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Original Research Article

Bacteriological Profile and antibiogram of isolates from tracheal secretions of patients in an intensive care unit

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ABSTRACT

Introduction: Hospital-acquired pneumonia (HAP) is defined as an infection in patients admitted in hospital for more than 48hours, and ventilator associated pneumonia (VAP) can be defined as infection occurring in patients admitted in ICU after 48hrs endotracheal intubation and mechanical ventilation. VAP has a mean of 7.3/1000 ventilator days for medical ICU patients and 13.2/1000 ventilator days for surgical ICU patients. The crude mortality rates for HAP are approximately 10% and are higher for VAP, ranging from 20% to 60%. The culture of endotracheal (ET) aspirates will help know the etiological agent and formulate the antibiotic policy for early treatment.

Objectives: To isolate the bacterial pathogens of ET secretions from patients with VAP and know their antibiotic susceptibility pattern.

Results: In the present study, out of 102 endotracheal secretions from cases of VAP, 88 samples (86.27%) were culture positive, and the remaining 14 samples (13.73%) were culture negative. Out of the 88 positive cultures, 62 samples (60.78%) showed growth of single isolates, and 26 samples (25.49%) showed multiple isolates. Out of the 114 isolates, 18 isolates (15.8%) were gram positive organisms, and 96 isolates (84.2%) were gram negative organisms. Of the gram positive isolates, the predominant organism was Methicillin resistant *Staphylococcus aureus* (MRSA) (55.56%), followed by Methicillin sensitive *Staphylococcus aureus* (MSSA) (22.22%) and *Streptococcus spp*(22.22%). Out of the gram negative isolates, the predominant organism was *Klebsiella pneumoniae* (36.46%), followed by Acinetobacter spp (25%), *Pseudomonas aeruginosa* (23.96%), *Escherichia coli* (12.5%), *Enterobacter spp* (1.04%), and Proteus mirabilis (1.04%). In the present study, Gram positives isolates showed the highest susceptibility to vancomycin and linezolid (100%), and gram negative isolates showed the highest susceptibility to polymyxin B (100%) and meropenem (47.92%).

Conclusion: The study gives insight into the bacterial pathogens and their antibiotic susceptibility patterns of isolates from endotracheal secretions of mechanically ventilated patients to prevent the mortality and morbidity of mechanical ventilation and VAP, helping in formulating an antibiotic policy for appropriate empirical therapy.

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1. Introduction

Hospital acquired pneumonia (HAP) is defined as an infection occurring in patients admitted in hospital for more than 48hrs, and ventilator associated pneumonia (VAP) can be defined as infection occurring in patients admitted in

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ICU after 48hrs endotracheal intubation and mechanical ventilation.[1–3]

Despite recent advances in antimicrobial therapy, supportive care, and also prevention measures, VAP remains a significant cause of mortality, morbidity, and healthcare cost.[4] The VAP rates have a mean of 7.3/1000 ventilator days for medical ICU patients and 13.2/1000 ventilator days for surgical ICU patients. The VAP rates vary according to the patient population, disease severity, method of diagnosis, and duration of mechanical ventilation. VAP is estimated to be 3% per day during the first five days and 2% per day after that.[5]

Crude mortality rates for HAP are approximately 10% and are higher for VAP which may range from 20%-60%.[6]

The risk factors for the development of VAP include the patient's endogenous flora, hospital staff, contaminated devices, microaspiration of contaminated oropharyngeal secretions, duration of ventilation, positioning of the patients, enteral feeding, and over sedation.[7]

VAP is categorized into early onset VAP (infection occurring during the first five days of the hospital stay) and late onset VAP (infection occurring after five days of the hospital stay). Early onset VAP is often caused by community acquired organisms in contrast to late onset VAP caused by multi drug resistant hospital strains.[7] Early onset VAP is caused by pathogens like *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Legionella pneumophila*, and anaerobic bacteria, and late onset VAP by *Pseudomonas aeruginosa*, *Acinetobacter spp*, MRSA, and also by some members of the Enterobacteriaceae family.[8,9]

In intubated patients, lower respiratory tract colonization may progress to ventilator associated tracheobronchitis (VAT), which is a precursor to VAP.[10] Clinical diagnosis of VAP can be done by the presence of at least two of the three features like 1) temperature >38^{o}c or hypothermia 2) leukocytosis /leukopenia 3) purulent tracheal secretions. Blood cultures are helpful in conditions like those infected with *Streptococcus pneumoniae* or *Staphylococcus aureus*.[7]

Several studies also show that the patients admitted to ICUs develop multi-bacterial infections due to their prolonged stay in the hospital. The changing floras also complicate the therapy by developing resistance to multiple antibiotics.[11]

Studies show that in mechanically ventilated patients, the folds of standard cuffed endotracheal tubes (ETT) are the permanent source of infection, bacterial colonization, and biofilm formation.[12]

The endotracheal tube also acts as a reservoir for infecting organisms. It harbors the microorganisms in the inferior of the first distal third, making them a significant risk factor for pulmonary infection leading to VAP. For the diagnosis and management of cases of VAP, detection of etiological agents is crucial, done by collecting the endotracheal aspirates, performing semi quantitative culture, and interpreting as moderate or heavy growth of the pathogens. Culture of endotracheal aspirates reported as few colony forming units of an organism represents tracheal colonization. Growth of > 10^{5-6} CFU/ml of an organism is consistent with the diagnosis of VAP.

Quantitative distal airway samples collected by broncho-alveolar lavage (BAL), protective specimen brush (PSB) have good diagnostic sensitivity and specificity than endotracheal aspirate samples. Still, they are not widely available, are expensive, require expertise, and are invasive. Most of the hospitals use the semi quantitative endotracheal aspirates for the diagnosis of VAP which is a noninvasive procedure. Biological markers like procalcitonin and soluble triggering receptors expressed on myeloid cells also help in the diagnosis of VAP. Still, they cannot determine the causative organisms and the associated patterns of antibiotic susceptibility.[13]

According to the literature, by 2050, approximately 10 million people will die each year due to antibiotic resistance and improper diagnosis.[14,15] The prevalent flora and antibiotic resistance patterns of the pathogens vary from region to region. Hence, it is mandatory to know about them, which can provide clinicians with prompt and empirical treatment with appropriate antibiotics in VAP cases and helps in revising the antibiotic policy for better management of the patients.[11,16]

So, a study was done to identify the bacterial etiology, antibiotic susceptibility patterns in cases of VAP by the culture of endotracheal secretions.

1.1. Inclusion criteria

ET tips and aspirates of all the patients who have been mechanically ventilated for more than 48hrs for various reasons.

1.2. Exclusion criteria

Patients already having respiratory infections and those who developed respiratory infections within 48hrs of mechanical ventilation and post-op ventilated patients.

2. Materials and Methods

This study is a descriptive cross-sectional study conducted at Dr.Pinnamaneni Siddhartha Institute of Medical Sciences and Research Foundation, Vijayawada, after obtaining approval from the institutional ethics committee (IEC) for one year. A total of 102 tracheal secretions collected from mechanically ventilated patients admitted to ICU were included in the study. Collection of tracheal aspirates was done by gently introducing a 22-inch suction catheter into the endotracheal tube, secretions aspirated, the catheter withdrawn from the ET tube, and the tip of the suction catheter along with tracheal secretions transferred to a sterile container. The samples were immediately transported

to the microbiology laboratory, where they were subjected to Gram's staining and semi quantitative cultures.[17]

Gram's stain of these specimens was done, which can provide clues of type of bacteria present and also whether the material is purulent or not (>25 neutrophils and < 10 squamous cells per lower power field).[18,19] The samples were inoculated onto blood agar, chocolate agar, and macconkey agar and incubated at 35-37^{o}C for 24hrs. The cultures were read the next day for positive and negative where the positive cultures were read semi quantitatively. The bacteria were identified by colony morphology, hemolysis on blood agar, lactose fermenter or non-lactose fermenterand gram's stain to confirm whether the isolate was gram positive or gram negative cocci or bacilli and further identification and confirmation was done by conventional biochemical tests.[20]

Antimicrobial susceptibility testing of the isolates was done on Mueller Hinton agar by Kirby Bauer disc diffusion technique, the plates were incubated at 35-37^{o}C for 24hrs and interpreted as sensitive (S), Intermediate (I), and resistant (R) according to clinical laboratory standards institute (CLSI) guidelines.[21]

Vancomycin (5μg), linezolid (30μg), Erythromycin (15 μg), tetracycline (30 μg), penicillin (10units), ceftriaxone (30μg), cephalothin (30μg), ciprofloxacin (5μg), Amoxyclav (20/10 μg), oxacillin (1μg), co trimoxazole (25μg), clindamycin (2 μg), gentamicin (10 μg) discs of Himedia labs, Mumbai were used for testing gram positive organisms.

Polymyxin-B(300μg),Imipenem (10μg), Meropenem (10 μg), piperacillin tazobactam (100/10μg), Cefoperazone Sulbactum (75μg), gentamicin(10μg) , ceftriaxone (30 μg) , cephalothin (30μg), ciprofloxacin(5μg), co trimoxazole (25μg), Amoxyclav (20/10μg), Amikacin (30μg), Ceftazidime (30μg),cefipime(30μg) discs of Himedia labs, Mumbai were used for testing gram negative organisms.

The results were analyzed and expressed in words, tables, and percentages.

3. Results

In the present study, a total of 102 endotracheal secretions samples were collected. Out of which 88(86.27%) samples were culture positive and the remaining 14 samples (13.73%) were culture negative.(Table 1)

Table 1: Shows no. of culture positive and negative from clinical specimens

Culture positives	88(86.27%)
Culture negatives	14 (13.73%)
Total	102(100%)

Out of 88 positive cultures, 62 showed growth of single isolate (60.78%), and multiple isolates were seen in 26 samples (25.49%).(Table 2)

Table 2: Shows no of isolates from clinical specimens

Single isolates	62 (60.78%)
Multiple isolates	26 (25.49%)
No bacterial growth	14 (13.73%)
Total	102 (100%)

In the positive cultures, the total number of isolates was 114(100%). Out of which 18 isolates were Gram positive organisms (15.8%) and 96 isolates were Gram negative organisms (84.2%).(Table 3)

Table 3: Shows no of Gram positive & Gram negative organisms isolated from clinical specimens

Gram positive organisms	18 (15.8%)
Gram negative organisms	96 (84.2%)
Total	114 (100%)

Out of 18(100%), Gram positive isolates Methicillin Resistant *Staphylococcus aureus* (MRSA) was the predominant isolate (55.56%) followed by Methicillin Sensitive *Staphylococcus aureus* (MSSA) (22.22%) and *Streptococcus* species (22.22%).(Table 4)

Table 4: Shows different types of Gram positive organisms isolated from clinical specimens

Organisms	**No of isolates**
Staphylococcus aureus (MRSA)	10(55.56%)
Staphylococcus aureus (MSSA)	4(22.22%)
Streptococcus spp	4(22.22%)
Total	18(100%)

Out of 96 (100%), Gram negative isolates *Klebsiella pneumoniae* was the predominant isolate (36.46%) followed by *Acinetobacter baumannii* (25%), *Pseudomonas aeruginosa* (23.96%), *Escherichia coli* (12.5%), *Enterobacter spp* (1.04%) and *Proteus mirabilis* (1.04%).(Table 5)

Table 5: Shows different types of Gram negative organisms isolated from clinical specimens

Organisms	**No. of isolates**
Klebsiella pneumonia	35 (36.46%)
Acinetobacter baumannii	24 (25%)
Pseudomonas aeruginosa	23 (23.96%)
Escherichia coli	12 (12.5%)
Enterobacter spp	1 (1.04%)
Proteus mirabilis	1 (1.04%)
Total	96 (100%)

In the present study among Gram positive organism, *Staphylococcus aureus* showed high susceptibility to Vancomycin & Linezolid (100%), Tetracycline & Cotrimoxazole (64.3%), Clindamycin (42.85%),

Gentamicin (35.71%), Amoxyclav, ceftriaxone & Cephalothin (28.6%), Erythromycin & Ciprofloxacin (21.43%), Penicillin (14.3%). Streptococcus spp isolated showed high susceptibility to Vancomycin, Linezolid, Penicillin & ceftriaxone (100%) followed by Erythromycin, Tetracycline & Ciprofloxacin (50%).(Table 6)

Among the Gram negative organisms, *Klebsiella pneumoniae* showed the highest susceptibility to Polymyxin B (100%) followed by Co-trimoxazole & meropenem (42.86%), Imipenem, Amikacin & Gentamicin (40%), Piperacillin tazobactam, Cefoperazone sulbactam & Amoxyclav (22.86%), Ciprofloxacin (20%), Ceftriaxone (17.14%), Cephalothin (14.29%).

Acinetobacter baumannii showed the highest susceptibility to Polymyxin B (100%) followed by Co-trimoxazole (29.2%), Cefoperazone sulbactam (25%), Meropenem, Imipenem & Ciprofloxacin (8.33%), Piperacillin tazobactam, Amikacin, Gentamicin, Amoxyclav, Ceftriaxone, Cephalothin, Ceftazidime (4.17%).

Pseudomonas aeruginosa showed the highest susceptibility to Polymyxin B (100%) followed by Meropenem, Imipenem & Piperacillin tazobactam (82.60%), Ciprofloxacin & Amikacin (73.91%), Gentamicin & Cefoperazone sulbactam (69.56%), Cefepime (65.21%), Ceftazidime (60.86%).

Escherichia coli showed the highest susceptibility to Polymyxin B (100%) followed by Gentamicin & Co-trimoxazole (75%), Meropenem, Imipenem & Amikacin (66.67%), Amoxyclav (50%), Cefoperazone sulbactam, Ciprofloxacin (41.67%), Piperacillin tazobactam, ceftriaxone, Cephalothin (25%).

Enterobacter spp & *Proteus mirabilis* showed 100% susceptibility to all the antibiotics.(Table 7)

4. Discussion

Endotracheal intubation and mechanical ventilation are lifesaving procedures done to prevent respiratory failure which may occur as a consequence of many conditions like sepsis, acute respiratory distress syndrome, cerebrovascular accidents, trauma, neurological dysfunctions etc. which may lead to life threatening respiratory infections by microorganisms getting access through exogenous or endogenous route resulting in ventilator associated pneumonia (VAP).[22]

The rate of nosocomial infection is steadily mounting in patients admitted to ICU due to invasive procedures being performed in these patients including artificial ventilator support. There is also constantly emerging resistance which is a serious situation due to which emphasis on the need to implement new regulations or antibiotic policies for the cautious use of antibiotics and also the hospital conditions are also to be refined to prevent exacerbation of resistance shown by the bacteria.[23]

In the present study out of 102 samples processed 88 samples (86.27%) were culture positive which correlated with other studies where according to Hasan Ahmad et al the culture positive rate of tracheal aspirates was 72.3%,[1] Vimal Shriram Rathos et al had a culture positive rate of 85%.[24] Neha Samal et al had a culture positive rate of 85.7%[17] and Masoum Khoshfetrat et al had a culture positive rate of 81.3%.[25]

In the present study 26/102 (25.49%) showed polymicrobial growth and 62/102 (60.78%) showed monomicrobial growth which correlates with a study reported by Vamsi C k et al[26] where Gram negative bacilli were the most predominant isolates in our study (84.2%) followed by Gram positive cocci (15.8%) correlating with studies by Neha Samal et al,[17] Chidambaram et al,[7] Khayyam et al[12] and Masoum et al.[25]

In the present study *Klebsiella pneumoniae* [35/96 (36.46%)] was the predominant Gram negative organism isolated followed by Acinetobacter spp [24/96 (25%)], *Pseudomonas aeruginosa* [23/96 (23.965)], *Escherichia coli* [12/96 (12.5%)], *Enterobacter spp* [1/96 (1.04%)] and *Proteus mirabilis*[1/96 (1.04%)] correlating with studies done by Ankita Patel et al[27] Malik et al,[28] Deepti C et al,[1] Vimal Shiram Rathod[24] in which *Klebsiella pneumoniae* was the predominant isolate. But in a study done by Neha Samal et al[17] *Acinetobacter baumannii* (46%) was the predominant isolate followed by *Pseudomonas aeruginosa* (17%) and *Klebsiella pneumoniae* (17%) and Khayyam et al[12] also reported that Acinetobacter spp was the predominant isolate (23.75%) followed by *Pseudomonas aeruginosa* (21.25%) and *Klebsiella pneumoniae* (16.25%).

In the present study, MRSA was the predominant isolate in Gram positive cocci 10/18 (55.56%) followed by MSSA 4/18 (22.22%) and Streptococcus spp 4/18(22.22%). According to Sarkar Mohammad et al,[16] Vimal Shiram Rathod et al,[24] Khayyam et al[12] with an isolation rate of 7.0%, 12.3%,15% respectively.

But according to Masoum et al[25] Staphylococcus epidermidis was the predominant Gram positive cocci isolated (50.3%) followed by *Staphylococcus aureus* (47.1%)

In the present study Gram positives including MRSA, MSSA, Streptococcus spp showed 100% susceptibility to vancomycin & linezolid which correlates with the findings of Hassan Ahmad et al[23] and Vimal Shiram Rathod et al[24] followed by Tetracycline, co-trimoxazole (64.3%), clindamycin (42.85%), gentamicin (35.71%), oxacillin, amoxyclav, ceftriaxone, cephalothin (28.6%), erythromycin, ciprofloxacin (21.43%) and the least susceptibility is seen with penicillin (14.3%). But according to Masoum et al,[25] the susceptibility rate for vancomycin was 89.65 and for Streptococcus spp, it was shown to be 66.7%.

Table 6: Shows the antibiotic susceptibility pattern of Gram positive organisms to various antibiotics

S. No.	Name of the antibiotic	Organism	
		***S.aureus* (MRSA& MSSA)**	***Streptococcus spp***
1	Vancomycin	14 (100%)	4 (100%)
2	Linezolid	14 (100%)	4 (100%)
3	Erythromycin	3 (21.43%)	2 (50%)
4	Tetracycline	9 (64.3%)	2 (50%)
5	Penicillin	2 (14.3%)	4 (100%)
6	Amoxyclav	4 (28.6%)	-
7	Cotrimoxazole	9 (64.3%)	-
8	Ceftriaxone	4 (28.6%)	4 (100%)
9	Cephalothin	4 (28.6%)	-
10	Ciprofloxacin	3 (21.43%)	2 (50%)
11	Gentamicin	5 (35.71%)	-
12	Oxacillin	4 (28.6%)	-
13	Clindamycin	6 (42.85%)	-

Table 7: Shows the antibiotic susceptibility pattern of Gram negative organisms to various antibiotics

S. No.	Name of the antibiotic	Organism					
		Klebsiella pneumoniae	***Acinetobacter baumannii***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Enterobacter spp***	***Proteus mirabilis***
1	Polymyxin B	35(100%)	24(100%)	23(100%)	12(100%)	1(100%)	1(100%)
2	Imipenem	14 (40%)	2(8.33%)	19(82.60%)	8(66.67%)	1(100%)	1(100%)
3	Meropenem	15 (42.86%)	2(8.33%)	19(82.60%)	8(66.67%)	1(100%)	-
4	Piperacillin tazobactam	8 (22.86%)	1(4.17%)	19(82.60%)	3(25%)	1(100%)	1(100%)
5	Cefoperazone sulbactam	8(22.86%)	6(25%)	16(69.56%)	5(41.67%)	1(100%)	1(100%)
6	Ciprofloxacin	7(20%)	2(8.33%)	17(73.91%)	5(41.67%)	1(100%)	1(100%)
7	Amikacin	14(40%)	1(4.17%)	17(73.91%)	8(66.67%)	1(100%)	1(100%)
8	Gentamicin	14(40%)	1(4.17%)	16(69.56%)	9(75%)	1(100%)	1(100%)
9	Amoxyclav	8(22.86%)	1(4.17%)	-	6(50%)	1(100%)	1(100%)
10	Ceftriaxone	6(17.14%)	1(4.17%)	-	3(25%)	1(100%)	1(100%)
11	Cephalothin	5(14.29%)	1(4.17%)	-	3(25%)	1(100%)	1(100%)
12	Cotrimoxazole	15(42.86%)	7(29.2%)	-	9(75%)	1(100%)	1(100%)
13	Ceftazidime	-	1(4.17%)	14(60.86%)	-	-	-
14	Cefepime	-	-	15(65.21%)	-	-	-

In the present study among Gram negative isolates, *Klebsiella pneumoniae* showed the highest susceptibility to polymyxin B (100%) correlating with findings of Deepti et al[1] 100% and 86.7% by Masoum et al[25] followed by meropenem showing susceptibility rate of 42.86% and 90.90% by Deepti et al,[1] 66.7% by Masoum et al[25] followed by Cotrimoxazole (42.86%), Imipenem, Amikacin & Gentamicin (40%), Piperacillin tazobactam, Cefaperazone sulbactam & Amoxyclav (22.86%), Ciprofloxacin (20%), Ceftriaxone (17.14%), Cephalothin (14.29%).

Among Acinetobacter spp isolated in the present study showed 100% susceptibility to polymyxin B correlating with findings of Deepti et al[1] 100% and 99.5% by Masoum et al[25] followed by meropenem showing less susceptibility rate (8.33%) and 71.42% by Deepti et al,[1] 3.7% by Masoum et al[25] followed by Cotrimoxazole (29.2%), Cefaperazone sulbactam (25%), Imipenem & Ciprofloxacin (8.33%), Piperacillin tazobactam, Amikacin, Gentamicin, Amoxyclav, Ceftriaxone, Cephalothin, Ceftazidime (4.17%).

Among Pseudomonas aeruginosa isolated in the present study showed 100% susceptibility to polymyxin B correlating with findings of Deepti et al[1] 100% and 91.7% by Masoum et al[25] followed by meropenem showing susceptibility rate (82.60%) and 80% by Deepti et al,[1] 30.5% by Masoum et al[25] followed by Imipenem & Piperacillin tazobactam (82.60%), Ciprofloxacin & Amikacin (73.91%), Gentamicin & Cefaperazone sulbactam (69.56%), Cefepime (65.21%), Ceftazidime (60.86%).

In the present study, *Escherichia coli* isolates showed 100% susceptibility to polymyxin B correlating with findings of Deepti et al[1] 100% and 92.3% by Masoum et al[25] followed by meropenem showing susceptibility

rate (66.67%) and 66.66% by Deepti et al,[1] 73.1% by Masoum et al[25] followed by Gentamicin & Co-trimoxazole (75%), Imipenem & Amikacin (66.67%), Amoxyclav (50%), Cefaperazone sulbactam, Ciprofloxacin (41.67%), Piperacillin tazobactam, ceftriaxone, Cephalothin (25%).

5. Conclusion

The present study gives insight into the bacterial pathogens and their antibiotic susceptibility patterns isolated from endotracheal secretions of mechanically ventilated patients in a tertiary care hospital. Gram positive isolates showed high susceptibility to vancomycin and linezolid and Gram negative organisms being the predominant isolates showed higher susceptibility to polymyxin B & meropenem. An updated antibiogram for each hospital and ICU based on local bacteriological patterns and their susceptibilities is always necessary to guide physicians to initiate empirical therapy. Performing the culture of ET secretions is an easy, cost-effective, and non-invasive method that helps in identifying the infective organism and also their antibiotic susceptibility pattern. The findings of this study may help the clinicians to formulate the first line empirical treatment regimens for the patients on mechanical ventilation.

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8. Conflicts of Interest

The authors declare no potential conflict of interest with respect to research, authorship, and/or publication of this article.

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