Characterisation of aerobic bacteria isolated from endotracheal aspirate in adult patients suspected ventilator associated pneumonia in a tertiary care center in Mangalore

Ramakrishna Pai Jakribettu, Rekha Boloor
Department of Microbiology, Fr. Muller Medical College, Mangalore, Karnataka, India

ABSTRACT

Background and Objectives: Despite advances in antimicrobial therapy, better supportive care modalities and use of a wide range of preventive measures, ventilator-associated pneumonia (VAP) continues to be an important cause of morbidity and mortality in intensive care unit (ICU). VAP requires a rapid diagnosis and initiation of appropriate antibiotic treatment, to prevent mortality and morbidity. Inappropriate and inadequate antibiotic treatment causes emergence of drug resistance in pathogens and poor prognosis in patients. Early detection of pathogens causing VAP helps to control their spread by administration of suitable antibiotics and proper infection control measures. The study was conducted to know the pathogens causing VAP in Fr. Muller Medical College Hospital, Mangalore, and their susceptibility pattern. Methods: A total of 100 patients, on mechanical ventilation for more than 48 h, who were suspected to have VAP were included in the study between December 2008 and November 2009. Their endotracheal aspirates (ETAs) were collected and processed. From 100 ETA, 138 isolates of count >10^5 CFU/mL were characterized and antibiogram was determined using standard antibiotics regime.

Results: Incidence of VAP was found to be 44.2% among the mechanically ventilated patients. Klebsiella pneumoniae (34%) was the most common pathogen isolated, followed by Pseudomonas aeruginosa (20%). Among them, most of the K. pneumoniae and P. aeruginosa isolates were resistant to penicillins, cephalosporins, fluoroquinolones was observed but were sensitive to piperacillin/tazobactum, cefaperazone/sulbactum, and carbapenems. All isolates were sensitive to amikacin. Interpretation and Conclusion: The present study shows prevalence of multidrug-resistant organisms in the study region. Klebsiella species was the most common pathogen isolated in ETA. Acinetobacter species were the most resistant pathogens prevailing in our ICU setup, leading to the increased mortality in the ventilated patients. Patients with chronic obstructive pulmonary disease is the most common predisposing factor for VAP in the study group.

Keywords: Hospital-acquired pneumonia, multidrug-resistant organisms, ventilator-associated pneumonia

Introduction

Nosocomial pneumonia are inflammatory conditions of the lung parenchyma caused by an infectious agent, not present or incubating at the time of admission and developed after 48–72 h of admission to the hospital. Ventilator-associated pneumonia (VAP), an important form of hospital-acquired pneumonia (HAP), specifically refers to pneumonia developing in a patient on mechanical ventilator for more than 48 h after intubation or tracheostomy. Despite the advancements in antimicrobial regimes, VAP continues to be an important cause of morbidity and mortality. VAP requires a rapid diagnosis and initiation of appropriate antibiotic treatment, as there is adverse effect of inadequate antibiotic treatment on patients’ prognosis and the emergence of multidrug-resistant (MDR) pathogens.

Access this article online

Quick Response Code:

Website: www.saudija.org
DOl: 10.4103/1658-354X.97022
The time of onset of pneumonia is an important risk factor for specific pathogens and outcome in patients with VAP. Early onset VAP, defined as occurring within first 4 days of hospitalization, usually caused by antibiotic-sensitive bacteria, that is, community acquired, whereas the late onset, that is, more than 5 days are associated with increased mortality in patients. The emergence of MDR pathogens is becoming a therapeutic challenge as the treatment alternatives are unavailable, toxic, and with poor outcome.

The aim of this study is to identify the bacterial pathogens causing VAP in our intensive care unit (ICU) setup and know their antibiotic profile.

**METHODS**

It was a prospective study done from December 2008 to November 2009 at ICU of selected 1200-bed tertiary care hospital, where patients were on mechanical ventilation for more than 48 h.

**Sampling technique**

The endotracheal aspirate (ETA) was collected by nonbronchoscopic method. 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 (ETT) for a distance of approximately 25–26 cm. Gentle aspiration was then performed without instilling saline, and the catheter was withdrawn from the ETT. After the catheter was withdrawn, 2 mL of sterile 0.9% normal saline was injected into it with a sterile syringe to flush the exudates into a sterile container for collection and transported to microbiology laboratory. ETA samples were immediately processed. The results of the Gram's stain were obtained within the first hour and quantitative cultures were performed immediately as proceeded by Rajashekar and co-workers.[5]

**Processing of sample**

Samples were mechanically liquefied and homogenized by vortexing for 1 min. The 0.01 mL of sample solution was then plated on sheep blood agar, chocolate agar (CA), MacConkey agar by using 4 mm Nichrome wire loop (Hi-Media, Mumbai, India). All plates were incubated overnight at 37°C and CA plates at 37°C in candle jar. All plates were checked for growth overnight and then after 24 and 48 h of incubation. For definite diagnosis of VAP, 10⁸ CFU/mL was considered as threshold. Growth of any organism below the threshold was assumed to be due to colonization or contamination. Any growth was characterized by colony morphology and Gram's staining from the plates. A detailed biochemical testing identified any significant growth, and antibiotic sensitivity testing was performed on Mueller–Hinton agar plates by Kirby—Bauer disc diffusion method.[6] *Escherichia coli* strain ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains.

**RESULTS**

A total number of 226 patients were on mechanical ventilator during the study period. Out of 226, only 100 patients were included in the study as their Clinical Pulmonary Infection Score (CPIS) > 6 after 48 h of MV. All together 138 bacteria were isolated from 100 ETA. Occurrence of VAP was common in men (68%) than women (32%) among the cases studied. Out of 100 VAP patients, 46 (46%) patients expired, and 41 improved and got discharged. This high mortality rate for the patients on ventilator may be contributed by the underlying disease rather than pneumonia in critically ill patients. So, VAP alone is not the cause for such a high mortality rate.[6] The maximum and minimum number of cases [Figure 1] were seen in the age group of 45–55 and 35–45 years, that is, 22 and 9, respectively. The youngest patient suffering from was 16 years and eldest being 94 years of age.

Table 1 shows that the occurrence of VAP was more common in patients with chronic obstructive pulmonary disease (COPD), followed by renal failure and diabetes mellitus (DM). Organo Phosphorus poisoning (9%) is one of the cause for respiratory failure requiring ventilator support in our ICU. *Klebsiella pneumoniae* is the most frequent isolate in VAP and the least being *Serratia* species [Figure 2]. *P. aeruginosa* and *Acinetobacter* species were the next common pathogens following *K. pneumoniae*. Figure 3 denotes that the cephalosporins were ineffective in >80% of the cases. Most of the pathogens were susceptible to amikacin (82.6%) and levofloxacin (77.5%). The carbapenems were effective in 82% of the pathogens.
DISCUSSION

While considering the nosocomial infections, our study on VAP has demonstrated it as one of the cause for increased morbidity and mortality in patients receiving mechanical ventilation in our Medical ICU. As mentioned earlier, MV itself is one of the risk factor for developing nosocomial pneumonia. The risk of developing pneumonia in ventilated patients increases as the number of days of ventilation. The risk of VAP is estimated to be 3%/day during the first 5 days of ventilation, 2%/day during 5–10 days of ventilation, and 1%/day thereafter. Therefore, patients on MV must be given extra care to prevent the development of VAP.

The occurrence of VAP in our study is 44.2%, which is similar to 45.4% as in study done by Dey et al.[6] [Table 2]. But the incidence is slightly higher than the other studies done on VAP.[7–13] The patients with a variety of predisposing factors, such as COPD, DM, multorgan failure, may be the cause for the increased occurrence in our study. We have seen the increased rates of VAP in patients on MV for 5–10 days [Figure 4]. Age group of 45–55 years showed increased incidence of VAP. This increased occurrence, may be as 45–55 years age group patients are more in number than other age group. In our study, K. pneumoniae (34%) was the most common isolate followed by P. aeruginosa (20%) and Acinetobacter species (18%). We had 3 isolates of MDR S. aureus (0.21%) from VAP, whose occurrence is very low in studies done in India compared with Western studies.[14–16]

All patients who were diagnosed to have VAP, blood culture was done, only 10% of the sample grew the pathogen.

Figure 1: Agewise distribution of incidence of ventilator-associated pneumonia

Figure 2: The distribution of pathogens isolated in ventilator-associated pneumonia

Figure 3: Percentage of bacterial resistance to various antibiotics

Figure 4: The duration of mechanical ventilation seen in patients with ventilator-associated pneumonia
isolated from the ETA. Hence blood culture was not helpful in diagnosing sepsis in VAP patients. Blood cultures have a low sensitivity for detecting the same pathogenic microorganism as respiratory sample in patients with VAP.[17] However, it is important to keep in mind not only that the sensitivity of blood cultures for the diagnosis of VAP is <25% but also that when positive, the organisms may originate from an extrapulmonary site of infection in as many as 64% of cases and even when VAP is present.[17]

In our study, we have isolated MDR pathogens from VAP. We have found that around 92% of the isolates are resistant to ampicillin, 1st and 2nd generation cephalosporins. Eighty percent of isolates are resistant to 3rd generation cephalosporins.

Among aminoglycosides, gentamicin was not effective in 58%, whereas amikacin is resistant only in 17.4% of isolates, but as most of the patients on ventilator had renal insufficiency. Hence, administration of amikacin was not recommended. Otherwise, amikacin had shown very good sensitivity among the MDR Gram negative bacilli.

Levofoxacin showed resistance only in 22.5% of the isolated compared with 56.5% ofloxacin among fluoroquinolones. Hence, levofoxacin may be used empirically in our MICU setup.

Carbapenems are the least resistant drug that are seen in our setup. Only 8% of the isolates showed resistance against carbapenems. All E. coli, P. aeruginosa, and K. pneumoniae except one isolate, were sensitive to carbapenems. The emergence of the resistance to carbapenems was noted during the end of the study period. Nowadays, metallo-beta-lactamas are the major cause of resistance in most of MICU setups. Hence, steps must be taken to prevent the spread of the resistance. Alterations and rotation in antibiotic prescribing patterns might decline the antibiotic resistance.[18,19] Thus, the present study gives importance of knowing the pathogens and their antibacterial susceptibility pattern, prevalent in the particular ICU, to initiate the empirical antibacterial therapy for patients on mechanical ventilation.

CONCLUSION

The following conclusions can be drawn from the present study:

- **Klebsiella** spp. and **P. aeruginosa** were the most common agents responsible for VAP
- The age group of 45–55 years was the most commonly associated VAP
- Most of the case of VAP cases was seen in the patients who were ventilated for >4 days
- Most common predisposing factors for developing VAP in our study was COPD, followed by DM, hypertension, ischemic heart disease, and renal failure
- Levofoxacin, amikacin, and carbapenems seem reasonable alternatives to cephalosporins for the treatment of VAP. A larger sample size study should be performed to confirm the present findings. It is the call of the day to identify the prevalent pathogens in each ICU setup and formulate antibiotic policy as well as protocol for the management of the various nosocomial infections.

REFERENCES

1. Strausbaugh LJ. Noscomial Respiratory Infection, Ch 301, in Mandell, Douglas and Bennett’s Principles and Practice of Infectious Disease, 6th ed, In: Mandell L, Bennett E, Editors Dolin, 2005;2:3362-70.
2. Rajasekhar T, Anuradha K, Suhasini T, Lakshmi V. The role of quantitative cultures of non-bronchoscopic samples in ventilator associated pneumonia. Indian J Med Microbiol 2006;24:107-13.
3. Miles RS, Amyes SG. Laboratory control of antimicrobial therapy, Chapter No. 8, in Mackie and McCartney Practical Medical Microbiology, in: Coller JG, Fraser aG, Marmion BP, Medical Microbiology, in: Coller JG, Fraser aG, Marmion BP, Simmons A, Editors, 14/e, New delhi, India: Elsevier Publication; 1996. p. 151-78.
4. Craven DE, Kunches LM, Kilinsky V, Lichtenberg DA, Make BJ, McCabe WR. Risk factors for pneumonia and fatality in patients receiving continuous mechanical ventilation. Am Rev Respir Dis 1986;133:792-6.
5. Cook DJ, Walter SD, Cook RJ, Griffith LE, Guyatt GH, Leasa D, et al. Incidence of and risk factors for ventilator-associated pneumonia in critically ill patients. Ann Intern Med 1998;129:440.
6. Dey A, Baity 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.

7. El-Ebiary M, Torres A, Gonzalez J, Bellacasa JP, Garcia C, Anta MT, et al. Quantitative cultures of endotracheal aspirates for the diagnosis of ventilator associated pneumonia. Am Rev Respir Dis 1993;148:1552-7.

8. Jourdain B, Novara A, Joly GM, Dombret MC, Calvat S, Trouillet JL, et al. Role of quantitative cultures of endotracheal aspirates in the diagnosis of nosocomial pneumonia. Am J Respir Crit Care Med 1995;152:241-6.

9. Chevret S, Hemmer M, Carlet J, Langer M. Incidence and risk factors of pneumonia acquired in intensive care units. Results from a multicenter prospective study on 996 patients. European Cooperative Group on Nosocomial Pneumonia. Intensive Care Med 1993;19:256-64.

10. Singhal R, Mohanty S, Sood S, Das B, Kapil A. Profile of bacterial isolates from patients with ventilator associated pneumonias in a tertiary care hospital in India. Indian J Med Res 2005;121:63-4.

11. Rakshit P, Nagar SV, Deshpande AK. Incidence, clinical outcome, and risk stratification of ventilator-associated pneumonia: A prospective cohort study. Indian J Crit Care Med 2005;9:211-6.

12. Goel N, Chaudhary U, Aggrawal R, Bala K. Antibiotic susceptibility pattern of the gram negative bacilli isolated from lower respiratory tract of ventilated patients in the intensive care unit. Indian J Med Crit Care Med 2009;13:148-51.

13. Ahmed SM, Choudhary J, Ahmed M, Arora V, Ali PS. Treatment of ventilator associated pneumonia with piperacillin/tazobactum and amikacin vs cefpime and levofloxacin: A randomized prospective study. Indian J Crit Care Med 2007;11:117-21.

14. Rello J, Torres A, Ricart M, Valles J, Gonzalez J, Artigas A, et al. Ventilator-associated pneumonia by Staphylococcus aureus: Comparison of methicillin-resistant and methicillin-sensitive episodes. Am J Respir Crit Care Med 1994;150:1545-9.

15. Fridkin SK. Increasing prevalence of antimicrobial resistance in intensive care units. Crit Care Med 2001;29:64-8.

16. Richards MJ, Edwards JR, Culver DH, Gaynes RP. Nosocomial infections in medical ICUs in the United States: National Nosocomial Infections Surveillance System. Crit Care Med 1999;27:887-92.

17. Luna CM, Videla A, Mattera J, Vay C, Famiglietti A, Vujacic P, et al. Blood cultures have limited value in predicting severity of illness and as a diagnostic tool in ventilator-associated pneumonia. Chest 1999;116:1075-84.

18. Fridkin SK, Gaynes RP. Antimicrobial resistance in intensive care units. Clin Chest Med 1999;20:303-16.

19. Niederman MS. Is ‘Crop rotation’ of antibiotics the solution to a ‘resistant’ problem in the ICU? Am J Respir Crit Care Med 1997;156:1029-31.

How to cite this article: Jakribettu RP, Boloor R. Characterisation of aerobic bacteria isolated from endotracheal aspirate in adult patients suspected ventilator associated pneumonia in a tertiary care center in Mangalore. Saudi J Anaesth 2012;6:115-9.

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

Staying in touch with the journal

1) Table of Contents (TOC) email alert
   Receive an email alert containing the TOC when a new complete issue of the journal is made available online. To register for TOC alerts go to www.saudija.org/signup.asp.

2) RSS feeds
   Really Simple Syndication (RSS) helps you to get alerts on new publication right on your desktop without going to the journal’s website. You need a software (e.g. RSSReader, Feed Demon, FeedReader, My Yahoo!, NewsGator and NewsCrawler) to get advantage of this tool. RSS feeds can also be read through FireFox or Microsoft Outlook 2007. Once any of these small (and mostly free) software is installed, add www.saudija.org/rssfeed.asp as one of the feeds.