

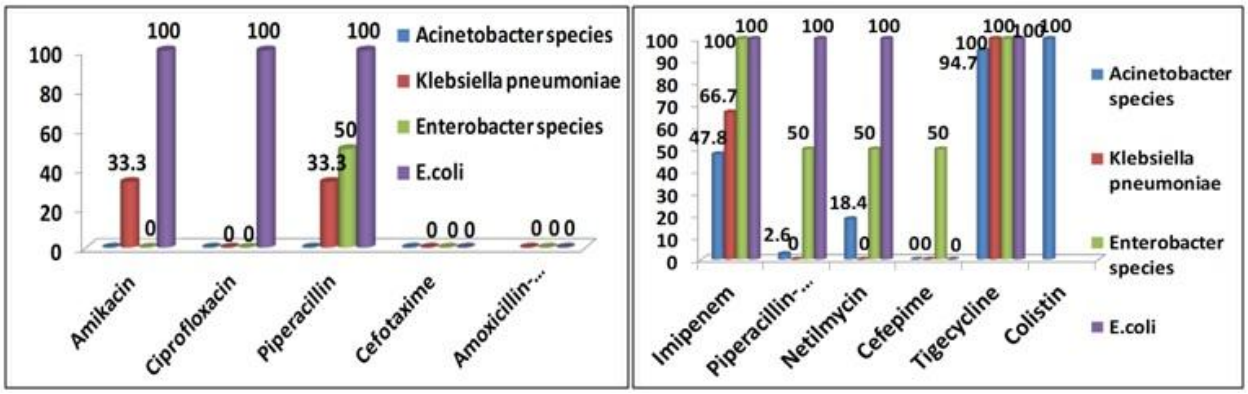

Figure 2: Antimicrobial susceptibility pattern of Gram negative bacilli except *Pseudomonas aeruginosa* in VAP cases to first and second line antibiotics

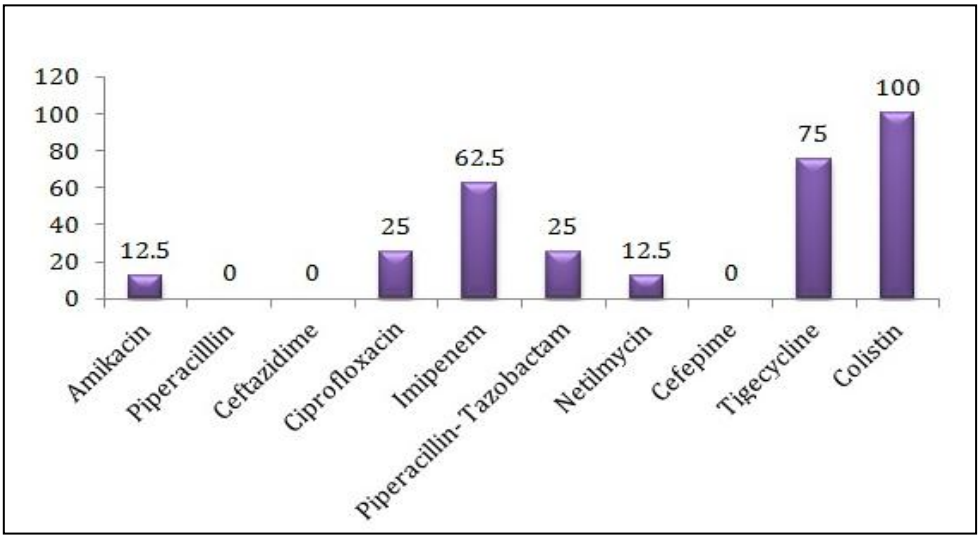

Figure 3: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* in VAP (%)

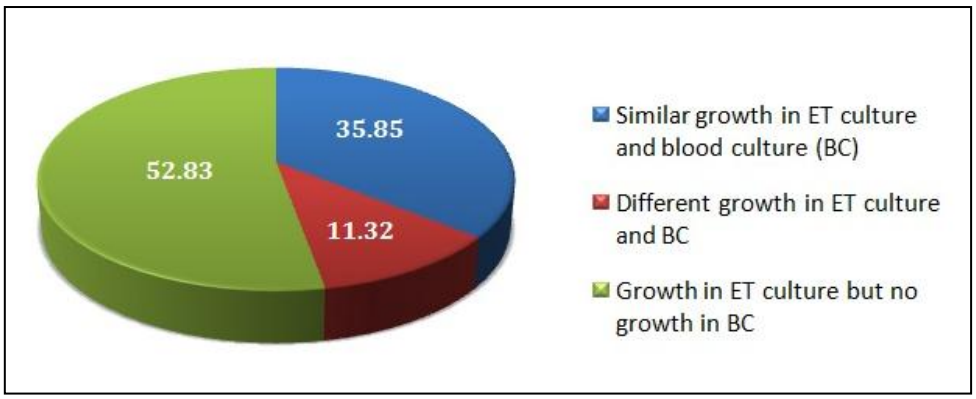

Figure 4: Correlation of the findings of endotracheal secretion cultures and blood cultures in VAP (%)

Table 2: Male predominance in different studies

Studies	Year	Percentage of males
Dey *et al.*[2]	2007	71.1%
Gadani *et al.*[6]	2010	62%
Gupta *et al.*[7]	2011	63.5%
Rakshit *et al.*[8]	2005	56.9%
Present study	2014	60%

Table 3: Common organisms isolated from cases of VAP in different studies

Studies	Isolates (%)			
	Acinetobacter sp.	Pseudomonas aeruginosa	Klebsiella sp.	Staphylococcus aureus
Gupta *et al.*[7]	20.0	30.0	23.3	26.7
Rakshit *et al.*[8]	5.9	32.0	20.0	17.6
Joseph *et al.*[13]	29.8	21.3	6.4	14.9
Goel *et al.*[16]	23.6	35.0	13.6	2.4
Saldanha *et al.*[17]	10.3	41.1	15.4	13.1
Rajasekhar *et al.*[20]	31.0	25.0	12.5	6.2
Present study	70.4	14.8	5.5	3.7

Table 4: Predominant organisms responsible for early and late onset VAP

Studies	Predominant organisms in early onset VAP	Predominant organisms in late onset VAP
Gupta *et al.*[7]	*Klebsiella* and *Acinetobacter*	*Pseudomonas* followed by MRSA
Rakshit *et al.*[8]	*Pseudomonas* followed by *Klebsiella*	*Pseudomonas* followed by *Klebsiella*
Joseph *et al.*[13]	*Acinetobacter* followed by *S. aureus*	*Acinetobacter* followed by *S. aureus*
Ibrahim *et al.*[14]	*Pseudomonas* followed by MSSA and MRSA	*Pseudomonas* followed by MRSA
Present study	*Acinetobacter* followed by *Pseudomonas aeruginosa*	*Acinetobacter* followed by *Pseudomonas aeruginosa*

Discussion

Nosocomial infections represent a major health problem because of the excess morbidity, mortality and cost. Ventilator associated pneumonia (VAP) is considered to be one of the most frequent and troublesome problems in intubated patients receiving mechanical ventilation in Intensive Care Units (ICUs). Because appropriate antimicrobial treatment of patients with VAP significantly improves the outcome, prompt identification of organisms in these patients and accurate selection of antimicrobial agents represent important clinical goals.

This study was conducted in 130 clinically suspected cases of VAP. Identification of bacterial agents and their susceptibility to different antibiotics, along with correlation of ET aspirate cultures with blood cultures were done.

In the present study, 60% of the cases were males and 40% were females which is almost similar to the study by Gadani *et al.*[6] (62% males). Similar male preponderance was also observed in other studies, as shown in Table 2.

The patients studied belonged predominantly to the age group of 21 – 40 years (42.3%), with a mean of 37.42 ± 16.95 years. Similarly, in a study conducted by Gupta *et al.*, most patients belonged to the age group of 21– 30 years.[7]

Patients from MICU and IRCU who were clinically suspected of VAP were enrolled in this study. Maximum patients (80%) were from MICU. The important clinical conditions found in this study were poisoning (17.7%), followed by meningoencephalitis and Acute Respiratory Distress Syndrome (12.3% each). In a study conducted by Rakshit *et al.*,[8] organophosphorous poisoning was found in 21.56% of the patients. Similarly, in a study by Gupta *et al.*,[7] though neuroparalytic snake bite predominated (22.43%), meningoencephalitis constituted 16.82% of the cases and poisoning in 12.15%.

In this study, about 11.54% of the patients had associated diabetes mellitus (DM) and almost 11% had Chronic Obstructive Pulmonary Disease (COPD). These conditions predispose to colonization and pneumonia because of disease associated impairment of host defense function. A study conducted by Alp *et al.*[9] showed that COPD and coma or reduced consciousness are independent risk factors for development of VAP. In a study by Fernando *et al.*,[10] 7.3% of patients had COPD and 16% had DM. COPD was present in 19.17% cases in a study by Arango *et al.*[11]

The systemic signs of pneumonia such as fever, dyspnoea, tachycardia and leukocytosis as observed in maximum number of patients in this study, are non-specific and they can be caused by any state that releases the cytokines, interleukin-1, interleukin-6, tumor necrosis factor alpha and gamma interferon in conditions such as trauma, surgery, the fibroproliferative phase of ARDS, deep vein thrombosis, pulmonary embolism and pulmonary infarction.[10] In a post-mortem study conducted by Fabregas *et al.*,[12] when findings on histologic analysis and cultures of lung samples obtained immediately after death was used as reference, the clinical diagnostic criteria of VAP which includes, a new and persistent (> 48 hours) infiltrate on chest radiograph plus two or more of the following three criteria (i) fever >38.3^{0}C (ii) leucocytosis of >12×10^9/ml, and/or (iii) purulent tracheobronchial secretions, had a sensitivity of 69% and a specificity of 75% for establishing the diagnosis of VAP.

Because of the poor specificity of the clinical diagnosis of VAP, Pugin *et al.*[3] developed a clinical score, called the Clinical Pulmonary Infection Score (CPIS), based on six variables. Thus, with a CPIS score of > 6, the probability of patient having VAP is more. In this study, out of the total 130 clinically suspected cases of VAP, 53 cases (40.77%) had a score > 6 and were considered as VAP patients and

the remaining 59.23% (77 cases) were classified as non-VAP patients. It was also observed that mechanical ventilation days, leucocyte count, CPIS, and PaO_2 were highly significant in VAP cases as compared to non-VAP cases. Gadani *et al.*[6] in a similar study reported that the incidence of VAP is directly proportional to the duration of ventilator support and a declining PaO2 /FiO2 ratio is an early predictor of VAP.

Qualitative culture of ETA was performed in all 130 cases. All VAP cases i.e. 53 cases showed growth in qualitative culture, whereas from 77 Non-VAP cases, growth was seen in 60 patients only. Thus, the sensitivity and specificity of qualitative culture of endotracheal aspirates were 100% and 22.08%, respectively. The positive and negative predictive values were 46.9% and 100%, respectively.

Late onset VAP was predominant in this study (81.13%), as also reported by Gadani *et al.*[6] and Joseph *et al.*[13] (70% and 66% respectively). Also, in a study from Lucknow,[7] early VAP was seen in only 13% cases whereas late onset VAP contributed to 87% of the cases. As opposed to the above studies, Ibrahim *et al.*[14] showed that 56% patients developed early VAP and 44% late VAP.

The organisms responsible for VAP vary according to diagnosis, institution, prior antibiotic exposure and duration of mechanical ventilation. In this study, the aerobic gram-negative bacilli predominated (96.3%), while gram-positive cocci were only 3.7%. The predominance of aerobic gram-negative bacilli was also high in two other Indian studies from Uttarakhand[15] and Haryana[16] – 89% and 97%, respectively. Studies by Gupta *et al.*[7] and Saldanha *et al.*[17] also showed that gram-negative bacilli were responsible in 77% and 81% of the cases of VAP and gram-positive cocci in 23% and 19% cases, respectively.

In the present study, the commonest bacteria isolated were *Acinetobacter species* (70.37%), followed by *Pseudomonas aeruginosa* (14.81%) and *Klebsiella pneumoniae* (5.56%). The predominant aerobic gram-negative bacteria in VAP cases are usually *Pseudomonas aeruginosa* and *Acinetobacter species* followed by *Klebsiella species* and *Escherichia coli*.[16] However, some investigators have reported gram-positive bacteria becoming increasingly common with *Staphylococcus aureus* as the predominant gram-positive isolate,[7] though in this study, only 3.7% of the isolates was *Staphylococcus aureus*. Table 3 shows the common organisms isolated from cases of VAP in different studies.

In this study, polymicrobial bacteria were seen in four cases (7.5%) and all of them were in the late onset VAP category. The incidence of these polymicrobial bacteria was well comparable with other studies. Study conducted by Thakuria *et al.*[15] and Goel *et al.*[16] showed polymicrobial growth to be 10.75% and 5.7% respectively. However, a study conducted in France[18] showed a higher rate of polymicrobial growth (48%) in cases of VAP. Table 4 shows the predominant organisms responsible for early and late onset VAP in different studies.

Goel *et al.*[16] showed that there was an increasing trend of resistance to amoxicillin-clavulanic acid and cephalosporins, which is also seen in the present study (almost 98% resistance to cefepime). This might be due to extensive usage of the above group of drugs. Though decreasing trend of resistance to aminoglycosides and carbapenems was observed by Goel *et al.*,[16] but in this study amikacin resistance was 94.23%, whereas overall imipenem resistance was 46.15% for all gram-negative bacilli. The latter data is almost similar to a study from Uttarakhand (50%).[15]

Thakuria *et al.*[15] showed that for gram-negative bacilli, tigecycline susceptibility was 96%, which is almost similar to the present study (92.3%). Cefepime and amikacin had very low susceptibility in their study, which is in concordance with this study.

In the present study, *Acinetobacter species* showed maximum susceptibility to colistin (100%) and tigecycline (94.74%). Imipenem susceptibility in this study was 47.37%, which is almost similar to a study by Gupta *et al* (50%).[7] Netilmycin susceptibility in Indian studies varied from 65%-89% in the last decade,[2], [19] but in the present study, it was only 18.4%. *Acinetobacter species* showed reduced susceptibility to aminoglycosides i.e. < 18% in all Indian studies,[2], [7], [16], [19] but this study shows 100% resistance. Ciprofloxacin susceptibility have reduced drastically to < 15% in other studies,[16], [19] with 100% resistance in this study.

Klebsiella pneumoniae susceptibility to amikacin varies from 28.5% – 66.7%[2], [7], [16], [19] and in this study it was 33%. Ciprofloxacin susceptibility though reported upto 33% by some authors,[2], [19] a study from Hyderabad[20] have reported 100%

resistance which is exactly similar to the present study. Though imipenem susceptibility has been reported to be 85% – 100% in *Klebsiella pneumoniae*,[2], [7], [19], [20] but in the present study, it was 66.7%. In this study, tigecycline susceptibility of *Klebsiella pneumoniae* was 100%.

In this study, *Pseudomonas aeruginosa* was susceptible to colistin (100%). Tigecycline and imipenem susceptibility was 75% and 62.5%, respectively similar to that reported by Gupta *et al*.[7] Though imipenem susceptibility in *Pseudomonas species* reported in other studies varies from 50% - 78%,[2], [7], [19] a study from Hyderabad[20] has reported the same in only 25%. Ceftazidime susceptibility varies from 31%−50% in different studies,[2], [7], [16], [19] but in this study, there was 100% resistance. *Pseudomonas aeruginosa* is showing reduced susceptibility to aminoglycosides in the recent years, varying from 16%−40%,[2], [7], [16], [19] and in this study amikacin susceptibility was only 12.5% (Figure 3). Piperacillin-tazobactam susceptibility varies from 50% - 70% in various Indian studies,[2], [7], [19] whereas in this study it was 25%.

In the present study, all *Acinetobacter species* were multidrug resistant (MDR) and with *Pseudomonas aeruginosa*, 75% MDR was seen. Joseph *et al*.[13] observed that MDR was seen in 78.7% of VAP pathogens, which included gram-negative bacilli and MRSA. Two *Acinetobacter species* showed Extended Spectrum β-lactamase (ESBL) production, while one *Acinetobacter species* and two *Pseudomonas aeruginosa* showed Metallo β-lactamase (MBL) production. MBL production in a study from Karnataka[19] in VAP cases was seen in 50% *Pseudomonas aeruginosa* and 21.74% of *Acinetobacter species*. A study conducted by Dey *et al*.[2] showed 80% of the isolates of *Escherichia coli* and 100% *Klebsiella pneumoniae* to be ESBL producers, though in this study none of the above two bacteria were ESBL producers.

Overall imipenem resistance in gram-negative bacilli in this study was 46.15%. In a study from Uttarakhand,[13] carbapenem resistance in gram-negative bacilli in cases of VAP was 48%, which is almost similar to the present study, but a study conducted by Dwivedi *et al*.[21] showed only 12.6% carbapenem resistant *Enterobacteriaceae* responsible for VAP.

In this study, *Staphylococcus aureus* were resistant to all baseline antibiotics, similar to a study by Rajasekhar *et al*.[20] Vancomycin and linezolid susceptibility was 100% in this study, similar to a study from Lucknow.[7] In the present study, no Vancomycin Resistant *Staphylococcus aureus* (VRSA) and Vancomycin Intermediate susceptible *Staphylococcus aureus* (VISA) were detected, similar to a study from Hyderabad.[21]

In this study, 47.17% growth was seen in blood cultures of VAP patients, of which *Acinetobacter species* (33.96%) was the most common isolate. This finding correlates well with the study of Modi *et al*.[22] with blood culture positivity of 44%. Similar growth in ET culture and blood culture, i.e. pulmonary origin bacteremia was seen in 76% patients, whereas different growth in ET culture and blood culture i.e. non-pulmonary origin bacteremia was seen in 24% patients. In 52.83% cases, growth was seen in ET culture only, but not in blood culture (Figure 4). This can be explained by the fact that VAP spreads to the blood or pleural space in <10% of cases.[23] The sensitivity of blood cultures for the diagnosis of VAP is less than 25%. When blood cultures are positive, the organisms may originate from an extrapulmonary site of infection in as many as 64% of cases, even when VAP is present.[23]

Overall mortality in cases of VAP was found to be 50.94% in this study. Other studies have shown that VAP is associated with mortality ranging from 32% to 62%.[7], [8], [15] While Gupta *et al*.[7] has reported mortality as low as 32.7%, Mukhopadhyay *et al*.[19] has reported as high as 61.9%. These variations in mortality rates could be explained by differences in patient characteristics, inadequate and improper antimicrobial treatment, increased length of mechanical ventilation and duration of hospital stay, antimicrobial resistance of the organisms responsible, severity of illness, co-morbid factors and host response factors.

Conclusion

VAP is an important nosocomial infection with increasing multidrug resistant bacteria. The knowledge of causative microbial flora in VAP with its antibiogram pattern is important to ensure more effective utilization of antibiotics and thereby a better outcome as it allows formulation of strategies to decrease the incidence of VAP.

Conflict of interest – None declared
Sources of support – Nil

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