Original Research Article

Ventilator-Associated Pneumonia : A Clinico-Micribiological study of Causative Organism and their Antibiotic Susceptibility Pattern

Kanhaiya Jha[1], Prakash Kumar Mishra[2], Mahadeo Mandal[3],MD. Shahnawaj Mehdi[4*]

[1]Associate Professor, Department of Microbiology, Jan Nayak Karpuri Thakur Medical College Madhepura, Bihar,India

[2]Assistant Professor, Department Of Microbiology, Jan Nayak Karpuri Thakur Medical College, Madhepura, Bihar,India

[3]Tutor, Department of Microbiology, Jan Nayak Karpuri Thakur Medical College, Madhepura, Bihar,India

[4]Tutor, Department of Microbiology, Jan Nayak Karpuri Thakur Medical College, Madhepura, Bihar,India

Received: 03-09-2021 / Revised: 16-10-2021 / Accepted: 03-12-2021

Abstract

Background: Ventilator Associated Pneumonia (VAP) is defined as pneumonia that occurs 48 hours or more after endotracheal intubation or tracheostomy, caused by infectious agents not present or incubating at the time mechanical ventilation started. High mortality and healthcare costs area associated with ventilator-associated pneumonia (VAP) due to Multidrug-Resistant (MDR) Pathogens. **Aim:** To identify the relation of risk factors for ventilator-associated pneumonia (VAP) and mortality with the drug resistance profile. **Materials and Method:** A total of 38 isolates from 35 VAP patients were collected during the study. They were processed following standard laboratory protocol. Antibiogram was done using appropriate antibiotics by Kirby-Bauer disc diffusion method and the occurrence of MRSA, ESBLs and MBLs was seen. **Results:** Males were most commonly affected, and Acinetobacter spp. Were the most common organism isolated. For MDR isolates most sensitive drug was Cefoperazone-sulbactum, followed by Piperacillin-tazobactam, Piperacillin and Cefoperazone. Whereas in non-MDR isolates Amikacin was most sensitive followed by both Cefoperazone-sulbactum and Gentamicin . Most common mechanism of resistance among MDR isolates was found to be Carbapenemase production, followed by AmpC, and ESBL. Diabetes mellitus was most common risk factor, followed by smoking, and alcohol. Majority of patients had leucocytosis and some were anaemic. **Conclusion:** Periodic analysis of Sputum culture and their antibiotic sensitivity report should be made to identify the changing trends in etiological and sensitivity patterns.

Keywords: Ventilator associated pneumonia, Multidrug resistant, Geriatric VAP, Extended Spectrum β-lactamases, Metallo- betalactamases

Introduction

Ventilator-associated pneumonia (VAP) is defined as pneumonia that occurs 48-72 hours or thereafter following endotracheal intubation, characterized by the presence of a new or progressive infiltrate, signs of systemic infection (fever, altered white blood cell count), changes in sputum characteristics, and detection of a causative agent [1]. VAP contributes to approximately half of all cases of hospital-acquired pneumonia [1], [2]. VAP is estimated to occur in 9-27 % of all mechanically ventilated patients, with the highest risk being early in the course of hospitalization [1], [3]. It is the second most common nosocomial infection in the intensive care unit (ICU) and the most common in mechanically ventilated patients [4], [5]. VAP rates range from 1.2 to 8.5 per 1,000 ventilator days and are reliant on the definition used for diagnosis [6]. Risk for VAP is greatest during the first 5 days of mechanical ventilation (3 %) with the mean duration between intubation and development of VAP being 3.3 days [1], [7]. This risk declines to 2 %/day between days 5 to 10 of ventilation, and 1 %/day thereafter [1], [8]. Earlier studies placed the attributable mortality for VAP at between 33-50 %, but this rate is variable and relies heavily on the underlying medical illness [1]. Over the years, the attributable risk of death has decreased and is more recently estimated at 9-13 % [9], [10], largely because of implementation of preventive strategies. Approximately 50 % of all antibiotics administered in ICUs are for treatment of VAP [2], [4]. Early onset VAP is defined as pneumonia that occurs within 4 days and this is usually attributed to antibiotic sensitive pathogens whereas late onset VAP is more likely caused by multidrug resistant (MDR) bacteria and emerges after 4 days of intubation [1], [4]. Thus, VAP poses grave implications in endotracheally intubated adult patients in ICUs worldwide and leads to increased adverse outcomes and healthcare costs. Independent risk factors for development of VAP are male sex, admission for trauma and intermediate underlying disease severity, with odds ratios (OR) of 1.58, 1.75 and 1.47-1.70, respectively [7]. The recent advances in medical technologies, usage of mechanical ventilator and other procedures like bronchoscopes, prior antibiotic prescription even before the availability of culture results and frequent admission to hospital lead to the bacterial colonization and infection.[20] With the emergence of antibiotic resistant bacteria, the role that hospitals play in the development and spread of organisms becomes an important factor for investigation. Therefore, this study was conducted to identify the relation of risk factors for ventilator-associated pneumonia (VAP) and mortality with the drug resistance

**Correspondence*
Dr. MD. Shahnawaj Mehdi
Tutor, Department of Microbiology, Jan Nayak Karpuri Thakur Medical College, Madhepura, Bihar,India
E-mail: drmehdi30@gmail.com

profiles of Multidrug-Resistant (MDR) Pathogens with detection of MRSA, ESBLs and MBLs in intensive care unit.

Materials and Method

This was a prospective, descriptive, cross-sectional, study. The study sample consisted of Lower respiratory tract samples of geriatric patients like Broncho-alveolar lavage (BAL) and Endo Tracheal Aspirate submitted to diagnostic Microbiology laboratory.

Inclusion criteria

Lower respiratory tract samples like BAL & ET Aspirate of patients aged 60 years or above.

Exclusion criteria

Patient on chronic suppressive antimicrobial therapy.

Patient diagnosed as pulmonary tuberculosis

Patient diagnosed as Retro positive.

Tracheal aspirate/ BAL - Most purulent portion of tracheal secretion was taken, 0.1 ml sample was diluted in 9.9 ml sterile physiological solution. 0.01 ml was seeded (calibrated loop) on MacConkey agar, blood agar & chocolate agar and Incubation at 35 ± 1ºC for 24 to 48h, (chocolate agar, in capnofilia (5% of CO_2) at 35 ± 1ºC for 24 to 48h). Plates were evaluated for growth at 24 and 48hours. Bacterial isolates grown in culture were identified by means of Gram's staining and biochemical reactions by standard microbiological techniques. Each colony corresponded to 20,000CFU/ml and it was considered ETA positive when the count was $\geq 10^5$CFU/ml.

Antibiotic susceptibility tests were done against antibiotics by using Standard Kirby Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) criteria. Every batch of Mueller-Hilton agar and antibiotic discs were tested by using following control strains: ATCC 25922 Escherichia coli, ATCC 27853 Pseudomonas aeruginosa and ATCC 25923 Staphylococcus aureus.

Extended Spectrum β-Lactamase (ESBL) was detected by Phenotypic disc confirmatory test

AmpC β-Lactamase was detected by AmpC Disk test. Carbapenamase and Metallo-β Lactase (MBL) was detected by Modified Carba NP test and EDTA synergy test respectively.

MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. In present study, a Gram negative bacterium was considered MDR when it is resistant to representative drug from these three groups of antibiotics, β-lactam (ceftazidime), aminoglycoside (gentamicin) and quinolone (ciprofloxacin).Cefotaxime (30µg) or Ceftazidime disks (30µg) with and without clavulanate (10µg) are used. A difference of ≥5mm between the zone diameters of either of the cephalosporin disks and their respective cephalosporin/clavulanate disk was taken to be phenotypic confirmation of ESBL production[20] The CLSI recommends that the disk tests be performed with confluent growth on Mueller-Hinton agar.Briefly, 0.5 McFarland suspension of Escherichia coli ATCC 25922 was inoculated on the surface of MHA plate. A 30µg Cefoxitin disk & a sterile plain disk inoculated with several colonies of the test organism was placed just beside the Cefoxitin disk almost touching it, with inoculated disk face in contact with the agar surface. After overnight incubation at 37^0C, the plates were examined for either an indentation or a flattening of the zone of inhibition, indicating enzymatic inactivation of Cefoxitin (positive result), or absence of a distortion (negative result).CNP A solution was prepared by adding phenol red (0.05%) and ZnSO4.7H2O (0.1 mmol/L) to Clinical Laboratory Reagent Water; pH was adjusted to 7.8 ± 0.1, and the solution was stored at 4ºC in amber-coloured bottles for up to 15 days. The B solution was freshly prepared by adding 12 mg/ml imipenem- cilastatin injectable form (doubling the amount to compensate the cilastatin component; equivalent to 6 mg/ml of imipenem standard grade powder) to A solution and stored at 40C till use. Two calibrated loops (10µl) of bacterial colony from 18 to 24 h growth culture from sheep blood agar were re-suspended in 200µl of 5 M NaCl solution and vortexed for 5 seconds. A 100µl of inoculum was added to two micro centrifuge tubes labelled "a" and "b." Reagents A and B were added to tubes a and b, respectively, incubated at 37ºC and read at 2hours. The test was considered positive when tube "a" was red and tube "b" was orange/yellow. In a negative test, both tubes remained red.

Combined disk synergy test (CDST) with 0.5 M ethylene diamine tetra acetic acid Two IPM (10µg) disks were placed 30mm apart from center to center on the surface of an agar plate, and 10µl 0.5 M EDTA solution was added to one of them to obtain the desired concentration of 750µg. If zone of inhibition of IPM-EDTA disk was ≥7 mm more than that of IPM disk alone, it was considered as MBL positive.

Results and Discussion

A total of 38 organisms (33-gram negative and 5-gram positive) were isolated from 35 patients who developed VAP. Among which 15 isolates were Multi-drug resistant from 12 Patients. Among the 12 patients, 9 (75%) yielded pure bacterial (mono-microbial) and 3(25%) yielded mixed infection (two organisms- polymicrobial).

Table 1: Age and Sex wise distribution of MDR Isolates (n=12)

Age group	Female	Male	Total
60-79	4 (33.3%)	6 (50%)	10
≥80	0	2 (16.6%)	02
Total	4 (33.3%)	8 (66.6%)	12

Among 12 patients, predominant were males accounting for 66.6% in which 50% were between 60-79 years and 16.6% were ≥80 years. 33.3% were females all belonging to 60- 79years. (Table -1)

Table 2: Distribution of Poly-microbial isolates

Organism	No	Age	Sex
Acinetobacter spp + Klebsiella pneumoniae	1	80	M
Pseudomonas aeruginosa + Escherichia coli	1	72	F
Acinetobacter spp + Escherichia coli	1	68	F
Total	3		

Table 3: Distribution of MDR phenotypes among tracheal aspirate & BAL

Organism	MDR	Percentage %
Klebsiella pneumoniae (n=7)	5	33.33
Acinetobacter spp. (n=15)	6	40
Pseudomonas aeruginosa (n=8)	2	13.33
Escherichia coli (n=3)	2	13.33
Total (n=33)	15	

Among Enterobacteriaceae, 33.33% of Klebsiella pneumoniae and 13.33% of Escherichia coli were MDR and in Non-Enterobacteriaceae 40% of Acinetobacter spp., and 13.33% Pseudomonas aeruginosa were MDR. Overall MDR among Gram negative isolates were 45.5%.

Table 4: Comparison of Antibiotic Resistance among Gram negative isolates (n=33)

Antibiotic	MDR (n=15)	%	Non-MDR (n=18)	%
Piperacillin	13	86.7	13	72.2
Ciprofloxacin	15	100	9	50
Cefoperazone	14	93.3	14	93.3
Ceftazidime	15	100	12	80
Piperacillin-tazobactam	12	80	7	38.9
Cefperazone-sulbactam	10	66.7	5	27.8
Aztreonem	14	93.3	12	80
Gentamycin	15	100	5	27.8
Imipenem	14	93.3	7	38.9
Meropenem	12	80	7	38.9
Amikacin	12	80	4	22.3

For MDR isolates most sensitive drug was Cefoperazone- sulbactum (25%), followed by Piperacillin-tazobactam (8.3%), Piperacillin (8.3%) and Cefoperazone (8.3%). Whereas in non-MDR isolates Amikacin (77.7%) was most sensitive followed by Cefoperazone-sulbactum and Gentamicin (72.2% each).

Table 5: Beta lactamase production among MDR Gram negative isolates (n=15)

Mechanism of resistance production		Frequency	Percentage (%)
ESBL		1	6.7
Carbapenamase	Metallo-βlactamase (n=3)	8	53.3
	Non-metallo- βlactamase (n=5)		
AmpC		2	13.3
ESBL+AmpC		1	6.7

Most common mechanism of resistance among MDR isolates was found to be Carbapenemase production (53.3%) {4 by Acinetobacter spp, 2 by Klebsiella pneumoniae, 1 each by Pseudomonas aeruginosa and Escherichia coli}, followed by AmpC (18.2%) {4-Klebsiella pneumoniae& 2- Escherichia coli}, and ESBL 3.3% by Klebsiella pneumoniae. Among Carbapenemase Metallo-betalactamase production was seen in 37.5% of isolates.

Table 6: Correlation with MDR and Carbapenemase among Acinetobacter spp

	MDR+	Non-MDR+	Total
Carbapenamase +	3 (75%)	1	4 (26.67%)
Non carbapenamase +	1 (25%)	10	11(73.33%)
Total	4	11	15

Among the 4 MDR positive Acinetobacter species, 3 isolates were Carbapenamase producers.

Table 7: Correlation with MDR and Carbapenemase among Pseudomonas aeruginosa

	MDR+	Non-MDR+	Total
Carbapenamase +	1	1	2 (25%)
Non carbapenamase +	1	5	6 (75%)
Total	2	6	8

Among the 2 MDR positive Pseudomonas aeruginosa, 1 isolate was Carbapenamase producer.

Table 8: Risk factors associated with MDR Positive VAP infections (n=12)

Risk factor		Percentage (%)
Diabetic	7	58.33
Smoking	6	50
Alcohol	5	41.67
Previous COPD	4	33.33
Poor oral hygiene	3	25
Cardiac diseases	2	16.67
Malnutrition	1	8.33
Renal disease	1	8.33
Hemiparesis	1	8.33
CA lung	1	8.33

Radiological correlation (n=12)

Correlation of chest X-ray was done in all patients, among which 5(41.67%) patients had pneumonic changes (consolidation) and 6(50%) patients had B/L alveolar or interstitial infiltration and 1 (8.33%) patient had consolidation with CA lung

Table 9: Laboratory correlation (n=15)

Investigation	Percentage (%) or Mean
Anaemia	17%
Mean Hb	10.02g/dl
Mean TLC	17348cells/mm^3
Leucocytosis	88%

Conclusion

Periodic analysis and their antibiotic sensitivity report should be made so that changing trends in the etiological and sensitivity patterns can be identified and therapy adjusted accordingly so that emergence of resistance will be prevented. Strict infection control measures should also be followed to contain hospital acquired infections.

References

1. Forbes BA, Sahm DF, Weissfeld AS, editors. Bailey & Scott‟s Diagnostic Microbiology. 12th ed. St. Louis (USA): Mosby Elsevier; 2007. Lower respiratory tract infection; 842-54.
2. Iginskiene AC, Dambrauskiene A, Rello J, Adukauskiene D. Ventilator-Associated Pneumonia due to Drug-Resistant Acinetobacter baumannii: Risk Factors and Mortality Relation with Resistance Profiles, and Independent Predictors of In-Hospital Mortality. Medicina. 2019; 55:49
3. C van de Nadort, HM Smeets, J Bont et al. Prognosis of primary care patients aged 80 years and older with lower respiratory tract infection. Br J Gen Pract. 2009; 59:261-65.
4. Koulenti D, Lisboa T, Brun-Buisson C, Krueger W, Macor A, Sole-Violan J, Diaz E, Topeli A, DeWaele J, Carneiro A, Martin-Loeches I, Armaganidis A, Rello J. EU-VAP/CAP Study Group Spectrum of practice in the diagnosis of nosocomial pneumonia in patients requiring mechanical ventilation in European intensive care units. Crit Care Med. 2009; 37:2360–2368.
5. Mouton CP, Bazaldua OV, Pierce B, Espino DV. Common infections in older adults. Am Fam Physician. 2001; 63(2):257-68.
6. Gavazzi G, Herrmann F, Krause HK. Aging and infectious diseases in the developing world. Clin Infect Dis. 2004; 39:83–91.
7. Mane AB. Ageing in India: Some social challenges to elderly care. J Gerontol Geriatr Res. 2016; 5:136.
8. Vijay S, Dalela G. Prevalence of LRTI in Presenting with Productive Cough and Their Antibiotic Resistance Pattern. J Clin Diagn Res. 2016; 10(1):09-12.
9. Trouillet JL, Chastre J, Vuagnat A, Joly-Guillou ML, Combaux D, Dombert MC, Gibert C. Ventilator- associated pneumonia caused by potentially drug- resistant bacteria. Am J Respir Crit Care Med. 1998; 157:531–539.
10. Bont J, Hak E, Hoes AW, Schipper M, Schellevis FG et al. A prediction rule for elderly primary-care patients with lower respiratory tract infections. Eur Respir J. 2007; 29:969-75.
11. GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause of death, 1980-2015: A systematic analysis for the global burden of disease study 2015. Lancet. 2016; 388:1459-544.
12. Chastre J, Fagon JY. Ventilator-associated pneumonia. Am J Respir Crit Care Med. 2002; 165:867–903.
13. Canadian Critical Care Trials Group A randomized trial of diagnostic techniques for ventilator-associated pneumonia. N Engl J Med. 2006; 355:2619–2630.
14. Chastre J.Conference summary:ventilator-associated pneumonia. Respir Care. 2005; 50:975–983.
15. American Thoracic Society Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med. 2005; 171:388–416.
16. Gavazzi G, Herrmann F, Krause HK. Aging and infectious diseases in the developing world. Clin Infect Dis. 2004; 39:83–91.
17. Goel N, Chaudhary U, Aggarwal R, Bala K. Antibiotic sensitivity pattern of gram negative bacilli isolated from the lower respiratory tract of ventilated patients in the intensive care unit. Indian J Crit Care Med. 2009; 13(3):148-51.
18. Gagneja D, Goel N, Aggarwal R, Chaudhary U. Changing trend of antimicrobial resistance among gram negative bacilli isolated from lower respiratory tract of ICU patients: A 5-year study. Indian J Crit Care Med. 2011; 15(3):164-67.
19. Kumari HBV, Nagarathna S, Chandramuki A. Antimicrobial resistance pattern among aerobic gram- negative bacilli of lower respiratory tract specimens of intensive care unit patients in a neurocentre. Indian journal of chest diseases and allied sciences. 2007; 49(1):19.
20. Jackson ML, Neuzil KM, Thompson WW. The burden of community-acquired pneumonia in seniors: results of a population-based study. Clin Infect Dis. 2004:39(11):1642-50.
21. Pereira FO, Menis FA, da Silva BL, Evandro W, Pereira CNC, Alessandro RM.Collection of tracheal aspirate: safety and microbiological concordance between two techniques. Rev. esc. enferm. 2014; 48(4):618-24.
22. Koenig SM and Truwit JD. Ventilator-Associated Pneumonia: Diagnosis, Treatment, and Prevention. Clin Microbiol Rev. 2006; 19(4):637–57.
23. J G Collee, W Marr. Specimen collection, culture containers, and media. In: Mackie and McCartney Practical Medical Microbiology. 14th edition. JG Collee, AG Fraser, 113 BP Marmion, A Simmons, editors. Churchill livingstone, Gurgaon Haryana, 2014, 95-100
24. Clinical and Laboratory Standards Institute (CLSI). M100-S24. Performance standards for antimicrobial susceptibility testing; 24th informational supplement: Wayne, PA: CLSI, 2014.
25. Rudresh SM, Ravi GS, Sunitha L, Hajira SN, Kalaiarasan E, Harish BN. Simple, rapid, and cost- effective modified Carba NP test for carbapenemase detection among Gram negative bacteria. J Lab Physicians. 2017; 9:303-07.
26. Panchal CA, Oza SS, Mehta SJ. Comparison of four phenotypic methods for detection of metallo-β- lactamase-producing Gram-negative bacteria in rural teaching hospital. J Lab Physicians. 2017; 9:81-83.

Conflict of Interest: Nil
Source of support:Nil