

Microbiological profile of ventilator-associated pneumonia in the intensive care unit of a tertiary hospital in Visakhapatnam, India

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Abstract

Introduction: Ventilator-associated pneumonia (VAP) is defined as pneumonia occurring more than 48 hours after the initiation of endotracheal intubation and it is the most common nosocomial infection in the intensive care unit (ICU). VAP is one of the important nosocomial infection that is the cause of significant morbidity and mortality among patients on mechanical ventilation.

Aim of the study: This prospective study was undertaken with an objective to determine the common etiological agents and their antimicrobial sensitivity patterns of VAP in our institute.

Results: Among the 134 mechanically ventilated patients and 56 of them developed VAP. Among the causative agents, *Pseudomonas* and *Escherichia coli* were most common among gram negatives and *Staphylococcus aureus* was common among the gram-positive isolates. Eighteen of the 56 isolates (32%) were polymicrobial. Thirty-eight isolates from VAP patients were multi-drug resistant (MDR) pathogens.

Conclusion: High incidence of VAP and the potential MDR pathogens are a real threat in our ICUs. Combined approach of judicious antibiotic usage and training programs to health care personnel might be of help in combatting high incidence of antibiotic resistance in our institute.

Keywords: Endotracheal aspirate, Intensive care units, Multi-drug resistant pathogens, *Pseudomonas*, Ventilator-associated pneumonia.

Introduction

Ventilator-associated pneumonia (VAP) is defined as pneumonia occurring more than 48 hours after the initiation of endotracheal intubation and mechanical ventilation including pneumonia developing even after the extubation.

Incidence of VAP in ICUs (Intensive Care Unit) ranges from 8% to 28% in intubated mechanically ventilated patients.¹

The most common mechanism of acquiring VAP is by micro aspiration of oral and pharyngeal flora into the lower respiratory tract. Other potential routes are less common, such as haematogenous spread of bacteria from distant foci of infection like catheter-related bloodstream infections, from the hospital environment, hands of health care workers or contaminated respiratory equipment, bronchoscopes, medical aerosols, water or air.²

Even with heightened vigilance and advances in diagnostic methods, the identification of VAP in ventilated patients is riddled with hurdles due to the lack of a 'gold standard' for diagnosis and also due to incomplete data on etiologic agents and epidemiology.

The etiologic agents of VAP depends on various factors such as the population of patients in an intensive care unit, duration of hospital stay, and prior antimicrobial therapy. Even with the advances of antimicrobial regimen, VAP continues to be an important cause of morbidity and mortality in ICU patients. VAP requires a rapid confirmation of diagnosis for early and appropriate instillation of therapy as inadequate antibiotic treatment on patient's prognosis

may lead to emergence of multidrug-resistant (MDR) pathogens.³

Detection of the causative organism is an essential tool for the diagnosis of VAP which is done by collecting the lower respiratory tract sample either by invasive methods like protected specimen brush (PSB) and broncho-alveolar lavage (BAL) or non-invasive techniques endotracheal aspirate (ETA). Isolation of the causative organism from the respiratory samples quantitatively or semi-quantitatively for the microbiological confirmation is essential.

The American Thoracic Society (ATS) guidelines recommend that quantitative cultures can be performed on ETA or samples collected either invasive or non-invasive.⁴ The recent studies have shown that there is no added advantage to invasive techniques like bronchoscopic cultures over quantitative ETA cultures when patient outcome was considered, and this makes quantitative ETA as a diagnostic tool more effective.^{5,6}

Our current study aims to investigate the common microbial etiology and their antimicrobial susceptibility patterns in clinically confirmed cases of VAP in patients admitted and on mechanical ventilation in the intensive care units in our tertiary hospital in Visakhapatnam.

Materials and Methods

Our study was carried out during the 10-months period between April 2016 and January 2017 in the Department of Microbiology and various ICUs of NRI Institute of Medical Sciences, Sangivalasa, Bheemunipatnam Mandal in the district of Visakhapatnam, Andhra Pradesh, India. The study

population included the patients admitted for mechanical ventilation in the various disciplines of ICUs like trauma ICU, post-operative ICU, cardiac ICU and neonatal ICUs.

Patients above the age of 18 years who were on mechanical ventilation for more than 48 hours were included in the study. The following data was collected at start of the patient's admission into the ICUs that were put on mechanical ventilation like age, gender, primary diagnosis at admission, underlying diseases, date of initiation of mechanical ventilation, any surgical procedures during the hospital stay and current antibiotic therapy. The approval of the institutional review board was obtained during the planning phase of the study and each patient (or his/her caregivers) gave informed consent prior to participation in the study.

Exclusion criteria for the study were patients with pneumonia or other respiratory disorders like Adult Respiratory Distress Syndrome (ARDS), cystic fibrosis, cavitary lung disease, primary or metastatic lesions in the lungs based on chest X-ray findings. During the study period, 134 patients who were on mechanical ventilation for more than 48 hours were analyzed and diagnosis of VAP was made using modified clinical pulmonary infection score (CPIS) > 6 (Table - 1).⁷ The endotracheal aspirates collected from the study subjects were sent to the laboratory and semi-quantitative cultures were performed.⁸

Ten μL of sample was inoculated on sheep blood agar and MacConkey agar. All plates were incubated overnight at 37°C. All plates were checked for growth overnight and then after 24 and 48 hours of incubation. Plates with growth were subjected to analysis for bacterial counts and were calculated as follows: number of colonies x inoculation factor and expressed as colony forming units per mL (CFU/mL). Therefore, presence of 1000 colonies per plate after inoculation with 10 μL of ETA was interpreted as more than 10⁵ CFU/mL.²¹

For definite diagnosis of VAP, along with clinical criteria a bacterial count of 10⁵ CFU/mL of ETA was considered significant.⁹ Cultures with lower colony count were considered as colonization or contamination. Identification of the bacterial isolates was done by various array of biochemical tests.²²

Antimicrobial sensitivity testing (AST) of the bacterial isolates was made by Kirby-Bauer's disk diffusion method. Interpretation were made according to the current Clinical and Laboratory Standards Institute (CLSI) guidelines.¹⁰

If the isolate was suspected to be an extended-spectrum beta lactamases (ESBL) producer, they were confirmed by double disk synergy test using Ceftriaxone (30 μg) and Ceftriaxone-Clavulanic acid (30 μg + 10 μg) as described in earlier studies.¹¹ A zone diameter difference of more than 5 mm with the double discs confirmed that the isolates were ESBL producers.

Isolates showing reduced susceptibility to carbapenems (imipenem/meropenem) were selected for

detection of metallo-beta lactamases (MBLs) enzymes by imipenem-EDTA disk method. This was performed by Imipenem & EDTA combined disc test. Two (10 μg) imipenem discs were placed on a plate inoculated with the test isolate, and 10 μL of 0.5 M EDTA solution was added to one of the two discs. An increase in zone diameter of $\geq 7\text{mm}$ around imipenem + EDTA disk in comparison to imipenem disk alone indicated production of MBL.¹²

For quality control of disc diffusion tests ATCC control strains of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 strains were used. Chi-square test was used to compare proportions of groups.

Results

Of the 134 patients admitted in the ICU for mechanical ventilation, 56 patients were diagnosed with VAP based on CPIS score more than 6. Among the 56 confirmed cases, 31 (39.24%) were men and 25 (45.45%) were women [Table 2].

In our study, VAP was predominantly more common among the age group of 46 – 65 years (44.64%) with a mean age of 52.13 \pm 15.92 years [Table 3].

The incidence of VAP in our study was 41.8%, 56 cases out of the 134 suspected ventilated ICU patients in the hospital were diagnosed with Ventilator associated Pneumonia based on a CPIS score of more than six. Cultures from the 56 confirmed cases of VAP, 38 (67.9%) were monomicrobial (one bacterial isolate in ETA) and 18 (32.1%) were polymicrobial (two or more bacterial isolates in ETA), thus yielding a total of 74 isolates [Fig. 1].

Of the 74 isolates, 60 were gram-negative bacilli. *Pseudomonas* spp was found to be the most predominant accounting for 28.4% followed by *E. coli* (24.3%), *Klebsiella* (12.3%) and *Acinetobacter* spp (9.5%) [Fig. 2]. Fourteen of the 74 isolates were gram-positive cocci and *Staphylococcus aureus* (14.9%) was the most common isolate [Fig. 3].

Twenty-four (40%) out of 60 gram-negative isolates were ESBL producers, 6 (10%) were MBL producers and 8 of the 11 *S. aureus* were MRSA (72.7%) [Fig. 4]. All the ESBL producing isolates were resistant to ampicillin, amoxicillin-clavulanic acid, ceftriaxone and ciprofloxacin. Antibiotic with highest sensitivity was meropenem (83%) followed by piperacillin-tazobactam (75%) among the ESBL producers. Non-ESBL producers showed 100% sensitivity for amikacin, netilmicin and piperacillin-tazobactam [Table 4]. All MBL producers were sensitive to colistin, 66.7% of the MBL producing isolates were sensitive to netilmicin and 16.7% were sensitive to piperacillin-tazobactam.

All the gram-positive isolates were resistant to penicillin and 57.1% of the gram-positive isolates were methicillin resistant. Eight (72.7%) of the 11 isolates of *Staphylococcus aureus* were MRSA (Methicillin-sensitive *Staphylococcus aureus*). All the gram-positive

cocci were sensitive to vancomycin and linezolid [Table 5].

Discussion

Early diagnosis and prompt administration of empirical antimicrobial therapy has been shown to have significant positive effect on mortality from hospital acquired pneumonia. The microbiological evidence prior to the instillation of treatment of VAP avoids unwanted over-treatment of colonizers from pathogens. There are investigative techniques like invasive bronchoscopy for biopsy and protective specimen brush from the site of infection that are highly specific for diagnosing VAP. However, they are invasive in nature and expensive but quantitative ETA culture showed similar results as that of invasive methods and it is affordable and non-invasive. Irrespective of what method was employed for the collection of sample for culture (bronchoscopic or endotracheal aspiration), some studies have shown that those patient outcomes were similar.⁵

The overall incidence of VAP was 41.79% in our study. This value falls under the range of 15-58% as reported by other investigators.¹³ The incidence was slightly higher than the incidence of reported in a study of 37 patients on ventilator therapy which was 37%.¹⁴ Even higher incidence rates were reported in other studies with 45.4%¹⁵ and even as high as 73%.⁸ This was probably because of the shorter duration or the smaller sample size of the study.

Patients belonging to the age group of 46-60 years in our study showed highest incidence of VAP in patients exposed to mechanical ventilation for more than 48 hours and this correlated with data from other studies.¹⁶ The incidence of VAP was higher in males (39.53%) compared to females (29.03%) which correlated with other studies.¹⁷

Etiological agents of VAP vary widely according to the different types of the patients in the ICUs, duration of hospital stay, prior antibiotic therapy and comorbid conditions of the patients. The most common bacterial isolates in our current study were *Pseudomonas* spp followed by *Escherichia coli*, which were also reported

as the commonest isolates by other studies^{17,18} whereas other studies have shown gram positive cocci mainly *Staphylococcus aureus* and *Streptococcus pneumoniae*¹⁹ as the most frequently isolated organism in early onset VAP which contrasts with our study.

Pseudomonas species which was the commonest isolate in our study and it has been isolated from the various respiratory therapy equipment, disinfectants, sinks, etc. Owing to its hardiness and ability to evade human immune (antibacterial) defenses, it is one of the most common nosocomial pathogens.²³

ESBL producers among Enterobacteriaceae were 56%, Meropenem resistance was high in this study which was 43% of *Acinetobacter* spp and 38% of *Pseudomonas* species showed to be multidrug resistant (MDR) [Table 4], which was similar to other studies,¹⁵ but some studies reported a lower incidence of meropenem resistance.¹⁷ Methicillin resistance among *Staphylococcus aureus* was 72.7% which correlates with other studies.²⁰

With the observations made in this study regarding the incidence of VAP in ventilated patients, various isolates from the ET aspirate and prevalence of antibiotic resistance among the isolates, it is necessary to step up the VAP prevention protocol bundle. VAP is a multifaceted diagnosis with many variables regarding etiology, epidemiology, diagnosis, comorbid conditions, prognosis and its management. However, universally accepted fact is that, a majority of the VAP cases could be prevented by improving the quality of care to the patients on ventilator.²⁴

Measures to prevent VAP includes ensuring adequate pressure in the endotracheal cuff, early extubation, timely subglottic drainage, oral intubation, drainage of the condensate from the ventilator circuits, and humidification using HME filters. Increasing nurse to patient ratio in the ICUs goes a long way as to reduce cross-contamination from health-care personnel to patients. Strict adherence of infectious disease prevention protocols by the health-care professionals along with judicious usage of antimicrobials for the patients in ICUs will reduce the incidence of VAP.²⁵

Table 1: Clinical pulmonary infection score (CPIS)

CPIS points	0	1	2
Tracheal secretions	Absent	Not Purulent	Abundant and Purulent
Leucocyte count (mm ³)	> 4,000 and < 11,000	< 4,000 and > 11,000	< 4,000 or > 11,000 plus band forms > 50%
Temperature (°C)	> 36.5 and < 38.4	> 38.5 and < 38.9	> 39 or < 36
PaO ₂ /FiO ₂ (mmHg)	> 240 or ARDS	-	≤ 240 and no ARDS
Chest radiograph	No infiltrate	Diffuse infiltrate	Localized infiltrate
Culture of endotracheal aspirate (Microbiological criteria)	Negative	-	Positive
Note: Microbiological criteria – Positive Gram stain (> 10 polymorphonuclear cells/low power field and > 1 bacteria/oil immersion field) and semi-quantitative endotracheal aspirate culture showing > 10 ⁵ CFU/ ml.			

Table 2: Gender-wise distribution of VAP cases

Gender	No. of VAP cases (56)	No. of cases VAP was absent (78)	Total no. of cases (134)
Male	31 (39.24%)	48 (60.75%)	79 (100%)
Female	25 (45.45%)	30 (54.55%)	55 (100%)

Table 3: Age-wise distribution of VAP cases

Age-groups (years)	No. of VAP cases
18 – 25	03 (5.4%)
26 – 45	18 (32.14%)
46 – 65	25 (44.64%)
> 65	10 (17.86%)
Total	56 (100%)

Table 4: Antimicrobial resistance pattern of gram negative bacilli

	Pseudomonas (n=21)	E. coli (n=18)	Klebsiella (n=9)	Acinetobacter (n=7)	Citrobacter (n=4)	Enterobacter (n=1)
Ampicillin	NT	12 (67%)	6 (67%)	NT	2 (50%)	1 (100%)
Amoxicillin – clavulanic acid	NT	12 (67%)	6 (67%)	NT	2 (50%)	1 (100%)
Cefotaxime	NT	11 (61%)	6 (67%)	NT	2 (50%)	1 (100%)
Ceftazidime	10 (48%)	NT	NT	3 (43%)	NT	NT
Ciprofloxacin	11 (52%)	12 (67%)	7 (78%)	5 (71%)	3 (75%)	1 (100%)
Gentamicin	9 (43%)	6 (33%)	4 (44%)	4 (57%)	1 (25%)	0 (0%)
Amikacin	9 (43%)	6 (33%)	3 (33%)	4 (57%)	1 (25%)	0 (0%)
Netilmicin	7 (33%)	4 (22%)	2 (22%)	4 (57%)	1 (25%)	0 (0%)
Piperacillin – tazobactam	9 (43%)	4 (22%)	1 (11%)	3 (43%)	1 (25%)	1 (100%)
Meropenem	8 (38%)	2 (11%)	1 (11%)	3 (43%)	1 (25%)	0 (0%)
Cefaperazone-sulbactam	6 (29%)	3 (17%)	1 (11%)	2 (29%)	1 (25%)	0 (0%)

Table 5: Antimicrobial resistance pattern of gram positive bacilli

Antibiotics	<i>Staphylococcus aureus</i> (n=11)	Coagulase-negative <i>Staphylococcus</i> species (n=3)
Penicillin	11 (100%)	3 (100%)
Amoxicillin-clavulanic acid	9 (82%)	1 (33%)
Cefoxitin	8 (73%)	0 (0%)
Erythromycin	6 (45%)	1 (33%)
Clindamycin	7 (64%)	0 (0%)
Linezolid	0 (0%)	0 (0%)
Vancomycin	0 (0%)	0 (0%)

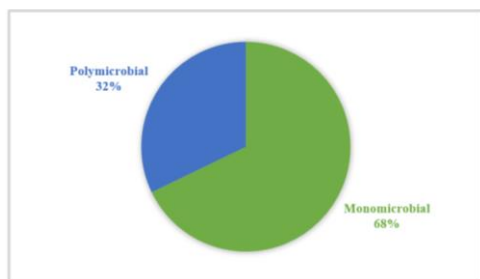


Fig. 1: Isolates

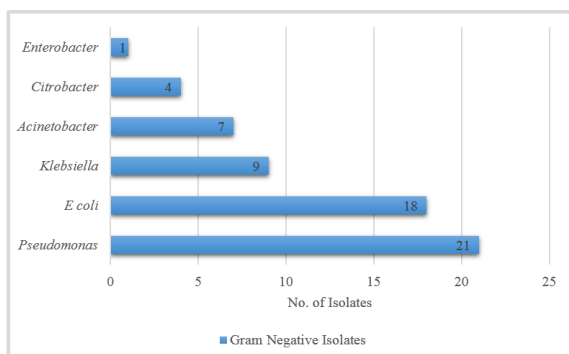


Fig. 2: Gram Negative Isolates

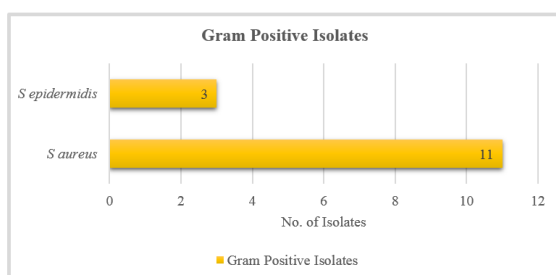


Fig. 3: Gram Positive Isolates

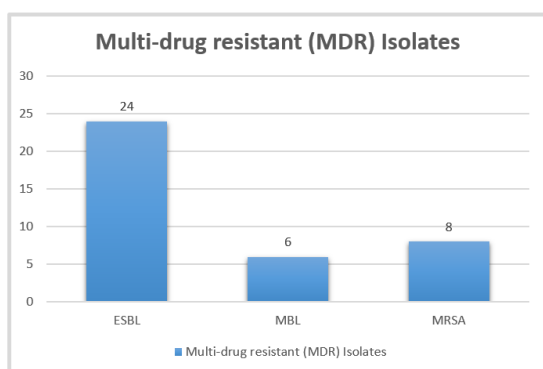


Fig. 4: Multidrug resistant Isolates

Conclusion

Prevention strategies like necessary barrier precautions, contact isolation of the patients with VAP and maintaining recommended nurse to patient ratio are to be emphasized in ICUs to reduce the burden of transmission of colonized MDR organisms in the hospitalized patients will impact the incidence of VAP in a positive manner. More detailed local studies form

developing countries on the risk factors of VAP, combined with the data of causative pathogens and their antimicrobial susceptibility patterns are potentially useful in formulating some multimodal preventive strategies.

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