Section: Microbiology

Original Research Article

BACTERIOLOGICAL PROFILE OF ISOLATES FROM SUSPECTED CASES OF VENTILATOR ASSOCIATED PNEUMONIA IN A TERTIARY CARE HOSPITAL

Ameena K.K.[1]*,Syed Mustaq Ahmed*[2]*, Sumita Rajeevan*[3]

[1]*Assistant Professor, Department of Microbiology, MES Medical College & Hospital, India*
[2]*Professor & HOD, Department of Microbiology, MES Medical College, India.*
[3]*Associate Professor, Department of Microbiology, MES Medical College, Palachode PO, Perintalmanna, Malappuram DT, Kerala, India.*

Received *: 16/04/2024*
Received in revised form *: 18/04/2024*
Accepted *: 20/04/2024*

Corresponding Author:
Dr. Ameena K.K.
Assistant Professor, Department of Microbiology, MES Medical College & Hospital, Palachode PO, Perintalmanna, Malappuram DT, Kerala, India.
Email: ameenanouka@gmail.com

DOI: 10.5530/ijmedph.2024.2.143

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2024; 14 (2); 746-750

ABSTRACT

Background: Ventilator-associated pneumonia (VAP) is one among the most common hospital acquired infections in the intensive care unit (ICU).VAP is often diagnosed late because of unawareness of health care staff about VAP prevention bundle. Timely detection of VAP and initiation of appropriate antibiotic treatment is necessary as delay in diagnosis will affect patient's prognosis and emergence of multi drug resistant pathogens. Aim of the present study was to investigate the microbiological profile of VAP caused by aerobic bacteria and their antibiotic susceptibility patterns in ICU patients.

Material and Methods: A cross sectional study conducted for a period of one year from 01/05/2022 to 31/04/2023.135 endotracheal aspirates collected from patients admitted in various ICUs of M.E.S Medical College Hospital who were on mechanical ventilator for more than 48 hrs & suspected for VAP were included in the study. Isolates were identified from the samples using conventional methods. Antimicrobial susceptibility testing done by Kirby - Bauer disc diffusion method as per latest CLSI guidelines.

Results: Out of 276 patients, 135 were on mechanical ventilator for more than 48 hours.105 samples showed culture positive and 111 bacterial isolates were obtained. Majority of the isolates were gram negative which are multi drug resistant.

Conclusion: VAP is a problem in the ICU setting due to multi drug-resistant pathogens. Clinicians should take preventive measures to minimize the incidence of VAP by focusing on the modifiable risk factors. An effective antimicrobial policy has to be initiated based on the resistance pattern of the pathogens prevalent in the respective Intensive care Unit.

Keywords: Ventilator associated penumonia, Klebsiella pneumoniae, Acinetobacter baumanii.

INTRODUCTION

Ventilator-associated pneumonia (VAP) is one of the most common hospital acquired infections affecting one-third of patients on mechanical ventilation in the intensive care unit (ICU).[1] Among 10-20 % of patients requiring mechanical ventilation may develop ventilator associated pneumonia with a high mortality.[2] An important risk factor is the time of onset of VAP which affect the outcome in patients with VAP. Early onset VAP which occurs within first 4 days of hospitalization caused by antibiotic susceptible bacteria and is community acquired whereas the late onset VAP more than 5 days associated with increased mortality in patients caused by multidrug resistant organisms[3].Timely detection of VAP and giving appropriate antibiotic treatment is necessary as delay in diagnosis and inappropriate antibiotic treatment will have adverse effect on patient's prognosis and will result in emergence of multidrug resistant pathogens. The most common aetiologic agents of VAP include hospital pathogens such as Enterobacterales, Pseudomonas, Acinetobacter and other non-fermenters and gram-positive pathogens such as

Staphylococci.[4,5] The aim of the present study is to investigate the microbiological profile of VAP caused by aerobic bacteria and their antibiotic susceptibility patterns among ICU patients.

MATERIAL AND METHODS

A cross sectional study was conducted for a period of one year from 01/05/2022 to 31/04/2023.The study was undertaken in Microbiology laboratory, Department of Microbiology, MES Medical College Hospital, Perintalmanna. Patients who were admitted in various ICUs of M.E.S Medical College Hospital who were on mechanical ventilator for more than 48 hrs & with clinical suspicion of VAP were included in the study. The study protocol was approved by the institutional ethics committee.

Sample collection and microbiological analysis

Around 135 endotracheal aspirates were collected from VAP suspected cases. Endotracheal aspirate collected by non bronchoscopic method using 22 inch Ramsons's 12 F suction catheter with a mucus extractor introduced through endotracheal tube for a distance of approximately 25-26 cm. Gentle aspiration was performed without instilling saline, and the catheter will be withdrawn from the endotracheal tube. After catheter withdrawal, 2ml of 0.9 % normal saline injected into catheter with a sterile syringe to flush out the exudates into a sterile container for collection and transported to Microbiology lab without delay.

Processing of sample: The endotracheal aspirates received in the Microbiology diagnostic lab were subjected to Gram stain and then to quantitative culture technique. A colony count of $\geq 10^5$ colony forming units (cfu)/ml considered to be significant.[6,7] Any growth with low colony count considered as colonization or contamination. After incubation for 18-24 hrs at 37^0C, plates were examined for growth and colony type. Further identification with biochemical tests were done as per the standard protocol. Antibiotic susceptibility testing done by Kirby - Bauer disc diffusion method as per the latest CLSI guidelines.[8] For the Enterobacteriaceae members and non-fermenters, the antibiotics used were Ampicillin (AMP), Amoxicillin-clavulanate (AXV), Cefotaxime (CTX), Cefepime (CPM), Ceftazidime (CTZ), Cefoperazone-sulbactam(CFS),Ciprofloxacin (CIP),Levofloxacin(LE),Co-trimoxazole (COT), Gentamicin (GEN),Amikacin(AK), Imipenem (IPM), Meropenem (MRP), Piperacillin-tazobactam (PTZ) and Colistin (CO). AMP, AXV, GEN, AMK were excluded from the panel for Pseudomonas isolates. For Gram-positive pathogens, the panel included AMP, AXV, Penicillin (PEN), Cefalexin (CEX) COT, CIP, GEN, Erythromycin (ERY), Clindamycin (CLI), Vancomycin (VAN) and Linezolid (LIZ). ESBL production detected among Gram negative isolates with combined disc test performed with Ceftazidime (CAZ 30µg) and Ceftazidime-Clavulanic acid. CXT (30 µg) disc was used as a surrogate marker for determining methicillin resistance amongst the Staphylococci. All carbapenem resistant Enterobacterales were tested for colistin resistance with disc elution test according to CLSI guidelines. ATCC Control strains of Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa ATCC 27853 were used for antimicrobial susceptibility testing. In case CLSI guidelines for disc diffusion technique was not available, the following strategies were adopted: (i) CLSI interpretative guideline for cefoperazone was used for CFS; minimum inhibitory concentration (MIC) test was performed using the MIC E-strip method for susceptibility of antimicrobials like vancomycin.[9]

Analysis

Data was analyzed using WHO NET antibiotic resistance surveillance software.

RESULTS

The study was conducted in the Microbiology lab from the samples received from patients who were on mechanical ventilator for >48 hrs in Intensive Care Units of Medicine, Surgery, Coronarycare and Neurosurgery in M.E.S Medical College Perintalmanna. A total number of 276 patients were on mechanical ventilator during the study period May 2022 to April 2023. 135 endotracheal aspirates received in the Microbiology lab were processed and 105 samples showed culture positive for aerobic bacterial isolates numbering 111 and these culture positive samples were included in the study as they were fulfilling CDC VAP guidelines. Sex wise distribution showed that males are more when compared to females in VAP cases. [Table 1]

Majority of the VAP cases were in the age group of 61-70 in this study. Maximum number of endotracheal aspirates were collected from patients admitted in Medical ICU followed by Surgical ICU. Most common co-morbidity observed in patients were Chronic obstructive pulmonary disease followed by Chronic kidney injury. [Table 2]

Out of 111 isolates, 109 gram negative bacteria and 2 gram positive bacteria were isolated. Among 109 gram negative isolates, the most common organism isolated was Klebsiella pneumoniae (48.7%) followed by Acinetobacter baumanii (22.5%) and Pseudomonas aeruginosa (20.7%). Other gram negative organisms isolated were Citrobacter freundi (5.4%) and Enterobacter aerogenes (0.9%). [Table3]

All Enterobacterales were tested for colistin resistance with disc elution test and MIC was detected to be intermediate. Two Gram positive isolates were Methicillin resistant Staphylococcus aureus (1.8%).

Table 1: Sex Wise Distribution

	Frequency	Percent
M	72	69
F	33	31
Total	105	100

Table 2:

SL No.	Co-morbid factors	No. of patients with VAP
1	COPD	35
2	CKD	20
3	Diabetes mellitus	18
4	Hypertension	15
5	CVA	10
6	RTA	4
7	Malignancy	2
8	OP poisoning	1

Table 3: Bacterial Isolates from VAP Cases

Organisms	Number	%
Klebsiella pneumoniae	54	48.7
Acinetobacter baumannii	25	22.5
Pseudomonas aeruginosa	23	20.7
Citrobacter freundii	6	5.4
Staphylococcus aureus	2	1.8
Enterobacter aerogenes	1	0.9
Total	111	100

DISCUSSION

Ventilator associated pneumonia is always a serious complication amongst critical care patients in any healthcare setting and its incidence is found to be highly variable among different hospitals and also in ICUs of the same hospital. Despite major advanced techniques in caring patients whose respiratory tracts are instrumented and the routine use of efficient disinfection procedures for the respiratory equipment, hospital acquired bacterial pneumonia definitely complicate the course of 7-41% of patients receiving continuous mechanical ventilation.[10]

Incidence of VAP in the our study was 38 % which was highly similar to the incidence in other studies.[11,12] VAP rate in developing countries varied from 10 to 41.7 per 1000 ventilator-days.[13] In this study, incidence of VAP is slightly on the higher side when compared to other studies, can be due to differences in the study population, co-morbidites in the patient and long duration of ventilation. High work load and low staffing level increase the risk for negative patient outcomes such as death and healthcare-associated infections.[13]

In this study with relation to gender, a male predominance is seen [69%] compared to females [31%] similar to the study by Diling Wu etal.[14] Incidence of VAP is more in patients with age group above 55 years [52%] in this study correlates with Chang etal study.[15] Comorbid factors found in patients with VAP in the present study are COPD [33%] followed by Renal disease, Diabetes mellitus and Hypertension. A study on risk factors of VAP done in Pakistan, COPD was found to be the commonest co-morbid factor in VAP patients supports this study.[16] Majority of the patients are from Medical ICU.

From 105 patients who were suspected to have VAP,111 organisms were isolated.Among 111 organisms,109 were gram negative & 2 were gram positive in nature as also reported in a study by Anitha Gunalan etal where Gram negative bacilli were found to be the major cause for VAP than gram positive.[12] Klebsiella pneumonia was the most frequently isolated among all isolates (48.7%) which is similar to study by Girish N et al.[17] Majority of Indian studies Nonfermenters like A. baumanii and P. aeruginosa were found to the most frequently isolated organisms from VAP cases.[12] In the present study, A.baumanii and P.aeruginosa isolated were 22.7% and 20.7% respectively.2 isolates of Methicillin resistant Staphylococcus aureus (MRSA) were isolated in the study (1.8%) whose occurrence is low in Indian studies compared to Western studies.[18]

Analysing the antibiotic sensitivity pattern in the present study, majority of the organisms were found to be resistant to beta lactam antibiotics.80% of organisms were resistant to first, second, third and fourth generation cephalosporins.

Among Aminoglycosides, tested Gentamicin showed highest resistance (46.4%) whereas Amikacin showed the least resistance (36.9%). Even though 63.1% of isolates show sensitivity to Amikacin, not recommended on patients on ventilator since majority of them have renal insufficiency.

Analysing the sensitivity pattern of isolates to combination drugs like Piperacillin/Tazobactum [PIT] and Cefaperazone/ Sulbactum [CFS], 63.6% of isolates showing resistance to PIT and 61.5% showing resistance to CFS. The high resistance can

be due to prophylactic use of these antibiotics in the respective ICU.

Multidrug resistant strains such as extended spectrum beta lactamase (ESBL) producers and Carbapenemase producers were isolated in the present study. Among all gram negative isolates 45.9% were observed to be ESBL producers. Among 25 isolates of A.baumanii,[19] 50 % were observed to be ESBL producers.

Multidrug resistant organisms and Carbapenemase producers were chiefly responsible for late onset VAP. The rate of carbapenemase producers in the present study is 12.8% among Enterobacterales which is similar to study done by Sangla et al.[21] Among Enterobacteriacae, percentage of isolates resistant to Carbapenem were Citrobacter freundi 33% & Klebsiella pneumonia 10% respectively, however Acinetobacter showed 28 % Carbapenem resistance activity. Emergence of C. freundii as a causative organism for VAP being a carbapenemase producer is also reported in a study by Thakuria etal where the rate of carbapenemase producing C.freundii is 53.3%.[10] [Table 4]

Analyzing the resistance pattern in Non-fermenters, A. baumanii, second mostly isolated organism in this study is showing multidrug resistance pattern and showed resistance to Carbapenems (28%) similar to other studies.[21] The emergence of A. baumannii as an important cause of nosocomial infections is favored by three major factors, like resistance to drying, disinfectants, and antimicrobial agents.[20] Pseudomonas aeruginosa, another common VAP causing organism as reported in many studies and usually shows a multidrug resistant pattern is sensitive to combination drugs and carbapenems in the present study. [Table 5]

During the study period, strict surveillance has been maintained in ICU s regarding care of patients who were on mechanical ventilator, sample collection, screening for multidrug resistant pathogens among health workers etc as routine hospital infection control programme. Thus the present study gives importance of knowing the pathogens and their antibacterial susceptibility pattern, prevalent in a particular ICU, to initiate the empirical antibacterial therapy for the patients on Mechanical ventilation.

Table 4: Antibiotic Susceptibility Pattern of Enterobacteriacae

Antimicrobial agents	Klebsiella pneumonia (%)	Citrobacter freundi (%)	Enterobacter aerogens (%)
AMP	0	0	0
CTX	15	0	0
CPM	16	0	0
PIT	35	0	100
CFS	37	0	100
MRP	91	83	100
IMP	89	83	100
GEN	53	50	100
AMK	68	67	100
CIP	41	60	0
LEV	68	60	100
CO	100	100	100

Table 5: Antibiotic Susceptibility Pattern among Non-Fermenters

Antimicrobial agent	P.aeruginosa (%)	A.baumanii (%)
AMP	-	0
AXV	-	0
CTX	-	0
CAZ	55	0
CPM	66	0
CIP	53	63
PIT	87	57
CFS	87	58
IMP	91	70
MRP	95	80
CO	100	100

CONCLUSION

Ventilator associated pneumonia among intubated patients is a major problem in the ICU setting, due to high percentage of multidrug-resistant pathogens. Clinicians should be cautious about reducing the incidence of VAP through preventive techniques. The focus should be addressing modifiable risk factors such as endotracheal and nasogastric tubes, tracheostomy, reintubation, enteral nutrition, corticosteroid administration, gastric pH-modifying agents, supine positioning, prior antibiotic usage, poor infection control practice, and contaminated respiratory equipment, medications or water. Nursing staff should be adequately trained on following the VAP prevention bundle. Repeated training and interactive educational sessions should be done regularly to assess the competency of health care workers regarding the VAP bundle. Quantitative cultures should be carried out immediately if there is a suspicion of VAP. Surveillance of HAI should be stringently carried out by the Hospital infection control team to detect VAP at the earliest. Knowledge of local

antibiograms should guide the choice of antibiotics, in addition to likelihood of organisms (early- or late-onset VAP). The resistance pattern of the pathogens along with their profile mentioned in this study can help the institution to formulate effective antimicrobial policy for VAP based on evidence of the local scenario, along with the necessary infection control measures and thus improve the outcomes for a common and serious medical complication seen in ICU among mechanically ventilated patients.

Ethical Approval: The study was approved by the Institutional Ethics Committee of MES Medical College, Perintalmanna, Malappuram, Kerala, India.

Acknowledgements: Not applicable

Authors' Contribution: First author of the study Ameena K.K contributed conceptual design, literature search, collected the data, analyzed the data, and wrote the first draft of the manuscript. The second author Syed Mustaq Ahmed guided the work and corrected the manuscript. The third author Sumita Rajeevan helped in the literature survey.

Conflicts of Interest: The study declared "no conflicts of interest"

Funding: The study did not receive any funds and grants.

REFERENCES

1. Van Vught LA, Klowenberg PM, Spitoni C, et al, for the MARS Consortium. Incidence, risk factors, and attributable mortality of secondary infections in the intensive care unit after admission for sepsis. JAMA 2016;315(14):1469–79
2. Chaudhury A, Rani AS, Kalawat U, Sumant S, Verma A, Venkataramana B. Antibiotic resistance & pathogen profile in ventilator-associated pneumonia in a tertiary care hospital in India. Indian J Med Res. 2016 Sep; 144(3):440-446. doi: 10.4103/0971-5916.198679. PMID: 28139543; PMCID: PMC5320850
3. Patro S, Sarangi G,Das P, Mahapatra A, Mohapatra D, Paty BP,et al. Bacteriological profile of ventilatorassociated pneumonia in a tertiary care hospital.Indian J Pathol Microbiol 2018;61:375-9
4. Chung DR, Song JH, Kim SH, Thamlikitkul V, Huang SG, Wang H, So TM, Yasin RM, Asian Network for Surveillance of Resistant Pathogens Study Group. High prevalence of multidrug-resistant nonfermenters in hospital-acquired pneumonia in Asia. Am J Respir Crit Care Med. 2011 Dec 15; 184(12):1409-17. doi: 10.1164/rccm.201102-0349OC. Epub 2011 Sep 15. PMID: 21920919.
5. Charles-Edouard Luyt, Guillaume Hekimian, Despoina Koulenti, Jean Chastre.Microbial cause of ICU-acquired pneumonia: hospital-acquired pneumonia versus ventilator-associated pneumonia. Current Opinion in Critical Care, Lippincott, Williams & Wilkins, 2018, 24 (5), pp.332-338.
6. Steven M. Koenig, Jonathon D. Truwit, Ventilator-Associated Pneumonia: Diagnosis, Treatment and Prevention. Clin Microbiol Rev. 2006 Oct; 19(4): 637–657
7. Joseph NM, Sistla S, Dutta TK, Badhe AS, Parija SC. Ventilator-associated pneumonia in a tertiary care hospital in India: incidence and risk factors. J Infect Dev Ctries. 2009 Dec 15;3(10):771-7
8. CLSI M-100, Performance Standards for Antimicrobial Susceptibility Testing, 33rd Edition
9. Winn W, Jr,, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, et al. Koneman's colour atlas and textbook of diagnostic microbiology. 6th ed. Lippincot: 2006.
10. Iregui M, Ward S, Sherman G, Fraser VJ, Kollef MH. Clinical importance of delays in the initiation of appropriate antibiotic treatment for ventilator-associated pneumonia. Chest. 2002; 122:262–8
11. Mathai AS, Phillips A, Isaac R. Ventilator-associated pneumonia: A persistent healthcare problem in Indian Intensive Care Units! Lung India 2016; 33(5):512–516. DOI: 10.4103/0970-2113.188971
12. Gunalan A, Sastry AS, Ramanathan V, Sistla S. Early- vs Late-onset Ventilator-associated Pneumonia in Critically Ill Adults: Comparison of Risk Factors, Outcome, and Microbial Profile. Indian J Crit Care Med 2023;27(6):411–415
13. Arabi Y, Al-Shirawi N, Memish Z, Anzueto A. Ventilator-associated pneumonia in adults in developing countries: a systematic review. Int J Infect Dis. 2008;12(5):505-12
14. Wu D, Wu C, Zhang S, Zhong Y. Risk Factors of Ventilator-Associated Pneumonia in Critically III Patients. Front Pharmacol. 2019 May 9; 10:482. doi: 10.3389/fphar.2019.00482. PMID: 31143118; PMCID: PMC6521332
15. Chang, L., Dong, Y., and Zhou, P. (2017). Investigation on risk factors of ventilatorassociated pneumonia in acute cerebral hemorrhage patients in intensive care unit. Can. Respir. J. 2017:7272080. doi: 10.1155/2017/7272080
16. Karatas M, Saylan S, Kostakoglu U,Yilmaz.An assessment of ventilator-associated pneumonias and risk factors identified in the Intensive Care Unit.Pak J Med Sci. 2016;32(4): 817–822
17. Girish N, Rajendran R.Bacteriological Profile of Ventilator Associated Pneumonia in a Tertiary Care Hospital and their Antibiotic Resistance Pattern.IJMMTD,Oct-Dec, 2015;1(1):1-5
18. Turkovic TM, Grginic AG, Cuculic BD, Gaspar B, Siranovic M, Peric M. Microbial profile and antibiotic susceptibility patterns of pathogens causing Ventilator associated pneumonia at Intensive Care Unit in Croatia. Acta Clin Croat 2015; 54:127-13520.
19. Goel V, Hogade SA, Karadesai SG. Ventilator associated pneumonia in a medical intensive care unit: microbiological aetiology, susceptibility patterns of isolated organisms and outcome. Indian J Anasth 2012; 56(6):558–562. DOI: 10.4103/0019-5049.104575
20. Huang Y, Zhou Q, Wang W, Huang Q, Liao J, Li J, Long L, Ju T, Zhang Q, Wang H, Xu H, Tu M. Acinetobacter baumannii Ventilator-Associated Pneumonia: Clinical Efficacy of Combined Antimicrobial Therapy and in vitro Drug Sensitivity Test Results. Front Pharmacol. 2019 Feb 13; 10:92. doi: 10.3389/fphar.2019.00092. PMID: 30814950; PMCID: PMC6381041
21. Sangale A, Vivek B, Kelkar R, Biswas S. Microbiology of Ventilator-associated Pneumonia in a Tertiary Care Cancer Hospital. Indian J Crit Care Med. 2021 Apr; 25(4):421-428. doi: 10.5005/jp-journals-10071-23790. PMID: 34045810; PMCID: PMC8138642.