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Microbiological Profile of Ventilator Associated Pneumonia in A Tertiary Care Intensive Care Unit

KEYWORDS: Ventilator-associated pneumonia, Intensive care unit, Gram Negative bacilli.

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ABSTRACT *Introduction: Ventilator associated pneumonia, (VAP) an important form of hospital acquired pneumonia, specifically refers to pneumonia developing in mechanically ventilated patients for more than 48 hours after tracheal intubation or tracheostomy.VAP remains to be the commonest cause of hospital morbidity and mortality in spite of advances in diagnostic techniques and management.*

Aim of the study: The aim of the study to find out organisms associated with VAP and their antimicrobial susceptibility pattern.

Materials and methods: A prospective study was performed over a period of one year from March 2013 to February 2014 in patients undergoing mechanical ventilation (MV) for >48 h. Endotracheal aspirates (ETA) were collected from patients with suspected VAP cases and quantitative cultures were performed on all samples. Identification and antimicrobial susceptibility pattern of the isolates was done by using the VITEK® 2 compact system, an automated identification (ID) and susceptibility (AST) system (bioMériux – France).

Results: Incidence of VAP was found to be 36.84% among the mechanically ventilated patients, out of which 29.17% had early-onset (<5 days MV) VAP and 70.84% had late-onset (>5 days MV) VAP. Gram negative organisms were the major pathogens (n=30, 88.23%) in our study. The most common organism isolated was found to be Acinetobacter baumannii 14 (41.18%), followed by Pseudomonas aeruginosa and Klebsiella pneumoniae 9 (26.47%) and 5(14.71%) respectively. while among gram positive organisms, 4(11.76%) Staphylococcus aureus was isolated.

Conclusion: Gram negative organisms were the primary cause of VAP in our study. The knowledge of prevalent local pathogens and their antibiogram will help the clinician to choose the appropriate antimicrobial agent for effective and rationale treatment.

INTRODUCTION

Ventilator associated pneumonia, (VAP) an important form of hospital acquired pneumonia, specifically refers to pneumonia developing in mechanically ventilated patients for more than 48 hours after tracheal intubation or tracheostomy[1]. The incidence of VAP is high, ranging from 6 to 52% and can reach 76% in some specific settings [2].

It is a common condition, difficult to diagnose accurately, and expensive to treat. Its development prolongs a patient's stay in the intensive care unit (ICU), and is associated with significant morbidity and mortality. Most cases seem to result from aspiration of pathogenic material that commonly colonises the oropharyngeal airways of the critically ill [3].

VAP is one of the most common infections in the intensive care units (ICUs), increasing the length of stay of patients in these units, the cost of the treatment, and the risk of death [4].

The etiology of VAP depends on multiple factors such as time of ventilation, prior administration of antibiotics, and presence of chronic obstructive pulmonary disease, coma, and local factors[5]. Various microbial agents such as non fermentative Gram-negative multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* have been described, over the last decades, as agents that cause this type of infection [6].

A local surveillance program is essential at each centre, as the knowledge of local resistant patterns is vital for selecting appropriate agents for treating infections. So, the present study was undertaken to assess the microbiological profile and susceptibility pattern of isolates in the patients who developed VAP in our settings.

MATERIALS AND METHODS

A hospital based observational and descriptive study was conducted in the Department of Microbiology, S.M.S. Medical College and Attached Hospitals, Jaipur, Rajasthan. A total number of 150 Ventilated cases which fulfilled study's inclusion and exclusion criteria were studied over a period of one year from March 2013 to February 2014. The patients fulfilling both the clinical and microbiological criteria were diagnosed as VAP cases and the remaining were categorized as non-VAP cases. Microbiological criteria included positive Gram stain (>10 polymorphonuclear cells / low power field and ≥1 bacteria/ oil immersion field), while clinical criteria included modified Clinical Pulmonary Infection Score (CPIS) > 6 developed by Pugin et al[7].

Inclusion Criteria:

Patients on mechanical ventilation for more than 48 hours in the ICU.

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Exclusion Criteria:

- Patients on mechanical ventilation for less than 48 hours.
- Patients having pulmonary infiltrate prior to MV
- **Collection of Endotracheal Aspirates (ETA).** [8]

A trained respiratory therapist collected ETA every time. The ETA was collected using a 22-inch Ramson's 12 F suction catheter with a mucus extractor, which was gently introduced through the endotracheal tube for a distance of approximately 25–26 cm. Gentle aspiration was then performed without instilling saline, and the catheter was withdrawn from the endotracheal tube. After the catheter was withdrawn, 2 ml of sterile 0.9% normal saline was injected into it with a sterile syringe to flush out the exudates into a sterile container. ETA samples were immediately taken to the laboratory for processing. The samples were first subjected to Gram's staining and then quantitative cultures were performed.

Quantitative Culture

Samples were mechanically liquefied and homogenized by vortexing for 1 min and then serially diluted in 0.9% sterile normal saline solution with final dilutions of 10^{-2}, 10^{-3} and 10^{-4}. Primary inoculation of the samples was done blood agar (BA), and MacConkey agar (MA) by using 4 mm Nichrome wire loop, which holds 0.01 ml of sample. All plates were incubated overnight at 37°C and observed for growth after 24 hr. For definite diagnosis of VAP in this study, quantitative culture threshold[9] was considered as 10^5cfu/ml. Growth of any organism below the threshold was assumed to be due to colonization or contamination. Significant Isolates characterized by colony morphology and Gram stain.

Identification and determination of antimicrobial susceptibility[10] Identification and antimicrobial susceptibility pattern of the isolates was detected by using the VITEK® 2 compact system, an automated identification (ID) and susceptibility (AST) system (bioMériux – France). Strain characterization was performed with using the ID card – GN for both Gram-negative fermenting and non-fermenting bacilli, ID card GP for Gram-positive cocci. Antimicrobial susceptibility testing was performed with AST- **GN-25, GN-N090,** cards, in accordance with the manufacturer's instructions.

Statistical analysis

Chi square test was done for comparison of proportions The level of significance was set as 5% in all analysis All Statistical test were performed using Graphpad Prism version 6.

Results

During one year study period, 76 patients enrolled for the study according to the inclusion and exclusion criteria. Quantitative culture results showed significant growth ($\geq 10^5$ cfu/ml) for pathogenic organisms causing VAP in 28 (36.84%) patients, while 48 (63.17%) patients showed insignificant growth ($\leq 10^5$ cfu/ml) considered as NON VAP.

Table No. 1: Characteristics of Patients developing VAP (n= 28)

Characteristics	Patients developing VAP n= 28
Cases	
VAP	28(36.84%)
NON VAP	48(63.16%)
Sex	
Male	17 (60.71%)
Female	11 (39.28%)
VAP onset	
Early (< 5days)	9(26.47%)
Late (>5days)	25(73.53%)
Infection	
Polymicrobial	8(23.53%)
Monomicrobial	26(76.47%)

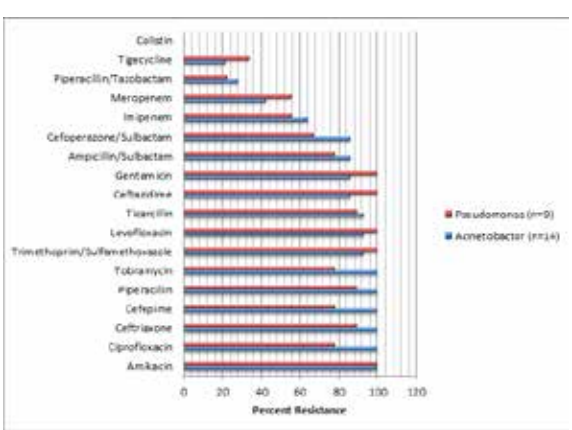
Table No. 2: Initial Admitting Diagnosis in 28 VAP Cases.

S.N.	Diagnosis	Pt. No.(%)
1	Chronic Lung Diseases	6(21.43%)
2	Cardiovascular Diseases	5(17.86%)
3	Neuromuscular Disorders	5(17.86%)
4	PUO / Fever with Altered Sensorium	4(14.29%)
5	Complicated cases of (Malaria, Dengue, Scrub Typhus)	3(10.71%)
6	Chronic Kidney Diseases	3(10.71%)
7	Suspected Poisoning	1(3.57%)
8	Others	1(3.57%)

Table No. 3: Organisms Isolated from Early and late Onset VAP Cases

Isolates	Total No. (%)	E-VAP (%)	L- VAP (%)	P Value
Aci. baumannii	14(41.18)	3(21.43)	11(78.57)	0.003
Ps. aeruginosa	9(26.47)	2(22.22)	7(77.78)	0.056
Kleb. pneumoniae	5(14.71)	2(40)	3(60)	1.000
S. aureus	4(11.76)	1(25)	3(75)	0.479
E. coli	1(2.94)	1(100)	0	0.157
Proteus mirabilis	1(2.94)	0	1(100)	0.157
Total	34(100)	9(26.47)	25(73.53)	

Table No. 4: Antimicrobial Resistance Pattern of Acinetobacter baumannii and Pseudomonas aeruginosa. (%R)



DISCUSSION

Ventilator-associated pneumonia (VAP) is an important nosocomial infection among ICU patients receiving mechanical ventilation (MV). It is the second most common noso-

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comial infection in the intensive care unit (ICU) and the most common nosocomial infection in mechanically ventilated patients.[11,12] Despite major advances in techniques for the management of ventilator-dependent patients and the routine use of effective procedures to disinfect respiratory equipment, ventilator-associated pneumonia continues to complicate the course of 8 to 28% of the patients receiving mechanical ventilation.[13] It is a common condition, difficult to diagnose accurately and expensive to treat. Its development prolongs patient's stay in the intensive care unit, and is associated with significant morbidity and mortality.[14] A favorable outcome seems to be more likely if appropriate antibiotics are given in a timely manner.

Incidence of VAP in our study was found to be 36.84%. Similar incidence has been reported by H. Gadani et al (37%), S. Golia et al (34.14%), and Saldanha et al (38.5%)[15,16,17] . However Rajasekhar *et al.*[18] reported higher incidence (73%) in their study which may be due to their smaller sample size. Divergence of incidence can be attributed to several factors such as differences in the study population, differences in the definition of VAP, e.g. clinical versus microbiological definition and possibly, to the use of preventive strategies.[2, 19, 20]

In the present study Incidence of VAP was found to be higher in males (60.71%) as compared to females (39.28%). Other studies also reported higher incidence of VAP in males then females these are by S. Dominic et al[17], Kotgire S. A[21] et al and N. Ranjan et al [22]

Present study observed that Chronic Obstructive Pulmonary Diseases (COPD) 6(21.43%), was the most common underlying conditions as shown in Table No. 2. Similar finding was reported by Vijay Hada et al[23], who observed that 22.8% COPD cases were associated with VAP.

Out of 28 VAP cases, 9(26.47%) were categorized under early-onset VAP and 25(73.53) % under late-onset VAP which was in concordance with studies conducted by Gadani et al[15], S Golia[16] et al. However with the late-onset VAP cases, the isolates of *Acinetobacter baumannii* were significantly associated as showed in Table - 3 (*P* value < 0.05), which co-relates to the study conducted by N M Joseph[23] et al.

In our study 8(23.53%) cases were found to have polymicrobial growth and 26(76.47%) had monomicrobial growth. Rates of polymicrobial infection vary widely.

We observed that non-fermenters 23(67.65%) such as *Acinetobacter baumannii*. and *Pseudomonas aeruginosa* were the most predominant VAP pathogens, followed by members of Enterobacteriaceae (20.59%), and S aureus 4 (11.76%). Microbial profile of VAP is shown in the Table No.3. The pathogens which are responsible for VAP vary, depending on the duration of the mechanical ventilation, prior antibiotic exposure and the length of stay in the hospital. Airway intubation is associated with increased frequency of Gram-negative bacterial colonization of upper and lower respiratory tract with subsequent overgrowth and pneumonia.

In the present study *Acinetobacter baumannii* was found to be the commonest 14 (41.18%) isolate, which co-relates to the study conducted by A. Dey et al [8], and N. Ranjan et al[22], Earlier reports had showed that among the gram-negative organisms, *Pseudomonas aeruginosa* was the commonest causative agent for VAP.[19,25] The increase of *Acinetobacter baumannii* infections could be due to its greater resistance to the environment which enables its spread, its extraordinary ability to develop resistance to commonly used antimicrobials and its spread by aerosols.[26]

Regarding the susceptibility profiles of etiological agents of VAP, *Acinetobacter baumannii* showed higher resistance to 14 of the 18 antibiotics tested with the exception of Colistin, Tigecycline, Piperacillin/Tazobactam combination and Meropenem which showed 100%, 78.57%, 72%, and 58% sensitivity respectively. High antibiotics resistance among *Acinetobacter baumannii* also reveled in other studies conducted by M. Medell et al,[27] Naveed et al,[28] and Namita et al[29]

In Pseudomonas *aeruginosa* Colistin was found to be most effective antibiotic followed by piperacillin/tazobactum combination.

S. aureus was found to be 100% resistant to Oxacillin, similar result was observed in a study conducted by A. Gupta et al[30] which indicating the high prevalence of MRSA as a cause of VAP in their setting. Vancomycin and Linezolid were found to be most effective drugs among Gram Positive Cocci (100% sensitive). Other studies of Thakuria et al[31] S. Goila et al[16] reported 100% sensitivity to the same.

Conclusion

Acinetobacter baumannii and *Pseudomonas aeruginosa* were the main microorganisms that affected patients with VAP while Acinetobacter *baumannii* isolates were significantly associated with LVAP. Colistin was the only antibiotic fully effective against of both *Acinetobacter baumannii* and *Pseudomonas aeruginosa* strains. Knowledge of the susceptibility pattern of the local pathogens should guide the choice of antibiotics, in addition to the likelihood of organisms, as there is an increasing prevalence of MDR pathogens in VAP cases.

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