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Original Research Article

Identification of multidrug-resistant pathogens and their antibiotic susceptibility in ventilator-associated pneumonia patients in a rural tertiary care hospital, India

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ABSTRACT

Background: The present study aimed to determine the microbiological profile and their antibiotic susceptibility isolated from the endotracheal secretions of the ventilator-associated pneumonia (VAP) patients, which will help to know the etiological agents associated with VAP and formulate the antibiotic policy for early treatment. This would help clinicians select the most appropriate antibiotics for empirical therapy in patients with VAP.
Methods: A total of 56 endotracheal aspirated samples were collected from the 56 clinically suspected cases of VAP patients. The samples were inoculated on sheep blood agar, chocolate agar, and MacConkey agar and incubated for 24 to 48 hours at 37°C. The isolates with significant colony count were identified by standard laboratory procedures and antibiotic susceptibility testing was done by Kirby Bauer disc diffusion methods.
Results: Out of 56 endotracheal secretions samples, 49 (87.5%) samples showed significant growth with culture positivity being 87.5%. In the positive cultures, the total number of isolates were 63. 48 (76.19%) were Gram negative bacteria and 15 (23.80%) were Gram positive bacteria. Among Gram negative isolates, most predominate isolate was Acinetobacter baumannii 14 (29.16%) and among Gram positive isolates, Methicillin Resistant Staphylococcus aureus was the predominant isolate 7(46.66%). The most effective antibiotic against Gram negative bacteria was colistin and the most effective antibiotic against Gram positive organisms were vancomycin and linezolid.
Conclusions: Pathogens isolated from VAP patients showed high incidence of resistance to commonly used antibiotics. The study will help to know the pathogens associated with VAP and in formulate the antibiotic policy for empirical therapy.

Keywords: Endotracheal secretions, Ventilator-associated pneumonia, Multi-drug-resistant pathogens

INTRODUCTION

Ventilator associated pneumonia (VAP) accounts for 15% of nosocomial infections and is the second most common cause of hospital acquired infection with a high rate of morbidity and mortality.[1]

VAP is common in the intensive care unit (ICU), affecting up to 27% of mechanically ventilated patients.[2] VAP can be defined as infection of lung parenchyma in patients after 48 hours of endotracheal intubation and mechanical ventilation.[3] The intubation for mechanical ventilation is one of the life saving practice and it is associated with high risk of acquiring ventilator associated pneumonia. Intubation lowers the natural barrier between the oropharynx and trachea and thereby helps the bacteria to enter into the lung by aspiration and leakage of contaminated secretions around the endotracheal tube cuff.[4]

According to the American Thoracic Society (ATS) and the Infectious Disease Society of America, patients developing VAP are identified by new progressive radiographic infiltrates and the presence of at least two of three clinical features, including fever (>38°C of body temperature), purulent secretions, and leukocytosis or leukopenia. These clinical manifestations of VAP are taken as a criterion for initiating empiric antibiotic therapy and a delay in treatment can potentially lead to septic shock or acute respiratory distress syndrome.[2]

The risk factors for the developing of VAP include the patient's own flora, microaspiration of contaminated oropharyngeal secretions, duration of ventilation, positioning of the patients, enteral feeding, over sedation, hospital staff and contaminated devices.[5] In mechanically ventilated patients, the folds of endotracheal tube cuff are the potential source of infection and bacterial colonization and formation of biofilm in the endotracheal tube can act as a reservoir for infectious microorganisms, which subsequently migrate to the distal airways and alveoli, thus increasing the risk for developing severe pneumonia.[6]

The most predominant pathogens that cause VAP includes *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus*. Other microbial pathogens such as *proteus spp*., *Providencia spp*., *Enterobacter spp.*, *Stenotrophomonas maltophilia*, coagulase negative *Staphylococcus*, and *Enterococcus spp*. are less frequently associated with VAP7.[8]

The mortality rate among VAP patients is very high due to increase in the number of Multidrug resistant (MDR) bacteria such as Multidrug resistant Gram negative bacilli including extended spectrum beta lactamase (ESBL) producers, Carbapenem resistant organisms and Colistin resistant organisms and Methicillin resistant Staphylococcus aureus (MRSA). Delay in antibiotic administration is strongly associated with increased mortality in VAP patients and inappropriate antibiotic administration can be responsible for treatment failure and antibiotic resistance.[7,8]

Hence, the present study aimed to determine the microbiological profile and their antibiotic susceptibility isolated from the endotracheal secretions of the VAP patients, which will help to know the etiological agents associated with VAP and formulate the antibiotic policy for early treatment. This would help clinicians select the most appropriate antibiotics for empirical therapy in patients with VAP.

METHODS

This prospective observational cohort study was conducted in the intensive care unit (ICU) of Adichunchanagiri Institute of Medical Sciences (AIMS), BG Nagara, Karnataka, India, a rural tertiary care hospital from May 2024 to April 2025. This study was approved by the institutional ethical committee, AIMS.

In this study all patients on mechanical ventilation for > 48 hours were included. Patients who were diagnosed with pneumonia within 48 hours of mechanical ventilation and patients with lower respiratory infections such as pulmonary tuberculosis, chronic obstructive pulmonary disease, acute respiratory distress syndrome, or bronchial asthma were excluded from the study.

Sample collection and microbiological testing

A total of 56 endotracheal aspirated samples (endotracheal secretions) were collected from the 56 clinically suspected cases of VAP patients in a sterile and labelled container and transported to the laboratory without delay. The samples (endotracheal secretions) were inoculated on sheep blood agar, chocolate agar, and MacConkey agar and the plates were incubated for 24 to 48 hours at 37°C.[9]

In positive cultures, a colony count of ≥105 colony-forming unit/ml (cfu/ml) was taken as significant and counts less than this were considered insignificant and if no growth was observed after 48 hours of incubation, cultures were reported as no growth. The isolates with significant colony count were identified by standard laboratory procedures. Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion methods as per Clinical Laboratory Standards Institute (CLSI) guidelines 2023.[10]

Data analysis

The frequency of resistant isolates was calculated as the sum of resistant isolates relative to the total number of isolates tested. The susceptibility results for any antimicrobial that were based on <0.05 were considered to be significant. SPSS (release 20.0, SPSS Inc., Chicago, U.S.) and OpenEpi (Version 2.2, www.OpenEpi.com. com, Atlanta, GA, U.S.) software were used for all statistical analyses.

RESULTS

In the present study, a total of 56 endotracheal aspirated samples (endotracheal secretions) were collected from the 56 clinically suspected cases of VAP patients, of whom 47(84%) were male and 9 (16%) were female (Table 1).

Table 1: Sex distribution of patients.

Sex	No. of patients	Percentage
Male	47	84
Female	9	16
Total	56	100

Age distribution of the patients includes, 2 (3.57%) were in the age group <1 year, 6 (10.7%) of 1-10 years, 6

(10.7%) of 11-20 years, 7 (12.5%) of 21-30 years, 9 (14.48%) of 31-40 years, 4 (7.14%) of 41-50 years, 10 (17.85%) of 51-60 years, 5 (8.92%) of 61-70 years, 6 (10.71%) of 71-80 years and 1 (1.78%) of >80 years, majority of the patients were in the age group of 51-60 years (Table 2).

Table 2: Age distribution of patients.

Age (years)	No. of patients	Percentage
<1	2	3.57
1-10	6	10.7
11-20	6	10.7
21-30	7	12.5
31-40	9	14.48
41-50	4	7.14
51-60	10	17.85
61-70	5	8.92
71-80	6	10.70
>80	1	1.78
Total	56	100

In this study, out of 56 endotracheal secretions samples, 49(87.5%) samples showed significant growth and 7 (12.5%) samples showed no growth with total percentage of culture positivity being 87.5% (Table 3).

Table 3: Culture positivity.

	No. of samples	Percentage
Growth	49	87.5
No growth	7	12.5
Total	56	100

Culture positivity found more common in male patients 44 (89.79%) as compared to female patients 5 (10.2%) and most common in age group of 51-60 years followed by 71-80 years (Table 4).

Table 4: Sex distribution of culture positivity.

Sex	No. of samples	Percentage
Male	44	89.79
Female	05	10.21
Total	49	100

Out of 49 positive cultures, 38 (77.5%) were monomicrobial showed growth of single isolate and 11 (22.5%) were polymicrobial showed growth of multiple isolates. A total of 63 bacterial pathogens were isolated from 49 positive culture samples.

Table 5: Pathogens isolated from the samples.

Isolates	No. of isolates	Percentage
Gram negative bacteria	48	76.19
Gram positive bacteria	15	23.80
Total	63	100

In the positive cultures, the total number of isolates were 63. Out of 63 isolates, 48 isolates (76.19%) were Gram negative bacteria and 15 isolates (23.80%) were Gram positive bacteria (Table 5).

Table 6: Gram negative bacteria isolated from the samples.

Isolates	No. of isolates	Percentage
Acinetobacter baumannii	14	29.16
Klebsiella pneumoniae	12	25
Pseudomonas aeruginosa	07	14.58
Enterobacter spp.	06	12.5
Citrobacter spp.	05	10.41
Escherichia coli	04	8.33
Total	48	100

Among Gram negative isolates (48), most predominate isolate was *Acinetobacter baumannii* 14 (29.16%), followed by *Klebsiella pneumoniae* 12 (25%), *Pseudomonas aeruginosa* 7 (14.58%), *Enterobacter spp*. 6 (12.5%), *Citrobacter spp.* 5 (10.41%) and *E. coli* 4 (8.33%) (Table 6).

Among Gram positive isolates (15), methicillin resistant *Staphylococcus aureus* (MRSA) was the predominant isolate 7 (46.66%) followed by Methicillin resistant coagulase negative *Staphylococci* (MRCONS) 5 (33.33%) and *Enterococci faecalis* 3 (20%) (Table 7).

Antibiotic sensitivity pattern of Gram positive and Gram-negative organisms was studied. In the present study among Gram negative bacteria, *Acinetobacter baumannii* showed highest susceptibility to tigecyclin and colistin (100%), followed by amikacin (28.57%), tobramycin (14.28%), levofloxacin, ofloxacin and cefepime (7.15% each). *Klebsiella pneumoniae* showed 100 % sensitive to tigecyclin and colistin followed by 16.16% to meropenem, imipenem, piperacillin-tazobactam, gentamicin, amikacin and tobramycin. *Pseudomonas aeruginosa* was 100% sensitive to colistin, followed by 42.85% to meropenem, piperacillin-tazobactam, gentamicin, amikacin and tobramycin.

Enterobacter spp. showed highest sensitivity to tigecycline and colistin (100%), followed by gentamicin and amikacin (66.66%), tobramycin (50%), meropenem and piperacillin-tazobactam (33.34%) and cefepime (16.16%). *Citrobacter spp*. was 100% sensitive to tigecycline and colistin. *E. coli* was 100% sensitive to tigecycline and colistin, followed by 75% to meropenem, gentamicin, amikacin and tobramycin, 25% to ofloxacin and levofloxacin (Table 8).

The most effective antibiotic against Gram negative bacteria were colistin and tigecycline followed by meropenem and amikacin.

Table 7: Gram positive bacteria isolated from the samples.

Isolates	No. of isolates	Percentage
Methicillin resistant *Staphylococcus aureus* (MRSA)	07	46.66
Methicillin resistant coagulase negative *Staphylococci* (MRCONS)	05	33.33
Enterococci faecalis	03	20
Total	15	100

Table 8: Antibiotic sensitivity pattern of Gram negative bacteria.

Antibiotic	*Acinetobacter spp.* n=14 (29.1%)	*Klebsiella spp.* n=12 (25%)	*Pseudomonas aeruginosa* n=7 (14.5%)	*Enterobacter spp.* n=6 (12.5%)	*Citrobacter spp.* n=5 (10.4%)	*E. coli* n=4 (8.33%)
Colistin	100%	100%	100%	100%	100%	100%
Tigecycline	100%	100%	0%	100%	100%	100%
Imipenem	0%	16.6%	28.57%	0%	0%	0%
Meropenem	0%	16.6%	42.85%	33.34%	0%	75%
Piperacillin- Tazobactam	0%	16.6%	42.85%	33.34%	0%	
Ofloxacin	7.14%	0%	28.57%	0%	0%	25%
Levofloxacin	7.14%	0%	28.57%	0%	0%	25%
Ciprofloxacin	0%	0%	28.57%	0%	0%	0%
Amikacin	28.5%	16.6%	42.85%	66.66%	0%	75%
Gentamicin	0%	16.6%	42.85%	66.66%	0%	75%
Tobramycin	14.28%	16.6%	42.85%	50%	0%	75%
Cefotaxime	0%	0%	0%	0%	0%	0%
Ceftriaxone	0%	0%	0%	0%	0%	0%
Ceftazidime	0%	0%	28.57%	0%	0%	0%
Cefepime	7.14%	8.33%	28.57%	16.16%	0%	0%
Amoxicillin-clavulanic acid	0%	0%	0%	0%	0%	0%
Ampicillin	0%	0%	0%	0%	0%	0%

Table 9: Antibiotic sensitivity pattern of Gram positive bacteria.

Antibiotic	MRSA n=7 (46.66%)	MRCONS n=5 (33.33%)	*Enterococci faecalis* n=3 (20%)
Vancomycin	100%	100%	100%
Linezolid	100%	100%	100%
Clindamycin	50%	60%	0%
Azithromycin	75%	60%	0%
Tetracycline	75%	60%	0%
Gentamicin	75%	40%	0%
Amikacin	75%	40%	0%
Ciprofloxacin	25%	0%	0%
Ofloxacin	25%	0%	0%
Levofloxacin	25%	0%	0%
Erythromycin	25%	0%	0%
Cotrimoxazole	0%	0%	0%
Chloramphenicol	50%	60%	0%
Cefotaxime	0%	0%	0%
Amoxicillin-clavulanic acid	0%	0%	80%
Ampicillin	0%	0%	80%
Penicillin	0%	0%	0%
High level Gentamicin	-	-	33.33%

In the present study among Gram positive bacteria, *Staphylococcus aureus* showed high susceptibility to vancomycin and linezolid (100%) followed by gentamicin, amikacin and tetracycline (75%), clindamycin and chloramphenicol (50%) coagulase negative *Staphylococci* was 100% sensitive to vancomycin and linezolid, 60% to azithromycin, clindamycin, chloramphenicol and tetracycline and 40% to gentamicin and amikacin. *Enterococcus faecalis* showed highest sensitivity to vancomycin and linezolid (100%) followed by ampicillin and amoxicillin-clavulanic acid (80%) and high-level gentamicin (33.31%) (Table 9).

The most effective antibiotic against Gram positive organisms were vancomycin and linezolid (100% each) followed by clindamycin and tetracycline (60%)

In the present study, majority of the isolates showed high resistance to commonly used antibiotics belongs to penicillins (ampicillin and amoxicillin-clavulanic acid), cephalosporins (cefotaxime, ceftriaxone and ceftazidime), fluoroquinolones (ciprofloxacin, ofloxacin and levofloxacin), aminoglycosides (gentamicin and amikacin) and to reserve antibiotics belonging to carbapenems (imipenem and meropenem).

DISCUSSION

Ventilator-associated pneumonia (VAP) is the most serious conditions among ICU patients receiving mechanical ventilation. VAP associated with high morbidity and mortality which are attributed to the presence of multidrug-resistant bacterial infections, hence timely detection, identification and antimicrobial susceptibility testing of pathogens causing ventilator-associated pneumonia are important. The present study gives information about pathogens causing ventilator-associated pneumonia and also provides information about antibiotic sensitivity pattern that plays an important role in management of ventilator-associated pneumonia cases

In this study, total 56 endotracheal secretions samples were collected, out of which 49(87.5%) samples showed significant growth and 7 (12.5%) samples showed no growth with culture positivity being 87.5%. Similar findings are observed in study conducted by Saritha et al, Rathos et al, Samal et al, Khoshfetrat et al, Gohell, Chandra et al and Malik et al and Rajesh et al.[11-16]

In the present study, culture positivity found more common in male patients 44 (89.79%) as compared to female patients 5 (10.2%) indicating males are more susceptible to disease. Our study in concordance with studies of Rajesh et al, Faraag et al, Rana et al, Samal et al and Daef et al.[16-20] Males are more susceptible to VAP may be related to differences in sex hormone with higher testosterone levels which suppress the immune system making them more prone to infections and differences in gender related gene variation.[14]

In this study, VAP is most common in the age group of 51-60 years (20.4%) which is in comparison with study conducted by Rajesh et al, Girish et al.[16,21] In the study of Sharma et al, and Shete et al incidences of VAP were more common in the age group 61-70.[22,23] However, based on the data, age does not contribute significantly in acquiring VAP as it affects patients in various age groups.[16]

In the present study, out of 49 positive cultures, 38 (77.5%) were monomicrobial and 11 (22.5%) were polymicrobial which correlates with a study reported by Saritha et al, et al, Samal et al, Chidambaram et al and Khayyam et al and Kapaganty et al, but in study by Vaidhyswaran et al and Golia et al, Samaddar et al polymicrobial organism distribution was higher (71.8%) than monomicrobial distribution (28.1%).[5,11,24-29]

In the positive cultures, the total number of isolates were 63. Out of 63 isolates, 48 isolates (76.19%) were Gram negative bacteria and 15 isolates (23.80%) were Gram positive bacteria.

Similar findings are observed in study by Samal et al, Chidambaram et al, and Khayyam et al, Chandra et al, and Gupta et al.[5,14,24,25,30] In contrast to our study, Quartin et al from New York reported 63.4% of identified organisms were gram-positives and 36.6% of Gram-negative bacteria.[31]

As per worldwide data in Western countries Gram-positive organisms are major cause of VAP. Potential reasons include the use of indwelling catheters, local environmental conditions, and the administration of specific antibiotic agents, especially as prophylaxis.[31]

According to the Indian studies, Gram-negative bacteria are the major cause of VAP. This can be due to colonization of the gut and exposure to antimicrobials. The critically ill patients within 24-48 hours of hospitalization get colonized exogenously from exogenous sources, such as contaminated respiratory instruments, infective aerosols from the ICU environment, and contaminated hands of the healthcare workers. or endogenously with hospital flora and the oral flora shifts to a predominance of hospital microbial flora that is aerobic gram-negative pathogens. Pulmonary aspiration of these oropharyngeal flora increases the risk for VAP. Also, critically ill patients are on broad-spectrum empirical antibiotics, which cause selection pressures on these colonizers and leads to emergence of resistant strains of gram-negative pathogens.[32]

In this study, among Gram negative isolates (48), Acinetobacter baumannii was the most predominant organism isolated (29.16%), followed by *Klebsiella pneumoniae* (25%), *Pseudomonas aeruginosa* (14.58%). Our result in concordance with Verma et al (41.18%), Shreshta et al (43.47%) and Goel et al (36.82%). The study by Dey et al, Rajasekhar et al, and Goel et al also reported *Acinetobacter baumannii* was the most predominant

organism isolated.[13,32,33] But in study conducted by Saritha et al and Malik et al *Klebsiella pneumoniae* was predominant isolate followed by *Acinetobacter spp*. and *Pseudomonas aeruginosa*.[11,15] Another study by Chi et al reported the most common organism being *Pseudomonas* species.[34] The higher rate of isolation of *Acinetobacter spp*. among Gram-negative bacteria may be due to their ubiquitous nature in the hospital environment and inadequate infection control practice.

In the present study, among Gram positive isolates (15), methicillin resistant *Staphylococcus aureus* (MRSA) was the predominant isolate (46.66%) followed by methicillin resistant coagulase negative *Staphylococci* (MRCONS) (33.33%) and *Enterococci faecalis* (20%). Similar findings were reported by Dey et al, Rajasekhar et al, Goel et al and Vaidhyswaran et al.[13,32,33,35] But according to Masoum et al *Staphylococcus epidermidis* was the predominant Gram-positive cocci isolated (50.3%) followed by *Staphylococcus aureus.*[12]

In this study, *Acinetobacter baumannii* and *Klebsiella pneumoniae* showed highest susceptibility to Tigecyclines and Colistin (100%), which is in comparison with other studies by Rit et al and Goel et al.[13,36] Pseudomonas aeruginosa is 100% sensitive to Colistin which is similar to the study conducted by Gohel et al.[13] In contrast, Dey et al showed that *Acinetobacter* species were more susceptible to imipenem and tigecycline.[33]

In the present study MRSA, MRCONS and *Enterococcus spp.* showed 100% susceptibility to vancomycin and linezolid which correlates with the findings of Ahmad et al and Rathod et al followed by Tetracycline, co-trimoxazole (64.3%) and clindamycin (42.85%).[8,37]

In the present study, majority of the isolates showed high resistance to commonly used antibiotics belongs to penicillins (ampicillin and amoxicillin-clavulanic acid), cephalosporins (cefotaxime, ceftriaxone and ceftazidime), fluoroquinolones (ciprofloxacin, ofloxacin and levofloxacin) and aminoglycosides (gentamicin and amikacin), carbapenems (imipenem and meropenem) and this is in comparison with other studies. This might be due to continuous exposure of these bacteria to a variety of β-lactams that leads to the production of beta-lactamases. Moreover, plasmid and chromosomal gene mediated beta-lactamase enzymes are major reasons for antibiotic resistance. The greatest threat with MDR and carbapenem resistant Gram negative bacteria is that the infections are usually untreatable due to the limited options of the antibiotics available, resulting into increased mortality

Acinetobacter baumannii showed 100% resistant to ampicillin, amoxicillin-clavulanic acid, cefotaxime, ceftriaxone, ceftazidime, cefepime, piperacillin-tazobactam, imipenem, 92% resistant to ciprofloxacin, ofloxacin, levofloxacin, gentamicin and tobramycin, 85% resistant to meropenem and 71% to amikacin.

Klebsiella pneumoniae showed 100% resistant to ampicillin, amoxicillin-Clavulanic acid, cefotaxime, ceftriaxone, ceftazidime, cefepime, ciprofloxacin, ofloxacin and levofloxacin. 84% resistant to meropenem, imipenem, amikacin, gentamicin, tobramycin and piperacillin-tazobactam.

Pseudomonas aeruginosa showed 100% resistant to ampicillin, amoxicillin-clavulanic acid, cefotaxime, ceftriaxone. 71.5% resistant to ceftazidime and cefepime, ciprofloxacin, ofloxacin and levofloxacin, imipenem. 51% resistant to meropenem, amikacin, gentamicin, tobramycin and piperacillin-tazobactam.

The greatest threat with MDR and carbapenem-resistant Gram-negative bacteria is that the infections are usually untreatable due to the limited options of the antibiotics available, resulting in increased mortality. Although we found that VAP is most predominant among 51-60 years of age, the VAP was also found in all other age groups. Further studies required to understand whether VAP affects specific age groups.

The main factors causing the increase in antimicrobial resistant bacteria are poor infection control practices and inappropriate use of antibiotics. Another factor that leads to antibiotic resistance is the dysbiosis of the lung microbiota due to altered oropharyngeal microbiome, which translocate from ETT into deeper lungs. Further, impair cough reflexes and secretion clearance, decreasing bacterial elimination in ICU patients enhances bacterial burden. Strict infection control measures along with antibiotic policy for judicious antibiotic therapy should be implemented in the hospitals as control measures against VAP and to check the emergence of resistance.[35]

Efforts should be directed on the prevention of VAP using good infection control practices, hand hygiene, ventilator care bundle approach, and appropriate empirical antibiotic therapy.

CONCLUSION

Ventilator associated pneumonia (VAP) is the second most common cause of hospital acquired infection with a high rate of morbidity and mortality. Acinetobacter baumannii was the most predominant organism isolated followed by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and also noted high incidence of resistance to commonly used antibiotics such as ampicillin, amoxicillin-clavulanic acid, cefotaxime, ceftriaxone, ceftazidime, ciprofloxacin, ofloxacin and levofloxacin gentamicin amikacin, imipenem and meropenem. The present study provides information on the spectrum of pathogens causing VAP and their antimicrobial susceptibility profile, helping the clinicians in selecting the most appropriate antibiotics for the empirical therapy in the management of VAP and thereby decreasing mortality and morbidity due to life-threatening VAP.

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