



## Original Research Article

# Bacteriological profile and antimicrobial susceptibility pattern of endotracheal tube secretion of patients in ICUs of a tertiary care hospital in Punjab

Kiranjeet Kaur<sup>1,\*</sup>, Shavetika Jindal<sup>1</sup><sup>1</sup>Dept. of Microbiology, Adesh Institute of Medical Sciences and Research, Bathinda, Punjab, India

## ARTICLE INFO

## Article history:

Received 18-06-2021

Accepted 30-07-2021

Available online 22-09-2021

## Keywords:

Antimicrobial resistance

Health care infections

Ventilator Associated Pneumonia (VAP)

Acinetobacter baumannii

## ABSTRACT

**Introduction:** Healthcare associated pneumonia (HAP) is second most common HCAs that occur in 27% critically ill patients. Eighty-six percent of HAP are associated with mechanical ventilation and termed as ventilator associated pneumonia (VAP). VAP due to multidrug resistant *Acinetobacter baumannii* has also increased in recent past.

**Objective:** To isolate and identify the bacterial pathogens in endotracheal tubes aspirates of ICUs patients and study their antimicrobial susceptibility pattern.

**Materials and Methods:** A prospective longitudinal study was conducted in the Microbiology laboratory of a tertiary care hospital over a period of six months after clearance from institutional Research Committee and Ethical Committee. All the samples of ETT secretions received in Clinical Microbiology lab from ICU patients and fulfilling the criteria for VAP were included in this study. Samples were processed as per standard protocol and organisms were identified on the basis of gram staining, colony characters and biochemical tests. Antibiotic sensitivity was performed by Kirby Bauer disc diffusion method as per CLSI guidelines.

**Results:** A total of 100 samples of ET secretions were collected and proceeded for culture. Out of 100 samples, 76 (76.0%) were positive for bacterial growth. Among 76 positive cultures, a total 80 bacterial isolates were obtained as some cultures were showing polymicrobial growth. Five (6.26%) isolates were Gram Positive bacteria and 75 (93.7%) were Gram negative. The most frequent isolates were *Acinetobacter baumannii* 35 (43.7%) followed by *Klebsiella pneumoniae* 25 (31.2%) *Pseudomonas aeruginosa* 7 (8.75%), *Acinetobacter baumannii* isolates were sensitive to colistin while resistant to ampicillin and amoxiclav. *Klebsiella pneumoniae* isolates were sensitive to colistin and resistant to ampicillin, amoxiclav, ciprofloxacin, ceftixime, piperacillin tazobactam. *Pseudomonas aeruginosa* isolates were sensitive to colistin while resistant to ampicillin, amoxiclav, ceftazidime and piperacillin tazobactam.

**Conclusion:** In our study antimicrobial pattern of isolated bacteria shows multidrug resistant pathogens which are associated with VAP and limit therapeutic options.

This is an Open Access (OA) journal, and articles are distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License](https://creativecommons.org/licenses/by-nc-sa/4.0/), which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: [reprint@ipinnovative.com](mailto:reprint@ipinnovative.com)

## 1. Introduction

Health care-associated infections (HCAIs) are the infections which occur in hospitals during health care. HCAIs are usually developed in hospitals and appear after 48 hours of hospital admission, or within 30 days after getting health

care services.<sup>1</sup> The US Center for Disease Control and Prevention identifies that nearly 1.7 million hospitalized patients annually acquire HCAIs and more than 98,000 patients (1 in 17) die worldwide due HCAIs.<sup>2</sup> Commonly acquired HCAIs are infections of surgical wounds, urinary tract infections and respiratory tract infections like Hospital Acquired Pneumonia (HAP).<sup>3</sup> HAP is second most common HCAIs that occur in 27% critically ill patients.<sup>4</sup>

\* Corresponding author.

E-mail address: [kiransandhu0802@gmail.com](mailto:kiransandhu0802@gmail.com) (K. Kaur).

HAP can be defined as pneumonia that occurs after 48 hour or more after admission to the hospital but did not appear to be incubating at time of admission.<sup>5</sup>

These ICUs are equipped with mechanical ventilators to assist breathing through an endotracheal tube (ETT) or by a tracheotomy tube. Eighty-six percent of HAP are associated with mechanical ventilation (MV) and are termed as ventilator associated pneumonia (VAP).<sup>6</sup> VAP is defined as bacterial pneumonia developing in patients after at least 48 hours of Mechanical ventilation but not present at the time of intubation or admission in hospital.<sup>7</sup> Early onset VAP (EOVAP) is defined as VAP occurring within 0-4 days of endotracheal intubation while Late onset VAP (LOVAP) is defined as VAP occurring after 5 or more days of intubation. VAP is commonest complication in ICUs patients reported at the rate of 1-3% per day of Mechanical Ventilation(MV) and prevalence rate ranges from 10% to 65% in tertiary care hospitals.<sup>8</sup>

Risk of VAP increases with increase in duration of mechanical ventilation, so accelerate weaning and by using non-invasive ventilation can reduce the risk.<sup>9</sup> Supine (0°) patient positioning also facilitates aspiration, which can be decreased by changing position to semirecumbent (45°) position.<sup>10</sup> Enterally feeding has been considered as a risk factor for development of VAP because of an increase risk of aspiration. Non-modifiable risk factors include male gender, head trauma, preexisting pulmonary disease, AIDS, coma and multi-organ system failure. Other risk factors for the development of VAP include- tracheostomy, dialysis, reintubation, tube thoracostomy, sedatives, corticosteroids, inotropic drugs, presence and duration of central venous and arterial catheters.<sup>11</sup>

Microorganisms causing pneumonia can be endogenous (digestive system or nose and throat), or exogenous from contaminated respiratory equipment which colonizes in the upper airway and bronchi and can cause infection in the lungs (pneumonia). Both Gram-positive and Gram-negative bacteria are implicated in VAPs, but most commonly found are *Acinetobacter baumannii*, *Pseudomonas aeruginosa* followed by *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Enterobacter species*.<sup>12</sup> Studies highlighted that frequent and unselective usage of broad spectrum antibiotics without consideration of culture and Susceptibility reporting leads to the development of multidrug resistant microorganisms (MDRs).<sup>13</sup> These MDRs are frequently colonized through endogenous or exogenous sources on life saving instruments such as Mechanical Ventilators in ICUs.<sup>14</sup> Bacteriological examination of respiratory secretions offer helping hand to clinician in diagnosing VAP and also helps him to initiate early antibiotic regimen. The rapid availability of cytological data including inflammatory cells and gram stain are useful in initial therapeutic decisions. Every possible effort should therefore be made to obtain reliable pulmonary

specimens for direct microscopic examination and cultures from each patient clinically suspected of having developed VAP before new antibiotics are administered.

## 2. Aims and Objective

To isolate and identify the bacterial pathogens in endotracheal tubes aspirates of ICUs patients and study their antimicrobial susceptibility pattern.

## 3. Materials and Methods

A prospective longitudinal study was conducted in the Microbiology laboratory of a tertiary care hospital over a period of six months. All the samples of ETT secretions received in Clinical Microbiology lab from ICU patients and fulfilling the criteria for VAP were included in this study. This present study was carried out for 06 months after clearance from institutional Research Committee and Ethical Committee.

Direct smear staining was performed for each sample and organisms were identified on the basis of morphology, arrangement and Gram's reaction. The samples were inoculated on Blood agar and MacConkey agar plates. The plates were then incubated overnight at 37°C for 24 hours. The growth of the organisms were observed on Blood agar medium and MacConkey agar medium. The colonies were identified from colony characters like size, shape, surface, edges, margin, consistency, emulsifiability, opacity, colour and any odour. Further growth was confirmed by Gram staining, biochemical reactions and other specific confirmatory tests. Antimicrobial susceptibility testing was performed on Mueller Hinton Agar (MHA) by Kirby Bauer disc diffusion method as per CLSI guidelines.<sup>15</sup>

Different antibiotics disks of HIMEDIA were used according to bacterial isolate. Sensitivity was recorded by measuring the diameter of zone of inhibition in reference to CLSI. Clinical data was collected from patient's file by visiting ICU.

## 4. Results

Total 100 samples were enrolled in the present study of which 67(67.0%) were of male and 33(33.0%) of female patients (Table 1). The age wise distribution showed 13 (13.0%)ETT secretions samples were received from 0-20 year's age group, 14(14%) from 21-40 year age group, 29(29.0%) from 41-60 years age group and 44(44.0%) from >60 years age group (Table 2). Total 20(20.0%) samples of ETT had the history of early onset of VAP and 80(80.0%) samples belong to late onset of VAP. Total 70(70.0%) patients has the prior history of antibiotics, 44(44.0%) has the clinical representation to Prior Hospitalization, 15(15.0%) were belong to Aspiration, 18(18.0%) patients has clinical history of Re-Intubation, 34(34.0%) samples patients has prior history of Diabetes Mellitus, 30(30.0%)

patients has clinical picture of Hypertension and 07(7.0%) were on immunosuppressant therapy (Table 3). In our study, 76 (76.0%) ET samples were showing the significant bacterial growth with a total 80 isolates obtained. Among these 80 bacterial isolates, 05(6.26%) were gram positive and 75(93.7%) were gram negative. The most frequent isolates were *Acinetobacter baumannii* 35(43.7%) followed by *Klebsiella pneumoniae* 25(31.2%) *Pseudomonas aeruginosa* 7(8.75%), *Escherichia coli* 6 (7.5%), *Staphylococcus aureus* 3(3.7%), *Enterococcus faecalis* 2 (2.5%) and other gram negative isolates 02 (2.5%) (Table 4). Antibiotic susceptibility pattern of isolated bacteria was as shown in Tables 5 and 6.

**Table 1:** Gender wise distribution of patients with VAP

Gender	Distributions	Percentage (%)
Male	67	67%
Female	33	33%
Total number of cases (n) = 100		

**Table 2:** Age wise distribution of patients with VAP

Age in years	Distributions	Percentage
0-20	13	13%
21-40	14	14%
41-60	29	29%
>60	44	44%
Total Number of Cases = 100		

**Table 3:** Distribution of cases according to risk factors and co-morbidities

Risk Factors/Co morbidities	Distributions	Percentage
Prior antibiotics	70	70%
Prior hospitalization	44	44%
Aspiration	15	15%
Re-intubation	18	18%
Diabetes mellitus	34	34%
Hypertension	30	30%
Immunosuppressant	07	07%

## 5. Discussion

The risk for development of VAP depends upon several factors such as Host immunity, duration of stay in hospitals, exposure to potential pathogens, re-intubation and Diabetes mellitus.<sup>16</sup> In our study among 100 samples 67(67%) were from male (Table 1) patients which were similar to study by Neha Samal et al. This may be due to more admission of male patients and also male are more prone to accidental trauma.<sup>17</sup> In this study maximum patients were belong to the age group >60 years. Similar finding was also reported by Mukesh Dube et al. in his study in 2018.<sup>18</sup> This

**Table 4:** Bacterial isolates among positive ETT secretion

Name of Organism	Isolates Obtained	Percentage
<i>Acinetobacter baumannii</i>	35	43.7%
<i>Klebsiella pneumoniae</i>	25	31.3%
<i>Pseudomonas aeruginosa</i>	07	8.7%
<i>Escherichia coli</i>	06	7.5%
<i>Staphylococcus aureus</i>	03	3.7%
<i>Enterococcus faecalis</i>	02	2.5%
Other bacterial isolates	02	2.5%
Total number of isolates obtained= 80		

indicates that patients with higher age group are highly prone to VAP. This is because of the fact that patients with higher age groups were having lower immunity, decrease mucociliary clearance of secretions and co-morbid conditions like diabetes mellitus and hypertension. In our study maximum cases of VAP were late onset compare to early onset this is because of prolonged hospital stay increases cross infection and HAIS among patients. Our results were similar to that of ElipsGiantsou while a study by cook et al. found higher incidence of early onset VAP.<sup>19</sup> Major risk factors associated with VAP in our study were history of prior antibiotics, prior hospital admission, re-intubation, diabetes and hypertension (Table 3). In a similar study Tedja R has analysed 107 samples out of which 49 were having the history of home antibiotics.<sup>20</sup> The reason behind this is production of MDRs by unselective use of antibiotics. These MDRs are potential risk of VAP. The prevalence of VAP in our study was 76%. Similar high prevalence 53% of VAP was also noted by Pooja Gupta et al. In our study Gram negative isolates were predominate over Gram positive isolates.<sup>21</sup> Our study results indicate *Acinetobacter baumannii* is major pathogenic bacteria followed by *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (Table 4). A study conducted by Zorgani A et al. also found major pathogens as *Acinetobacter baumannii* and *Klebsiella pneumoniae*. All *Acinetobacter baumannii* isolates were sensitive to colistin while resistant to ampicillin and amoxiclav (Table 5). Sensitivity to tigecyclin was 82.2%, imipenem and meropenem 11.4% and piperacillin tazobactam 5.7% (Table 5). All *Klebsiella pneumonia* isolates were sensitive to colistin and resistant to ampicillin, amoxiclav, ciprofloxacin, cefixime, piperacillin tazobactam. Sensitivity to tigecyclin was 44% amikacin 28%, imipenem and meropenem 16% and gentamicin 12% (Table 5). All *Pseudomonas aeruginosa* isolates were sensitive to colistin while resistant to ampicillin, amoxiclav, ceftizidime and piperacillin tazobactam. Sensitivity to amikacin and gentamicin was 42.8%, ciprofloxacin, imipenem and meropenem is 28.5% each.(Table 5) In our study

**Table 5:** Antimicrobial susceptibility pattern of Gram negative isolate

Organisms	Sensitivity	A	A- CLAV	AK	G	CF	CZ	CM	PCTZ	IMP	MRP	TC	CT
<i>Acinetobacter baumannii</i>	S*	00	00	01 (2.85%)	05 (14.2%)	02 (5.7%)	02 (5.7%)	02 (5.7%)	02 (5.7%)	04 (11.4%)	04 (11.4%)	29 (82.8%)	35 (100%)
	R**	35 (100%)	35 (100%)	34 (97.1%)	30 (85.7%)	33 (94.2%)	33 (94.2%)	33 (94.2%)	33 (94.2%)	31 (88.5%)	31 (88.5%)	06 (17.1%)	00
<i>Pseudomonas aeruginosa</i>	S	00	00	03 (42.8%)	03 (42.8%)	02 (28.5%)	00	01 (14.2%)	00	02 (28.5%)	02 (28.5%)	00	07 (100%)
	R	07 (100%)	07 (100%)	04 (57.1%)	04 (57.1%)	05 (71.4%)	07 (100%)	06 (85.7%)	07 (100%)	05 (71.4%)	05 (71.4%)	07 (100%)	00
<i>Klebsiella pneumonia</i>	S	00	00	07 (28%)	03 (12%)	00	00	00	00	04 (16%)	04 (16%)	11 (44%)	18 (72%)
	R	25 (100%)	25 (100%)	18 (72%)	22 (88%)	25 (100%)	25 (100%)	25 (100%)	25 (100%)	21 (84%)	21 (84%)	14 (56%)	07 (28%)
<i>Escherichia coli</i>	S	00	01 (16.6%)	05 (83.3%)	02 (33.3%)	02 (33.3%)	01 (16.6%)	01 (16.6%)	01 (16.6%)	04 (66.6%)	04 (66.6%)	05 (83.3%)	06 (100%)
	R	06 (100%)	05 (83.3%)	01 (16.6%)	04 (66.6%)	04 (66.6%)	05 (83.3%)	05 (83.3%)	05 (83.3%)	02 (33.3%)	02 (33.3%)	01 (16.6%)	00

\* Sensitive, \*\*Resistant  
A-Ampicillin, A-CLAV-Amoxicillin Clavulanic acid, AK-Amikacin, G-Gentamicin, CF-Ciprofloxacin, CZ-Ceftazidime, CM-Cefixime, PCTZ-Piperacillin Tazobactam, IMP-Imipenem, MRP-Meropenem, TC-Tigecycline, CT-Colistin

**Table 6:** Antimicrobial susceptibility pattern of Gram positive isolates

Organisms	Sensitivity	A- CLAV	CN	CF	LF	E	G	C	Cl	Co	Va	Tp	LZ	Tc
<i>S.aureus</i>	S	02 (66.6%)	03 (100%)	01 (33.3%)	02 (66.6%)	02 (66.6%)	03 (100%)	01 (33.3%)	-	02 (66.6%)	03 (100%)	-	03 (100%)	3 (100%)
	R	01 (33.3%)	-	02 (66.3%)	01 (33.3%)	01 (33.3%)	-	02 (66.6%)	03 (100%)	01 (33.3%)	-	03 (100%)	-	-
<i>E.faecalis</i>	S	02 (100%)	01 (50%)	02 (100%)	-	-	01 (50%)	-	-	-	2 (100%)	02 (100%)	2 (100%)	2 (100%)
	R	-	01 (50%)	-	02 (100%)	02 (100%)	01 (50%)	02 (100%)	02 (100%)	02 (100%)	-	-	-	-

A-clav-Amoxicillin Clavulanic acid, CN-Cefotaxim, CF-Ciprofloxacin, LF-Levofloxacin, E-Erythromycin, G-Gentamicin, C-Clindamycin, Cl-Chloramphenicol, Co-Cotrimoxazole, Va-Vancomycin, Tp-Teicoplanin, LZ-Linezolid, Tc-Tigecycline

antimicrobial pattern of isolated bacteria shows multidrug resistant pathogens are associated with VAP. Similar type of Multidrug resistance among VAP pathogen was also noted by another studies.<sup>17,22</sup> The detection of Multi Drug Resistant isolates in VAP patients further limit therapeutic options and necessitating the role of culture and sensitivity. A combined clinical, microbiological, infection control strategies which include proper diagnosis and appropriate antibiotic can lead to proper patient management. Every hospital should have appropriate antibiogram to start the empirical antibiotic treatment.

## 6. Conclusion

The presence of devices in airway, prevents the cough, impairs mucocilliary clearance, permit micro aspiration of contaminated sub-glottic secretions around the cuff and allow the formation of intraluminal biofilms by bacteria. These Gram negative and Gram Positive bacteria are major source of Ventilator associated pneumonia. Every hospital should have appropriate antibiogram to start the empirical antibiotic treatment. Appropriate training of health care staff regarding different measures to prevent spread of multidrug resistance should be done time to time decrease the incidence of VAP and to fight MDR pathogens.

## 7. Source of Funding

Nil.

## 8. Conflict of Interest

Nil.

## References

- Haque M, Sartelli M, Mckimm J, Bakar MA. Health care-associated infections-an overview. *Infection and drug resistance*. *Infect Drug Resist*. 2018;11:2321–33.
- Santos SB, Cunha AP, Macedo M, Nogueira CL, Brandão A, Costa SP, et al. Bacteriophage-receptor binding proteins for multiplex detection of Staphylococcus and Enterococcus in blood. *Biotechnol Bioeng*. 2020;117(11):3286–98.
- Liu JY, Wu YH, Cai M, Zhou CL. Point-prevalence survey of healthcare-associated infections in Beijing, China: a survey and analysis in 2014. *J Hosp Infect*. 2016;93(3):271–9.
- Evans S. Could a risk-assessment tool prevents hospital-acquired pneumonia? *British Journal of Nursing*. 2018;27(7):402–404.
- Russell CD, Koch O, Laurenson IF, O'Shea DT, Sutherland R, Mackintosh CL, et al. Diagnosis and features of hospital-acquired pneumonia: a retrospective cohort study. *J Hosp Infect*. 2016;92(3):273–9.
- Khademi G, Lotfi M, Bakhtiari E, Imani B, Aelami MH. Minor Diagnostic Factors in Ventilator Associated Pneumonia in Children. *Int J Pediatr*. 2018;6(7):8015–23.
- Zakharkina T, Martin-Loeches I, Matamoros S, Pova P, Torres A, Kastelijan JB, et al. The dynamics of the pulmonary microbiome during mechanical ventilation in the intensive care unit and the association with occurrence of pneumonia. *Thorax*. 2017;72(9):803–10.
- Ramírez-Estrada S, Lagunes L, Peña-López Y, Vahedian-Azimi A, Nseir S, Arvaniti K, et al. Assessing predictive accuracy for outcomes of ventilator-associated events in an international cohort: the EUVAE study. *Intensive Care Med*. 2018;44(8):1212–20.
- Comellini V, Pacilli AM, Nava S. Benefits of non-invasive ventilation in acute hypercapnic respiratory failure. *Respiology*. 2019;24(4):308–17.
- Michetti CP, Prentice HA, Rodriguez J, Newcomb A. Supine position and nonmodifiable risk factors for ventilator-associated pneumonia in trauma patients. *Am J Surg*. 2017;213(2):405–12.
- Rose DD, Pezzotti P, Fortunato E, Sordillo P, Gini S, Boros S, et al. Clinical predictors and microbiology of ventilator-associated pneumonia in the intensive care unit: a retrospective analysis in six Italian hospitals. *Eur J Clin Microbiol Infect Dis*. 2016;35(9):1531–9.
- Wąlaszek M, Kosiarska A, Gniadek A, Kołpa M, Wolak Z, Dobro W, et al. The risk factors for hospital-acquired pneumonia in the Intensive Care Unit. *Przegl Epidemiol*. 2016;70(1):15–20.
- Sakai AM, Iensue TN, Pereira KO, DeSouza NA, Silva CM, Salvador MSA, et al. Colonization by multidrug-resistant microorganisms of hospitalized newborns and their mothers in the neonatal unit context. *J Infect Dev Countr*. 2020;14(07):765–71.
- Tedja R, Nowacki A, Fraser T, Fatica C, Griffiths L, Gordon S, et al. The impact of multidrug resistance on outcomes in ventilator-associated pneumonia. *Am J Infect Cont*. 2014;42(5):542–5.
- Clinical and Laboratory Standards institute (CLSI). Performance standards for antimicrobial susceptibility testing: 23rd informational supplement; 2013.
- Rit K, Chakraborty B, Saha R, Majumder U. Ventilator associated pneumonia in a tertiary care hospital in India: Incidence, etiology, risk factors, role of multidrug resistant pathogens. *Int J Med Public Health*. 2014;4(1):1–13.
- Samal N, Padhi S, Paty BP. Bacteriological profile and antimicrobial sensitivity pattern of endotracheal tube aspirates of patients admitted in ICU. *J Dr NTR Univ Health Sci*. 2020;9(3):151.
- Dube M, Goswami S, Singh A. pattern and incidence of ventilator associated pneumonia among mechanically ventilated patients. *IJNM*. 2018;5(2):442–5.
- Giantsou E, Efrainidou E, Efrainidou E, Panopoulou M, Alepopoulou E, Kartali-Ktenidou S, et al. Both early-onset and late-onset ventilator-associated pneumonia are caused mainly by potentially multiresistant bacteria. *Intensive Care Med*. 2005;31(11):1488–94.
- Cook DJ, Walter SD, Cook RJ. Incidence of risk factors ventilator associated pneumonia in critically ill patients. *Ann Internal Med*. 1998;129(6):433–40.
- Zorgani A, Abofayed A, Gliha A. Prevalence of device-associated nosocomial infections caused by Gram negative bacteria in a tertiary intensive care unit in Libya. *Oman Med J*. 2015;30(4):270–5.
- Swati A, Yamini K, Rajkumar RV. Microbiological spectrum and antimicrobial susceptibility patterns of various isolates from endotracheal tubes aspirates in tertiary care hospital. *Telangana IJMR*. 2018;5(2):202–209.

## Author biography

**Kiranjeet Kaur**, Associate Professor

**Shavetika Jindal**, MSc Student

**Cite this article:** Kaur K, Jindal S. Bacteriological profile and antimicrobial susceptibility pattern of endotracheal tube secretion of patients in ICUs of a tertiary care hospital in Punjab. *Indian J Microbiol Res* 2021;8(3):224-229.