Original Research Paper

Volume-9 | Issue-12 | December - 2019 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Microbiology

BACTERIOLOGICAL PROFILE AND ANTIBIOGRAM OF ENDOTRACHEAL ASPIRATES IN INTUBATED PATIENTS AT A TERTIARY CARE HOSPITAL

Gupta Veetheeanveshana	Junior resident Department of Microbiology, Dr. Rajendra Prasad Govt. Medical College & Hospital Kangra at Tanda (H.P.)
Chaudhary Anuradha*	Senior resident Department of Microbiology, Dr. Rajendra Prasad Govt. Medical College & Hospital Kangra at Tanda (H.P.) *Corresponding Author
Tamarkar Meenakshi	Junior resident Department of Microbiology, Dr. Rajendra Prasad Govt. Medical College & Hospital Kangra at Tanda (H.P.)
Rana Aditya	Junior resident Department of Microbiology, Dr. Rajendra Prasad Govt. Medical College & Hospital Kangra at Tanda (H.P.)
Jaryal S. C.	Professor and Head Department of Microbiology, Dr. Rajendra Prasad Govt. Medical College & Hospital Kangra at Tanda (H.P.)
Sood Anuradha	Associate professor Department of Microbiology, Dr. Rajendra Prasad Govt. Medical College & Hospital Kangra at Tanda (H.P.)

ABSTRACT **Background:** Endotracheal intubation is a life-saving procedure, but it is associated with a high risk of acquiring respiratory infections. [1]Several factors like new mutations, selection of resistant strains and suboptimal infection control along with the use high level antibiotics influences the rapid spread of extensively drugs resistant bugs in these intubated patients.. These infections are associated with significant rise in morbidity, mortality and healthcare cost.[2,3]
It is very essential for the clinicians to be aware of local bacteriological flora and their susceptibility pattern to encourage rational use of antibiotics.
Aim: The aim of our study was to present a data that represents the local bacterial trend in respiratory secretions of the ventilated patients and their antibiotic susceptibility patterns in this tertiary care centre.
Materials and Methods: This was a retrospective descriptive analytic study of endotracheal (ET) samples of patients on mechanical ventilation done over a period of 2 years from January2017 to December 2018 .This was done to analyse bacteriological profile and their antibiotic susceptibility. Analysis of Endotracheal Aspirates of 110 intubated patients were cultured on Blood, MacConkey and Chocolate Agar. Isolation and identification was done using conventional techniques and Biochemical reactions. Antibiotic Sensitivity testing was done by Kirby-Bauer disc diffusion method as per CLSI guidelines.
Results: Out of 110 samples 98(89%) were culture positive. 35 cases were found to be polymicrobial. 12 samples showed no growth. Extensively drug-resistant (XDR) *Acinetobacter baumannii* was the most common organism (20.3.%), and it had occurred endemically throughout the year. *Pseudomonas aeruginosa*was the next most common organism (18.8%). In Enterobacteriaceaefamily *Citrobacter spp*.(13.2%) ,*Klebsiella* sp.(11%) *E. coli* (7.6%) were the most common detected isolates. Amongst gram positive bacteria *S*. aureus (15.3%) and CoNS (4.8%) were commonly detected. Most isolates of *Acinetobacter spp*. were multi drug resistant and showed sensitivity to imipenem and Colistin only. The gram Negative bacilli were mostly sensitive to Imipenem, Meropenem, Cefoperazone plus sulbactum, Piperacillin Tazobactam. The Gram Positive cocci were mostly sensitive to Vancomycin and Linezolid.
Conclusion: Endotracheal intubation is a major risk factor in causing iatrogenic infections to patients which lead to an increase in the morbidity and mortality. Knowledge of local bacteriological trend of the antibiotic susceptibility pattern is a must to initiate a judicious antibiotic treatment.

KEYWORDS : ventilator-associated pneumonia (VAP), Endotracheal tube (ET), Intensive Care Units (ICU), and extensively drug resistant (XDR),Coagulase negative Staphylococcus (CoNS).

INTRODUCTION

Endotracheal intubation and mechanical ventilation are life-saving procedures needed in clinical conditions like sepsis, acute respiratory distress syndrome and neurological dysfunctions. Patients on mechanical ventilation are at higher risk of acquiring hospital acquired infection due to interplay of compromised host defence, virulent organism and presence of invasive device. These invasive therapeutic and diagnostic methods may lead to nosocomial infections particularly in Intensive Care Units'(ICU) and Critical Care Units'(CCU). [1]

Ventilator-associated pneumonia (VAP) is the second-most common hospital-acquired infection (HAI), accounting for 15% of HAIs and has the highest morbidity and mortality. According to a recent review by Morehead *et al*., (2000) the incidence of ventilator associated pneumonia was 9 to 24% for patients incubated longer than 48hrs.[4] Bypassing of the upper respiratory tract and imperfect functioning of mucociliary escalator (due to insertion of tube in trachea) impair the immune system. Besides, leakage of secretion around the tube and opening of the binding site for gram negative bacteria may have cause high rate of colonization.[2,3]

The etiologic agents widely differ according to the population of patients in an intensive care unit, duration of hospital stay and prior antimicrobial therapy. The tracheostomized patients are colonized mostly by gram negative bacteria which may cause either tracheobronchitis or bronchopneumonia the predominant Gram negative bacteria are *Pseudomonas aeruginosa*, *Acinetobacter spp.* and *Klebsiella pneumoniae*. [3,6,7]

Timely surveillance for local microbiological data is extremely important in predicting the type of resistance that may be present in the etiologic agent causing a clinical infection. There is a dire need of epidemiological studies for ventilated patients, to know the local microbial flora and their antibiotic profiles for rational use of antibiotics. [8,9] Hence, this study was undertaken to determine the prevalence of pathogenic bacteria in respiratory secretions of ventilated patients and their antibiotic susceptibility patterns in our tertiary care hospital. We also aimed to review available therapeutic options for the treatment of resistant organisms causing VAP, based on evidence from the literature.

Aims & Objectives:

1. To study the bacterial pathogens in the endotracheal tubes of the intubated patients. 2. To study the Antimicrobial Susceptibility pattern of bacterial isolates.

MATERIAL AND METHODS:

The study was conducted in the department Of Microbiology

atDRPGMC. Medical College, Kangra at Tanda. Retrospective analysis of Endotracheal Aspirates of 110 intubated patients over a period of 2yr (January2017toDecember2018) was done. The samples were collected aseptically and processed immediately following collection. The clinical samples were cultured Sheep's Blood Agar, MacConkey, and Chocolate Agar for routine bacterial isolation following the standard operating procedures. Isolates were identified using conventional methods based on their reaction in biochemical tests.[10] Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method strictly adhering to the standards stipulated in CLSI 2015 guidelines.[11] The following antibiotics (Hi-Media Disc in µg) were tested for AST - Amikacin (30µg), Co-trimoxazole (20/10µg), Cefepime (30µg), Cefoxitin (30µg), Cefotaxime (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), Imipenem (10µg), Meropenem (10µg), Piperacillin plus tazobactum (100/10µg), Ampicillin plus sulbactum (25µg), Cefoperazone plus sulbactum (30/10µg),Colistin(10µg), Vancomycin (30µg), Linezolid (10 µg) and Levofloxacin (5µg).

ATCC strains of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* (MSSA) ATCC 25923, MRSA ATCC 33591 and *Pseudomonas aeruginosa* ATCC 27853 strains were used as quality control.

RSSULTS:

A total of 110 samples were processed, out of these 72(65.5%) were male and 3834.5%) were female [Figure1]. Maximum patients were in 30-50 age group (Table1) Out of the 110 samples 98(89%)were culture positive [Figure.2]. 35 out of 98 positive samples were found to be polymicrobial and 12 samples showed no growth. Among these143 clinical isolates*Acinetobacter baumanii*29(20.2%)was the most common isolate followed by *Pseudomonas aeruginosa27*(18.8%), *Citrobacter spp.*19(13.2%), *Klebsiella pneumoniae16*(.11%) *Escherichia coli11*(7.6%), *Staphylococcus aureus 22*(15.3%) and Coagulase Negative *Staphylococcus* (CoNS) 7(4.8%),*Streptococcus* spp.4(2.8%)and Candida3(2.1%) [Figure2].

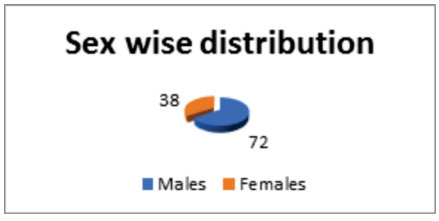

Figure 1 Sex wise distribution

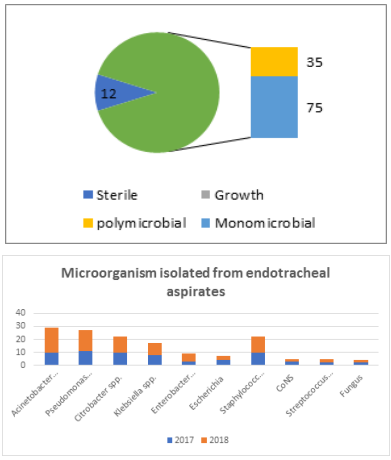

Figure 2 Samples distribution

Table1 : Age wise distribution

Age	Number=110	percentage
<1 YR	5	4.5 %
1-10	4	3.6%
11-20	5	4.5%
21-30	14	12.7%
31-40	22	20%
41-50	25	22.7%
51-60	16	15.5%
61-70	9	8.1%
71-80	6	5.5%
>80	4	3.6%

Antibiotic Susceptibility Profile

Amongst gram negative bacteria, E. *coli* was the least resistant organism being sensitive to aminoglycosides and cotrimoxazole followed by *P. aeruginosa.* On the other hand, *Acinetobacter spp.* and *Citrobacter spp*. isolates were resistant to lower antibiotics namely cephalosporins, aminoglycosides and fluoroquinolones. While some *Citrobacter* spp. isolates showed susceptibility to carbapenems, A. *baumannii*was maximum resistant to all but sensitive to colistin. Amongst gram positive bacteria, 100% susceptibility was shown to higher antibiotics viz. linezolid and Vancomycin, while resistance was observed towards beta lactam antibiotics. The resistance was high towards fluoroquinolones for both gram positive and gram-negative isolates (Table 1).

Table 2 Antibiogram of Isolates

Organism isolated	Highly sensitive	Intermediate	Resistant
Acinetobacter spp.	colistin,	Imipenem,meropenem	Levofloxacin, ceftazidime, piperacilin+tazobactamCefoperazone+sulbactam
Pseudomonas spp.	colistin, meropenem, imipenem	Piperacilin +tazobactam,	Levofloxacin, cefoperazone+sulbactam,
Citrobacter spp.	colistin, meropenem, imipenem	Levofloxacin Amikacin cefoperazone+sulbactam,	Cefixime,ceftazidime Ceftriaxone, Ceftazidime plus sulbactum
Klebsiella spp.	Colistin, meropenem, imipenem	Levofloxacin Amikacin Ceftazidime plus sulbactum	Ceftriaxone,ceftazidime, cefotaxime
Methicillin resistant staph aureus	Vancomycin, linezolid	Clindamycin, levofloxacin,	amoxicillin+clavulanate, erythromycin

DISCUSSION:

Ventilator associated pneumonia (VAP) is a major problem and it is one of the most frequently encountered hospital acquired infection in the ICU. The microbial profile of pathogens causing VAP may differ between hospitals and ICUs. [1,3]

Our study showed 89% growth from endotracheal aspirates which is concurrent with the studies of Bhaskar Thakuria et al.[13]SantoshKhanal et al, [14] and Koirala et. al [15](78-92%) and higher than the study of Vadivoo et. al. [16](73%) and Shalini S et.al. [17]. Out of 143 isolates clinical 77% were Gram negative Bacilli and only 23% were Gram positive cocci. Similar results were shown by Fugan *et al.*[18]who reported an incidence of 75 % of gram negative bacilli and Smseke*t al.*[19]who reported an incidence of 72 % of gram negative bacilli.

The common pathogens were *Acinetobacter spp.,Citrobacter spp.,Pseudomonas aeruginosa, Klebsiella pneumoniae* and gram-positive cocci like *Staphylococcus aureus.* There have been many studies done in the Indian subcontinent that have identified the aetiological agents of VAP as well as the susceptibility pattern which has been showing increasing resistance. Most common VAP pathogens are *P. aeruginosa*, *Acinetobacter spp., E. coli*, *K. pneumoniae* and *S. aureus* Summaiya M et al,[20]while assessing biofilm formation by VAP aetiological agents found that the most common isolates are *Pseudomonas aeruginosa* and *Acinetobacter spp.* While Trilok Patil [21]study at Aurangabad found *Pseudomonas aeruginosa* to be the most common organism (37.4%) followed by *Klebsiella pneumoniae* (28.5%).George P et al [22]study had Acinetobacter as the most common isolate (21%) followed by Pseudomonas (17%) and Citrobacter(15%). In Anusha et al study[23], Klebsiella was the most common bacteria followed by *Pseudomonas, Acinetobacter* and *E. coli*. As per Deepti et al study, Gram negative enteric aerobic bacteria were isolated from most of the patients, most common being Klebsiella species (32.35%) followed by Acinetobacter and Pseudomonas.[24]

In our study *Acinetobacter* spp resistance remains very high for most of the antimicrobials ranging from 50 percent to 100 percent except for Imipenem An increase in resistance was shown by *Pseudomonas aeruginosa* forAmikacin, levofloxacin and Ceftazidime. For Amikacin 20percent to 40percent and for Imipenem 15 percent to 28percent.

Among the Enterobacteriacea49% of *Citobacter*spp and 32.00 % of Klebsiella spp. were found to be probable ESBL producers and multi-drug resistant .The emergence of extended spectrum betalactamase (ESBLs) necessitated the increase use of carbapenems, but this increased use of drugs may be contributing to the emergence of multidrug resistant Gram negative bacilli. All the ESBL producing isolates were sensitive to Imipenem in this study.

VAP due to Gram positive bacteria (23%) and amongst them MRSA is another global problem, this study showed among all *Staphylococcus aureus* isolates, 22 (76.00%) isolates were methicillin resistant *S. aureus* (MRSA). It correlates with the study of Naouel Mandani 78.3 % were resistant to Methicillin. More than forty percent of the CONS (Coagulase negative *Staphylococcus aureus*) were resistant to cefotaxime, ciprofloxacin and erythromycin and all the isolates were resistant to Penicillin.

However all the gram positive cocci were sensitive to vancomycin. Hence Vancomycin should be part of regimen because *Staphylococcus aureus* is the most frequent gram positive isolates with high methicillin resistance rates Most of the isolates are becoming resistant to meropenem which is an alarming trend. Resistance to beta lactam class of antibiotic is a common occurrence and pan-drug-resistant strains are beginning to emerge.

In our series, *A. baumannii* was the most common organism causing VAP, and carbapenems were the most widely used antimicrobial class. .However ,almost all the *A. baumannii*i solates in our study were resistant to carbapenems, yet were still susceptible to colistin . Thus, intravenous CST is the onlyremaining therapeutic option for such cases. Yet, it is well known for its nephrotoxic and neurotoxic profiles. The use of adjunctive therapies with additive or synergistic effects to CST was profoundly discussed in the 2016 IDSA/ATS guidelines. The panel recommended the use of adjunctive aerosolized CST, since it improved clinical outcomes without increasing harms.[5,6]

Adequate timing of antibiotic administration, ideally within the first hour, is an essential element in determining the outcome of critically ill patients with infection. Combination antibiograms are important tools to optimize empiric therapy in the ICU through identifying which antimicrobial combinations give the highest likelihood of having at least one active agent against all likely causative pathogens in a specific disease state (VAP), thereby minimizing prolonged delays in instituting appropriate antimicrobial therapy.[8]

CONCLUSION :

The optimum strategy for the management of VAP is a subject of research and debate Good management strategies for VAP like adequate infection control practices include hand washing by hospital personal, basic cleaning of all surface levels, increased barrier precautions, early accurate diagnosis and more specific antimicrobial use may significantly improve patients' outcome.

A multidisciplinary approach, coordinated participation of microbiologist, clinician, nursing personal and hospital infection control team is necessary for management of this nosocomial infection.

REFERENCES :

1. Kalanuria, A. A., Zai, W and Mirski, M. (2014). Ventilator-associated pneumonia in the ICU. Critical care, 18:208.
2. Erbay R H, Yalcin A N, Zencir M, Serin S, Atalay H. Costs and risk factors for ventilatorcontrol study. BMC Pulm Med2004;4:3.
3. Pawar, M., MehtaY., Khurana P., Chaudhary A., Kulkarni V and Trehan, N. Ventilator-associated pneumonia: incidence, risk factors, outcome, and microbiology. Journal of cardiothoracic and vascular anesthesia,2003;17:22-8.
4. Ramakrishna PaiJakribettu and RekhaBoloor, "Characterisation of aerobic bacteria isolated from endotracheal aspirate in adult patients suspected ventilatorassociated pneumonia in a tertiary care center in Mangalore", Saudi J Anaesth. 2012 ; 6: 115–9.
5. Kalil AC, Metersky ML, Klompas M, et al. Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 clinical practice guidelines by the Infectious Diseases Society of America andthe American Thoracic Society. Clin Infect Dis. 2016:63:61–111.
6. Ferrer M, Ioanas M, Arancibia F, Marco MA, de la Bellacasa JP, Torres A. Microbial airway colonization is associated with noninvasive ventilation failure in exacerbation of chronic obstructive pulmonary disease. Critical care Medic.2005;33:2003-9.
7. Nishat Hussain Ahmed, Tabish Hussain, and InduBiswal, "Antimicrobial resistance of bacterial isolates from respiratory secretions of ventilated patients in a multispecialty hospital", Avicenna J Med. 2015 JulSep;5:74–8..
8. Clec'h, C., Timsit, J. F., De Lassence, A., Azoulay, E., Alberti, C., Garrouste- Orgeas, M and Cohen, Y. Efficacy of adequate early antibiotic therapy in ventilator-associated pneumonia: influence of disease severity. Intensive care medicine.2004;301327-33 Kollef, M. H and Schuster, D. P. Ventilator-associated pneumonia: clinical considerations. AJR. American journal of roentgenology, 1994;163:1031- 35.
9. Leroy, O., Meybeck, A., d'Escrivan, T., Devos, P., Kipnis, E and Georges, H. Impact of adequacy of initial antimicrobial therapy on the prognosis of patients with ventilator-associated pneumonia. Intensive care medicine, 2003;29:2170-73.
10. Collee JG, Marr W. Culture of bacteria. In: Marmion BP, Fraser AG, Simmons A. (eds). Mackie and Mccartney's practical medical microbiology. 14th edn. New York: Churchill Livingstone, 1996:113–30.
11. Miles RS, Amyes SG. Laboratory control of antimicrobial therapy. Mackie and McCartney practical medical microbiology. 1996;14:151-78.
12. Clinical and laboratory standards institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty seventh informational supplement. CLSI document 2017:M100-S26.
13. BhaskarThakuria, Preetinder Singh, Sanjay Agrawal, Veena Asthana, "Profile of infective microorganisms causing ventilator associated pneumonia: A clinical study from resource limited intensive care unit", Journal of Anaesthesiology Clinical Pharmacology.2013; 29:361-66.
14. Santosh Khanal, Dev Raj Joshi, Dwij Raj Bhatta, UpendraDevkota, and Bharat Mani Pokhrel, -Lactamase-Producing Multidrug-Resistant Bacterial Pathogens from Tracheal Aspirates of Intensive Care Unit Patients at National Institute of Neurological and Allied Sciences, Nepal", ISRN Microbiology. 2013. 47569, 5 pages.
15. Koirala P, Bhatta DR, Ghimire P, Pokhrel BM and Devkota U. "Bacteriological Profile of Tracheal Aspirates of the Patients Attending a Neuro-hospital of Nepal". Int J LifeSci,2010;4:60-5.
16. N. ShanmugaVadivoo, PriyaSantharam, K. Sudha, G. Kalaiselvi, B.K. Padmavathi, B. Usha, Amar Kumar, Nitesh Kumar Jaiswal, "Dynamic bacterial profile of endotracheal aspirates and its sensitivity pattern –a cause of concern", Int J Cur Res Rev, 2014; 06 :112-9.
17. Shalini S, Kranthi K, Gopalkrishnabhat K. "The Microbiological Profile of Nosocomial Infections in the Intensive Care Unit" Journal of Clinical and Diagnostic Research. 2010 ;4:3109-12.
18. Fagon, J. Y., Chastre, J., Domart, Y., Trouillet, J. L., Pierre, J., Darne, C and Gibert, C. Nosocomial pneumonia in patients receiving continuous mechanical ventilation: prospective analysis of 52 episodes with use of a protected specimen brush and quantitative culture techniques. American Review of Respiratory Disease, 1989;139: 877.
19. Simsek, S., Yurtseven, N., Gercekogalu, H., Izgi, F., Sohtorik, U., Canik, S and Ozler, A. Ventilator-associated pneumonias in a cardiothoracic surgery centre postoperative intensive care unit. Journal of Hospital Infection,2001;47:321-24.
20. Mulla Summaiya A. Assessment of biofilm formation by the causative organisms of ventilator associated pneumonia at intensive care unit of a tertiary care hospital. NatJ.Medic Res2012;2:15-8.
21. Patil, T. The Study of the organisms colonising trachea in mechanically ventilated patients admitted in the intensive care unit (ICU). International J Med Sci Edu.2014;1:6.
22. George, P, Sequiera, A. Antimicrobial sensitivity pattern among organisms isolated from the endotracheal aspirates of patients with ventilator associated pneumonia. J ClinDiag Res.2010;4:3397-01.
23. Anusha N, Madhu KP, Arun BJ, et al. Microbiological profile and sensitivity pattern of endotracheal secretions in mechanically ventilated patients in ICU. Journal of EvidenceBased Medicine And Healthcare2014;1:1177-84.
24. Chandra D, Laghawe A, Sadawarte K, et al. Microbiological profile and antimicrobial sensitivity pattern of endotracheal tube aspirates of patients in ICU of a tertiary care hospital in Bhopal, India. Int J CurrMicrobiol App Sci2017;6:891-5.
25. Foglia E, Meier M, El-ward A. Ventilatorassociated pneumonia in neonatal and pediatric intensive care units. Clin Microbiol Rev.2007;20:409-25.
26. Sanjai, N, Paul, B, Stephen, B, Michael D, Mark, L, Gary D. Sampling variability in the microbiological evaluation of expectorated sputa and endotracheal aspirates. J. Clin.Microbiol. 2001;39:2344-7.
27. Tripathi S, Malik GK, Jain A. Study of ventilator associated pneumonia in neonatal intensive care unit: characteristics, risk factors and outcome. Internet J Med Update.2010;5:12-9.
28. Deja M, Spies C. Prevention measures of ventilatorassociated pneumonia. Crit CareMed.2009;37:330- 2.
29. Ahmed NH, Hussain T, Biswal I. Antimicrobial resistance of bacterial isolates from respiratory secretions of ventilated patients in a multi-specialty hospital. Avicenna J Med 2015;5:74–8.