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A Study to Assess the Bacterial Pathogens of Ventilator Associated Pneumonia in the Intensive Care Unit Patients in a Tertiary Care Hospital in South India

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Abstract: ***Pneumonia is a common illness affecting approximately 450 million people a year and occurring in all part of the world. It is the major cause of death among all age groups, resulting in 1.4 million deaths in 2010. One of the distinct subgroups of Pneumonia is ventilator-associated pneumonia (VAP). It refers to the development of parenchymal lung infection after a patient has undergone intubation and received mechanical ventilation (MV) for ≥ 48 hours. In spite of the recent advances in the diagnosis and management of VAP it continues to be a major cause of hospital morbidity and mortality. This study will attempt to identify the bacterial pathogens causing VAP in the ICU and to determine their Antimicrobial Susceptibility profile. Local data collected in this study can assist in making informed treatment choices. Knowledge of incidence of VAP, their causative microbial flora in a local setting along with information on the susceptibility patterns will help in selection of the appropriate antibiotics for therapeutic use and a better outcome.***

Keywords: Ventilator-associated pneumonia, bacterial pathogens, susceptibility, morbidity and mortality.

1. Introduction

Pneumonia is an inflammatory condition of the lungs affecting the distal air spaces from the alveolar ducts to the alveolar sacs [1]. The alveoli are filled with pus and fluid, which makes breathing painful and limits oxygen exchange [2]. Globally, pneumonia claimed the lives of more than 9 lakhs children under the age of 5 in 2015. Of these deaths, about 1.8 lakhs occurred in India [3].

The burden of severe pneumonia in terms of morbidity and mortality is unknown in India especially at sub-national level. It is recognized that for India, which accounts for 23% of the global pneumonia burden and 36% of the WHO regional burden, national estimates may hide significant sub-national disparities.

Pneumonia is classified into Community Acquired Pneumonia (CAP) and Hospital Acquired Pneumonia (HAP). CAP is defined as pneumonia acquired outside the hospital by an immunocompetent individual. CAP is well recognized to be a leading cause of death among the infectious diseases. It is to be distinguished, on the basis of a wider spectrum of pathogens, from nosocomial pneumonia (which arises more than 48 hours after hospital admission or within 3 months of discharge) and from pneumonia in an immune-compromised host (e.g., in the setting of neutropenia, iatrogenic immune suppression with drugs, status post organ or stem-cell transplantation, HIV infection, or a congenital immune deficiency).

Hospital Acquired Pneumonia (HAP) is defined as pneumonia that develops 48 hours or more after hospital admission. HAP is distinct from VAP, which arises more than 48–72 h after endotracheal intubation. Nosocomial pneumonia is the most frequent hospital acquired infection and along with primary bacteremia, it is the leading cause of death from infection acquired in the hospital [4].

Ventilator-associated pneumonia (VAP) refers to the development of parenchymal lung infection after a patient has undergone intubation and received mechanical ventilation (MV) for ≥ 48 hours [5].

Mechanical ventilation is an essential, life saving therapy for patients with critical illness and respiratory failure. These patients are at high risk for complications such as ventilator associated pneumonia (VAP) with a prevalence ranging from 6.6% to 32%. VAP is a significant problem among pediatric intensive care units and that it is the second most common hospital-acquired infection after bloodstream infection [6]. The main route for acquiring VAP is gross or micro aspiration of oropharyngeal organisms into the distal bronchi, either directly or secondarily by reflux from the stomach into the oropharynx.

The VAP was classified into two types:

Early-onset VAP, defined as that occurring within the first 4 days of hospitalization, usually carry a better prognosis, and are more likely to be caused by antibiotic sensitive bacteria. Late-onset VAP (5 days or more) are more likely to be caused by multidrug resistant (MDR) pathogens, and are associated with increased patient mortality and morbidity.

Detection of the causative organism is crucial for the diagnosis of VAP. This is done by collecting the lower respiratory tract sample either by invasive (protected specimen brush (PSB)) or Bronchoalveolar lavage (BAL) or non-invasive Endotracheal aspirate (ETA) techniques and culturing quantitatively or semi-quantitatively. The

American Thoracic Society (ATS) guidelines recommend that quantitative cultures can be performed on ETA or samples collected either bronchoscopically or non-bronchoscopically. The microbiological differentiation between early onset and late onset VAP has been implicated in the selection of broad spectrum antimicrobial coverage for MDR pathogens. Because appropriate antimicrobial treatment of patients [7] with VAP significantly improves outcome, more rapid identification of infected patients and accurate selection of antimicrobial agents represent important clinical goals.

Currently, the diagnosis of VAP is based on a combination of clinical, radiological, and microbiological criteria. There are a wide range of clinical conditions that mimic VAP in ventilated patients, including acute respiratory distress syndrome (ARDS), pulmonary edema, pulmonary contusion, tracheo-bronchitis, and thrombo-embolic disease. Some of the clinical features used to define a VAP (e.g. change in tracheal secretions) are subjective and are subject to inter- and intra-observer variation. Invasive and non-invasive sampling techniques are used to obtain microbiological specimens to diagnose VAP. Invasive techniques include Bronchoalveolar lavage (BAL) and protected specimen brushings (PSB), while less invasive techniques include mini BALs. Tracheal aspirates are the least invasive to obtain but the most likely to be contaminated with oropharyngeal colonizing bacteria. Quantitative cultures are often used to differentiate between colonization and infection. The diagnostic threshold for BALs is 104 colony forming units per milliliter (CFU/ml−1) and this is often the gold standard against which other diagnostic criteria are compared.

Local data collected in this study can assist in making informed treatment choices. Knowledge of incidence of VAP, their causative microbial flora in a local setting along with information on the susceptibility patterns will help in selection of the appropriate antibiotics for therapeutic use and will aid a better outcome.

2. Materials and Methods

A prospective observational study was conducted in the Department of Microbiology, Saveetha Medical College and Hospital from May 2018 to October 2018. Ethical committee approval was obtained from the Institutional Ethics Committee on 13/03/2018, (number SMC/IEC/2018/03/029).

2.1 Sample Collection

Patients who received mechanical ventilation for more than 48 h were included in this study. The patients included in this study were of both sexes, i.e., male and female, aged more than 18 years of age, on mechanical ventilation for more than 48 hours. The patients who were excluded from this study were patients who died within 48 hours, patients who developed pneumonia within 48 hours, patients who had pneumonia on admission and patients with acute respiratory distress syndrome. Data were collected from patients who satisfied the inclusion criteria.

Modified Clinical Pulmonary Infection Score (CPIS) was followed as a screening method to clinically diagnose VAP. 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 is defined as the number of VAPs/1,000 ventilator days. The diagnosis of VAP was based on clinical and microbiological criteria. A clinical suspicion of VAP was made in patients with modified CPIS score >6.

The samples were collected under strict aseptic precautions in sterile containers, labeled and were transported to the laboratory in appropriate conditions and processed within one hour of collection. The diagnosis was confirmed when significant growth was obtained in the samples.

Endotracheal aspirate (ETA) and Bronchoalveolar lavage (BAL) samples were collected. BAL samples were collected by using a fiber optic bronchoscope. Small amount of physiological saline was infused and the reaspirated sample was collected in a sterile container. Endotracheal aspiration samples were obtained by sterile means using Suction catheter for patients with endotracheal tube.

2.2 Isolation and Identification

Hucker's Gram stain preparations were made from all ETA (Endotracheal lavage) and BAL (Bronchoalveolar lavage) samples and examined under oil immersion field (×100 objective).The relative number of pus cells, the relative number of microorganisms and their morphology were recorded.

All the samples were inoculated on blood agar, MacConkey agar and chocolate agar. Semi-quantitative cultures were done. The MacConkey agar, blood agar and chocolate agar were incubated for 18-24hours at 37°C in the presence of 5-10% carbon dioxide.

After the specified period and condition of incubation, the number of colonies grown in the culture plate was counted and the CFU/ml was calculated using the following formula,
CFU/ml = Number of colonies X dilution factor

Growth which was >105 CFU/ml was taken as the cut-off threshold for endotracheal aspirates.

Growth which was >104 CFU/ml was taken as the cut-off threshold for Bronchoalveolar lavage. Showing growth less than these thresholds were assumed to be due to colonization or contamination. Isolates were identified by standard microbiology techniques in case of significant growth.

Identification and Antimicrobial Susceptibility Testing were carried out by Vitek 2 compact automated machine.

3. Results and Discussion

Our study was carried out in the department of Microbiology, Saveetha Medical College and Hospital. Out of the154 respiratory samples, 60 (39%) were BAL samples and 94 (61%) were ETA samples

Of the 154 respiratory samples tested, 94 samples showed bacterial growth. Of these 94 samples 26 (28%) were Bronchoalveolar lavage (BAL) samples and 68 (74%) were Endotracheal aspirate (ETA) samples. A total of 94 patients fulfilled the clinical and microbiological criteria for the diagnosis of VAP.

Table :1 show the number of patients with VAP in different age groups the patients. Patients above the age of 18 years were included in this study and it was found that the most number of VAP cases occurred in the 51- 70 years age group followed by in patients between 31 to 50 age group.

Table 1: Patients Developing VAP In Different Age Groups (In Year):

S.No.	*AGE IN YEARS*	*NUMBER*
1	18 – 20	8
2	21 – 30	3
3	31 – 40	11
4	41 – 50	12
5	51 – 60	27
6	61 – 70	26
7	71 – 80	5
8	81 – 90	2

Table: 2 show the percentage of different organisms isolated. Most isolates were gram negative bacteria (n=91) (96.8%.) The most common bacterial pathogen isolated was *Klebsiella pneumoniae* (n= 37) (40%), followed by *Acinetobacter baumannii* (n=32) (34%) and *Pseudomonas aeruginosa* (n=16) (17%). Other bacterial pathogens isolated were *Escherichia coli* (n=1) (1.01% , *Enterobacter aerogenes* (n=4) (4.25%), *Methicillin Resistant Staphylococcus aureus* (n=1) (1.01%), *Methecillin Sensitive Staphylococcus aureus* (n= 1) (1.01%) , *Enterococcus faecalis* (n=1) (1.01%), *and Morganella morganii* (n=1) (1.01%). 3 gram positive bacteria were isolated (4%), 1 *Enterococcus faecalis,* 1 Methicillin resistant *Staphylococcus aureus,* and 1 Methicillin sensitive *Staphylococcus aureus* . In 7 patients more than one organism was isolated and all the rest had monomicrobial growth.

Table 2: Microbial growth from respiratory samples

S.No	*Organisms*	*Number*	*BAL*	*ETA*
1	*Klebsiella pneumoniae*	37	9	24
2	*Pseudomonas aeruginosa*	16	5	11
3	*Acinetobacter bumannii*	32	8	24
4	*Enterococcus faecalis*	1	1	
5	*Escherichia coli*	1		1
6	*Methecillin Resistant Staphylococcus aureus*	1	1	
7	*Enterobacter aerogenes*	4	2	2
8	*Morganella morganii*	1		1
9	*Methicillin Sensitive Staphylococcus aureus*	1		1
	Total	94	26	68

Klebsiella pneumoniae, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* were resistant to many antimicrobial drugs commonly used. Of the Klebsiella pneumoniae isolates 54% were multi drug resistant (MDR), 60% of Acinetobacter baumannii were multi drug resistant and 19% of *pseudomonas aeruginosa* were multi drug resistant.

Table: 3 show the antimicrobial resistance of the 3 most common isolates. Among the *Klebsiella pneumoniae* isolates, 35% were resistant to Cefriaxone, 62% of them showed resistance to Aminoglycosides and Meropenem, 64% of the isolates were resistant to Imepenem, 68% of the isolates were resistant to Ciprofloxacin, 86% of them were resistant to Cefepime and 76% of them showed resistance to Cefoperazone sulbactam.

Among the *Acinetobacter baumannii* 56% were resistant to Cotrimoxazole, 15% were resistance to Amikacin, 65% of the isolates were resistant to Gentamicin, 81% were resistant to Imepenem, 75% of the isolates were resistant to Meropenem, 67% were resistant to Ciprofloxacin, 73% of them were resistant to Cefepime and 64% of them showed resistance to Cefoperazone sulbactam.

Among the *Pseudomonas aeruginosa* 19% were resistance to Ceftazidime, 25% of them showed resistance to Amikacin and Ciprofloxacin, 19% of them showed resistance to Gentamicin and Cefoperazone sulbactam, 31% of them showed resistance to Imepenem, Meropenem and Cefepime.

Table 3: Antimicrobial resistance of the 3 most common isolates

Drugs	*Acinetobacter bumannii*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
Amikacin	15%	62%	25%
Gentamicin	65%	62%	19%
Ciprofloxacin	67%	68%	25%
Cotrimaxazole	56%	-	-
Ceftazidime	-	-	19%
Cefatoxime		35%	
Imipenem	81%	64%	31%
Meropenem	75%	62%	31%
Cefepime	73%	86%	86%
Cefaperazome sublactum	64%	76%	76%

Table: 4 shows the risk factors and isolates in patients with COPD, CKD, and Head injury *Klebsiella pneumoniae* were the commonest isolates. Most *Acinetobacter baumannii* isolates were from COPD patients. 19% of patients with COPD and 19% of patient with CVA had *Pseudomonas aeruginosa* growth

Table 4: Risk factors and isolates

S.No.	*Risk factors*	*Acinetobacter bumannii*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
1.	COPD	38%	34%	19%
2.	CKD	16.2%	9.3%	6.25%
3.	Aspiration Pneumonia	14%	22%	-
4.	CVA	8%	-	19%
5.	Laporotomy	5%	-	-
6.	Tracheatomy	5%	-	-
7.	Head injury	3%	9.3%	-
8.	SAH/Craniotomy	3%	3.1%	-
9.	ARDS	-	9.3%	-
10.	C4 Spinal cord compression	-	6.2%	6.25%

11.	Septic shock	-	3.1%	-
12.	Urosepsis	-	3.1%	6.25%
13.	RTA	-	-	19%
14.	Hemiplegia	-	-	6.25%

In this study, *Klebsiella pneumoniae* was the most common bacterial pathogen isolated, followed by *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, causing VAP in ICU patients. Other bacterial pathogens were *Enterobacter aerogenes*, *Escherichia coli*, *Morganella morganii*, *Enterococcus faecalis*, Methicillin Resistant Staphylococcus *aureus*, and Methicillin Sensitive *Staphylococcus aureus.* In a study by Frauke Mattner *et al*. multi resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* was the most commonly isolated pathogen causing VAP. VAP is a fatal disease with a high mortality.

A etiological agents of VAP widely differ according to the population of the patients in the intensive care unit, duration of hospital stay and prior antimicrobial therapy [8]. A quantitative culture test of lower respiratory specimens like BAL (Bronchoalveolar lavage) and ETA (Endotracheal aspiration specimen) has been reported to have high sensitivity and specificity. *Acinetobacter baumannii* infections is due to its great resistance to the environment which enables it to spread, its virulence and its extraordinary ability to develop resistance to all the antimicrobials and its ability to spread by aerosols. Multi drug resistant (MDR) organisms are a major threat to VAP patients[9]. Most of the gram negative bacilli showed multidrug resistance. Infection by the multidrug organisms have increased in the intensive care unit[10].

In this study VAP rate were found be 61%, and out of this 34% were early onset VAP and 66% were late onset VAP. This is similar to the study by Amit Khelgi *et al*. In our study, 96.8% of VAP cases were due to gram negative bacilli and 3.1% were due to gram positive organisms.

Our study showed that *Klebsiella pneumoniae* was the commonest isolate (39.3%) followed by *Acinetobacter baumannii* (34%) and *Pseudomonas aeruginosa* (17%). It differs from other studies, such as a study by Dey *et al*. and Amit Khelgi *et al*. which shows that the *Acinetobacter* spp. were the commonest isolate followed by *Pseudomonas aeruginosa*.

In our study 75%-80% of *Acinetobacter baumannii* isolates showed carbapenem resistance. 35% of *Klebsiella pneumoniae* isolates were resistant to cefotaxime. Around 62%-64% of *Klebsiella pneumoniae* isolates were resistant to carbapenem whereas 62% of them were resistant to aminoglycosides.

To conclude, in spite of the recent advances in the diagnosis and management of VAP, it continues to be a major cause of hospital morbidity and mortality. Local data collected in this study can assist in making informed treatment choices. Knowledge of incidence of VAP, their causative microbial flora in a local setting along with information on the susceptibility patterns will help in selection of the appropriate antibiotics for therapeutic use and will aid a better outcome. The components of VAP bundle interventions should be implemented together with standard precautions as well as adequate disinfection and maintenance of equipment and devices will go along way in reducing the incidence of VAP.

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