



BACTERIOLOGICAL PROFILE AND ANTIBIOGRAM OF AEROBIC BACTERIA ISOLATED FROM PATIENTS WITH VENTILATOR ASSOCIATED PNEUMONIA ADMITTED IN CRITICAL CARE UNIT OF A MEDICAL COLLEGE IN WEST BENGAL

Microbiology

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ABSTRACT

Culture sensitivity of deep tracheal suction samples collected from intubated patients has paramount importance in getting a proper antibiogram which can prevent morbidity and mortality and reduce the duration of ICU stay of the patients. Objectives are to find out the bacteriological profile of isolates from deep tracheal suction samples at zero hours and at 48 hours of intubation, along with their antibiotic sensitivity pattern. 51 participants whose samples showed no growth at zero hours of intubation were included. Among the remaining 51 samples, 36 were found culture positive at 48 hours of intubation, where male participants were the majority with >60 years age group being maximally affected. Samples were collected in ICU, sent immediately to the microbiology laboratory followed by direct gram staining, and after plating, incubation, motility test and various biochemical identification methods, ABST was performed. Incidence of causative bacteria revealed gram negative bacilli to be more than gram positive organisms. *Klebsiella pneumoniae* showed maximum sensitivity to amikacin with other drugs being partially sensitive. *Acinetobacter baumannii* complex showed maximum sensitivity to levofloxacin and amikacin, while being completely resistant to all other drugs. Amikacin and piperacillin tazobactam served the best combination therapy against *Pseudomonas aeruginosa*, while MRSA showed maximum sensitivity to linezolid. Antibiogram is thus an important tool in choosing selected antibiotics in order to prevent emergence of bacterial resistance in ventilator patients.

KEYWORDS

VAP, Antibiogram, CCU.

INTRODUCTION:

Ventilator-associated pneumonia (VAP) is defined as pneumonia occurring 48 hours or more after endotracheal intubation/initiation of mechanical ventilation or pneumonia developing even after extubation.¹ VAP may be caused by a wide spectrum of bacterial pathogens which may be polymicrobial, and rarely can be due to viral or fungal pathogens in immune competent hosts², which is the major cause of hospital morbidity and mortality, despite recent advances in diagnosis and accuracy of management.³ Kollef, M. H., and co-workers did a prospective survey among 1873 mechanically ventilated patients in 56 ICUs across 11 countries of Europe, United States, Latin America and the Asia Pacific region and found that, VAP prevalence was 15.6% (293/1,873) globally, 13.5% in the United States, 19.4% in Europe, 13.8% in Latin America, and 16.0% in Asia Pacific ($p = 0.04$).⁴ Patro S and co-workers stated that in order to combat high antibiotic resistance in ITU setup, combined approaches of rational antibiotic therapy might be beneficial.² So knowledge of susceptibility pattern of local pathogens can guide the clinicians to choose appropriate antibiotics according to the likelihood of organisms of early or late-onset VAP⁵ and also about multidrug resistance.^{6,7,8} Therefore, *aim* is to find out the bacteriological profile and antibiogram of aerobic bacteria isolated from patients with ventilator associated pneumonia. **Objectives** are to find out the bacteriological profile of isolates from deep tracheal suction samples at zero hours and at 48 hours of intubation, along with antibiotic sensitivity pattern of the isolates among ventilated patients admitted in critical care unit.

METHODOLOGY:

After achieving IEC clearance and informed consent from the patient relatives, this descriptive cross-sectional study was conducted over a period of 1 year on ICU patients who were on intubation and mechanical ventilation in a medical college of West Bengal. Adults of both sexes who developed pneumonia at 48 hours or more on intubation and mechanical ventilation were included. First day of deep tracheal suction material positive cases and patients whose relatives refused to give consent for the study were excluded. Deep tracheal suction sample was collected under strict aseptic precautions from adult patients by using 12 to 14 F suction catheter at zero and at 48 hours of intubation and mechanical ventilation. After collection, all the samples were transported to the microbiology laboratory immediately without delay. The samples were first subjected to direct gram stain and examined under oil-immersion objective to look for the presence of bacteria and pus cells. Samples were then inoculated on Nutrient agar, Blood agar, and MacConkey agar followed by incubation at 37°C. After overnight incubation, growth was identified by studying the colony morphology, gram staining from colony and performing various biochemical tests such as catalase test and coagulase test for gram positive cocci and citrate utilization test, urease test, triple sugar iron test, indole test and oxidase test for gram negative bacilli. Motility of

bacteria was observed by Hanging Drop preparation method. Antibiotic sensitivity testing was performed by Kirby Bauer disk diffusion method on Mueller Hinton agar and results were tallied according to Clinical Laboratory Standards Institute (CLSI). All the data were entered into a microsoft excel spreadsheet and analyzed using standard statistical software. Categorical variables were expressed as frequencies and percentages. Continuous variables were expressed as mean, standard deviation and in percentage, and were compared using student's t test. All analysis were two-tailed and $p < 0.05$ was considered statistically significant.

RESULTS:

A total of 102 deep tracheal suction samples from 51 patients were included for analysis. 51 samples of those patients at zero hours of intubation showed negative culture. Among the rest 51 samples, 36 were found culture positive at 48 hours or more of intubation and mechanical ventilation (70.59%; $p < 0.05$).

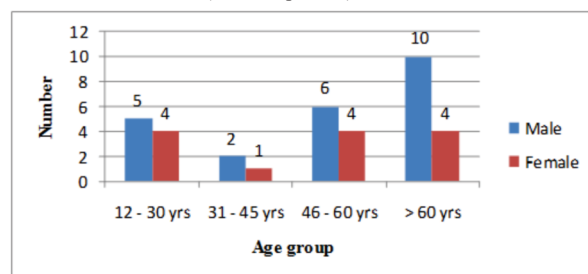


Figure 1: Age and sex wise distribution of patients with culture positive samples

Figure 1 shows age and sex wise distribution of patients with culture positive samples, where 23 (63.89%) samples were from male and 13 (36.11%) were from female patients.

Table 1: Different isolates obtained from culture positive deep tracheal suction samples

Isolates	Total no of isolates	Percentage
<i>Klebsiella pneumoniae</i>	20	55.56%
MRSA	8	22.22%
<i>Pseudomonas aeruginosa</i>	6	16.66%
<i>Acinetobacter baumannii</i> complex	2	5.56%

Table 1 shows different isolates obtained from culture positive deep tracheal suction samples where, out of 36 isolated organisms, 20 (55.56%) were *Klebsiella pneumoniae*, 8 (22.22%) were Methicillin resistant staphylococcus aureus (MRSA), 6 (16.66%) were *Pseudomonas aeruginosa* and 2(5.56%) were *Acinetobacter*

baumannii complex.

Table 2: Antibiotic susceptibility pattern of isolates from deep tracheal suction samples

SL NO	PATHOGENS	ANTIBIOTIC	Sensitive No. (%)	Resistant No. (%)
01	Klebsiella pneumoniae	Amikacin	7(35%)	13(65%)
		Gentamicin	1(5%)	19(95%)
		Ciprofloxacin	4(20%)	16(80%)
		Levofloxacin	5(25%)	15(75%)
		Cotrimoxazole	3(15%)	17(85%)
		Meropenem	1(5%)	19(95%)
		Imipenem	0(0%)	20(100%)
		Ceftriaxone	2(10%)	18(90%)
		Ceftazidime	0(0%)	20(100%)
		Cefepime	1(5%)	19(95%)
		Cefixime	1(5%)	19(95%)
		Piperacillin tazobactam	5(25%)	15(75%)
02	Methicillin resistant Staphylococcus aureus (MRSA)	Amoxyclav	1(5%)	19(95%)
		Aztreonam	1(5%)	19(95%)
		Cefoxitin	0(0%)	8(100%)
		Cotrimoxazole	2(25%)	6(75%)
		Azithromycin	0(0%)	8(100%)
		Gentamicin	0(0%)	8(100%)
		Ciprofloxacin	1(12.5%)	7(87.5%)
		Levofloxacin	0(0%)	8(100%)
		Linezolid	8(100%)	0(0%)
		Meropenem	3(50%)	3(50%)
		Imipenem	0(0%)	6(100%)
		Amikacin	4(66.67%)	2(33.33%)
03	Pseudomonas aeruginosa	Gentamicin	0(0%)	6(100%)
		Ciprofloxacin	1(16.67%)	5(83.33%)
		Levofloxacin	0(0%)	6(100%)
		Ceftazidime	0(0%)	6(100%)
		Cefepime	1(16.67%)	5(83.33%)
		Piperacillin Tazobactam	4(66.67%)	2(33.33%)
		Aztreonam	0(0%)	6(100%)
		Gentamicin	0(0%)	2(100%)
		Amikacin	1(50%)	1(50%)
		Levofloxacin	1(50%)	1(50%)
		Ciprofloxacin	0(0%)	2(100%)
		Meropenem	0(0%)	2(100%)
04	Acinetobacter baumannii complex	Imipenem	0(0%)	2(100%)
		Cefepime	0(0%)	2(100%)
		Ceftazidime	0(0%)	2(100%)
		Ceftriaxone	0(0%)	2(100%)
		Piperacillin Tazobactam	0(0%)	2(100%)

Table 2 shows the antibiotic susceptibility pattern of isolates from deep tracheal suction samples. *Klebsiella pneumoniae* showed maximum sensitivity (35%) to amikacin, 25% to levofloxacin and piperacillin tazobactam, 20% to ciprofloxacin, 15% to cotrimoxazole, 10% to ceftriaxone and 5% sensitivity each to gentamicin, cefixime, cefepime, amoxyclav, meropenem and aztreonam, while it showed 100% resistance to ceftazidime and imipenem. Methicillin resistant *Staphylococcus aureus* (MRSA) showed maximum sensitivity (100%) to linezolid, 25% to cotrimoxazole and 12.5% sensitivity to ciprofloxacin, while it showed 100% resistance to cefoxitin, gentamicin, levofloxacin and azithromycin. *Pseudomonas aeruginosa* showed maximum sensitivity (66.67%) to amikacin and piperacillin tazobactam, 50% to meropenem and 16.67% sensitivity each to ciprofloxacin and cefepime, while it showed 100% resistance to gentamicin, levofloxacin, ceftazidime, imipenem and aztreonam. *Acinetobacter baumannii* complex showed sensitivity only to levofloxacin and amikacin (50% each) while it was 100% resistant to meropenem, imipenem, gentamicin, ciprofloxacin, ceftriaxone, ceftazidime, cefepime and piperacillin tazobactam.

DISCUSSION:

Sample received by deep tracheal suction came out as satisfactory practice in identifying the causative agents in prevention and treatment of VAP which is similar to the research work of Davis KA coworkers¹ and patro S coworkers². Similar to the study of Hina Gadani and co-workers, this study also states that every one of the critical care unit should understand the factors that place the patients at risk of VAP³. Mathai AS and co-workers revealed that per 1000 mechanical ventilation days, a total of 95 (38%) patients developed VAP, incidence being 40.1. Study by Somi Patro and co-workers stated that prevalence of VAP was 35%. In the present study, 36 out of 51 patients (70.59%) showed bacteriological positivity in deep tracheal suction samples at 48 hours of intubation and mechanical ventilation. Mathai AS and co-workers revealed that the predominant organisms in their study were Gram-negative organisms, especially *Acinetobacter* species (58 isolates, 53.2%).⁶ Kollef MH and co-workers concluded that ventilator-associated pneumonia remains a common ICU infection with *Pseudomonas aeruginosa* being one of the most common causative pathogens.⁴ Results of research work of Valerio Monteiro and co-workers revealed that the main pathogens of VAP are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Enterobacteriaceae* members.⁷ Study by Somi Patro and co-workers stated that enterobacteriaceae (66.66%) and *Staphylococcus aureus* (20%) were common in early-onset VAP, while nonfermenters (50%) and enterobacteriaceae (40.61%) were predominant from late-onset VAP.² C. Su Young and co-workers revealed that the most frequent pathogens of VAP were *Staphylococcus aureus* and *Acinetobacter baumannii*.⁸ In the present study, distribution of different isolates from 36 culture positive deep tracheal suction samples was *Klebsiella pneumoniae* being 20 (55.56%), Methicillin resistant *Staphylococcus aureus* (MRSA) being 8 (22.22%), *Pseudomonas aeruginosa* being 6 (16.66%) and 2 (5.56%) being *Acinetobacter baumannii* complex. *Klebsiella pneumoniae* was the most commonly isolated organism (55.56%). Study by Somi Patro and co-workers stated that among the *Staphylococcus aureus* isolates, 75% were cefoxitin resistant.⁷ In this study, all *Staphylococcus aureus* isolates (100%) were methicillin resistant which is similar to the study of C. Su Young and co-workers. Similar to the study of Chastre and co-workers⁵ this study revealed that MRSA showed maximum sensitivity to linezolid; whereas 100% resistance was seen against gentamicin, levofloxacin, cefoxitin and azithromycin. Linezolid has demonstrated high penetration into the epithelial lining fluid of patients with ventilator-associated pneumonia and has also shown statistically superior clinical efficacy. Mathai AS and co-workers revealed that multidrug resistance was demonstrated by 27.3% of *Acinetobacter baumannii* isolates.⁶ C. Su Young and co-workers revealed that imipenem resistance rate of *Acinetobacter baumannii* was 69%.⁸ In the present study, *Acinetobacter baumannii* complex showed maximum sensitivity to levofloxacin and amikacin, while being 100% resistant to meropenem, imipenem, gentamicin, ciprofloxacin, ceftriaxone, ceftazidime, cefepime, piperacillin and tazobactam. *Klebsiella pneumoniae* showed maximum sensitivity to Amikacin, while ceftazidime and imipenem showed 100% resistance. All other drugs showed partial sensitivity. *Pseudomonas aeruginosa* showed maximum sensitivity to amikacin and piperacillin tazobactam, while it showed 100% resistance to gentamicin, levofloxacin, ceftazidime, imipenem and aztreonam.

CONCLUSION:

This study concluded with increased incidence of growth of gram-negative organisms in deep tracheal suction samples at 48 hours of intubation and mechanical ventilation. Development of VAP may be due to aspiration of oropharyngeal secretions, or it may be a nosocomial infection, and presents as a major diagnostic challenge. Early and appropriate use of antibiotics may result in reducing mortality among patients on mechanical ventilation. Moreover, it is important to find out an appropriate guideline for antibiotic therapy in patient management, which must be based on local prevalence and microbiological data. Thus, there is paramount importance of doing culture sensitivity of deep tracheal suction samples for getting a proper antibiogram suitable for proper management of the patient in order to prevent morbidity and mortality, reduce the duration of ICU stay and also to prevent emergence of bacterial resistance.

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