VENTILATOR ASSOCIATED PNEUMONIA IN RESPIRATORY INTENSIVE CARE UNIT: MICROBIAL AETIOLOGY, SUSCEPTIBILITY PATTERNS OF ISOLATED MICROORGANISMS AND OUTCOME

P. V. Shiva[1], G. Rajendra[2], Deepraj Bias[3]

[1]Associate Professor, Department of Anaesthesia, Government Medical College, Nizamabad.
[2]Associate Professor, Department of Anaesthesia, Government Medical College, Nizamabad.
[3]Professor, Department of Anaesthesia, Government ENT Hospital, Hyderabad.

ABSTRACT

AIM

The aim of the study is to isolate, identify and quantitate bacteria and to perform the antibiotic susceptibility testing from the endotracheal aspirates in RICU patients with suspected Ventilator associated Pneumonia.

MATERIALS AND METHODS

This study is a prospective study conducted on 80 patients during the period of February 2011 to January 2012 in the Department of Anaesthesia & Dept. of Microbiology in Govt. Chest and General Hospital, Hyderabad, in patients, who were suspected to have ventilator associated pneumonia.

INCLUSION CRITERIA

Patients above 18 years, who received mechanical ventilation for more than 48 hours and clinically suspected of having contracted VAP were included in this study. Clinical pulmonary infection score (CPIS, was used to diagnose VAP which was evaluated on a daily basis until the patient was on ventilator support. CPIS of >6 was used as diagnostic criteria for VAP till clinically diagnosed ventilator-associated pneumonia was observed.

EXCLUSION CRITERIA

Patients who were suspected to have clinical and radiological pneumonia when admitted & paediatric patients were excluded.

RESULTS

97 patients taken up for the study were meeting eligibility criteria and were on mechanical ventilation for more than 48 hours, out of which 65 developed VAP. There were 40 males and 25 females. 69 (92%) were multidrug resistant out of the total 75 isolates, and it was observed that not even a single isolate was sensitive to all the drugs tested. Some of this resistance can be because of the presence of various degradative enzymes like ESBLs, AmpC β-lactamase and MBLs within these pathogens. Out of the total 28 isolates of Acinetobacter spp., (45%) isolates produced AmpC β-lactamase. In P. aeruginosa, it was seen to be produced by 20.1% isolates, in K. pneumoniae by 25.7% isolates, and in C. freundii it was seen to be produced by 64.2% isolates. The ESBL production was highest in case of E. coli (98%). It was also produced by 63.8% of Enterobacter spp. isolates, 15.9% of C. freundii and 12.2% of K. pneumoniae isolates. The MBL production was maximum in case of P. aeruginosa (25.9%). In case of Acinetobacter spp., it was 19.9% and in K. pneumoniae only 7.8% isolates produced MBL.

CONCLUSION

Almost all the pathogens were multidrug resistant and in all the isolates resistance was due to presence of ESBL, MBL, AmpC β lactamase. Thus, the intensivists can choose the antibiotics based on the bacteriological review of management of VAP. This study has shown that it is helpful in diagnosis of ventilator associated pneumonia and early specific treatment of these patients.

KEYWORDS

Ventilator Associated Pneumonia.

HOW TO CITE THIS ARTICLE: Shiva PV, Rajendra G, Bias D. Ventilator associated pneumonia in respiratory intensive care unit: Microbial aetiology, susceptibility patterns of isolated microorganisms and outcome. J. Evid. Based Med. Healthc. 2016; 3(56), 2880-2885. DOI:10.18410/jebmh/2016/628

INTRODUCTION: One of the most commonly associated encountered hospital acquired infections in respiratory intensive care unit is ventilator associated pneumonia (VAP) and it is associated with significant morbidity and high costs.

Financial or Other, Competing Interest: None.
Submission 24-06-2016, Peer Review 30-06-2016,
Acceptance 08-07-2016, Published 13-07-2016.
Corresponding Author:
Dr. P. V. Shiva,
#5-3-318, Jeera, M. G. Road,
Lane Opposite Buddha Bhavan, Secunderabad,
E-mail: shivavputcha@gmail.com
DOI: 10.18410/jebmh/2016/628

VAP is a common complication in patients receiving ventilatory support for patients with acute respiratory failure and is associated with increased morbidity and mortality. Ventilator-associated pneumonia (VAP) is defined as pneumonia occurring more than 48 hours after patients are intubated and ventilated mechanically.[1] VAP is the most common nosocomial infection in the intensive care unit (ICU) with an incidence ranging from 8% to 28% in intubated and mechanically ventilated patients.[2,3]

The course of patients is complicated by Ventilator associated pneumonia receiving ventilation mechanically in spite of major advances in techniques for its diagnosis and treatment. VAP is assumed to be diagnosed more accurately by bronchoscopic sampling and microbiological cultures of the lower respiratory tract in the absence of a gold standard. Bronchoscopy, being invasive, is not uncommonly associated with complications, especially the patients who are 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.[4,5] per millilitre of exudates that is considered as optimal for the microbiological confirmation of VAP. In the diagnosis of VAP, detection of causative organisms and their antibiotic susceptibility is crucial in order to initiate the appropriate antibiotic treatment thereby reducing the adverse effects of inadequate antibiotic treatment on the patient prognosis. The second most common nosocomial infection in the United States is pneumonia and the leading cause of death from hospital-acquired infections, with a crude mortality rate ranging from 20% to 50%. In the intensive care unit, it is the single most common infection which occurs. The aim of the study is to isolate, identify and quantitate bacteria and to perform the antibiotic susceptibility testing from the endotracheal aspirates of the clinically suspected patients of VAP.

MATERIALS AND METHODS: This study is a prospective study conducted on 80 patients during the period of February 2011 to January 2012 in the Department of Anaesthesia and Dept. of microbiology in Govt. Chest and General Hospital who were suspected to have ventilator associated pneumonia.

Inclusion Criteria: Patients above 18 years who have undergone ventilation mechanically for more than 48 hours and clinically suspected of having contracted VAP were included in this study. Clinical pulmonary infection score (CPIS, was used to diagnose VAP which was evaluated on a daily basis until the patient was on ventilator support. CPIS of >6 was used as diagnostic criteria for VAP till clinically diagnosed ventilator-associated pneumonia was observed.

Exclusion Criteria: Patients who were suspected to have clinical and radiological pneumonia on admission and paediatric patients were excluded. When significant growth was obtained in the culture of the samples, the diagnosis was confirmed. Endotracheal aspirates (ETA) and bronchoalveolar lavage (BAL) samples of the patients were collected and sent immediately to the laboratory for microbiological processing.

After making smears of the samples and the samples were then inoculated on blood agar, MacConkey agar and chocolate agar, Gram staining was done. Semi-quantitative cultures were done. Incubation of the MacConkey plates were done at 37˚C while incubation of blood agar and chocolate agar were done at 37˚C in presence of 5-10% carbon dioxide.

Growth which was >105 CFU/mL was taken as the cut-off threshold for endotracheal aspirates while growth which was >104 CFU/mL was taken as cut-off for BAL. Samples showing growth less than these thresholds were assumed to be due to colonisation or contamination. Isolate was identified using standard microbiological techniques in case of significant growth. Antibiotic testing was done by Kirby Bauer disk diffusion method for each isolate. If it was resistant to at least three classes of antimicrobial agents, an isolate was considered as MDR. ESBL was detected by combination disk method. If there was ≥5 mm increase in zone diameter of ceftazidime-clavulanate disk as compared to zone diameter of disk containing ceftazidime alone, organism was considered to be ESBL producer. Amp C β-lactamases detection was done by Amp C disk method. In the vicinity of the test disk, a positive test appeared as flattening or indentation of the cefoxitin inhibition zone. MBL detection was done by imipenem-EDTA combined disk method.

It was considered as MBL positive, if the increase in inhibition zone with the imipenem and EDTA disk was ≥7 mm than the imipenem disk alone. MRSA detection was done by cefoxitin disk diffusion method. The isolate was considered MSSA, if the inhibition zone around the cefoxitin disk was >22 mm and if the zone was <21 mm, then it was considered as MRSA. Organisms were identified and the antimicrobial susceptibility tests of the following drugs were determined by the Kirby-Bauer disk 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) (5 µg), 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) (HiMedia Laboratories, Mumbai). Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as quality control strains.

Isolates showing reduced susceptibility to either ceftazidime (30 µg) or cefotaxime (30 µg) disks were considered as screen positive for AmpC beta lactamases and selected for detection of plasmid-mediated AmpC by the AmpC disk test. As per CLSI 2011 guidelines, when using the new interpretive criteria, routine ESBL testing is no longer necessary before reporting results (i.e., it is no longer necessary to edit results for cephalosporins, aztreonam, or penicillins to resistant). Isolates showing reduced susceptibility to imipenem were selected for detection of metallo-beta lactamases (MBL) enzymes by Imipenem-EDTA combined disk method. Chi-square test was used to compare proportions of groups.

RESULTS: 97 patients were enrolled who were meeting eligibility criteria of who were on mechanical ventilation for more than 48 hours, out of which only 65 developed VAP. There were 40 males and 25 females. One was MRSA which was resistant to cephalexin, doxycycline and ciprofloxacin, out of the two isolates of S. aureus.

The only isolate of Enterococcus spp. was found to be resistant to vancomycin, gatifloxacin, pristinamycin. Gram-negative bacteria were found to be highly resistant to various drugs such as co-trimoxazole, doxycycline, amikacin, ciprofloxacin, ceftazidime, aztreonam, meropenem, piperacillin/tazobactam. Colistin, polymyxin-B and cefoperazone/sulbactam combination were found to be quite effective.

Bacterial Isolates	Number	Percentage
Gram + ve Staphylococcus aureus	3	4
Enterococcus Species	2	2.66
Gram-ve Acinetobacter baumannii	24	32
Acinetobacter lwoffii	4	5.33
Pseudomonas aeruginosa	18	24
Klebsiella pneumonia	14	18.66
Citrobacter freundii	5	6.66
Enterobacter species	3	4
Escherichia coli	2	2.66
Total	**75**	**100**

Table 1: Shows the Distribution of Organisms Isolated from Samples in VAP Patients

Bacterial Isolates	No. of Isolates	E	Ce	Cd	Cf	Va	Do	Lz	Cp	Gf	Ac	Pm
Staphylococcus aureus	3	0	1	0	0	1	0	1	0	2	0	0
Enterococcus	2	-	1	0	1	0	0	0	1	1	0	0

Table 2: Shows Antibiotic Susceptibility Pattern of Gram-positive Bacteria Isolated from VAP Patients

Cefoxitin (Ce), Erythromycin (E), Clindamycin (Cd), Gentamicin (G), Ciprofloxacin (Cf), Vancomycin (Va), Doxycycline (Do), Linezolid (Lz), Cephalexin (Cp), Gatifloxacin (Gf), Amoxyclav (Ac), and Pristinamycin (Pm).

Bacterial Isolates	No. of isolates	Co	Do	Ak	Cf	Ca	Ao	Mr	Pt	Pb	Cl	Cfs
Acinetobacter baumannii	24	21	18	18	20	19	18	17	0	0	6	2
Acinetobacter lwoffii	4	8	8	10	14	14	0	0	3	2	0	0
Pseudomonas aeruginosa	18	5	3	4	5	4	6	0	0	2	0	0
Klebsiella pneumonia	14	1	0	0	1	0	1	0	0	0	0	1
Citrobacter freundii	5	0	0	0	1	1	0	1	0	0	1	0
Enterobacter Species	3	0	0	2	0	1	0	2	1	0	1	0
Escherichia coli	2	0	1	0	1	0	1	1	1	0	0	0

Table 3: Shows Antibiotic Susceptibility Pattern of Gram-negative Bacteria Isolated from VAP patients

Co-trimoxazole (Co), Doxycycline (Do), Amikacin (Ak), Ciprofloxacin (Cf), Ceftazidime (Ca), Aztreonam (Ao), Meropenem (Mr), Piperacillin/Tazobactam (Pt), Polymyxin B (Pb), Colistin (Cl), Cefoperazone/ Sulbactam (Cfs), and Ticarcillin/clavulanate (Tc).

Bacterial isolates	AmpC %	ESBL %	MBL%
Acinetobacter Spp.	45	0	19.9
Pseudomonas aeruginosa	20.1	0	25.9
Klebsiella pneumonia	25.7	12.2	7.8
Citrobacter freundii	64.2	15.9	0
Enterobacter species	22.6	63.8	0
Escherichia coli	0	98	0

Table 4: Shows Distribution of AmpC, ESBL, MBL in Bacterial Isolates from VAP Patients

69 (92%) were multidrug resistant out of the total 75 isolates, and it was observed that not even a single isolate was sensitive to all the drugs tested. Some of this resistance can be because of the presence of various degradative enzymes like ESBLs, AmpC β-lactamase and MBLs within these pathogens. Out of the total 28 isolates of Acinetobacter spp., (45%) isolates produced AmpC β-lactamase.

In P. aeruginosa, it was seen to be produced by 20.1% isolates, in K. pneumoniae by 25.7% isolates, and in C. freundii, it was seen to be produced by 64.2% isolates. The ESBL production was highest in case of E. coli (98%). It was also produced by 63.8% of Enterobacter spp. isolates, 15.9% of C. freundii and 12.2% of K. pneumoniae isolates. The MBL production was maximum in case of P. aeruginosa (25.9%).

In case of Acinetobacter spp., it was 19.9% and in K. pneumoniae only 7.8 isolate produced MBL.

DISCUSSION: Many studies have been reported regarding ventilator associated pneumonia. Varun Goel et al[6] have done a study which was a prospective study which was performed over a period of one year in a tertiary care hospital, enrolling patients on mechanical ventilation (MV) for ≥48 hrs. Endotracheal aspirates (ETA) were collected from patients with suspected VAP, and direct Gram 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. The results were that 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-betalactamases (MBLs) were produced by 47.06% (8/17) of Pseudomonas aeruginosa and 62.96% (17/27) of Acinetobacter baumannii. This study concluded that 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 cut-off point of 10^5 cfu/mL is one of the alternative to bronchoscopy in the diagnosis of clinically suspected ventilator associated pneumonia. Ajeet Kumar et al[7] have done a study which was a prospective cross sectional study was conducted in ICU at JMCH, Korangi, Karachi from Jan 2012 to Jan 2013. Patients, who received mechanical ventilation >48 hours, were prospectively followed for occurrence of VAP. The clinical diagnosis of VAP was made on the basis of CPIS criteria and confirmed by quantitative culture of tracheal secretion. The results were 275 patients meeting inclusion criteria included in the study, out of which 84 (30.5%) developed VAP. The common pathogens were Pseudomonas aeruginosa (63%), Acinetobacter lwoffii (22%) and Staphylococcus aureus (33%).

Increased ICU stay and overall mortality (59.5%) was observed in VAP group. This study concluded that the frequency of VAP in our ICU was comparable to other settings in our region, most common pathogens are Gram-negative bacilli which showed resistance to many antibiotics. Mortality was high in patients developing VAP when compared to patients on ventilator not developing pneumonia. Noopur Goel et al[8] aimed to critically review the incidence and outcome, identify various risk factors and conclude specific measures that should be undertaken to prevent VAP. We studied 40 patients randomly, kept on ventilator support for more than 48 hours. After excluding those who developed pneumonia within 48 hours, VAP was diagnosed when a clinical pulmonary infection score of ≥ 6 was obtained. Endotracheal aspirates (ETA) were collected from patients with suspected VAP. The finding of this study was that incidence of VAP was found to be 70% of which 50% were polymicrobial. Multidrug resistant bacteria, mainly Acinetobacter baumannii, and Pseudomonas aeruginosa were the most common pathogens isolated while Klebsiella pneumoniae has emerged as the most resistant strain showing sensitivity only to Polymyxin B of the drugs tested.

This study interpreted that occurrence of VAP in patients on mechanical ventilation with no other predisposing factors is still a major concern in developing countries. Repeated monitoring of the endotracheal aspirates for early diagnosis, and its antibiogram will be a guide for an approach to targeted treatment; thus assist in tackling the serious problem of MDR faced in ICU environment. Lila Bouadma et al[9] compared VAP rates during a 45-month baseline period and a 30-month intervention period in a cohort of patients who received mechanical ventilation for 148 hrs. VAP was diagnosed on the basis of quantitative cultures of distal specimens. VAP incidence density rates were expressed as total VAP episodes over total mechanical ventilation duration and as first VAP episodes over mechanical ventilation duration at VAP or hospital discharge. We used segmented regression analysis and a Cox proportional hazard model to assess the impact of the program on first VAP occurrence.

The results were baseline and intervention VAP rates were 22.6, and 13.1 total VAP episodes over total mechanical ventilation duration per 1000 ventilation-days, respectively, and 26.1 and 14.9 first VAP episodes over mechanical ventilation duration at VAP or hospital discharge per 1000 procedure-days respectively (P .001). VAP rates decreased by 43% in both statistical analyses and remained significant after adjustment for confounders (Cox adjusted hazard ratio, 0.58; 95% confidence interval, 0.46–0.72; P .001). Daily VAP hazard rates on ventilation days 5, 10, and 15 were 2.6%, 3.5%, and 3.4%, respectively, during the baseline period and 1.4%, 2.3%, and 2% respectively, during the intervention period. This study concluded that our preventive program produced sustained VAP rate decreases in the longterm.

However, VAP rates remained substantial despite high compliance with preventive measures, suggesting that eliminating VAP in the intensive care unit may be an unrealistic goal. Wajahat Ahmed et al[10] in their descriptive study, nasobronchial lavages (NBL) were obtained by using a suction tube. These samples were processed using standard microbiological techniques. The frequency of the causative organisms was obtained by culturing the NBL samples on suitable media. The patients were analysed by age, gender, the causative bacteria and their antibiotics susceptibility pattern. The results were a total of 48 cases of VAP isolated. Monomicrobial infections were diagnosed in 32 patients and polymicrobial infections were diagnosed in 16 patients. Common causative agents were Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus and the least common were Proteus mirabilis, Klebsiella oxytoca, Acinetobacter johnsonii, Staphylococcus coagulase negative, Serratia odorifera, Serratia marcescens, Burkholderia cepacia, Citrobacter freundii and Enterobacter cloacae. From this study, it can be concluded that the aetiological agents of VAP vary from common organisms to very resistant pathogens.

With the increasing incidence of multidrug resistant organisms; its early isolation, detection, diagnosis and specific antibiotics are required to avoid hazardous outcomes.

From Zeina A Kanafani et al[11] study, it can be inferred that it is a prospective study. All patients were admitted to the intensive care and respirator y care units from March to September 2001, and who had been receiving mechanical ventilation for at least 48 hours, were included in the study. Results of samples submitted for culture were recorded and antimicrobial susceptibility testing of isolated pathogens was per formed. The results were that seventy patients were entered into the study. The incidence of VAP was 47%. Gram-negative bacilli accounted for 83% of all isolates. The most commonly identified organism was Acinetobacter anitratus, followed by Pseudomonas aeruginosa. Fifty percent of all Gram-negative bacterial isolates were classified as antibiotic resistant. Compared with patients without VAP, patients with VAP remained intubated for a longer period and stayed in the intensive care unit longer. VAP was not associated with an increased mortality rate.

Compared with other studies, the results from this referral centre in Lebanon indicate a higher incidence of VAP and a high prevalence of resistant organisms. These data are relevant because they direct the choice of empiric antibiotic therapy for VAP. Neelima Ranjan et al[12] have reported a prospective study which was carried out over a year to know the various aetiological agents of VAP and their drug susceptibility patterns. ESBL, MBL and AmpC B-lactamases were detected in various isolates by combination disk method, imipenem-EDTA combined disk method and AmpC disk method respectively. The results were that the majority of bacterial isolates causing VAP were found to be Gram-negative bacilli. Acinetobacter spp. accounted for 34.28% of VAP cases followed by Pseudomonas aeruginosa which was responsible for 25.71% cases.

Other Gram-negative bacilli isolated were Klebsiella pneumoniae, Citrobacter freundii, Enterobacter spp., and Escherichia coli. Out of the total 70 isolates, 67 (95.7%) were multidrug resistant and not even a single isolate was sensitive to all the drugs tested. This study concluded that most of the pathogens causing VAP in our institute were multidrug resistant and in many isolates this resistance was due to production of ESBL, MBL, and AmpC β-lactamases. Polymyxin-B and Colistin were found to be highly effective against multidrug resistant Acinetobacter spp. and P. aeruginosa. Urmi Jathwani et al[13] have conducted a study which aimed to study the causative organisms and determine the antibiotic susceptibility pattern of the lower respiratory tract isolates from patients admitted to ICU. Endotracheal aspirates from 200 patients admitted to the ICU were cultured, identified and antimicrobial susceptibility testing was performed by standard methods.

The results were that from 200 specimens, 69 (34.5%) were culture positive. Total 96 isolates were recovered, from these 92(96.87%) isolates were Gram-negative bacilli (GNB). In 34.78% specimens, two isolates were recovered. The most common Gram-negative organism being Acinetobacter spp. (31.25%) followed by Klebsiella spp. (21.87%), E-coli (21.87%) and Pseudomonas Spp. (17.7%). All GNBs were 100% sensitive to polymyxin B and Colistin and resistant to piperacillin, ceftazidime and cotrimoxazole.

50% E. coli and 38% of Klebsiella pneumoniae strains were ESBL (Extended-spectrum β-lactamase) producers. It concluded the trend in antimicrobial susceptibility pattern of Gram-negative bacilli in intensive care unit. It is the most important for specific treatment of ventilator associated pneumonia patients and to generate local data periodically to decide empiric antimicrobial therapy.

CONCLUSION: Almost all the pathogens were multidrug resistant, and in all the isolates resistance was due to presence of ESBL, MBL, AmpC β lactamase. Thus, the intensivists can choose the antibiotics based on the bacteriological review for the management of VAP. This study has shown that it is helpful in diagnosis of Ventilator associated pneumonia and its appropriate management.

REFERENCES

1. Koenig SM, Truwit JD. Ventilator-associated pneumonia: diagnosis, treatment, and prevention. Clin Microbiol Rev 2006;19(4):637–657.
2. Chastre J, Fagon JY. Ventilator-associated pneumonia. Am J Respir Crit Care Med 2002;165(7):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(6):725-741.
4. Cook D, Mandell L. Endotracheal aspiration in the diagnosis of ventilator-associated pneumonia. Chest 2000;117(4):195S–197S.
5. Fujitani S, Yu VL. Quantitative cultures for diagnosing ventilator-associated pneumonia: a critique. Clin Infect Dis 2006;43(Suppl 2):S106-S113.
6. 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(6):558-562.
7. Kumar A, Ghauri MI, Razzaque S, et al. Ventilator associated pneumonia; prevalence and microbial patterns. Jinnah Medical College Hospital, Karachi 2014.
8. Goel N, Nautiyal S, Jauhari S, et al. Microbiological profile of ventilator associated pneumonia cases in a tertiary care hospital, in Dehradun India. Indian Journal of Applied Research 2015;5(12):49-52.
9. Bouadma L, Deslandes E, Lolom I, et al. Long-term impact of a multifaceted prevention program on ventilator-associated pneumonia in a medical intensive care unit. Clin Infect Dis 2010;51(10):1115-1122.

10. Ahmed W, Rana MN, Muzaffar NA, et al. Microorganisms related with ventilator associated pneumonia and their antibiotic sensitivity pattern. Journal of Rawalpindi Medical College 2014;18(1):45-48.
11. Kanafani ZA, Kara L, Hayek S, et al. Ventilator-associated pneumonia at a tertiary-care center in a developing country: incidence, microbiology, and susceptibility patterns of isolated microorganisms. Infection Control and Hospital Epidemiology 2003;24(11):864-869.
12. Ranjan N, Ranjan KP, Chaudhary U, et al. Antimicrobial resistance in bacteria causing ventilator-associated pneumonia in a tertiary care hospital: one year prospective study. Int J Res Med Sci 2014;2(1):228-233.
13. Jethwani U, Shah N, Trivedi P. Antibiotic sensitivity pattern of Gram-negative bacilli isolated from the lower respiratory tract of ventilated patients in the intensive care unit. Indian Medical Gazette 2014:180-184.