

Original Research Article

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Etiology, Antibigram and Quantitative Endotracheal Aspirate Cultures in Ventilator Associated Pneumonia Patients in a Tertiary Care Hospital

Mir Salman Ali, Sreekanth Basireddy*, Manisha Singh and Vasanti Kabra

Department of Microbiology, Kurnool Medical College, Kurnool, Andhrapradesh, India

*Corresponding author

ABSTRACT

Ventilator associated Pneumonia (VAP) is the second most common nosocomial infection in hospitals. Timely diagnosis and appropriate treatment will greatly decrease the mortality and morbidity. To potentially improve the specificity of the diagnosis of VAP and the consequent unnecessary antibiotic use and its associated problems, the role of quantitative cultures of respiratory secretions is pivotal. Aims and objectives: To diagnose ventilator associated pneumonia by using quantitative endotracheal aspiration cultures and to identify the bacterial pathogens and determine the antimicrobial susceptibility pattern of those pathogens. Materials and methods: A prospective study was done which included 183 suspected cases of Ventilator associated pneumonia in SVS Medical College and Hospital for a period of one year and nine months. Quantitative endotracheal aspirate culture was done for all the samples, etiological agents were identified and antibiotic susceptibility was determined. Results: Out of 183 samples 102 samples (55.7%) have shown significant number of colonies by quantitative cultures. Gram negative organisms isolated predominantly (89.2%) followed by gram positive organisms (10.7%). Most common organism isolated was *Acinetobacter baumannii* (31.3%) followed by *Klebsiella pneumoniae* (24.5%). In early onset VAP *Klebsiella pneumoniae* was the most common while in late onset *Acinetobacter baumannii* was the most common. Multidrug resistance was common in all the isolates with *Acinetobacter baumannii* topping the list. Carbapenem resistance was observed in 40.6% of *Acinetobacter baumannii* whereas in *K. pneumoniae* it is only 12%. Polymyxin B and colistin are the most susceptible drugs for *Acinetobacter* and *Pseudomonas* whereas in enterobacteriaceae (*E. coli* and *K. pneumoniae*) tigecycline was the best drug (0% resistance) followed by Imipenem (12% and 13.3% resistance respectively). Among *S. aureus*, Methicillin resistance was observed in 66.6% of cases all the *S. aureus* were sensitive to vancomycin, linezolid and tigecycline. Conclusion, Ventilator associated pneumonia continues to be an important challenge to the critical care physician as well as microbiologist both for treatment and diagnosis. A high index of clinical suspicion along with early and prompt diagnosis by using various parameters that include quantitative endotracheal cultures is of utmost importance in appropriate management of the patients, which further decrease the mortality and morbidity along with decrease in the cost associated with the treatment of these infections.

Keywords

Ventilator associated
Pneumonia (VAP)
Nosocomial infection

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Introduction

Hospital acquired infections are the biggest burden in terms of mortality, morbidity as well as economically in any healthcare setup. Ventilator associated Pneumonia is the second most common nosocomial infection in critically ill patients, affecting one fourth of all hospital acquired infections¹. The mortality attributable to VAP has been reported to range between 0 and 50%^{1,2}.

The etiology of VAP primarily include organisms like *Pseudomonas*, *Klebsiella*, *Acinetobacter*, *Serratia*, *Enterobacter* and *Stenotrophomonas* and MRSA etc, which are all multidrug resistant pathogens and augments the toll of mortality if not diagnosed and treated promptly.

Ventilator-associated pneumonia is defined as pneumonia occurring more than 48 hrs after patients have been intubated and kept on mechanical ventilation. Diagnosing VAP requires a high clinical suspicion combined with bedside examination, radiographic examination, and microbiologic analysis of respiratory secretions, which involves a diverse group of specialized medical personnel including the microbiologist who plays a vital role in diagnosis of ventilator associated pneumonia, but it is always difficult to differentiate simple colonization of the endotracheal tube from significant infection. Though many invasive procedures like BAL (bronchoalveolar lavage), PSB (protected specimen brush samples) are having high sensitivity and specificity in the diagnosis, non-invasive and non-bronchoscopy techniques like quantitative endotracheal aspirate cultures, having advantages like less compromise of oxygenation, ventilation and respiratory mechanics during the procedure. Many studies conducted by different researchers have shown that quantitation of endotracheal

aspirate is a promising tool in the diagnosis of ventilator associated pneumonia when the count $>10^5$ cfu/ml^{3,4}.

Due to poor specificity and poor positive predictive value, non quantitative analysis will result in over diagnosis and therefore unnecessary treatment of VAP. Such an approach will result in excess antibiotic use with its attendant cost, potential toxicity, and selection of drug-resistant organisms.

To potentially improve the specificity of the diagnosis of VAP and the consequent unnecessary antibiotic use and its associated problems, the role of quantitative cultures of respiratory secretions is pivotal.

To diagnose ventilator associated pneumonia by using quantitative endotracheal aspiration cultures and to identify the bacterial pathogens and determine the antimicrobial susceptibility pattern of those pathogens.

Materials and Methods

A prospective study was done enrolling patients from the multidisciplinary ICUs of tertiary care hospital (SVS hospital, mahabnagar) from October 2013 – July 2015

Inclusion criteria

Intubated patients (irrespective of age) who are mechanically ventilated for more than 48 hrs, with a clinical suspicion of pneumonia.

Exclusion criteria

Patients with pre-existing pneumonia

A total of 183 clinically diagnosed Ventilator Associated Pneumonia cases were selected and data such as name, age, gender, date of admission into Intensive care unit, chief complaints, risk factors involved and duration

of mechanical ventilation were obtained. Data related to general physical examination, radiological and hematological investigations were collected.

Specimen collection and processing

Endotracheal aspirate (ETA) was collected under all aseptic precautions. A sterile 22 inch suction catheter was introduced into respiratory tract for a distance of 20-25 cms and the specimen was aspirated into a sterile container. The sample was transported to the laboratory immediately for processing.

The ETA so collected was subjected to Gram's stain. The specimen was further diluted using sterile saline (1 in 100) and the dilution factor was noted (Fig. 1).

The specimen was then inoculated for quantitative culture using standard techniques onto MacConkey agar, Blood agar and Chocolate agar plates using a 4mm sterile Nichrome loop (HiMedia), and then incubated at 37°C. The plates were examined for growth at 24 hrs, if there was no growth, it was further incubated for another 24hrs. The CFU/ml was then calculated, considering the dilution factor.

Bacterial growth with $>10^5$ CFU/ml was given significance as a pathogen, and was further identified using appropriate biochemical tests. The antibiotic susceptibility testing was performed according to the recommended CLSI procedures.

Interpretation of results

The diagnostic thresholds for endotracheal aspirate (ETA) were taken as 10^5 CFU/ml. Growth below the threshold was assumed to be due to colonization or contamination.^{3,5} Descriptive statistical analysis has been carried out in the present study Data was

analyzed statistically by computing percentage, mean and standard deviation. Wherever necessary, the results are depicted in the form of graphs and charts.

Results and Discussion

A total of 183 suspected cases were included in the study. Majority of the patients were males (60.1%) followed by females (39.8%). The male to female ratio was 1.5:1 indicating the male predominance

Majority of the patients were in the age group of 21 to 40 yrs (37.1%) followed by 41 to 60 yrs (26.7%), least number of patients were observed below the age of 20 yrs (15.8%) (Fig. 2).

Out of 183 endotracheal aspirates cultured, no growth was observed in 36 samples and was considered bacteriologically sterile. Growth was observed in 147 samples out of which in only 102 samples significant numbers of colonies were observed

Acinetobacter baumannii was the most common isolate observed (31.3%) followed by *Klebsiella pneumonia* (24.5%). In early onset VAP *K pneumonia* was the predominant isolate whereas in late onset VAP *A. baumannii* was the predominant. *S aureus* was most commonly isolated from late onset VAP cases.

Multidrug resistance was commonly observed in *A. baumannii* with majority of the isolates (40.6%) being resistant to Imipenem. *K. pneumonia* and *E. coli* were absolutely resistant to amoxyclav (100%) and also showed high resistance towards ceftriaxone (92% and 80%). Tigecycline has shown the highest sensitivity in enterobacteriace members whereas polymyxin group of antibiotics have shown the highest sensitivity in non fermenting gram negative bacilli. Among *Staphylococcus aureus* isolates,

MRSA was observed in 66.6% of the patients and MSSA in the remaining, All the *S.aureus* were sensitive to vancomycin and linezolid.

VAP is one of the major complications in the ICU patients, the mortality rates from which can range from 25 to 50%⁶. The bacteriological approach for the management of VAP avoids the problem of over treatment by separating colonizers from infecting pathogens.

Several methods to obtain good respiratory samples have been extensively investigated. each technique having its own diagnostic threshold and methodological limitations. The choice of method depends on expertise, experience, availability and cost. Studies showed that the results of quantitative cultures of endotracheal aspirates were comparable to

those using invasive bronchoscopic methods⁷. Our study showed occurrence of VAP to be 55.7%, which was in close agreement with many other studies. In Ranjan *et al.*,⁸ study the incidence of VAP was 57.14%, which is similar to our study.

The patients studied belonged predominantly to the age group of 21-40 years (37.1%). This type of age dominance of VAP was also noticed in Gadani *et al.*, study⁹ where majority of the patients were in the same age group with a mean age of 34 yrs. In the present study it was found that 60.1% of the cases were males and 39.8% were females. The male predominance can be attributed to high prevalence in males of other co-morbid conditions, risk factors like COPD and high susceptibility to road traffic accidents (RTA) (Table 1-6).

Table.1 Clinical distribution of patients studied

Clinical distribution	Number of Patients	Percentage
OP – Poisoning	49	26.7%
Trauma (RTA)	37	20.2%
COPD- Acute exacerbation	29	15.8%
CRF	21	11.4%
Sepsis	16	8.7%
DKA	13	7.1%
Stroke	8	4.3%
Burns	6	3.2%
CAD	4	2.1%

Table.2 Risk factors

Risk Factors	Number of patients	Percentage
Diabetes Mellitus	57	31.1%
Advancing age (> 60yrs)	49	26.7%
COPD	38	20.7%
Steroids	32	17.4%
Head Trauma	31	16.9%
CRF	20	10.9%
HIV	2	1%

Table.3 Quantitative culture report

Quantitative culture report	Frequency in patients	Percentage
No organism grown	36	19.6 %
Significant growth (> 10 ⁵ cfu/ml)	102	55.7 %
Insignificant growth (<10 ⁵ cfu/ml)	45	24.5 %

Table.4 Categorization – VAP

Categorization	Frequency
Early Onset	32.3%
Late Onset	67.6%

Table.5 Profile of the bacterial isolates

Isolates	Early Onset (n=33)	Late Onset (n=69)	Total (n=102)
<i>Acinetobacter baumannii</i>	8 (24.2%)	24 (34.7%)	32(31.3%)
<i>Pseudomonas aeruginosa</i>	4 (12.1%)	13 (18.8%)	17(16.6%)
<i>Klebsiella pneumonia</i>	11 (33.3%)	14 (20.2%)	25 (24.5%)
<i>Escherichia coli</i>	6 (18.1%)	9 (13%)	15 (14.7%)
<i>Staphylococcus aureus</i>	2 (6%)	4(5.7%)	6 (5.8%)
<i>Enterococcus spp</i>	2 (6%)	3 (4.3%)	5 (4.9%)
<i>Stenotrophomonas</i>	-	2 (2.8%)	2 (1.9%)

Table.6 Antibiotic resistance of GNB

Antimicrobial agent	<i>Acinetobacter baumannii</i> (n=32)	<i>Pseudomonas aeruginosa</i> (n=17)	<i>Klebsiella pneumonia</i> (n=25)	<i>Escherichia coli</i> (n=15)	<i>Stenotrophomonas</i> (n=2)
Amikacin	23(71.8%)	6 (35.2%)	10 (40%)	5 (33.3%)	1 (50%)
Ceftazidime	32 (100%)	14 (82.3%)	-	-	2 (100%)
Imipenem	13 (40.6%)	4 (23.5%)	3 (12%)	2 (13.3%)	2 (100%)
Piperacillin tazobactam	24 (75%)	11 (64.7%)	14 (56%)	8 (53.3%)	1 (50%)
Amoxyclav	32 (100%)	-	25 (100%)	15 (100%)	-
Doxycycline	15 (46.8%)	-	13 (52%)	7(46.6%)	0 (0%)
Ciprofloxacin	26 (81.2%)	8 (47%)	16 (64%)	8 (53.3%)	0 (0%)
Tigecycline	4 (12.5%)	-	0 (0%)	0 (0%)	0 (0%)
Colistin	2 (6.2%)	0 (0%)	-	-	-
Cotrimoxazole	23 (71.8%)	-	20 (80%)	13 (86.6%)	0 (0%)
Polymyxin B	-	0 (0%)	-	-	-
Ceftriaxone	-	-	23 (92%)	12 (80%)	-

Fig.1 Gram Staining from endotracheal aspirate showing Pus cells &

Gram negative coccobacilli

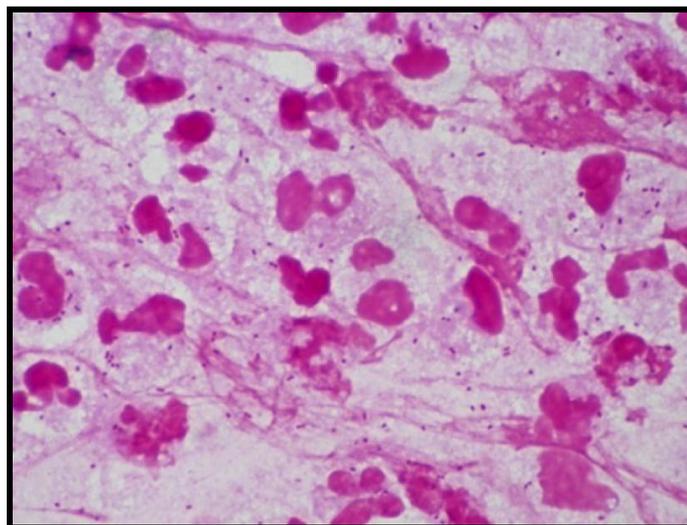
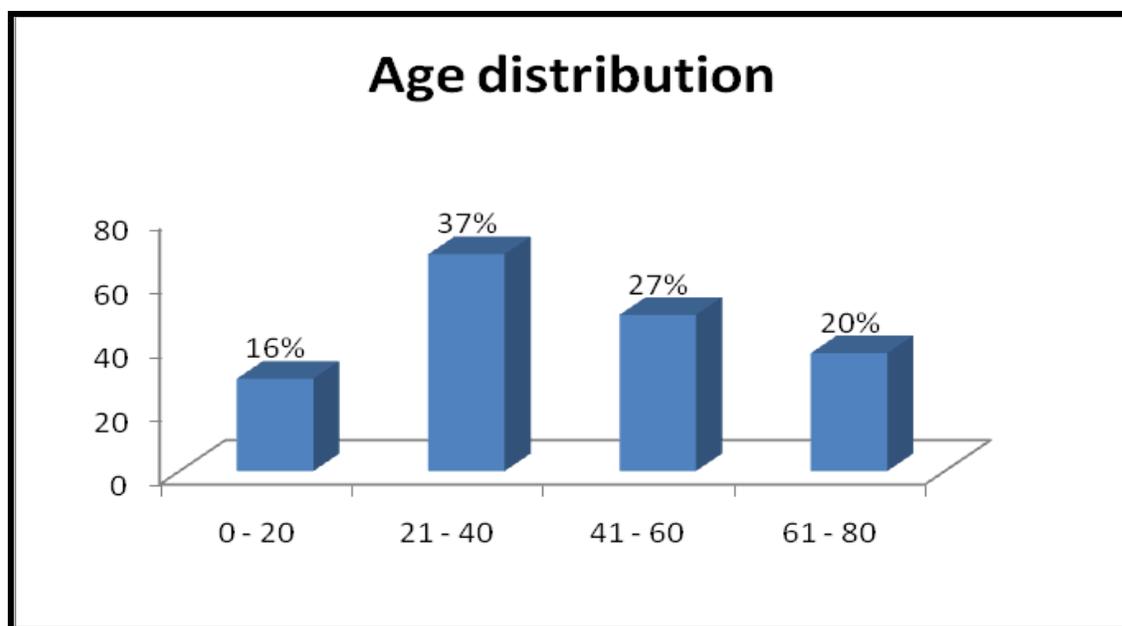


Fig.2 Age distribution



In our study, about 31.1% of the patients had associated Diabetes mellitus, 26.7% were in advancing age (>60 years), 20.7% had COPD and 16.9% had suffered head trauma. These conditions predispose to colonisation and pneumonia because of disease associated impairment in host defense function.¹⁰ These were considered as important risk factors for the development of VAP, as shown by

various studies.¹¹

In our study, the incidence of aerobic gram-negative bacteria was 89.2% and this high incidence correlated with other studies.⁷ The predominant aerobic gram negative bacteria are *Acinetobacter spp.* and *Klebsiella spp.* followed by other aerobic gram negative bacteria like *Escherichia coli* and

Pseudomonas aeruginosa.

In our study we found a significantly high percentage of *Acinetobacter baumannii* (31.3%), which was the most predominant organism followed by *Klebsiella* which is similar to other studies like Kumari *et al.*,¹² and Singhal *et al.*,⁴

The third common isolate in the present study was *Pseudomonas aeruginosa* accounting to 16.6% of the total isolates. Our findings are intermediate between Tripathy *et al.*,¹³ and Kumari *et al.*,¹² study where the isolation rate was 11% and 21.5% respectively. In Tripathy *et al.*,¹³ it is the fourth common isolate whereas in Kumari *et al.*,¹² study it is the second most common isolate

In the present study gram-positive isolates constitute only 10.7% of the total isolates. The Gram positive isolates were *Staphylococcus aureus* and *Enterococcus spp* accounting for 5.8% and 4.9% respectively. In a study conducted by Kumari *et al.*,¹² the isolation rate of *S aureus* was 7.8%, which was close to our findings. In a study conducted by Singhal *et al.*,⁴ *S aureus* was the least common organism isolated

In the present study *Acinetobacter baumannii* was highly resistant to most commonly used empirical drugs like ceftazidime (100%), ciprofloxacin (81.2%), piperacillin/tazobactam (75%) and amikacin (71.8%). These findings are similar in other studies too¹⁴. In the present study Imipenem resistance was noted to an account of 40.6% similar to Joseph *et al.*, study¹⁴.

In the present study *Acinetobacter baumannii* was least resistant to colistin (6.2%) followed by tigecycline (12.5%). Colistin is an age old drug which has been abandoned decades back because of its potential nephrotoxicity but with the recent increase in the carbapenem

resistant *Acinetobacter* isolates, this drug has gained its importance as a potential therapeutic agent despite its toxicity.

Pseudomonas was highly resistant to ceftazidime, the anti-pseudomonal cephalosporin with 82.3% of the isolates being resistant.

The anti pseudomonal penicillin which is most commonly used in clinical practice is piperacillin/tazobactam, our study shows that 64.7% of the isolates are resistant to this drug which is in accordance with Modi *et al.*,¹⁵ study showing resistance of 57%.

All the pseudomonal isolates in our study were uniformly sensitive to Polymyxin B making this antibiotic as a highly effective drug against these infections.

In the present study gram positive isolates constituted only a minority of the total isolates with *Staphylococcus aureus* showing predominance. Two thirds of the *S aureus* isolates are ceftazidime resistant (MRSA). All the Staphylococci are uniformly sensitive to vancomycin, linezolid and tigecycline

To conclude, Ventilator associated pneumonia continuous to be an important challenge to the critical care physician as well as microbiologist both for treatment and diagnosis.

A high index of clinical suspicion along with early and prompt diagnosis by using various parameters that include quantitative endotracheal cultures is of utmost importance in appropriate management of the patients, which further decrease the mortality and morbidity along with decrease in the cost associated with the treatment of these infections.

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