Original Research Article

Bacteriological profile and antimicrobial sensitivity pattern of Ventilator associated pneumonia in a tertiary care hospital

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Abstract

Background: Ventilator-associated pneumonia (VAP) is the second most common nosocomial infection and accounts for 15-20% of the total hospital acquired infections. It is the most common cause of death in ICU's with a mortality rate of up to 40%.VAP rate varies from 1.0 to 46.0 per 1000 mechanical ventilation days, depending on the ICU facility and the hospital.**Aims and Objectives:**1. To determine the aerobic bacterial pathogens of the patients diagnosed with VAP. 2. To study the antibiogram of isolated bacteria and to detect the drug resistance in the pathogens.**Material and methods:**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 as per the CLSI guidelines.**Results:** A total of 120 clinically suspected VAP patients were enrolled for the study who fulfilled our study′s predefined criteria. Among 120 patients, 52 patients (43.3%) showed significant growth of $\geq 10^5$CFU/ml growth indicating pathogenic bacteria causing VAP and 44 patients (36.6%) with $<10^5$CFU/ml classified under NO-VAP group and 24 (20%) showed no growth. This consists of 44 male patients and 8 female patients. Out of which 42/52 (80.7%) showed monomicrobial growth and 10/52 (19.2%) showed polymicrobial growth. The isolation rate of Gram negative bacilli in this study was 54/120 (45%) and Gram positive cocci isolated was 8/120(6.6%).Out of 54 Gram negative bacilli the predominant organism was Klebsiella species 34/54 (62.9%) followed by Acinetobacter sp.10/54 (18.5%) and Pseudomonas aeruginosa 6/54 (11.1%) and Escherichia coli 4/54 (7.4%). Out of 8 Staphylococcus aureus species 4 isolates were MRSA. Out of 54 GNB 28(51.8%) were ESBLs & 10(18.5%) were MBLs.**Conclusion:** A local antibiogram pattern for each hospital, based on bacteriological profile and susceptibilities is essential for prompt initiation of empirical antimicrobial treatment.Injudicious prophylactic use of antibiotics is not recommended in cases of VAP because exposure to antibiotics is a significant risk factor for colonization and infection with nosocomial multidrug resistant pathogens. The rational use of antibiotics may reduce patient colonization and subsequent VAP with multidrug pathogen.

Key words: Ventilator associated pneumonias, endotracheal aspirate, mechanical ventilation.

Introduction

VAP is defined as pneumonia occurring ≥ 48 hrs of intubation and the start of mechanical ventilation. [1]VAP is most common nosocomial infection in the intensive care unit (ICU) with an incidence ranging from 8 to 28% in intubated mechanically ventilated patients.[2-4]The risk of VAP is highest early during course of hospital stay and is estimated to be 3% per day during the first 5 days of ventilation, 2% per day during days 5–10 of ventilation, and 1% per day after this. [5]The risk of pneumonia is increased 3 to 10 folds for the intubated patient receiving mechanical ventilation. [6] The mortality with VAP is considerably high, varying from 24 to 50% and can reach as high as 76% in some specific settings or when lung infection is caused by high risk pathogens. [7] The etiologic agents widely differ according to the population of patients in an intensive care unit, duration of hospital stay, prior antimicrobial therapy and co-morbid conditions. [8]Despite the advancements in antimicrobial regimes, VAP continues to be an important cause of morbidity and mortality. VAP requires a rapid diagnosis and initiation of appropriate antibiotic treatment, as there is adverse effect of inadequate antibiotic treatment on patient's prognosis and the emergence of multidrug-resistant (MDR) pathogen. Inadequate antimicrobial therapy, such as inappropriate antimicrobial coverage, or delayed initiation of antimicrobials has been associated with higher hospital mortality in subjects with hospital acquired pneumonia (HAP) or VAP. The principal risk factors for the development of VAP is endotracheal tube, which predispose to micro aspiration of contaminated oropharyngeal secretions.Duration of mechanical ventilation, supine patient positioning, enteral feeding, modifiable factors associated with prolonged intubation such as oversedation or lack of protocol driven weaning increases the risk of developing pneumonia. 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). [9]VAP may be caused a wide spectrum of bacterial pathogens. Common pathogens include Pseudomonas spp., Acinetobacter spp., Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus, with varying prevalence). [10]Pseudomonas spp., Acinetobacter spp. and even Enterobacteriaceae are quite often multidrug-resistant (MDR) due to production of extended spectrum β-lactamases (ESBL), AmpC β-lactamases or metallo-β-lactamases (MBL) [11,12]. Inadequate antimicrobial therapy, such as inappropriate antimicrobial coverage, or delayed initiation of

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antimicrobials has been associated with higher hospital mortality in subjects with hospital acquired pneumonia (HAP) or VAP. Therefore, the aim of this study was to analyze the microbiological and clinical profile of VAP in our hospital, risk factors and prevalence of multi-drug resistant bacteria to implement effective prevention strategies.

Material and methodology

The present study was conducted in the Department of Microbiology, NRIIMS, Visakhapatnam,from December 2019 to Feb 2021.

All critically ill adult patients above the age of 18 years who were on mechanical ventilation for more than 48 hours were included in this study.

Inclusion criteria: All patients who were diagnosed with pneumonia 48-72 hrs after being admitted.

Exclusion criteria: Patients who were diagnosed to have pneumonia during the time of or within 48 hours ofadmission-those who did not give consent.

Under strict aseptic precautions the endotracheal aspirates sent to the lab were processed immediately. The samples were first subjected to Gram's staining and then quantitative cultures were performed. All samples were plated on MacConkey agar (MAC), Blood agar (BA), Chocolate agar (CA) using sterilized standard 4mm Nichrome wire loop (Hi-media, Mumbai, India), which holds 0.01ml of ETA. Plates were incubated overnight at 37°C. All plates were checked for growth overnight and then after 24 and 48 hours of incubation.

Quantitative culture threshold of $\geq 10^5$cfu/ml is considered to diagnose VAP in our study. All those samples which yielded quantitative culture threshold of $\geq 10^5$cfu/ml on culture plates were considered and categorized under VAP group. Growth of any organism below the threshold was assumed to be due to colonization or contamination. [13]Those samples which showed no growth was also categorized under non-VAP group in the study. Any growth was characterized by colony morphology and Gram's staining from the plates. A detailed biochemical testing identified any significant growth, and antibiotic sensitivity testing was 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. [14]For detection of methicillin resistance in Staphylococcus spp., 30 µg cefoxitin disc was placed on the lawn culture of the test organism on MHA and the plate was incubated for 16–18 h. For Staphylococcus aureus, zone of inhibition (ZOI), For extended-spectrum beta-lactamase (ESBL) detection, disc diffusion method was performed on Muller Hinton agar (MHA) with cefotaxime (30 µg) and cefotaxime- clavulanic acid (30/10 µg). A ≥5 mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanate vs. zone diameter of the agent when tested alone was identified as ESBL producers. and the isolates showing reduced susceptibility to carbapenems (imipenem and meropenem) were selected for detection of metallo-beta lactamases (MBLs) enzymes by imipenem (10 mcg) + imipenem-EDTA (10/750 mcg) disk method.

Antimicrobials tested for Gram-negative isolates were Gentamicin (10 µg), cefepime (30 µg), Amikacin (30 µg) Ciprofloxacin (5µg), cotrimoxazole(25 µg) piperacillin-tazobactam (100/10 µg), Netilmicin,imipenem (10 µg). Antimicrobials tested for Gram-positive isolates were Clindamycin (2µg),Cefotaxime, Linezolid(30 µg), Vancomycin(30µg) Azithromycin (15 µg), Cotrimoxazole(25 µg) and Cefoxitin (30 µg). Interpretation of the zone diameters was done as per clinical laboratory and standards institute (CLSI) guidelines 2019.

Results

A total of 120 clinically suspected VAP patients were enrolled for the study who fulfilled our study′s predefined criteria. Among 120 patients, $\geq 10^5$CFU/ml growth indicating pathogenic bacteria causing VAP were seen in only 52 patients (43.3%) and 44 patients (36.6%) with $<10^5$CFU/ml classified under NO-VAP group and 24 (20%) showed no growth (Table No.1). This consists of 44 male patients and 8 female patients (Table No.2). Out of which 42/52 (80.7%)showed monomicrobial growth and 10/52 (19.2%) showed polymicrobial growth (Table No.4). The isolation rate of Gram negative bacilli in this study was 54/120 (45%) and Gram-positive cocci isolated was 8/120(6.6%) (Table No.5).The incidence of VAP was more common in the age group of 18-30 years 24/52 (46.1%) followed by 31-45 years 12/52 (23.07%) and 46-60 years 12/52 (23.07%) respectively. (4/52)7.6% were in the age group of 61-85 years. (Table No.3)Out of 54 Gram negative bacilli the predominant organism was Klebsiella species 34/54 (62.9%) followed by Acinetobacter sp.10/54 (18.5%) and Pseudomonas aeruginosa 6/54 (11.1%) and Escherichia coli 4/54 (7.4%) (Table No.6). Out of 8 Staphylococcus aureus species 4 were MRSA isolates. (Table No.9)

Susceptibility pattern for various antibiotics against the Gram negative bacilli were Imipenem (80%), Cefepime (70%), Piperacillin +Tazobactam (78%),Amikacin (74%), Gentamicin (70%), Netilmicin (70%),Ciprofloxacin (62%), and cotrimoxazole (52%) (Figure 1).Susceptibility pattern for various antibiotics against the Gram positive cocci were Vancomycin(100%), Linezolid (100%), Tetracycline(75%),Cefoxitin(59%),Azithromycin(56%),Cefotaxime(54%),Clindamycin(52%) and Cotrimoxazole(51%) (Figure 2).

Table 1: Culture positivity among total samples

S.NO	Culture Report	Number	Percentage
1.	Significant Growth	52	43.3%
2.	Insignificant Growth	44	36.6%
3.	No Growth	24	20%

Table 2: Gender Distribution among total samples

S.NO	Gender	Number
1.	Male	44
2.	Female	8

Table 3: Age group Distribution of the samples received

S.NO	Age Group	Number	Percentage
1.	18-30	24	46.1
2.	31-45	12	23.07
3.	46-60	12	23.07
4.	61-85	4	7.6

Table 4: Distribution of Growth pattern among samples

S.NO	Growth Pattern	Number	Percentage
1.	Monomicrobial	42	80.7
2.	Polymicrobial	10	19.2

Table 5: Distribution of Culture isolates

S.NO	Culture Organism	Number	Percentage
1.	Gram negative bacilli	54	45%
2.	Gram positive cocci	8	6.6%

Table 6: Distribution of pure isolates of Gram Negative Bacilli

S.NO	Organism	Number	Percentage
1.	Klebsiellasp	34	62.9
2.	Acintobactersp	10	18.5
3.	Pseudomonas aeruginosa	6	11.1
4.	Escherichia coli	4	7.4

Table 7: ESBL producers among various Gram-negative isolates

S.NO	Organism	No. of Isolates	Number	Percentage
1.	Klebsiella	34	22	64.7%
2.	Pseudomonas	6	4	66.6%
3.	Escherichia coli	4	2	50%

Table 8: MBL producers among various Gram-negative isolates

S.NO	Organism	No. of Isolates	Number	Percentage
1.	Acinetobacter	10	4	40%
2.	Klebsiella	34	6	17.6%

Table 9: Distribution of MRSA and MSSA isolates among *Staphylococcus aureus*

S.NO	Organism	Number	Percentage
1.	MRSA	4	50%
2.	MSSA	4	50%

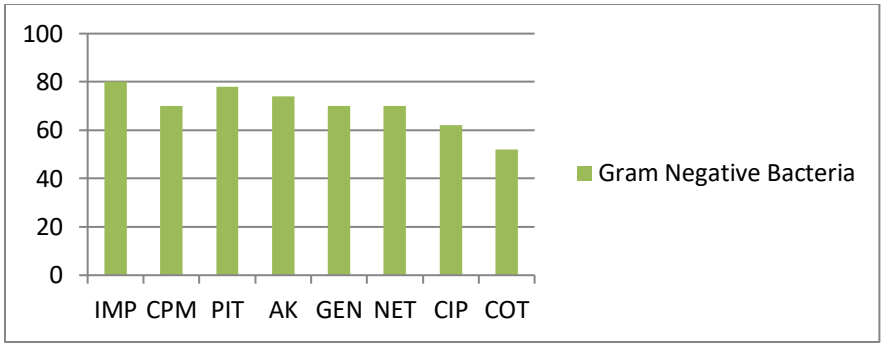

Fig 1: Antibiotic sensitivity pattern of Gram negative bacilli

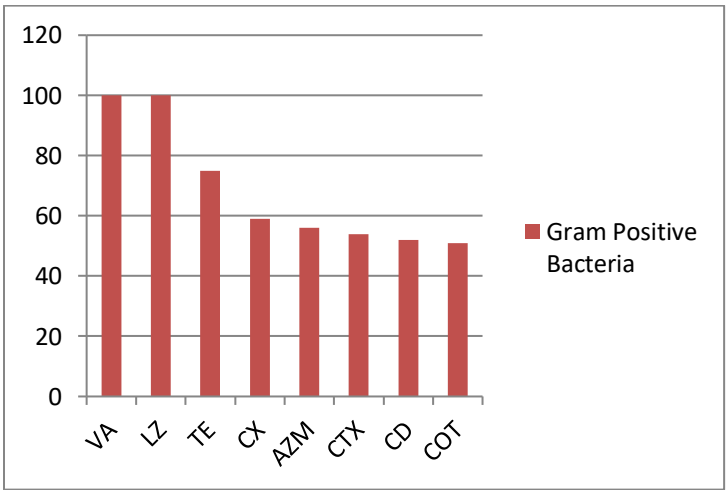

Fig 2:Antibiotic sensitivity pattern of Gram positive cocci

Discussion

VAP remains a major cause of morbidity and mortality in Intensive care units. The incidence of VAP, its etiology and susceptibility patterns may not only vary from hospital to hospital but also within the same hospital or ICU over time. Changes in pathogen distribution and antimicrobial resistance pattern complicate antibiotic treatment and care of the patients.VAP is an important nosocomial infection among ICU patients receiving mechanical ventilation. The risk of VAP is approximately 3%/day during the first 5 days of ventilation, gradually decreasing to 2% during 5–10 days of ventilation and approximately 1%/day thereafter, thus highest during the early course of hospital stay. [15]

The principal factor for the pathogenesis of VAP is reported to be due to aspiration of oropharyngeal pathogens and the leakage of secretions containing bacteria around the endotracheal tube.The bacteriological approach for the management of VAP avoids the problem of over treatment by separating colonizers from infecting pathogens). [13]Quantitative cultures of endotracheal aspirate or broncho-alveolar lavage is recommended by the American Thoracic Society for confirmation of VAP). [16] Endotracheal tube aspirate is comparatively less expensive compared to BAL and hence is widely preferable in most of the hospital settings. Investigators have also reported quantitative cultures of ETA to be of equal diagnostic accuracy to other invasive techniques.

In our study among 120 patients, $\geq 10^5$CFU/ml growth indicating pathogenic bacteria causing VAP were seen in only 52 patients (43.3%) and 44 patients (36.6%) with $<10^5$CFU/ml classified under NO-VAP group and 24 (20%) showed no growth. Culture positivity in our study is 43.3%(Table No.1). This finding correlated with with the study by ArindamDey et al [3]and ChiranjayMukhopadhyay et al [17]with an incidence of 45.4% and 42% respectively but varies with the study done by T. Rajasekar et al [13]at and ShaliniTripathi et al [18]with an incidence of 73.3% and 30.6% respectively. The difference could be due the different methods used for the diagnosis, sample size and the underlying disease state requiring ventilator support. High risk pathogens figure prominently in our study. It correlates with various studies done by Rajasekhar et al [13]ArindayDey et al [3]Rates of polymicrobial infection vary widely. Polymicrobial infection was seen in 19.2% cases of VAP in our study which is less as compared to study done Dr. Kotgire Santosh A.et al. [19] There is high antibiotic resistance in Gram negative pathogens which are isolated from ICUs that are resistant to ceftazidime, cefotaxime, ciprofloxacin, gentamicin and amikacin. Resistance to carbepenems is on a rise all over the world due to the production of metallo β Lactamase. Recent studies have shown the increasing incidence of multidrug resistant pathogens among patients with VAP. A study by Dey et al [3]showed the increased incidence of MDR organisms in the ICU,ESBL producing organisms, MBL, and MRSAs are of increasing clinical concern; thus, they have to be documented for epidemiological and infection control point of view, as they are challenging to the clinicians. In our study, ESBL producers were seen in 64.75% of *Klebsiella* spp. which correlated with 64% of ESBL producers in *Klebsiella* spp. in a study by Swati *et al*. [20]We have reported ESBL production in 66.6%% of *Pseudomonas* spp and E. coli is 50% while ESBL-producing Enterobacteriaceae colonization was identified among 5% to 30% of ICU-admitted patients in different studies.[21,22]MRSA producers in our study was 50%, while it was 86% in a study by Swati *et al*. [20]and 75% in Patel *et al*. [22]

Conclusion

It is very important to have the knowledge of organisms likely to be present and also the local resistance pattern in the respective hospital ICU.Any individual study may not necessarily reflect the same situation in other centers as incriminating organisms vary among hospitals.Injudicious prophylactic use of antibiotics is not recommended in cases of VAP because exposure to antibiotics is a significant risk factor for colonization and infection with nosocomial multidrug resistant pathogens.The rational use of antibiotics may reduce patient colonization and subsequent VAP with multidrug pathogen.

As incriminating pathogens vary among hospital it is very important to know the incidence of VAP and the associated local microbial flora in each setting so as to guide more effective and rational utilization of antimicrobial agents. Hence, we recommend a combined clinical and microbiological prevention strategies which include accurate investigation, invaluable input from the microbiological laboratory, rational and early antibiotic therapy, timely surveillance, strict infection control measures, monitoring risk factors and finally the knowledge of the treating physicians about the local epidemiological data and susceptibility pattern of isolates.

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