Original Article

Microbiological profile of Ventilator associated pneumonia at ICU of rural based teaching hospital.

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ABSTRACT

BACKGROUND AND OBJECTIVES: Ventilator-associated pneumonia (VAP) is an important intensive care unit (ICU) infection in mechanically ventilated patients. VAP occurs approximately in 9-27% of all intubated patients. Due to the increasing incidence of multidrug resistant organisms (MDR) in the ICU, early and correct diagnosis of VAP is an urgent challenge for optimal antibiotic treatment. The aim of the study was to assess the bacteriological and clinical profile of VAP, risk factors, etiology and prevalence of MDR in clinically suspected VAP cases in the ICU setting. METHODS: This prospective study was conducted in the period from September 2012 to August 2013, at Department of Microbiology, SBKS MI & RC Piparia, enrolling patients undergoing mechanical ventilation (MV) for >48 h. admitted at in ICU of Dhiraj Hospital Endotracheal aspirates (ETA) were collected from patients with suspected VAP, and quantitative cultures were performed on all samples. VAP was diagnosed by the growth of pathogenic organism ≥ 105 cfu/ml. RESULTS: Out of 50 patients, 28 (56%) had bacteriologically proven VAP. Out of 50 patients 23(46%) had early-onset (<96 hours MV) VAP and 27 (54%) had late-onset (>96 hours MV) VAP. Multi drug-resistant bacteria, mainly Klebsiella pneumoniae (20%), Acinetobacter spp.s (16%) and Pseudomonas spp.s (14%) were the most commonly isolated pathogens in both types of VAP. ESBL was produced by 67% and 20% of Klebsiella pneumoniae and Escherichia coli respectively. Metallo-beta lactamases (MBLs) were produced by 29% of Pseudomonas spp.s. INTERPRETATION AND CONCLUSION: High prevalence (56%) of VAP and the potential multidrug-resistant organisms are the real threat in our ICU. Combined approaches of rotational antibiotic therapy and education programs might be beneficial to fight against these MDR pathogens and will also help to decrease the incidence of VAP.

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1. Introduction

Ventilator-associated pneumonia (VAP), an important form of hospital-acquired pneumonia (HAP), refers to the development of lung parenchymal infection after a patient has undergone intubation more than 48 hours and received mechanical ventilation for more than 48 hours or tracheostomy. (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. (2-3,4)

The risk of VAP is highest early in the 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 intubation process itself contributes to the risk of pneumonia. (6) The risk of pneumonia is increased 3 to 10 folds for the intubated patient receiving mechanical ventilation. (7) 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. (8)

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. (9) 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 pathogen [5]. 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 [6].

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 so as to implement effective prevention strategies.

Material and Methods

A prospective study was conducted at the Department of Microbiology SBKS MI &RC over a period of September 2012 to August 2013. The study cases were from ICU of Dhiraj Hospital attached to SBKS MI &RC, Waghoria, Vadodara. Clinically suspected patients according to CDC criteria [11] were scored by the Chronic Pulmonary Infection Score (CPIS) according to the clinical, microbiological and radiological signs.

Patient details and clinical presentation was noted down. In each patient, risk factor assessment, co-morbid conditions, radiological findings, outcome, empirical and final use of antibiotics as well as other data were noted.

Inclusion criteria: All critically ill adult patients (age ≥18 years) who were on mechanical ventilation for more than 48 hours.

Exclusion criteria: Patients with pneumonia prior to mechanical ventilation or within 48 hours of mechanical ventilation and patients having with lower respiratory tract infection.

During this period 50 patients who were on mechanical ventilation for more than 48 hours were studied. Microbiological criteria included positive Gram stain (>10 polymorphonuclear cells / low power field and ≥ 1 bacteria/ oil immersion field). [12,13] Clinical criteria included Clinical Pulmonary Infection Score (CPIS) > 6. [14]

Patients on mechanical ventilation for less than 4 days (48-96 hours) were included in the early-onset VAP group and more than 96 hours were included in the late onset VAP group. [15]

Collections of Endotracheal aspirate (ETA):-

Under strict aseptic precautions endotracheal aspirate was collected by using suction catheter for adults. Suction catheter was gently introduced through the endotracheal tube, gentle aspiration was then performed and the catheter was withdrawn from the endotracheal tube. The results of the Gram’s stain were obtained within the 1 hour and quantitative cultures were performed immediately.

Microbiological processing:-

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) and Saboraud’s dextrose agar (SDA) using sterilized standard 4mm Nichrome wire loop (Hi-media, Mumbai, India), which holds 0.01ml of ETA. Plates were incubated overnight at 37°C and SDA plate was kept at room temperature. All plates were checked for growth overnight and then after 24 and 48 hours of incubation. SDA plates were checked for any growth up to one week. [12,13]

Quantitative culture threshold of ≥ 10⁵ cfu/ml is considered to diagnose VAP in our study. All those samples which yielded quantitative culture threshold of ≥ 10⁵ 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. [10] 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. [10] All methicillin resistant Staphylococcus aureus (MRSA) were confirmed by using cefoxitin (30 mcg). Suspected extended-spectrum beta lactamases (ESBLs) were identified by the double disk synergy test, by using ceftazidime (30 mcg) and the ceftazidime and davanolic acid (30/10 mcg) combination 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.[12,13]

Result

A total of 50 clinically suspected VAP patients were enrolled for the study who fulfilled study’s predefined inclusion criteria. Out of 50 VAP patients, CPIS scoring was >6; and among them, by quantitative culture of ETA, 28 (56%) patients showed colony count ≥10⁵ cfu/ml.

The occurrence of VAP was more common in the age group of 61-70 years 9 (18%). (Figure 1)
In present study, out of total 50 Gram stain smears - 21(42%) showed plenty of pus cells, 7 (14%) showed few pus cells and 22(44%) showed absolutely no pus cells. All the 21 samples, showing presence of plenty of pus cells, yielded quantitative culture threshold ≥ 10^6 cfu/ml on culture plates. Statistically also the association between the presence of plenty of pus cells in Gram stain and occurrence of VAP was found to be highly significant ($\chi^2=50$, d.f=2 and $p \leq 0.001$). (Table-1)

**TABLE 1: GRAM STAIN OF ENDOATRACHEAL ASPIRATE SHOWING PRESENCE OF PUS CELLS AND ITS ASSOCIATION WITH VAP AND Non-VAP CASES**

<table>
<thead>
<tr>
<th>GramStain</th>
<th>VAP</th>
<th>Non VAP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plenty pus cell</td>
<td>21</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Few pus cell</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>No pus cell</td>
<td>0</td>
<td>22</td>
<td>22</td>
</tr>
</tbody>
</table>

In a total of 50 clinically suspected VAP cases, 15 cases (30%) showed monomicrobial (one bacterial species in ETA) growth, 13 cases (26%) showed polymicrobial (two or more bacterial species in ETA) growth pattern and 22 cases (44%) showed no growth. Here the association between growth pattern and VAP cases was found to be highly significant ($\chi^2=50$, d.f=2 and $p \leq 0.001$). (Table-2)

**TABLE 2: ASSOCIATION OF GROWTH PATTERN IN VAP**

<table>
<thead>
<tr>
<th>Gram</th>
<th>VAP (n=28)</th>
<th>Total (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomicrobial</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Polymicrobial</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

All the 50 VAP cases, 23 (46%) were categorized under early onset VAP and 27 (54%) under late onset VAP.

Out of total 43 microorganisms isolated, *Klebsiella pneumoniae* 10 (20%) causing both early and late onset pneumonia, *Acinetobacter spps.*-8 (16%), *Pseudomonas spps.*-7 (14%) and *Candida non-albicans*-6 (12%) were the most common organisms isolated followed by *Staphylococcus aureus* and *Candida albicans* 4 (8%) each, *E.coli* 3 (6%) and *Proteus mirabilis* 1 (4%). (Figure 2)

**Figures:** Figure 1: Association of Age with VAP and Non-VAP Cases

![Figure 1](image1)

All the 10 (100%) isolates of *Klebsiella pneumoniae* were resistant to 3rd generation cephalosporin i.e. Cefotaxime (Ctx), Ceftazidime (Caz) and Amoxicillin + Clavulanic acid (Amc).

Among the 8 isolates of *Acinetobacter spps*. 2 (28%) were resistant to all antibiotics tested in this study, including carbapenem. All the 8 (100%) isolates of *Acinetobacter spps.* were multidrug resistant (MDR) i.e. resistant to three or more class of antibiotics. *Pseudomonas spps.* 6 (85.71%) out of the 7 were resistant to Aztreonam (AO), Piperacillin (Pi), Piperacillin + Tazobactum (PIT), Ceftazidime (Caz), Cefepime (Cpm) and Ciprofloxacin(Cip) 4 (57.14%). (Figure 3)

**Figure 2: Organism isolated in Early and Late onset of VAP**

![Figure 2](image2)

**Figure 3: Antibiotic resistance pattern of Gram negative bacilli**

![Figure 3](image3)
All strains of *Staphylococcus aureus* 4 (100%) were resistant to Penicillin (P). However, all the strains were sensitive to Vancomycin (VA). (Figure 4)

**Figure 4: Antibiotic resistance pattern of Gram positive cocci**

ESBL was produced by 20% and 67% of *E. coli* and *K. pneumoniae* respectively. Out of the 7 *Pseudomonas spps.*, 2 (29%) were MBL producing strains whereas 4 of the 3 (75%) *Staphylococcus aureus* were MRSA. (Figure 5)

**Figure 5: Different enzymes produced by the isolate strains**

### Conclusion

This study confirms the magnitude of the problem of ventilator associated pneumonia in our setup and also the emergence of MDR *Klebsiella pneumoniae, Acinetobacter spps.* and *Pseudomonas spps.*, as potential pathogens causing VAP in our ICU. The best approach to manage this problem seems to be adaptation of preventive strategies. 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.

In our study, age has significant role in the development of VAP which is line with Arindam Dey et al.[4] study which showed that patients of age > 30 years are more prone to get VAP. Comorbid conditions like Type-II Diabetes mellitus (DM), Hypertension (HTN), Chronic obstructive pulmonary disease (COPD) and Alcohol very present in our study group patients which is again similar to Arindam Dey et al.[14] study.

Rates of polymicrobial infection vary widely. Polymicrobial infection was seen in 13 (26%) cases of VAP in our study which is less as compared to study done Dr. Kotgire Santosh A.et al.[18]

*Klebsiella pneumoniae* (16%), *Acinetobacter spps.* (16%) and *Pseudomonas spps.* (9%) were the commonest isolates obtained in both early and late onset VAP cases, which were also reported as the commonest isolates by T.Rajshekar et al.[15] and Veena Krishnamurthy 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 carbapenems 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 showed the increased incidence of MDR organisms in the ICU.[14]

ESBLs are of increasing clinical concern. ESBLs are most commonly produced by *Klebsiella species* and *Escherichia coli* but may also occur in other gram negative bacilli. In our study ESBL producers were common among Enterobacteriaceae *Klebsiella pneumoniae* 2/10 (67%) and *Escherichia coli* 2/3 (20%) similar results have been reported by Dey et al.[14]

Although *Acinetobacter spp.* are generally less virulent than *P. aeruginosa*, these have nonetheless become problem pathogens because of increasing resistance to commonly used antimicrobial agents.[14] In this study, *Acinetobacter species* showed multi-drug resistance (MDR), even carbapenems, which is in concordance with Dey et al. More than 85% of isolates are susceptible to carbapenems, but resistance is increasing due either to IMP-type metalloenzymes or carbapenemases of the OXA type.[14] In our study, 2 (29%) of *Pseudomonas spps.* were plasmid-mediated metallo-beta lactamases enzyme producing strains, less as compared the studied by Dey et al (50%) [14] and Varun G et al (47.06%) [20].
We got a 4 (10%) isolates of *Staphylococcus aureus* in our setup of which 3 (75%) were methicillin-resistant *Staphylococcus aureus* (MRSA), more as compared to Veena Krishnamurthy et al (18.15%) [19].

Fungal pathogens are also not significant agents causing VAP. Among our cases, we isolated *Candida albicans* 4 (10%) and *Candida non-albicans* 6 (14%) and the colony count was also very low (<10⁵ cfu/ml), which determines that Candida was tracheal colonizer. It is probable that the association between MDR bacteria and Candida colonization reported by Hamet et al. [21] was more likely due to shared risk factors than causal association.

In the present study, both the prior antibiotic therapy and ICU length of stay could be important factors associated with the isolation of MDR organisms from patients who have suspected VAP. Although Trouillet et al suggested that previous antibiotic use (OR, 13.5) and previous use of broad-spectrum antibiotics (OR, 4.1) were associated with increased risk of VAP caused by organisms that were potentially drug-resistant. [22]

The rational use of appropriate antibiotics may reduce patient colonization and subsequent VAP with MDR pathogens. Similarly, unnecessary prolonged hospitalization of the patients should be avoided as far as possible. But it may not be feasible in most situations as the patients' condition may demand prolonged hospital stay. However, the knowledge of this risk factor should suggest the possibility of infection due to MDR pathogens in patients developing VAP after hospitalization for five days or more.

**Conclusion**

This study confirms the magnitude of the problem of ventilator associated pneumonia in our setup and also the emergence of MDR *Klebsiella pneumoniae, Acinetobacter spp*, and *Pseudomonas spp.*, as potential pathogens causing VAP in our ICU. The best approach to manage this problem seems to be adaptation of preventive strategies. 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.

**Reference**


