Section: Microbiology

Original Research Article

A STUDY ON BACTERIOLOGICAL PROFILE AND THE ANTIBIOTIC SENSITIVITY PATTERN OF VENTILATOR ASSOCIATED PNEUMONIA IN INTENSIVE CARE UNITS IN A TERTIARY CARE HOSPITAL

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Abstract

Background: Aim: To study the bacteriological profile of ventilator associated pneumonia and their antibiotic sensitivity pattern in Intensive care units in Chengalpattu Medical College and Hospital. **Materials and Methods:** 150 samples were collected from patients above 14 years of age and on mechanical ventilation for > 48hrs, and processed to determine the various etiological agents causing VAP and their antibiotic sensitivity pattern. **Results:** Incidence of VAP was 44 per 1000 ventilator days and accounted for 45% of the total 150 patients. Males were more affected. Klebsiella was the most common pathogen isolated of which majority 38% were multi drug resistant Klebsiella. Most cases of VAP was late onset 61% among them Pseudomonas constitute 33% which results in higher mortality rate 54%. Emergency intubation, re-intubation, Prior antibiotic therapy and hospitalization of more than five days were common risk factors associated with VAP. **Conclusion:** Gram Negative organisms were the predominant organism isolated, and they showed 100% susceptibility to carbapenem drugs (especially meropenem). This drug can be kept in reserve for serious infections. Piperacillin-tazobactam and Gentamicin showed promising susceptibility. The study showed 30% resistance to extended spectrum cephalosporins. Emergence of Extended spectrum beta lactamases (ESBLs) in a hospital set up is of increasing concern. The antimicrobial susceptibility pattern of the isolates obtained in this study showed that most of the organisms were Multi Drug Resistant (MDR) pathogens. Increasing prevalence of MDR pathogens in patients with late onset VAP indicate that appropriate broad spectrum antibiotics should be used to treat them. Implementing simple and effective preventive measures including precaution during emergency intubation, minimizing the occurrence of re-intubation, and judicious use of antibiotics is useful.

INTRODUCTION

Ventilator Associated Pneumonia is one of the most important nosocomial infections in ICU causing significant morbidity and mortality.[1] The incidence of VAP depend on the definition used to diagnose VAP, the hospital or ICU type, the study population and the level of exposure to antibiotics.[2]

The diagnosis of pneumonia in mechanically ventilated patients is based on the combination of clinical, radiological and microbiological criteria. The lower respiratory tract samples obtained either by bronchoscopic (eg.BAL, PSB) or non-bronchoscopic methods (Endotracheal aspirates) are used in the diagnosis. The endotracheal aspirates are easy to collect and have a high sensitivity.

Studies have estimated that more than 300,000 patients receive mechanical ventilation among them risk for complications and poor outcomes including death is very high. Patients on mechanical ventilation have been estimated to have high mortality when VAP is associated with Multi Drug Resistant (MDR) pathogens like Acinetobacter, Klebsiella, Pseudomonas and Escherichia coli.[3]

India has an overall mortality of 40% attributable to infection alone.[4] Emergence of multidrug resistance contributes to the increase in morbidity and mortality (60%). There is increased risk of acquiring pneumonia when duration of mechanical ventilation increases.[3]

Ventilator Associated Events definition is for use in surveillance; it is not a clinical definition algorithm and is not intended for use in the clinical management of patients.[5] Rational antibiotic therapy for treatment of VAP will be beneficial to combat the increase in VAP caused by MDR. Early diagnosis and proper use of antibiotics can reduce length of hospital stay and mortality.

Most cases of VAP were caused by Gram-negative bacteria. Pseudomonas aeruginosa (21.3%) and Acinetobacter baumannii (21.3%) were the most common Gram-negative bacteria associated with VAP and Staphylococcus aureus (14.9%) was the most common Gram-positive bacteria.

Our Institution is a tertiary care hospital providing critical care facilities, where many patients routinely undergo assisted mechanical ventilation. The present study is undertaken to detect bacterial agents commonly associated with VAP in our hospital Intensive care unit and also to study their antibiotic susceptibility patterns.

MATERIALS AND METHODS

This cross-sectional study was conducted over a period of 1 year, from January 2020 to December 2020 in Chengalpattu Medical College and Hospital

Inclusion and Exclusion Criteria

All patients on mechanical ventilation for more than 2 days and above 14 years of age were included. Patient's relatives not giving informed consent and within 48 hours of mechanical ventilation were excluded. Approval was obtained from the Institutional ethics committee at Chengalpattu Medical College before commencement of the study (approval no CHMC/IEC/192/2022)

Specimen Collection

After getting informed consent from their relatives, Non repetitive, consecutive 150 samples were taken. Following universal precautions and the wearing of appropriate personal protective equipment, samples were collected under strict aseptic precautions. The samples received were divided into two parts: one for Direct Gram Stain and the other for quantitative culture [5] and was subjected to preliminary identification by Direct Gram staining and motility testing by the hanging drop method.

Bacterial Culture

After preliminary identification and within one hour of receiving the samples, they were plated onto blood agar and MacConkey agar media and incubated for 18-24 hours at 37°C for isolation. Growth was checked after overnight incubation, and species identification was done by standard biochemical techniques.

Antimicrobial susceptibility testing

The isolated organisms were subjected to antimicrobial susceptibility testing by disk diffusion method using the modified Kirby-Bauer technique. Three to four morphologically similar colonies were suspended in peptone water and incubated at 37°C for two hours. The turbidity of the test suspension was standardized to 0.5 McFarland units. The suspension was inoculated on a Mueller-Hinton agar plate with a sterile cotton wool swab by the lawn culture method. After brief drying, antibiotic disks (about five disks per 90 mm plate) were placed with sterile precautions. Then, the plate was incubated at 37°C for 24 hours and interpreted the next day as per the Clinical and Laboratory Standards Institute guidelines. All the above tests were performed in conjunction with positive, negative, and test controls as necessary according to the standard guidelines. The panel of drugs used for antibiotic susceptibility testing for GPC was penicillin 10 U, erythromycin 15 µg, clindamycin 2 µg, cotrimoxazole 1.25/23.75 µg, Ciprofloxacin 5 µg, cefoxitin 30 µg

For GNB, the following were used: ampicillin 10 µg, gentamicin 10 µg, amikacin 30 µg, ceftazidime 30 µg, cotrimoxazole 1.25/23.75 µg, ciprofloxacin 5 µg, piperacillin-tazobactam 100/10 µg, Amoxicillin-clavulanate 20/10 µg and meropenem 10 µg. Interpretation of the zone of inhibition was done according to CLSI standard M100, 30th edition, 2021 guidelines[6]

Statistical Analysis

We recorded the study data in Microsoft Excel 2019 (Microsoft Corporation, Redmond, WA). The frequencies and percentages of the organisms isolated were tabulated.

RESULTS

A total of 150 samples were collected from patients on mechanical ventilation after 48hrs of intubation. This study includes both the gender (89 males and 61 females) of age group above 14 years. Of the 89 male patient samples 45 (50.60%) showed positive growth, while 44 (49.40%) did not. Out of 61 female patient samples 22 (36%) showed positive growth, while 39 (64%) did not

Table 1: Out of 150 samples 67 (45%) showed growth of pathogens >105and 23 (15%) showed growth <104 and 60 (40%) showed No Growth. All 23 patients with <104 organisms got discharged

Table 2: Patients with CPIS score of >6 were diagnosed as VAP patients7. CPIS Score > 6: 67 patients and CPIS Score <6: 83 patients

Of the 67 VAP Positive, 26 (39%) were Early onset and 41(61%) were Late onset VAP

In the total 67 isolates 63 (94%) were Gram negative bacteria, 3 (4%) were Gram positive bacteria and 1 (2%) was fungi. Thus it was observed that more cases of ventilator associated pneumonia were caused by Gram negative organisms, than Gram positive organisms.

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Figure 1: On further genus identification 29 (43%) were Klebsiella species, 20(30%) were Pseudomonas aeruginosa, 13(19%) were Acinetobacter species, 3(4%) were Staphylococcus aureus, 1(2%) was Morganella morganii, 1(2%) was Candida species

Figure 2: Staphylococcus aureus showed 33% resistant to Erythromycin Inducible Clindamycin Resistant, 33% resistant to Clindamycin, 33% resistant to Cotrimoxazole and 100% resistant to Ampicillin

Figure 3: Of the Staphylococcus aureus isolated -2 (66.7%) were Methicillin Sensitive Staphylococcus aureus and 1(33.3%) was Methicillin Resistant Staphylococcus aureus

Pseudomonas aeruginosa showed resistant to Cotrimoxazole (85%) Ceftriaxone (60%), Ciprofloxacin (30%), Gentamicin (30%), Piperacillintazobactam (15%), Amikacin (5%) and (80%) resistant to Ampicillin and 100% sensitive to Meropenam

Figure 4: Acinetobacter species showed resistant to Cotrimoxazole (85%) Ceftriaxone (69%), Gentamicin (69%), Ciprofloxacin (31%), Piperacillintazobactam (31%), Amikacin (15%) and 100% sensitive to Meropenam, Ampicillin and Cotrimoxazole

Figure 5: Enterobacteriaceae - Klebsiella species showed resistant to Cotrimoxazole (60%) Ceftriaxone (53%), Gentamicin (47%), Ciprofloxacin (27%), Ampicillin (23%), Piperacillin tazobactam (20%), Amikacin (3%) and 100% sensitive to Meropenam

Figure 6: Multi Drug Resistance pattern of VAP Pathogens Out of 67 VAP Pathogens: 39 were MDR Pathogens, of which Pseudomonas aeruginosa 13 (33%), Acinetobacter species 10 (26%), Klebsiella species 15 (38%), Morganella morganii (3%).

Of the 39 patients with MDR patients: 24 died and the distribution of isolates was Pseudomonas aeruginosa 54%, Acinetobacter species 29%, Klebsiella species 17%. Mortality rate increased in patients got infected with Pseudomonas aeruginosa which is Multi Drug Resistant organism.

Based on the onset of VAP, late onset >5 days of intubation, 33% of infection is caused by Pseudomonas aeruginosa followed by Acinetobacter species 23% & Klebsiella species 10% In early onset <4 days of intubation, 28% was caused by Klebsiella species followed by Acinetobacter species 3% and Morganella morganii 3%

In Table 3 it is observed that more deaths occur in late onset VAP.

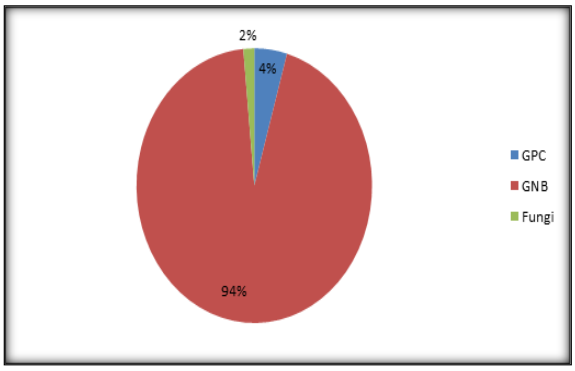

Figure 1: Microbiological Profile

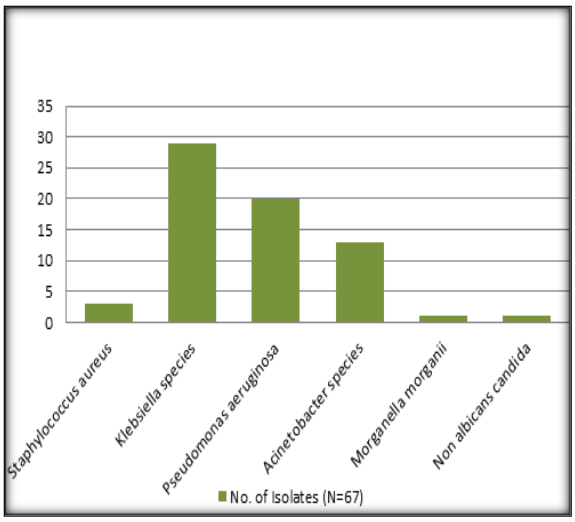

Figure 2: Culture Isolates

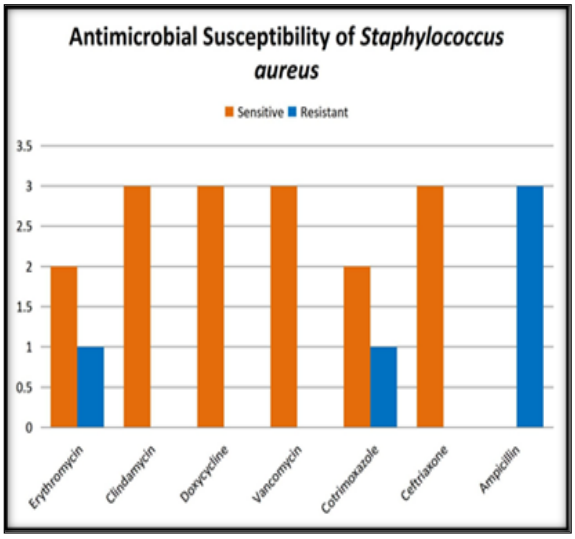

Figure 3: Antimicrobial Susceptibility of Staphylococcus aureus

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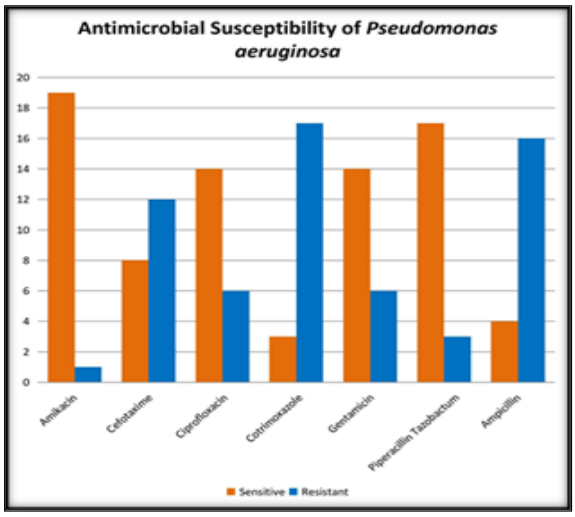

Figure 4: Antimicrobial Susceptibility of Pseudomonas aeruginosa

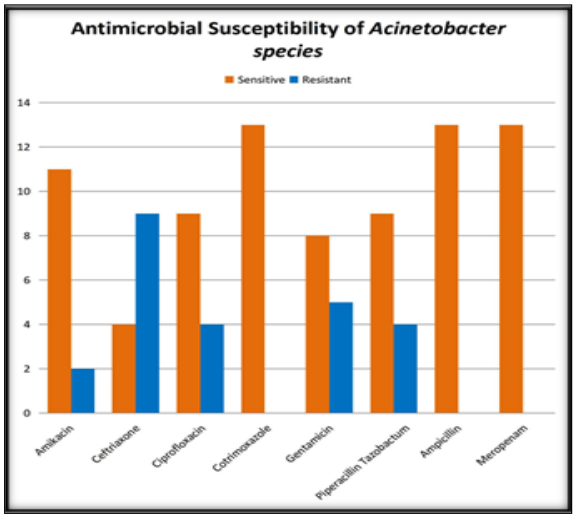

Figure 5: Antimicrobial Susceptibility of Acinetobacter species

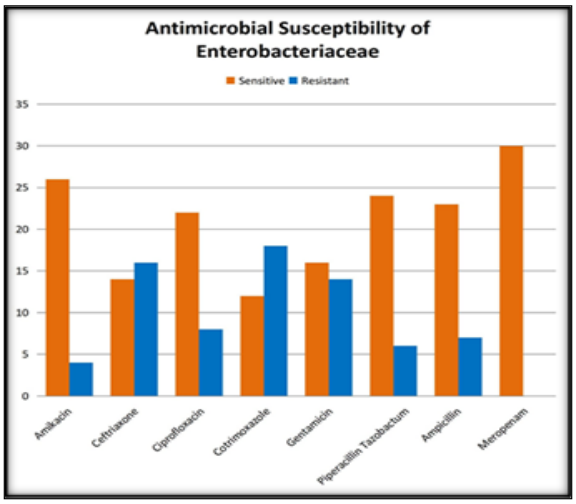

Figure 6: Antimicrobial Susceptibility of Enterobacteriaceae

Table 1: Age wise distribution of VAP

Age wise distribution	VAP Positive	VAP Negative	Total no of cases
Up to 20years (n =27)	8(40%)	12(60%)	20 (100%)
21 – 40years (n =45)	28(54%)	24(46%)	52 (100%)
41 – 60years (n =50)	16(32%)	34(68%)	50 (100%)
Above 61years (n =28)	15(54%)	13(46%)	28 (100%)
Total (n =150)	67	83	150

Table 2: Percentages of positive and negative growth

Result	No of patients	Percentage
Pathogens (>105)	67	45 %
<104Coloniser	23	15 %
No Growth	60	40%
Total	150	100 %

Table 3: Association between onset of VAP and death

Onset of VAP	Death	Discharged	Total
Late Onset 41	31 (76%)	10 (24%)	41 (100%)
Early Onset 26	1 (4%)	25 (96%)	26 (100%)

DISCUSSION

In this study out of 150 samples - 67 (45%) of patients got the growth in culture > 105 and 23 (15%) showed growth < 104 and 60 (40%) showed No Growth. Various studies have reported the frequency of VAP ranging from 19 %4 to 78%8. Of

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the 67 patients who developed growth (VAP) in this study 67% were male and 33% were female.

Of the VAP cases 39% were categorized as early onset and 61% categorized as late onset VAP. This categorization of VAP is more important for starting initial empirical antibiotic therapy. The late onset VAP is commonly associated with Multi Drug Resistant pathogens and hence it should be treated with broad spectrum antibiotics.[9]

In the present study 63 (94%) were Gram negative bacteria, 3 (4.5%) were Gram positive bacteria and 1 (1.5%) was fungi. Majority of VAP Pathogens was Gram Negative organism (94%). But this differs from the study conducted by Taleie10, where GPC Staphylococcus aureus was common 58.7%. This different distribution pattern of etiologic agents between early and late-onset VAP is also linked to the frequent administration of prior antimicrobial therapy in many patients with late-onset VAP.

Among the GNB isolates Klebsiella species 43%; Pseudomonas aeruginosa 30%; Acinetobacter species 19 %; Staphylococcus aureus 4%; Morganella morgagnii 2%; Candida 2%,[8,11] and differ from studies.[12,13] The common organism isolated is Klebsiella pneumoniae, Hence, the knowledge of difference in microorganisms causing VAP in different ICU settings will guide the prescription of appropriate empirical antibiotics and treatment of the infection adequately.

Out of 67 VAP Pathogens 39 were MDR Pathogens, which is similar to 78% MDR Organism12 & 43.65% MDR organism.[14] The prevalence of MDR organisms is variable between institutions and also within institutions

Of the 39 MDR pathogens isolated: individual isolates were Klebsiella,[15] (38%), Pseudomonas aeruginosa 13(33%), Acinetobacter species 10(26%), Morganella morganii (3%). In this present study Klebsiella species followed by Pseudomonas aeruginosa was found to be the most common organism causing Multi Drug Resistance.

Of the 39 patients with MDR isolates,[13] had early onset VAP and 26 had late onset VAP. MDR Isolates are more common in Late Onset VAP14. The higher incidence of VAP and MDR in this study could be attributed to a lack of infection control practices and the irrational use of antibiotics; this shows the importance of strictly following the protocols of antibiotic stewardship programs.

Based on the onset of VAP: In early onset <4 days of intubation, 28% was caused by Klebsiella species followed by Acinetobacter species 3% and Morganella morganii 3%. In late onset >5 days of intubation, 33% of infection is caused by Pseudomonas aeruginosa followed by Acinetobacter species 23% & Klebsiella species 10%

Pseudomonas aeruginosa 33% is most commonly associated with late onset VAP, followed by Acinetobacter species.[14,15]

Pseudomonas aeruginosa is important in causing nosocomial outbreaks and readily spreads from one patient to another patient. This appears to be due to their ability to survive on hands of health care workers and inanimate environmental surfaces and their intrinsic resistance to common antibiotics rather than any potent virulence factors aimed at host defense.

Staphylococcus aureus shows 33% resistant to erythromycin (Inducible clindamycin resistance), clindamycin and cotrimoxazole, whereas ampicillin is 100% resistant. Among them 2 (66.7%) were Methicillin Sensitive Staphylococcus aureus and 1(33.3%) was Methicillin Resistant Staphylococcus aureus.[12] All isolates were sensitive to Vancomycin which was detected by E test method .

In Pseudomonas aeruginosa 60% of isolates show resistant to cephalosporins, 85% to cotrimoxazole, 30% each to ciprofloxacin and gentamicin. They are 100% sensitive to carbapenam and 85% sensitive to piperacillin tazobactum.

In Acinetobacter species 69% resistant to cephalosporins, 85% to gentamicin, 31 % to piperacillin tazobactum as well as fluoroquinolones

Enterobacteriaceae are predominant pathogen isolated in this study and show 53% resistant to first line drugs like cefotaxime, 60% to cotrimoxazole, 47% to gentamicin and 27% resistant to fluoroquinolones. They are 100% sensitive to carbapenams and 80% sensitive to piperacillin tazobactum. They showed 30% resistance to extended spectrum cephalosporins. Emergence of Extended spectrum beta lactamases (ESBLs) in a hospital set up is of increasing concern. The antimicrobial susceptibility pattern of the isolates obtained in this study showed that most of the organisms are Multi Drug Resistant.

In the present study, it was found 32 patients died of VAP. Among them 24 persons (62%) died of Multi Drug Resistant organisms. Of the total 24 MDR patients died, 54% is due to Pseudomonas aeruginosa, followed by 29% due to Acinetobacter species, 17% due to Klebsiella species. Mortality rate increased in patients who got infected with Pseudomonas aeruginosa which is Multi Drug Resistant organism

41 persons developed late onset VAP patients, among them 76% died1, 16. Late-onset VAP had poor prognosis regarding mortality (97%) as compared to the early-onset type (3%). It is seen that the mortality was significantly high in patients with late-onset VAP caused by multi drug resistant Pseudomonas and Acinetobacter infection.

Of the total death, 41% is due to Pseudomonas aeruginosa, 34% due to Klebsiella species, 22% due to Acinetobacter species, 3% due to Staphylococcus aureus.

The higher incidence of ESBL and MDR organisms obtained in the study are a major concern which has to be addressed in terms of reducing inadvertent use of antibiotics and designing an effective infection control policy.[4] These findings confirm the importance of diagnosing VAP at early stage and initiating appropriate antibiotic treatment as tools for preventing adverse outcomes. The pathogens

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that are responsible for VAP vary according to the duration of mechanical ventilation, patient's prior antibiotic exposure and length of hospital stay.

CONCLUSION

Ventilator associated pneumonia is predominant in males; the common age group is 21 to 40 years. Gram negative bacilli were the significant contributor to the development of VAP, among which Enterobacteriaceae – *Klebsiella species* followed by nonfermentors like *Pseudomonas aeruginosa* followed by *Acinetobacter baumannii* were the commonest pathogens isolated. The causative organisms for early onset VAP is different from that of late onset VAP. Increase in association of MDR pathogens with late onset VAP indicates that appropriate broad spectrum antibiotics should be prescribed. MDR patients were more prone to mortality. This study gives knowledge on the causative organisms and the prevailing drug sensitivity pattern of our Intensive Care Unit, which will benefit in improving the active surveillance programs aimed towards an effective hospital infection control strategy.

Limitations

The current study contains relatively fewer numbers of isolates; this can be overcome by continuing to monitor this aspect as a part of quality indicator determining control of hospital acquired infections. This result cannot be applied to other institute, as the bacteriological agents and the factors causing VAP may vary from institution to institution. This study included patients from ICU and is single center study, so the findings and interpretation of the results cannot be generalized to other institutes or other ICUs.

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