

**BACTERIOLOGICAL PROFILE AND ANTIBIOTIC-SUSCEPTIBILITY PATTERN OF ISOLATES FROM ENDOTRACHEAL SECRETIONS OF MECHANICALLY VENTILATED PATIENTS ATTENDING A TERTIARY CARE HOSPITAL IN CENTRAL INDIA****Dr. Krishna Lekha T***

Senior Resident *Corresponding Author

Dr. P.M. Neethu

Senior Resident

Dr. Sonal Chavan

Associate Professor

ABSTRACT

Introduction: Ventilator associated pneumonia (VAP) is the second most common HAI and accounts for 15-20% of total HAIs. VAP is characterized by worsening of oxygenation, systemic symptoms like fever and leukopenia. According to CPIS criteria (Clinical Infection pulmonary Score) microbiological detection of pathogen plays a pivotal role. Our study aims to identify the bacterial causes of Ventilator associated pneumonia. **Methods:** Identifying bacterial pathogens using Standard bacteriological technique and antimicrobial susceptibility testing was done by CLSI 2024 guidelines. **Results:** out of 86 patients 73% of them were males. out of 90 bacterial isolates, 98% were gram-negative bacteria and 2% were gram-positive bacteria. The most common gram-negative bacteria isolated was *Acinetobacter* spp. (35.5%) followed by *Klebsiella pneumoniae* (28.8%). The least common gram-negative isolates were *E. coli* and *Citrobacter* Spp. (6.7% and 5.5% respectively). *Staphylococcus aureus* was the only gram-positive bacteria isolated (2.2%) (pie diagram no. 1). All gram-negative isolated were multi-drug resistant. All *Staphylococcus aureus* found to be Methicillin resistant but sensitive to Vancomycin. **Conclusion:** All the gram-negative isolates found to be multi-drug resistant, with detection of ESBL (78.37%), Amp-C (13.63%) and MBL (31.81%). The only gram-positive isolates in our study all *Staphylococcus aureus* and found to be methicillin resistant (100%) is really concerning Our study will help physician give awareness about the locally prevalent pathogens and their sensitivity pattern.

KEYWORDS : VAP (Ventilator Associated Pneumonia), Endotracheal Secretion (ET Secretion)**INTRODUCTION**

Ventilator associated pneumonia is the second most common HAI and accounts for 15-20% of total HAIs. Ventilator-associated pneumonia (VAP) means pneumonia where the patient is on mechanical ventilation for > 2 consecutive calendar days on the date of event, with day of ventilator placement being Day 1, and the ventilator was in place on the date of event or the day before. If the ventilator was in place prior to inpatient admission, the ventilator day count begins with the admission date to the first inpatient location. Ventilator is any device used to support, assist, or control respiration (inclusive of the weaning period) through the application of positive pressure to the airway when delivered via an artificial airway, specifically an oral/nasal endotracheal or tracheostomy tube. And it is characterized by presence of new progressive infiltrate, signs of systemic infection (fever, altered white blood cells, worsening of oxygenation, changes in the sputum characteristic. Microbiological detection of pathogen plays a pivotal role in the diagnosis of VAP. (1) Our study mainly aims to detect the locally prevalent bacterial pathogens and their antibiotic resistance pattern and specific patterns in the drug resistance

MATERIALS AND METHODS

It's a cross-sectional study conducted over a period of 6 months, June - December 2024 in the Department of Microbiology, G.M.C. Nagpur. Ethical Committee Approval was been taken from Institutional Ethics Committee. Informed consent was taken from patients participating the study. Endotracheal aspirates from patients admitted in various ICUs on mechanical ventilation were collected. Samples from 86 patients were collected. collected samples been subjected to gram stain and semiquantitative culture. And antibiotic-sensitivity tests been done by CLSI 2024 guidelines by KB-DD. MRSA detection was done by Cefoxitin disk diffusion test (where cefoxitin acts as the surrogate marker). All Methicillin resistant *Staphylococcus aureus* (MRSA) were subjected to Vancomycin sensitivity tested using E-strip method. ESBL (Extended spectrum Beta-lactamase) detection done by double disk synergy test (DDST). For Amp C (Ambler's Class -C) detection Cefoxitin(30µg) screen test was performed and confirmed by Disk antagonism test using Cefoxitin (30µg) as an inducer of Amp C and Cefotaxime (third generation cephalosporin) as the substrate. Blunting of the inhibitory zone of the substrate towards the inducer considered as positive test. MBL(Metallo-beta-lactamases) identified using Meropenem -EDTA disk (10/75 µg), increase in zone diameter >5 mm than the Meropenem (10 µg) was considered as positive test. (2)

RESULTS

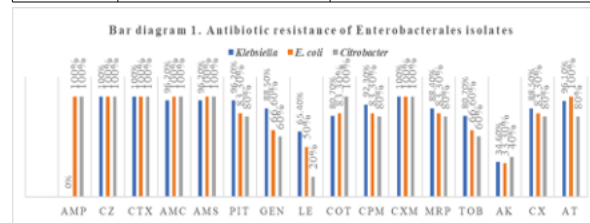
Table No. 1 explains the demographic data. Among 86 patients, 73% of them were males and 27% were females. The predominant age group affected was 41-50 years (21% of the total study population). Higher

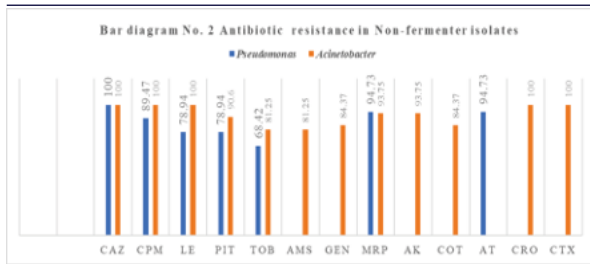
number of cases were reported from TICU -34 (39%) followed by MICU- 28 (33%). Least number of cases reported from NICU-14 (16%) and PICU-10 (12%). In present study that out of 90 bacterial isolates, 98% were gram-negative bacteria and 2% were gram-positive bacteria. The most common gram-negative bacteria isolated was *Acinetobacter* spp.- 32 (35.5%) followed by *Klebsiella pneumoniae*-26 (28.8%) and *Pseudomonas* 19 (21%). The least common gram-negative isolates were *E. coli*-6(6.7%) and *Citrobacter* Spp.-5 (5.5%) respectively). *Staphylococcus aureus*- 2 (2.2%) was the only gram-positive bacteria isolate.

All *Staphylococcus aureus* found to be Methicillin resistant but sensitive to Vancomycin. All gram-negative isolated were multi-drug resistant. (Bar diagram 1&2). *Klebsiella pneumoniae* being the predominant Enterobacterials isolate showing high level drug resistance to all beta-lactam antibiotics including carbapenems so as *E. coli* and *Citrobacter*. All Enterobacteriale isolates were showing low resistance to Fluoroquinolone like LE and Aminoglycoside like AK. In case of non-fermenter isolates, both *Acinetobacter* and *Pseudomonas* isolates were highly resistant to all Beta-lactam antibiotics, Aminoglycosides and fluoroquinolones.

Table No.1 Gender, Age Wise Distribution of Patients (n=86)

	Number of patients	Percentage
Gender		
Male	63	73%
Female	23	27%
Age group		
1-10	14	16%
11-20	10	12%
21-30	8	9%
31-40	12	14%
41-50	18	21%
51-60	14	16%
61-70	9	11%
71-80	1	1%





Ampicillin (AMP), Cefazolin (CZ), Cefotaxime (CTX), Cefuroxime (CXM), Ceftriaxone (CRO), Ceftazidime (CAZ), Cefoxitin (CX), Cefepime (CPM), Amoxicillin-Clavulanate (AMC), Ampicillin-sulbactam (AMS), Piperacillin-tazobactam (PIT), (MRP), Aztreonam (AT), Gentamycin (GEN), Tobramycin (TOB), Amikacin (AK), Levofloxacin (LE), Cotrimoxazole (COT)

Table no 2. Detection of ESBL, Amp C, and MBL in Gram-Negative Isolates

Isolate	Number	Percentage
ESBL Producers		
Klebsiella pneumoniae (n=26)	24	92.31%
E. coli (n=6)	4	66.66%
Citrobacter (n=5)	2	20%
Amp C Producers		
Klebsiella pneumoniae (n=26)	9	34.6%
E. coli(n=6)	2	33.3%
Citrobacter (n=5)	1	20%
MBL producers		
Klebsiella pneumoniae (n=26)	11	42.31%
E. coli (n=6)	3	50%
Pseudomonas (n=19)	14	73.68%
Acinetobacter (n= 32)	5	15.6%

DISCUSSION

Table no. 1 describes the age, gender wise distribution of patients. According to our findings, 73% of the affected population were male. Males are more prone to develop VAP compared to females. According to Rao SV et al (3), out of 338 patients, 68% of them were males. Studies by Dey A et al (4) and Kanafani Za et al (5) were also showing the male predominance of 75% and 58.6% respectively. The most common age group affected was 45-50 years (21%) followed by 51- 60 years (16%). In a study by Natarajan K et al similar findings can be seen the mean age group of affected population was 52 years (with interquartile range of 18-87 years). (6) We found the incidence of VAP is more common in TICU (39%). The incidence of VAP in trauma patients can be due to the nature of trauma, prolonged mechanical ventilation and use of sedatives for the management of pain (7).

The major pathogens causing VAP is gram-negative bacilli (98%), Acinetobacter (35.56%) and Klebsiella pneumoniae (28.8%) were the predominant pathogen. In an observational study been conducted in a tertiary care center by Rao SV et al (3), the predominant pathogens were Klebsiella and Acinetobacter (36.29% each). Similar findings can be seen in study by Ramasubban S et al (8). According to their study the Klebsiella and Acinetobacter were isolated as predominant bacterial pathogens causing VAP(42.9% and 33.3% respectively). In our study the only gram-positive pathogen was Staphylococcus aureus (2.2%) in accordance with studies by Natarajan K et al, the isolation rate of Staphylococcus aureus was 3.3%. (6)

Bar diagram No. 1 showing percentage of drug resistance in the Enterobacterials isolates. All are showing 100% resistance to AMP, CZ, CTX, CXM. Klebsiella, E. coli and Citrobacter were showing high level resistance both fourth generation beta-lactam antibiotic like CPM (92.3%,83.3% and 80% respectively) and BL-BLI combination like PIT (96.2%, 83.3% and 80% respectively). Resistance to Carbapenem like MRP was 88.4% and Sulfamethoxazole like COT was 80.7%. Klebsiella, E. coli and Citrobacter were showing low resistance to Aminoglycoside like AK (34.6%,33.3% and 40% respectively) and Fluroquinolones like LE (65.4%, 50% and 20%

respectively). In a study by Ramasubban S et al (8) Klebsiella was the predominant. Enterobacterials isolate, showing high level resistance to Carbapenem like Imipenem (IMP) and Sulfamethoxazole like COT (90% each) which are almost similar to our findings. But the resistance to Amikacin was very high (85%).

In the present study (Bar diagram No. 2) all Acinetobacter isolates were 100% resistant to Beta-lactam antibiotics like CRO, CTX, CAZ, CPM. Resistance to PIT was 78.94% and MRP was 93.75%. Aminoglycosides like GEN, TOB and AK showing resistance as follow 89.3%, 81.25% and 93.7% respectively. In the present study all isolates were 100% resistant to Levofloxacin. According to Sahib Noor Singh et al (9) resistance to Acinetobacter isolates were showing similar pattern with our findings. Very high-level resistance to PIT and MRP as follow 89.3% and 85.7%. Resistance to GEN and AK were as follow 89.3% and 85.7%. In their study also resistance to LE was 100%. In the present study Pseudomonas isolates were showing high level resistance to Beta-lactams like CAZ (100%), CPM (89.47%), Monobactam like AT (94.73%) and Carbapenem like MRP (94.73%). Resistance to Fluroquinolones like Levofloxacin (LE) and Aminoglycoside like TOB were relatively low (78.94% and 68.42% respectively). In accordance with our finding Farag AM et al's (10) study shows Pseudomonas aeruginosa has high resistance to several key antibiotics. They showed 100% resistance to AMP, CX, CTX, and CPM indicating these drugs are ineffective against this pathogen. Their study also shows P. aeruginosa demonstrated relatively better susceptibility to MRP (54.5%) and AK (68%), suggesting these could still be considered for treatment, although resistance remains significant. Similar to our finding, resistance to LE and TOB were relatively low (73% each) in their study.

In Table No. 2 Among Klebsiella pneumoniae, 92.3% of them were ESBL producers while 66.6% of E. coli were ESBL producers. Slavia Tet al (11) conducted the study on comparison of different phenotypic method of detection of drug resistance, observed major organisms producing ESBL by DDST were Klebsiella pneumoniae and E. coli (76.5% and 52.3% respectively). Similarly in the present study, Klebsiella is the major ESBL producer followed by E. coli (92.31% and 66.66% respectively). Inamdar DP (12) et al conducted a study Phenotypic detection of drug resistance, in which Amp C production in gram-negative organism by disk antagonism test, found Klebsiella and E. coli are the major pathogens (75% and 33% respectively). The present study states that Amp C production is common in Klebsiella and E. coli (34.6% and 33.3%). In our study the most common MBL producing gram-negative organisms were Pseudomonas, E. coli and Klebsiella (73.68%, 50% and 42.31% respectively) and least common in Acinetobacter (15.6%). Kambale D et al (13) has similar observation in their study, phenotypic detection of MBL Pseudomonas aeruginosa was the predominant organism (23.62%), followed by Acinetobacter (18.75%), E. coli (5.55%) and Klebsiella pneumoniae (9.09%).

CONCLUSIONS

In our study VAP is -predominant in male gender with high incidence in Trauma Intensive Care Units. Gram-negative bacilli are the most common causative agents, Acinetobacter and Klebsiella as the predominant pathogens. All the gram-negative isolates found to be multi-drug resistant, with detection of ESBL (78.37%), Amp-C (13.63%) and MBL (31.81%). The only gram-positive isolates in our study all Staphylococcus aureus and found to be methicillin resistant (100%) is really concerning. Occurrence of VAP can be reduced by emphasizing the infection control practices, implementing VAP bundle, judicious use of antibiotics. To implement the antibiotic policy for treatment of VAP, our study will help physician give awareness about the locally prevalent pathogens and their sensitivity pattern.

REFERENCES

1. Sastry SA, Deepashree R. Essentials of hospital infection control. Jaypee Brothers Medical Publishers; 2019 Feb 8.
2. Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute; 2024
3. Rao SV, Thilakchand KR, Boloor R, Suresh S, George T, Pais ML, et al. Antimicrobial resistance pattern in aerobic bacteria isolated from endotracheal aspirate in ventilator-associated pneumonia: Ten years observation from a tertiary care hospital. J Anaesthesiol Clin Pharmacol 2024;40:324-9.
4. Dey A, Bairy I. Incidence of multidrug-resistant organisms causing Ventilator associated pneumonia in a tertiary care hospital: A nine months' prospective study. Ann Thorac Med 2007;2:52-7
5. Kanafani ZA, El Zakhem A, Zahreddine N, Ahmadi H, Kanj SS. Ten-year surveillance study of ventilator-associated pneumonia at a tertiary care center in Lebanon. J Infect Public Health 2019;12:492-5
6. Natarajan K, Bahulikar A, Phalgune DS. Ventilator-associated Pneumonia: A Prospective Observational Study. J Assoc Physicians India 2025;73(5):21-24
7. Gouda, A.M.; Sileem, A.E.; Elnahas, H.M.; Tawfik, A.E.; Eid, R.A.; Shati, A.A.; Al-Qahtani, S.M.; Dawood, S.A.; Alshehri, M.A.; Eissa, M.; et al. Exploring Ventilator Associated Pneumonia: Microbial Clues and Biomarker Insights from a Retrospective Study. Medicina 2024, 60, 1346. <https://doi.org/10.3390/medicina60081346>

8. Ramasubban S, Sen S. A prospective study on clinical profile, severity, microbiology, and outcome of patients with ventilator associated infective complications admitted in intensive care unit of a tertiary care hospital. *IP Indian J Immunol Respir Med* 2024;9(1):15-25.
9. Cinical and Microbiological Profile of Ventilator-Associated Pneumonia in Intensive Care Unit of Tertiary Care Hospital Sahib Noor Singh1, GurinderMohan2, SarbjeetSharma
10. Farag AM, Tawfick MM, Abozeed MY, Shaban EA, Abo-Shadi MA. Microbiological profile of ventilator-associated pneumonia among intensive care unit patients in tertiary Egyptian hospitals. *The Journal of Infection in Developing Countries*. 2020 Feb 29;14(02):153-61.
11. Salvia T, Dolma KG, Dhakal OP, Khandelwal B, Singh LS. Phenotypic Detection of ESBL, AmpC, MBL, and Their Co-occurrence among MDR Enterobacteriaceae Isolates. *J Lab Physicians*. 2022 Apr 20;14(3):329-335. doi: 10.1055/s-0042-1744239. PMID: 36119416; PMCID: PMC9473942.
12. Inamdar DP, Anuradha B . Phenotypic methods for detection of Amp C β lactamases in Gram negative clinical isolates of a tertiary care hospital. *Indian J Microbiol Res* 2020;7(2):125-129.
13. Kamble D. Phenotypic detection of ESBL and MBL in Gram Negative bacilli isolated from clinical specimens . *Int J Med Res Rev* 2015;3(8):866-870