Clinical Investigation

Ventilator associated pneumonia in a medical intensive care unit: Microbial aetiology, susceptibility patterns of isolated microorganisms and outcome

Address for correspondence:
Dr. Varun Goel,
Department of Microbiology,
Jawaharlal Nehru Medical College, Belgaum - 590 010, Karnataka, India.
E-mail: drvarun21@gmail.com

Varun Goel, Sumati A Hogade, SG Karadesai
Department of Microbiology, Jawaharlal Nehru Medical College, Belgaum, Karnataka, India

Access this article online
Website: www.ijaweb.org
DOI: 10.4103/0019-5049.104575
Quick response code

ABSTRACT

Background: Ventilator-associated pneumonia (VAP) is a common complication of ventilatory support for patients with acute respiratory failure and is associated with increased morbidity and mortality. **Aim of the Study:** The present study was undertaken to do quantitative cultures of aerobic bacteria, perform the antibiotic susceptibility testing from the endotracheal aspirates and clinical outcome of the clinically suspected patients of VAP. **Methods:** A prospective study was performed over a period of one year in a tertiary care hospital, enrolling patients on mechanical ventilation (MV) for ≥48 hr. Endotracheal aspirates (ETA) were collected from patients with suspected VAP, and direct gram's stain criteria was used to accept the sample. Quantitative cultures of ETA were performed with the threshold for microbiological diagnosis of VAP was taken as $\geq 10^5$ colony forming units (cfu)/ml. **Results:** Out of 53 cases, 2 (3.77%) were polymicrobial. Multidrug resistant bacteria, mainly *Acinetobacter baumannii* 49.09% (27/55) and *Pseudomonas aeruginosa* 30.91% (17/55) were the most common pathogens isolated. Metallo-beta lactamases (MBLs) was produced by 47.06% (8/17) of *Pseudomonas aeruginosa* and 62.96% (17/27) of *Acinetobacter baumannii*. **Conclusion:** The bacteriological approach for the management of VAP helps the clinicians in choosing the appropriate antibiotics. This study showed that quantitative cultures of endotracheal aspirate at a cutoff point of 10^5 cfu/ml is one of the alternative to bronchoscopy in the diagnosis of clinically suspected ventilator associated pneumonia.

Key words: *Acinetobacter baumannii*, Endotracheal aspirate, metallo-beta lactamases, quantitative cultures, ventilator-associated pneumonia

INTRODUCTION

Ventilator-associated pneumonia (VAP) is defined as pneumonia occurring more than 48 hours after patients have been intubated and received mechanical ventilation.[1] VAP is the most common nosocomial infection in the intensive care unit (ICU) with an incidence ranging from 8% to 28% in intubated mechanically ventilated patients.[2,3]

Ventilator-associated pneumonia complicates the course of patients receiving mechanical ventilation inspite of major advances in techniques for its diagnosis and treatment. In the absence of a gold standard, VAP is assumed to be diagnosed more accurately by bronchoscopic sampling and microbiological cultures of the lower respiratory tract. Bronchoscopy, being invasive, is not uncommonly associated with complications, especially in patients on high respiratory supports. This has paved the way for less invasive tests such as endotracheal aspirates (ETA) and quantitative ETA cultures with a threshold of 10^5 to 10^6 bacteria per milliliter of exudates that is considered as optimal for the microbiological confirmation of VAP.[4-6]

The American thoracic society (ATS) guidelines recommend that quantitative cultures can be performed on ETA or samples collected either bronchoscopically or nonbronchosopically.[7] More

How to cite this article: Goel V, Hogade SA, Karadesai SG. Ventilator associated pneumonia in a medical intensive care unit: Microbial aetiology, susceptibility patterns of isolated microorganisms and outcome. Indian J Anaesth 2012;56:558-62.

importantly, recent small trials have repeatedly shown that there is no advantage of bronchoscopic cultures over quantitative ETA cultures when mortality was considered as the end-point further strengthening the case for quantitative ETA as a diagnostic tool.[8-11]

Detection of causative organisms and their antibiotic susceptibility is crucial for diagnosis of VAP in order to initiate the appropriate antibiotic treatment thereby reducing the adverse effects of inadequate antibiotic treatment on the patient prognosis.[12]

Hence, the present study is undertaken to isolate, identify and quantitate bacteria and to perform the antibiotic susceptibility testing from the endotracheal aspirates of the clinically suspected patients of VAP.

METHODS

The prospective study was carried out during the period from January 2010 to December 2010 in Department of Microbiology and Medical Intensive Care Unit (MICU) on 54 patients with clinical suspicion of ventilator-associated pneumonia. The approval of the institutional review board was obtained during the planning phase of the study and each patient (or his/her caregivers) gave informed consent prior to participation in the study.

Inclusion criteria

All the patients 18 years and above who were under mechanical ventilation for more than 48 hours and clinically suspected of having contracted VAP were included in this study. The diagnosis of VAP was established using clinical pulmonary infection score (CPIS), which was evaluated on a daily basis until the patient was on ventilator support. CPIS of greater than six was used as diagnostic criteria for VAP.[13] Clinically diagnosed ventilator-associated pneumonia were observed and clinical parameters were recorded from their medical records and bedside charts.

Exclusion criteria

All patients with clinical and radiological signs suggestive of pneumonia on admission.

Collection of endotracheal aspirates

Endotracheal aspirate (≥1 ml) was collected under aseptic precaution after 48 hours of intubation whenever patient was suspected to have developed VAP in MICU. The ETA was collected using a 22-inch Ramson's 12 F suction catheter with a mucus extractor, which was gently introduced through the endotracheal tube for a distance of approximately 25-26 cm. Chest vibration or percussion for 10 min was used to increase the retrieved volume (1 mL) in case the patient produced very little secretions. Only 1 ETA sample was collected from each patient and was immediately taken to the laboratory for prossessing.

Microbiological processing

The aspirate specimens showing presence of <10 squamous epithelial cells per low power field or organisms seen under oil immersion in the entire field on Gram stain were included in the study.[14,15] Samples were homogenized by vortexing for 1 min and centrifuged at 3000 rpm for 10 min. 1 ml of sample was diluted in 10 ml of 0.9% sterile saline solution with final log dilutions of 10^{-1},10^{-2}, 10^{-3}, 10^{-4}, 10^{-5},10^{-6}. The samples were then plated on sheep blood agar (SBA), chocolate agar (CA), and MacConkey agar (MA) by using 4 mm Nichrome wire loop (Hi-media, Mumbai, India), which holds 0.01 ml of solution. All plates were incubated overnight at 37°C and chocolate agar plates at 37°C in 5% CO_2 incubator. Plates were incubated 18-24 hours at 37ºC. Threshold for quantitative cultures of ETA was considered as 10^5 cfu/ml. Growth of any organism below the threshold was assumed to be due to colonization or contamination. Organisms were identified and the antimicrobial susceptibility tests of the following drugs were determined by the Kirby-Bauer disc diffusion method: Erythromycin (E) (15 μg), Clindamycin (Cd) (10 μg), Cotrimoxazole (CO) (25 μg), Cephalexin (Cp) (5 μg), Linezolid (Lz) (30 μg), Doxycycline (Do) (30 μg), Ciprofloxacin (Cip) (5μg), Ceftazidime (Caz) (30 μg), Amoxyclav (Amc) (30 μg), Vancomycin (Va) (30 μg), Amikacin (Ak) (30 μg), Imipenem (I) (10 μg), Cefotaxime (Ce) (30 μg), Piperacillin-tazobactam (Pt) (100 μg/10 μg) (Hi-media Laboratories, Mumbai). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains.[16] Isolates showing reduced susceptibility to either ceftazidime (30 μg) or cefotaxime (30 μg) discs were considered as'screen positive' for AmpC beta lactamases and selected for detection of plasmid-mediated AmpC by the AmpC disc test.[17]

As per CLSI 2011 guidelines, when using the new interpretive criteria, routine ESBL testing is no longer necessary before reporting results (ie, it is no longer necessary to edit results for cephalosporins, aztreonam, or penicillins to resistant).[16]

Isolates showing reduced susceptibility to imipenem were selected for detection of metallo-beta lactamases

(MBL) enzymes by Imipenem-EDTA combined disc method.[18] Chi-square test was used to compare proportions of groups.

RESULTS

This prospective study was done in the period from January 2010 to December 2010 in our hospital ICU. 54 patients were enrolled for the present study as per inclusion criteria. One sample was rejected as per the gram stain criteria. The mean±SD age of patients was 46.4±18.45 years (range 18 to 86 years), having a predominance of male population. Bimodal distribution of age was observed with the first peak between 18-28 years of age group and second peak at around ≥59 years of age(28.89%).

Distribution of total number of cases was done depending on the duration of mechanical ventilation into "early-onset VAP" developing within 4 days of intubation and "late-onset VAP" developing after 4 days of intubation. The percentage of patients with early onset VAP was 39.62% and late onset VAP was 60.38%.

In our study, *Acinetobacter baumannii* caused predominantly bilateral and right sided pneumonia whereas *Pseudomonas aeruginosa* caused more of bilateral bronchopneumonia [Table 1].

Of 53 patients diagnosed as VAP based on a CPIS score of more than six, 51 (96.23 %) patients had monomicrobial infection and 2 (3.77%) had polymicrobial infection. Among the 27 isolates of *Acinetobacter baumannii*, 1 (3.70%) was resistant to all group of antibiotics tested in the study, including carbapenems. Other 25 (92.59%) isolates of *Acinetobacter baumannii* were resistant to Cotrimoxazole, Ciprofloxacin and Amikacin, 24 (88.89%) to Imipenem, 23 (85.18%) to Ceftazidime, 11 (40.74%) to Doxycycline, 10 (37.04%) to Piperacillin-tazobactam. All the 27 (100%) isolates of *Acinetobacter baumannii* were multidrug resistant (MDR) i.e. resistant to three or more class of antibiotics.

Pseudomonas aeruginosa was resistant to Gentamicin (100%), Aztreonam (88.23%), Ciprofloxacin and Amikacin (82.35%), Imipenem (47.06%), Ceftazidime (35.29%) and Piperacillin-tazobactam (23.53%).

Figure 1 shows that late-onset VAP was more common than early-onset VAP. Among the early onset VAP common organisms isolated were *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Citrobacter freundii* and *Staphylococcus aureus* while in case of late onset VAP common organisms were *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*. Most of the isolates were multidrug resistant organisms (MDR).

Eight (47.06%) of the 17 *Pseudomonas aeruginosa* isolates were MBL producers. Among 27 isolates of *Acinetobacter baumannii*, 12 (44.44%) were AmpC producers while 14 (51.85%) were MBL producing strains. The co-existence of AmpC and MBL was reported in 9 (33.33%) isolates of *Acinetobacter baumannii* and 2 (11.76%) isolates of *Pseudomonas aeruginosa* [Figure 2].

In the present study it was found that the mortality rate was 45.28%.Rate of mortality was less in early-onset VAP (23.80%) as compared to late onset VAP group (59.37%) and the difference was found to be statistically significant (x_1^2=6.473; df=1; *P*=0.011).

DISCUSSION

Out of 53 cases of ventilator-associated pneumonia, 16 (30.19%) were female and 37 (69.81%) were male. Aetiological agents widely differ according to the population of the patients in the intensive care unit, duration of hospital stay and prior antimicrobial

Table 1: Chest X-ray finding in relation to the organism isolated

		Chest X-ray findings		
Organisms	**No. of isolates**	**Left side no. (%)**	**Right side no. (%)**	**Bilateral no. (%)**
Acinetobacter baumannii	27	6 (22.2)	10 (37.03)	11 (40.74)
Acinetobacter species	1	0	1 (100)	0
P. aeruginosa	17	2 (11.76)	5 (29.41)	10 (58.82)
K. pneumoniae	3	1 (33.33)	2 (66.67)	0
E. coli	2	1 (50)	1 (50)	0
C. freundii	1	1 (100)	0	0
S. aureus	4	2 (50)	1 (25)	1 (25)
Total	55	13	20	22

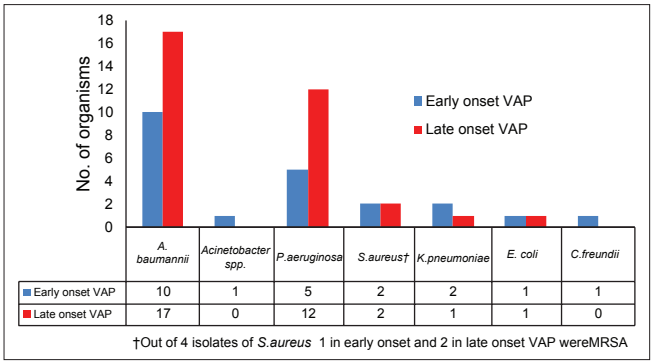

Figure 1: Organisms causing VAP (Early and late onset VAP)

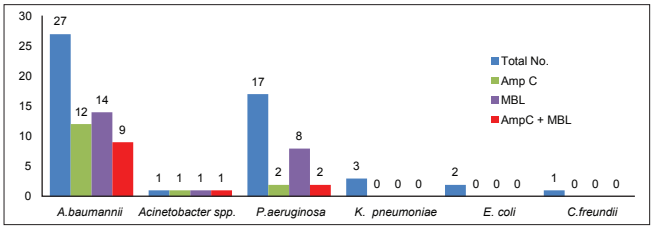

Figure 2: Different enzymes produced by the isolated strains

therapy. The increase of *Acinetobacter baumannii* (49.09%) infections is due to its great resistance to the environment which enables it to spread, its limited virulence and its extraordinary ability to develop resistance to all the antimicrobials and spread by aerosols.[19]

Two isolates of *Staphylococcus aureus* were isolated from early-onset VAP. One of them was methicillin-resistant *Staphylococcus aureus* (MRSA), but MRSA is generally associated with late onset VAP. This may be due the fact that this case showed mixed infection with predominant organism being *Citrobacter freundii* (1.8×10^7 cfu/ml) while MRSA was showing 2.1×10^6 cfu/ml in quantitative cultures. Secondly, the patient had history of prior antibiotic therapy which increases risk of MRSA.

Husni *et al.*[20] found X-ray pattern in *Acinetobacter baumannii* VAP is non-specific. In half of the cases it causes lung infiltration and a diffuse bilateral pattern is seen in other half of the cases. Some enzymes of *Pseudomonas aeruginosa* have invasive properties, causing thrombosis of pulmonary vessels and pulmonary infarction; this produces nodular bilateral lesions, predominantly in inferior lobes, and it is such a characteristic image that it should make the presence of *Pseudomonas aeruginosa* suspected.[21]

Multidrug-resistant (MDR) organisms are a major threat to VAP patients. The antibiotic resistance pattern of nonfermenters was almost the same in both early- and late-onset VAP. Many of the early-onset VAP cases had the risk factors such as prior antibiotic therapy and current hospitalization for five days or more for infection with MDR pathogens. That could be the reason for almost similar susceptibility pattern of the isolates from early- and late-onset VAP. Even the American Thoracic Society guidelines support the same reasoning by suggesting that patients with early-onset VAP who have received prior antibiotics or who had the prior hospitalization within the past 90 days are at greater risk for colonization and infection with MDR pathogens and should be treated similarly to patients with late-onset VAP.[22]

AmpC beta-lactamases are of increasing clinical concern in gram-negative bacteria. AmpC beta-lactamases hydrolyze cephamycins and are not inhibited by commercially available beta-lactamase inhibitors. These enzymes are typically associated with multiple antibiotic resistance, leaving few therapeutic options. Bacteria, mostly *Klebsiella pneumoniae* and *E. coli*, producing plasmid-mediated AmpC beta-lactamases have been responsible for nosocomial outbreaks of infection and colonization.[23,24] Twelve (44.44%) out of 27 isolates of *Acinetobacter baumannii* have shown production of AmpC beta lactamase enzyme, and no other Enterobacteriaceae showed production of AmpC beta lactamase.

Presently, there is concern about the acquisition of plasmid-mediated metallo-beta lactamases active against carbapenems, penicillins and cephalosporins. In this study, 14 (51.85%) isolates of *Acinetobacter baumannii* and 8 (47.06%) isolates of *Pseudomonas aeruginosa* were plasmid-mediated metallo-beta lactamases enzyme producing strains detected by imipenem-EDTA combined disc method which corresponds to a study were metallo-beta lactamases were produced by 21.74% of *Acinetobacter* spp. and 50% of *Pseudomonas aeruginosa*.[12]

Early-onset VAP in our study was found to be 39.62% while in various study it was found to be around 40%.[25] The low incidence of early onset VAP in our study may be due to antibiotic use before admission to the ICU. Studies[26] have shown that previous antibiotic use decreases early-onset VAP but markedly increases multidrug-resistant (MDR) pathogens, which is also reflected in our study.

In the present study, it was found that the mortality rate

was 45.28% which was in accordance with previous studies such as those by Gupta A *et al.*[27] (46.67%).

CONCLUSION

The bacteriological approach for the management of VAP avoids the problem of overtreatment by separating colonizers from infecting pathogens. This study showed that quantitative culture of ETA is a useful test for early diagnosis of VAP. The antibiotic susceptibility pattern of these isolates will also help the clinicians to choose the appropriate antimicrobial agents for prophylactic as well as treatment purposes.

REFERENCES

1. Koenig SM, Truwit JD. Ventilator-associated pneumonia: Diagnosis, treatment, and prevention. Clin Microbiol Rev 2006;19:637-57.
2. Chastre J, Fagon JY. Ventilator-associated pneumonia. Am J Respir Crit Care Med 2002;165:867-903.
3. Safdar N, Crnich CJ, Maki DG. The pathogenesis of ventilator-associated pneumonia: Its relevance to developing effective strategies for prevention. Respir Care 2005;50:725-39.
4. Cook D, Mandell L. Endotracheal aspiration in the diagnosis of ventilator-associated pneumonia. Chest 2000;117:195S-7.
5. Fujitani S,Yu VL. Quantitative cultures for diagnosing Ventilator-associated pneumonia: A critique. Clin Infect Dis 2006;43:S106-13.
6. Nair S, Sen N, Peter JV, Raj JP, Brahmadathan KN. Role of quantitative endotracheal aspirate and cultures as a surveillance and diagnostic tool for ventilator-associated pneumonia: A pilot study. Indian J Med Sci 2008;62:304-13.
7. American Thoracic Society; Infectious Diseases Society of America. 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.
8. Ruiz M, Torres A, Ewig S, Marcos MA, Alcon A, Lledo R, *et al*. Noninvasive versus invasive microbial investigation in ventilator associated pneumonia: Evaluation of outcome. Am J Respir Crit Care Med 2000;162:119-25.
9. Sanchez-Nieto JM, Torres A, Garcia-Cordoba F, El-Ebiary M, Carrillo A, Ruiz J, *et al*. Impact of invasive and noninvasive quantitative culture sampling on outcome of ventilator-associated pneumonia: A pilot study. Am J Respir Crit Care Med 1998;157:371-6.
10. Arango VM, Torres AM, Ordenana IJ, Lerma AF, Joaquinet CN, Casado HM, *et al*. Diagnostic value of quantitative cultures of endotracheal aspirate in ventilator-associated pneumonia: A multicenter study. Arch Bronconeumol 2003;39:394-9.
11. Wu CL, Yang D, Wang NY, Kuo HT, Chen PZ. Quantitative culture of endotracheal aspirates in the diagnosis of ventilator-associated pneumonia in patients with treatment failure. Chest 2002;122:662-8.
12. 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.
13. Pugin J, Auckenthaler R, Mili N, Janssens JP, Lew PD, Suter PM. Diagnosis of ventilator-associated pneumonia by bacteriologic analysis of bronchoscopic and nonbronchoscopic "blind" bronchoalveolar lavage fluid. Am Rev Respir Dis 1991;143:1121-9.
14. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic Microbiology, 12th ed. Missouri: Mosby Elsevier; 2007. p. 811.
15. Nagendra S, Bourbeau P, Brecher S, Dunne M, Larocco M, Doern G. Sampling variability in the microbiological evaluation of expectorated sputa and endotracheal aspirates. J Clin Microbiol 2001;39:2344-7.
16. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; 21th Informational Supplement (M100-S21). Wayne, Pa: Clinical and Laboratory Standards Institute; 2011.
17. Singhal S, Mathur T, Khan S, Upadhyay DJ, Chugh S, Gaind R, *et al*. Evaluation of methods for Amp C beta-lactamase in gram negative clinical isolates from tertiary care hospitals. Indian J Med Microbiol 2005;23:120-4.
18. Behera B, Mathur P, Das A, Kapil A, Sharma V. An evaluation of four different phenotypic techniques for detection of metallo-β-lactamase producing *Pseudomonas aeruginosa*. Indian J Med Microbiol 2008;26:233-7.
19. Brooks SE, Walczak MA, Rizwanullah H. Are we doing enough to contain *Acinetobacter* infections? Infect Control Hosp Epidemiol 2000;21:304.
20. Husni RN, Goldstein LS, Arroliga AC, Hall GS, Fatica C, Stoller JK, *et al*. Risk factors for an outbreak of multi-drug-resistant acinetobacter nosocomial pneumonia among intubated patients. Chest 1999;115:1378-82.
21. Valles J, Mariscal D. Pneumonia due to Pseudomonas aeruginosa. In: Rello J, editor. Nosocomial Pneumonia Strategies for Management, 1st ed. Great Britain: Wiley; 2007. p.100.
22. Joseph NM, Sistla S, Dutta TK, Badhe AS, Rasitha D, Parija SC. Ventilator-associated pneumonia in a tertiary care hospital in India: Role of multidrug resistant pathogens. J Infect Dev Ctries 2010;4:218-25.
23. Lagamayo EN. Antimicrobial resistance in major pathogens of hospital-acquired pneumonia in Asian countries. Am J Infect Control 2008;36:S101-8.
24. Black JA, Moland ES, Thomson KS. AmpC Disk Test for Detection of Plasmid-Mediated AmpC ß-Lactamases in *Enterobacteriaceae* Lacking Chromosomal AmpC ß-Lactamases. J Clin Microbiol 2005;43:3110-3.
25. Kollef MH. What is ventilator-associated pneumonia and why is it important? Respir Care 2005;50:714-21.
26. Park DR. The microbiology of ventilator-associated pneumonia. Respir Care 2005;50:742-63.
27. Gupta A, Agrawal A, Mehrotra S, Singh A, Malik S, Khanna A. Incidence, risk stratification, antibiogram of pathogens isolated and clinical outcome of ventilator associated pneumonia. Indian J Crit Care Med 2011;15:96-101.

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