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Antimicrobial susceptibility pattern of Gram negative bacterial isolates from cases of Ventilator Associated Pneumonia in a tertiary care institute

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Abstract

Introduction: Ventilator Associated Pneumonia (VAP) is the most commonly encountered health care associated infection among mechanically ventilated patients, which in turn contributed for raised morbidity, mortality and prolonged hospital stay. Early onset VAP is more common in comparison to late onset VAP.

Materials and Method: This study was conducted in a tertiary care teaching hospital for a period of one and half year ranging from January 2013 to June 2014. Clinical Pulmonary Infection score (CPIS) of more than six was used for the clinical diagnosis of VAP and only culture proven cases, out of clinically suspected were further evaluated.

Results: Overall rate of VAP was 19.87 per 1000 device days with (0.40) device utilization ratio. Among Gram negative bacterial isolates from VAP, *Pseudomonas aeruginosa* (50%) was the leading isolate followed by *Acinetobacter baumanii* (17.64%) and *E. coli* (14.70%). *Pseudomonas aeruginosa* exhibited (47.05%) resistance to ciprofloxacin and gentamicin followed by (41.17%) to cefepime, (35.29%) to piperacillin (17.64%) to Amikacin. *Acinetobacter baumanii* exhibited (100%) resistance to amoxycillin+clavulanic, piperacillin, piperacillin+ Tazobactum, followed by (83.33%) to amikacin.

Conclusion: Continuous surveillance data of Ventilator Associated Pneumonia will be helpful in reducing the number of cases of VAP and thus in reducing the associated adverse outcomes.

Keywords: Ventilator Associated Pneumonia, Mechanical Ventilation, *Pseudomonas aeruginosa, Acinetobacter baumanii.*

Introduction

Among several factors responsible for institutional morbidity and mortality Ventilator Associated Pneumonia (VAP) is a major contributing factor which in turn also responsible for extended hospital stay and raised economic burden on patients. Pneumonia of more than 48-72 hours of duration following mechanical ventilation along with altered leukocyte count, changed sputum characteristics, altered temperature, diffuse or localized infiltrate and culture proven detection of a microbial agent is known as Ventilator-associated pneumonia (VAP). Ventilator Associated Pneumonia (VAP) is the most commonly encountered health care associated infection among mechanically ventilated patients.[1-6] Ventilator-associated pneumonia (VAP) of less or equal of 4 days following mechanical ventilation is known as early onset VAP while of 5 or more days is known as late onset VAP. Microbes causing early onset VAP are more sensitive to antimicrobials while etiological agents of late onset VAP are multi drug resistant. VAP is associated with increased adverse outcomes and healthcare expenditure. Ventilator-associated pneumonia (VAP) may be caused by Gram positive and/or Gram negative bacteria and among Gram negative bacteria, *Pseudomonas aeruginosa* is a common isolate. Multidrug-resistant organisms are increasingly being reported especially with late onset VAP.[1-7]

The present study was designed to study the Antimicrobial susceptibility pattern of Gram negative bacterial isolates from cases of Ventilator Associated Pneumonia in a tertiary care institute. The purpose of the Antimicrobial Susceptibility Test (ABST) was to guide the choice of the antimicrobial for the treatment and to provide surveillance data to monitor the resistance trend.

Materials and Method

This study was conducted in Intensive Care Unit (ICU) (medical & surgical) of a tertiary care teaching hospital for a period of one and half year ranging from January 2013 to June 2014. Clinical Pulmonary Infection score (CPIS)[8] of more than six was used for the clinical diagnosis of VAP and only culture proven cases, out of clinically suspected were further evaluated. Endotracheal aspirate (ETA) was collected under aseptic precaution of VAP suspected patients. Only one sample was collected from each patient and after collection it transported immediately to the laboratory. Initially samples were screened by Gram staining and the specimens showed presence of organisms seen under 100x lens or <10 squamous epithelial cells per low power field were included in the study.[9,10] Samples were vertexed to homogenized and then centrifuged. Sample was diluted by mixing 1 ml of Endotracheal aspirate in 10 ml of 0.9% sterile saline solution and made final dilutions of 1/10, 1/100, 1/1000, 1/10000, 1/100000 & 1/1000000. By using a

caliberated nichrome wire loop of diameter of 4 mm carry 0.01 ml of samples was then streaked over blood agar (BA), Chocolate Agar (CA) and MacConkey agar (MA) followed by incubation of all plates at 37°C overnight. Next day plates were observed and bacterial count more than 10^5 cfu/ml was considered significant. Growth below the threshold limit was labeled as colonization or contamination. Identification of bacteria was based on the colony characteristics of the organism i.e. colony morphology, hemolysis on blood agar, changes in the physical appearance of the differential media and enzyme activities of the organisms, Gram staining and biochemical tests.[11-13] Antimicrobial susceptibility test was performed by Kirby-Bauer disk diffusion method on Muller Hinton agar plates.[14] Clinical and Laboratory Standards Institute (CLSI) disc zone interpretative criterion was used to label the isolates as susceptible, intermediate or resistant.[11,12,14] All the reagents and Antibiotic discs were procured from Hi-Media laboratories, Mumbai, India. Mechanical Ventilation (MV) utilization ratio were calculated by dividing the total number of device-days by the total number of patient days.[15] Rates of VAP per 1000 device-days were calculated by dividing the total number of culture positive VAP cases by the total number of device-days and multiplied by 1000.[16]

Results

This study was conducted in Intensive Care Unit (ICU) (medical & surgical) of a tertiary care teaching hospital for a period of one and half year ranging from January 2013 to June 2014. A total of 1315 patients were evaluated during the study period. Of these only 309 were mechanically ventilated for more than 48 hours hence they formed the study subjects. A total of 71 (22.9%) patients developed VAP during their stay in ICU. The incidence of VAP was 19.87 per 1000 ventilator days with (0.40) Mechanical ventilation utilization ratio. (Table 1) Among 71 culture positive VAP cases, 43 (61%) were male and 28 (39%) were female. Ventilator-associated pneumonia (VAP) of less or equal of 4 days following mechanical ventilation were labeled as early onset VAP while of 5 or more days were labeled as late onset VAP. In the present study incidence of late onset VAP (69%) was more in comparison to early onset (31%) VAP. Of 71 patients diagnosed as VAP all were monomicrobial infection with Gram negative bacterial isolates predominance over Gram positive bacterial isolates {64 (90.1%) vs 07 (9.9%)}. Of 64 Gram negative bacterial isolates *Pseudomonas aeruginosa*, 33 (51.56%) was the leading isolate followed by *Acinetobacter baumanii*, 12 (18.75%) and *E. coli*, 08 (12.5%). (Table 2) *Pseudomonas aeruginosa* exhibited (47.05%) resistance to ciprofloxacin and gentamicin followed by (41.17%) to cefepime, (35.29%) to piperacillin (17.64%) to Amikacin, and one (3.03%) of the isolates showed resistance against imipenem. *Acinetobacter baumanii* exhibited (100%) resistance to amoxycillin+clavulanic, piperacillin, piperacillin+ Tazobactum, cefepime, cefotaxime, cefoxitin, ceftazidime, gentamicin and ciprofloxacin followed by (83.33%) to amikacin and one (8.33%) of the isolates showed resistance against imipenem. (Table 4) *E.coli* showed 100% resistance to ampicillin, Amoxycillin+ Clavulanic acid, cegazolin, cefepime, cefotaxime, ceftazidime, cefuroxime and ciprofloxacin. None of the isolate of *E.coli* was resistant to imipenem. Amikacin was less resistant (20%) in comparison to Gentamicin (60%). *K. pneumoniae* showed 100% resistance to ampicillin, Amoxycillin+ Clavulanic, cefuroxime and ciprofloxacin. None of the isolate of *K. pneumoniae* was resistant to imipenem. (Table 3)

Table 1: Device utilization ratio and incidence and of VAP

Type of HAI	Type of device	Device-days	Patients days	utilization ratio	Culture positive VAP cases	Rate per 1000 device-days
VAP	MV*	3573	8845	0.40	71	19.87

* Mechanical Ventilation

Table 2: Gram negative bacterial isolates from VAP (n=64)

Microorganisms	Number	(%)
Pseudomonas aeruginosa	33	51.56
Acinetobacter baumanii	12	18.75
E. coli	08	12.5
Klebsiella pneumoniae	07	10.93
Klebsiella oxytoca	02	3.12
Citrobacter freundii	01	1.56
Citrobacter koseri	01	1.56

Table 3: Resistance pattern of common Gram negative bacterial isolates from cases of VAP (% resistance)

Antibiotics	*Pseudomonas aeruginosa* (n=33)	*Acinetobacter baumanii* (n=12)	*E. coli* (n=08)	*Klebsiella pneumoniae* (n=07)
Ampicillin	NA*	NA	100	100
Amoxycillin + Clavulanic acid	NA	100	100	100
Piperacillin	35.29	100	100	66.66
Piperacillin + Tazobactum	23.52	100	80	66.66
Cefazolin	NA	NA	100	100
Cefepime	41.17	100	100	66.66
Cefotaxime	ND	100	100	66.66
Cefoxitin	29.41	100	60	66.66
Ceftazidime	17.64	100	100	66.66
Cefoparazone	23.52	NA	NA	NA
Cefuroxime	NA	NA	100	100
Imipenem	3.03	8.33	00	00
Amikacin	17.64	83.33	20	66.66
Gentamicin	47.05	100	60	66.66
Ciprofloxacin	47.05	100	100	100
Trimethoprim/ Sulfamethoxazole	NA	83.33	20	33.33

*Not Applied

Observations

Ventilator Associated Pneumonia (VAP) is the most commonly encountered health care associated infection among mechanically ventilated patients.[1-6,17,18] in this study the incidence of VAP was (22.9%) or (19.87 per 1000 ventilator days) which is high but in accordance with the outcomes of other studies.[19-20] while results were not in accordance with the results of other workers.[21-23] The result of mechanical ventilation utilization ratio (0.40 vs 0.37[21]) recorded during the study is comparable with the mechanical ventilation utilization ratio reported by the US in the NNIS network.[21] MV utilization ratio range widely from (0.05 to 0.66).[17] In this study we observed lower rate of early onset VAP (31%) in comparison to the findings of other workers.[3,24] Prior administration of antibiotics may be the reason for lower rate of early onset VAP in the present study. Studies have shown that administration of antimicrobial before mechanical ventilation decreases early onset VAP markedly.[25] Cases of late onset VAP (69%) were slightly higher than the other studies.[13,19] Multidrug Resistant (MDR) Gram negative bacilli such as *A. baumannii*, *P. aeruginosa*, *K. pneumoniae* and *E. coli* are frequently isolated from the cases of VAP.[26-27] Usually VAP is a polymicrobial infection but in the present study we observed only monomicrobial VAP which is not in accordance with other studies.[13,19] In present study *P. aeruginosa* (51.56%) being the most common isolate followed by *A. baumannii* (18.75%) and *E. coli* in (12.50%) cases. These findings were in accordance with other study.[28] causative agents may differ according to the different health care facilities, duration of mechanical ventilation, duration of hospital stay and initiation of antimicrobials prior to the initiation of mechanical ventilation.[29-30] Antibiotic resistance is a major problem in VAP patients especially multidrug resistance among late onset VAP cases.[1-6] In the present study *Pseudomonas aeruginosa* showed 17.64% resistance to ceftazidime. Present study revealed 100% ceftazidime resistant *A. baumannii* and *E. coli* while 66.66% isolates of *Klebsiella* spp were resistant to ceftazidime. respectively. Other workers have reported lower rate of resistance (37- 67.5%) to ceftazidime.[31,32] Extensive and injudicious usage of third generation of cephalosporins may be the reason. In this study, 8.33% isolates of *Acinetobacter baumanii* and 3.03% isolates of *P. aeruginosa* were resistant to imipenem in contrast to another study, where resistance was found in 14.2% isolates of *A. baumannii* and 12-42.5% isolates of *P. aeruginosa*, respectively.[33,34] Present study documented 100% sensitivity to imipenem against *E. coli* and *K. pneumoniae*. These results are comparable with another study.[35] This finding advocates the judicious use of the carbapenems in future. Ciprofloxacin was resistant to 100% isolates of *Acinetobacter baumanii*, *K. pneumoniae* and *E. coli*. Different hospital setup, use of antibiotics and infection control policies among different institutes may be the reasons for varied results.

Conclusion

Data from present study indicates the need of more such kind of studies in the institution and continuous

watch on changing epidemiology, etiology and antimicrobial susceptibility will be helpful in reducing the number of cases of VAP after mechanical ventilation and thus in reducing the adverse outcomes associated with VAP.

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