ORIGINAL ARTICLE

ANTIBIOTIC RESISTANCE AMONG GRAM NEGATIVE BACILLI CAUSING VENTILATOR – ASSOCIATED PNEUMONIA

Aneela Altaf Kidwai, Salma Razzaq, Qaiser Jamal, Sabeen Aatif, Saleemullah Paracha

ABSTRACT

OBJECTIVE: To determine the microbial pattern and antimicrobial resistance among *Gram- negative bacteria (*GNB) recovered from patients with ventilator- associated pneumonia (VAP).

MATERIALS & METHODS: An observational case control study was conducted in medical intensive care unit (MICU), having 15 beds, from January 2010 to December 2010. All the patients who remained ventilated for $\geq$ 48 hours were prospectively followed for the occurrence of VAP. Deep tracheal aspirate were obtained for culture and Gram-stain and antibiotic resistance rate among *Gram negative bacilli* was documented.

RESULTS: Ventilator associated pneumonia was identified in 54 (42.8%) patients. *Gram negative bacilli* were isolated from 77% specimens. *Acinetobacter spp.* (34%) followed by *Pseudomonas aeruginosa* (18%) were the most frequently isolated pathogens. All *Acinetobacter spp.* were resistant to ceftazidime (100%), amikacin (91%), ciprofloxacin 88% and to imipenem 86%. Isolates of *Pseudomonas aeruginosa* were resistant to ceftazidime 83% and 77% to ciprofloxacin.

CONCLUSION: Ventilator associated pneumonia is predominantly caused by *Gram negative bacilli.* Ceftazidime, ciprofloxacin, imipenem and amikacin resistance rate were higher against Acinetobacter spp. Ceftazidime and ciprofloxacin resistant rates were also higher among other Gram- negative isolates.

KEY WORDS: Ventilators, Pneumonias, Gram negative bacteria, Antibiotic Resistance.

Medical unit-III, Abbasi Shaheed Hospital, Karachi.

INTRODUCTION

Ventilator-associated Pneumonia (VAP), defined as pneumonia occurring in more than 48 hours after endotracheal intubation and initiation of mechanical ventilation (MV), is the most frequent nosocomial infection among patients receiving mechanical ventilation[1]. VAP frequencies have been reported to be varied between 08 – 28%[1] and the reported mortality ranges from 20 – 70% [2]. Evidence suggests that rates of VAP are higher in countries with limited resources[3]. Significant organisms responsible for VAP are *Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter spp.* and *Enterobacteriaceae;* however the etiological agents may vary among different medical setups, population of patients in ICU, duration of mechanical ventilation, length of ICU stay and the method used for diagnosis of VAP.

In terms of mortality and healthcare cost, antibiotic resistance among the organisms causing VAP is a major concern. Prolonged hospital stay, increasing mortality rates & higher medical expenses have been associated with antimicrobial resistant strains. Among common pathogens causing VAP, Gram- negative bacilli have been reported to be the most common etiological agents [4,5]. With the emergence of increasing antimicrobial resistance among Gram- negative pathogens responsible for VAP, epidemiological information regarding the distribution and anti microbial resistant pattern is essential to the appropriate selection of empirical therapy. An updated data of local etiological agents and antimicrobial resistant pattern is of utmost importance to improve clinical outcomes. Present study was designed to analyze the trends in the distribution and antimicrobial resistant pattern of *Gram- negative* pathogens causing VAP in a tertiary care hospital.

MATERIALS & METHODS:

This observational case control study was conducted in the 850 bedded Postgraduate teaching tertiary care hospital, having 15 bedded Medical ICU, from January, 2010 to December, 2010. The study population consisted of all patients (14 years and above) who remained ventilated for 48 hours or more during ICU stay. Patients having respiratory tract infections at the time of admission in ICU were excluded. ICU admissions were from emergency room; patients shifted from medical wards and referred cases from other local hospitals in Karachi were also excluded.

All the eligible patients were prospectively followed by their attending physicians for the occurrence of VAP. Patients were followed until weaned from mechanical ventilation and discharged or died. Competent research assistances were assigned to collect data. A questionnaire was used to record demographics characteristics, duration of mechanical ventilation, length of ICU stay, associated co-morbidities and Acute Physiology and Chronic Health Evaluation (APACHE II score) . Chest radiographs and white blood cell counts were performed daily. Deep tracheal aspirate were obtained for Gram stain and culture in all suspected cases of VAP. Deep tracheal aspiration was performed using sterile suction catheter. Deep suctioning was defined as the insertion of a sterile suction catheter until resistence is met, followed by withdrawal of the catheter by 1 cm before application of negative pressure.

Suction tubes were sent to microbiology lab in sterile containers, secretions were inoculated according to standard CLSI recommended methods, and sensitivity was done by Kirlng baur disc diffusion method with standard MacFarland inoculums. Antibacterial agents included amikacin, betalactams (penicillin, third generation cephalosporin, imipenem), ciprofloxacin, and colistin / Polymyxin . Empirical antibiotic therapy (started after obtaining deep tracheal aspirates) was based on the knowledge of commonly indentified bacterial pathogens associated in VAP in Medical ICU.

Nosocomial Pneumonia was identified by using the Centers for Disease Control and Prevention (CDC) definition as follow [6]:

1. Radiological Signs of the definition of clinical diagnosis of Hospital Acquired Pneumonia are: (A) $\geq$2 serial chest radiographs with at least one of the following: 1: New or progressive & persistent infiltrate. 2: Consolidation. 3: Cavitation.

2. Clinical Signs of the definition of clinical diagnosis of Hospital Acquired Pneumonia are: At least one of the following: 1: Fever (Temperature > 38º without other recognized cause). 2: Leukopenia (< 4.010^9 Cells/L or leukocytosis (>12.010^9 Cells/L). 3: For adults $\geq$ 70 years of age, altered mental status with no other recognized caused. 4: New onset of purulent sputum, change in character of sputum, increased respiratory secretions or increased suctioning requirement. 5: New onset or worsening cough or dyspnea or tachypnea. 6: Rales or bronchial breath sounds. 7: Worsening gas exchange (e.g. oxygen desaturation ratio (PaO_2-FiO_2) $\leq$ 240, increase oxygen requirement, increase ventilation demand).

Ventilator Associated Pneumonia (VAP) was defined as nosocomial pneumonia in patients who remained on mechanical ventilation for $\geq$ 48 hrs.

Results were expressed as mean $\pm$ standard deviation for continuous variables and as percentages for categorical variables. Frequency of VAP was calculated by dividing the number of patients who acquired VAP with the total number of patients who received mechanical ventilation in during specified time period. Data was entered and evaluated on SPSS V.17. Continuous variables were compared by student's T-test. P. Value of < 0.05 was considered statistically significant.

RESULTS:

During the period of 01 year, 126 patients received mechanical ventilation for ≥ 48 hours, out of which 54 (42.8%) developed VAP. Twenty-two (40.7 %) were male and 32 (59.3 %) were females. Mean age was 36 ± 16.5 (range 14-82 years). A comparison of demographics characteristics, APACHE II score and duration of mechanical ventilation among patients who developed VAP (VAP group) & those who did not develop VAP (Non-VAP group) is shown in table I. Duration of mechanical ventilation was significantly higher among VAP group as compare to non-VAP (P-Value: 0.001)

Among 54 patients 105 micro-organisms were isolated as positive agents of VAP. Twenty three patients (44%) had monomicrobial infection while 30 (56%) patients had polymicrobial infection. Frequencies of micro organisms responsible for VAP are shown in table II. Gram- negative pathogens including *Acinetobacter spp*. (34%), *Pseudomonas aeruginosa* (18%), *Enterobacter* (14%) and *E.coli* (8.5%) were isolated from 77% tracheal aspirates where as Gram- positive pathogens including *Staphylococcus aureus* (20%) & *Streptococcus pneumonia* were isolated from 28% specimens. *Acinetobacter, Staphylococcus aureus* and *P.aeruginosa* were the most frequents pathogens included in polymicrobial infections. Regarding *Acinetobacter spp,* higher rates of resistance was observed for ceftazidime (100%), amikacin (91%), ciprofloxacin (88%) and imepenem (86%).

Multi drug resistance pattern (susceptible only to colistin/polymyxin-B) was observed in 42% of *Acinetobacter spp.* All isolates of *Acinetobacter* and *Pseudomonas* were sensitive to colistin/polymyxin. Distribution of antibiotic resistance among identified organisms is shown in table III.

DISCUSSION:

In Asian countries, incidence of VAP has been reported to be higher than Western countries. In Canadian ICU's, VAP has been documented in 18% of patients [7]. In contrast an Indian study showed 45.4% incidence of VAP [8]. Our study also reports higher incidence (41.2%) of VAP. The higher incidence of VAP in present study may be attributed to the higher proportion of referred cases from private sector, inadequate nursing staff as well as significantly longer duration of mechanical ventilation (Table I).

The higher rate of *Gram negative bacilli* (GNB) among etiological agents causing VAP has been increasingly reported and GNB have found to be responsible for more than 60% of VAP in several studies [1]. A study conducted by Erdem et al showed 72% of VAP were due to GNB [9.] In our study GNB represented 77% of the causative organisms. Our findings are in consistent with another local study from Karachi [10.]

Worldwide *Acinetobacter spp.* has been emerged as one of the most prevalent Gram- negative pathogens causing VAP. Chawla et al reported *Acinetobacter* to be the most common pathogens responsible for hospital acquired pneumonia & VAP in Asian countries including Pakistan, India, Malaysia and Thailand [11]. Present study and other local study from Karachi [10] also report *Acinetobacter* to be the most common etiological agent. In contrast to our findings, Wahid et al reported *Pseudomonas aeruginosa* and *Staphylococcus aureus* as the most prevalent organism[12]. *Pseudomonas* and *Staphylococcus aureus* were the next common isolated organisms in this study. Current study report significantly higher rates (56%) of poly microbial infections; other studies [10,13] also show similar results. *Acinetobacter, Staphylococcus aureus* and *Pseudomonas* were the predominant organisms identified in polymicrobial infection.

Increasing resistance of *Acinetobacter spp.* to commonly prescribe antibiotic is a growing concern [14]. *Acinetobacter* resistance to ceftazidime, ciprofloxacin and imipenem has been reported [9, 15]. Our study also shows high resistance to ceftazidime, amikacin, imipenem and ciprofloxacin (Table 03). In comparison to above mentioned antibiotic 58% of isolates were found to be resistant to cefoperazone/sulbactum where as all isolates were sensitive to colistin/polymyxin. Similar to our findings Khan et al (10) also reported higher resistance of Acinetobacter spp. to ceftazidime (89%) amikacin (78%) and meropenem (85%). Piperacillin-tazobactum, amikacin, imipenem and ceftazidime are commonly prescribed antibiotic against *Pseudomonas aeruginosa* in our setup. We found high resistance rate of piperacillin-tazobactum, amikacin and imipenem against *Pseudomonas aeruginosa* where as high resistance rate were also observed for ceftazidime and ciprofloxacin. We also report higher resistance rates of ceftazidime & ciprofloxacin against *Enterobacter spp.* and *E. coli.*

Multi-drug resistant (MDR) strains of *Acinetobacter spp.* have been reported by many studies [8, 16]. Emergence of *Acinetobacter spp.* resistant to major anti microbial drugs resulted in several outbreaks in ICUs [17, 18]. However, the resistance patterns are different in different parts of the world. . Day et al [8] reports 21.7% MDR *Acinetobacter* strains. In present study 42% of *Acinetobacter spp.* was MDR strains.

In conclusion, we identified predominant GNB causing VAP with higher frequency of polymicrobial infection. Ceftazidime, ciprofloxacin, imipenem and amikacin resistance rates were higher against *Acinetobacter spp,* which showed moderate resistance against cefoperazone/sulbactum. Ciprofloxacin and ceftazidime resistance rates were also higher against *Pseudomonas aeruginosa, Enterobacter spp.* and *E. coli.* We suggest administration of appropriate empirical antibiotic to reduce the emergence of antibiotic resistance among Gram- negative pathogens.

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TABLE I:

Demographics Characteristics of Patients

Characteristics	**VAP n=54**	**Non-VAP n=72**	**P-Value**	**95% C I**
Age (Mean ± SD)	36.4 ±16.5	40.25 ±19.18	0.33	- 9.48 – 3.28
Gender Male Female	 22 (40.7%) 32 (59.3%)	 48 (66.7%) 24 (33.3%)	0.006	1.13 – 2.25
Duration of Mechanical Ventilation	11.85 ±10.34	3.33 ± 3.21	0.001	5.06 – 11.43
APACHE II Score (Mean ± SD)	22.48 ± 9.85	24.92 ± 6.53	0.119	- 5.50 – 0.63

TABLE II:

Frequency of Isolated Organism:

Micro-organisms	**No (%)**
Acinetobacter spp.	36 (34%)
Staphylococcus aureus	21 (20%)
Pseudomonas aeruginosa	19 (18%)
Enterobacter spp.	15 (14%)
E. soli	09 (8.51%)
Streptococcus pneumonae	03 (2.8%)
Klebsella pneumonae	02 (2%)
Total (105)	(100%)

TABLE III:

Frequency of Antimicrobial Resistance of Isolated Pathogens:

PATHOGEN	ANTIBIOTIC	RESISTANCE No (%)
Acinetobacter spp.	Cefoperazone + Sulbactum	21/36(58%)
	Tazobactum / Piperacillin	25/36 (69.4%)
	Imepenem	31/36 (86%)
	Ceftazidime	36/36 (100%)
	Ciprofloxacin	32/36 (88.8%)
	Amikacin	33/36(91%)
	Polymyxin	0/36 (0%)
Pseudomonas	Tazobactum / Piperacillin	03/18(16.6%)
	Meropenem	03/18 (16.6%)
	Ceftazidime	15/18 (83%)
	Ciprofloxacin	14/18(77.7%)
	Amikacin	06/18 (33.3%)
	Polymyxin / colistin	0/18 (0%)
Enterobacter spp.	Amoxicillin / clav	04/15 (26.6%)
	Tazobactum / Piperacillin	03/15 (20%)
	Ceftazidime	15/15 (100%)
	Amikacin	07/15 (46.6%)
	Meropenem	0/15 (0%)
	Ciprofloxacin	09/15 (60%)
E. coli	Tazobactum / Piperacillin	01/09 (16.6%)

	Ceftazidime	09/09 (100%)
	Amikacin	1/09 (16.6)
	Meropenem	1/09 (16.6)
	Ciprofloxacin	09/09 (100%)