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Original Article

Ventilator associated pneumonia: bacterial isolates and its antibiotic resistance pattern.

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ABSTRACT

Aims: Ventilator Associated Pneumonia (VAP) is the most frequent intensive care unit (ICU) acquired infection. Due to the increasing incidence of multidrug resistant organisms (MDR) in the ICU, early and correct diagnosis of VAP is an urgent challenge for optimal antibiotic treatment. The aim of the study was to detect the bacterial pathogens and to know their susceptibility pattern among clinically suspected VAP cases. Materials and Methods: Patients admitted to ICU fulfilling the clinical criteria for VAP were included in the study. Endotracheal aspirate (EA) was collected aseptically and processed. Gram stain smear showing > 25 pus cells/ low power field were cultured and growth of > 10^5 cfu were considered as pathogens. The isolates were subjected to antibiotic sensitivity testing by Kirby Bauer disc diffusion method. Isolates resistant to 3rd generation cephalosporins were screened for the production of ESBL and AmpC ß lactamase. Isolates resistant to carbapenems and cefoxitin were screened for MBL and MRSA respectively. Results: A total of 806 patients were put on mechanical ventilation in different health care centres during Jan 2006- Dec 2010. At 48 hrs, 328 patients were weaned from ventilator support. The rate of VAP was 21%. The infection was polymicrobial in 20.29% cases. Gram negative bacilli were isolated in 351 cases (73.43%). *Acinetobacter baumannii* (22.17%) was the major pathogen isolated, followed by *Pseudomonas aerugionsa* (19.66%). Among the gram positive cocci isolated *Staphylococcus aureus* (11.44%) was predominant. *Klebsiella pneumoniae* (32.18%) and *Escherichia coli* (66.66%) were ESBL producers. *Pseudomonas aeruginosa* (2.19%) and *Acinetobacter baumannii* (0.9%) were Amp C ß lactamase producers. Conclusion: Rate of VAP in ICUs was high. MDR gram negative bacilli were the major pathogens. Judicious and timely use of appropriate antibiotic can reduce morbidity, mortality and high cost incurred because of longer ICU stay due to VAP.

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1. Introduction

Ventilator associated pneumonia(VAP) is defined as pneumonia occurring more than 48hrs after endotracheal intubation and initiation of mechanical ventilation (MV) including pneumonia developing even after extubation [1]. Risk factors such as patient related, infection related and intervention related predispose to VAP which occurs in 9 - 27% of all intubated patients [2]. It is the most common nosocomial infection which affects patients in the intensive care units [3].

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Early onset VAP, which occurs during the first four days of MV, usually is less severe, associated with better prognosis and are likely to be caused by antibiotic sensitive bacteria. Late onset VAP, which develops five or more days after initiation of MV is caused by multidrug resistant (MDR) pathogens and is associated with increased morbidity and mortality [4]. The incidence of multidrug resistant strains which cause VAP vary from hospital to hospital, among the types of ICU patients with antibiotic use and among different patient population and co-morbid conditions [3, 5].

In addition non-availability of proper diagnostic enterprise contributes to the poor prognosis seen in these cases. Appropriate sample collection and ideal bacteriological examination by an experienced clinical microbiologist is of major help in the management of VAP. It posses great management challenge to the physician because of multidrug resistant (MDR) nature of the causative agents such as *Methicillin Resistant Staphylococcus aureus* (MRSA), quinolone and carbapenem resistant Pseudomonas aeruginosa, carbapenem resistant Acinetobacter species and ß lactamase producing *Klebsiella pneumoniae* and *Escherichia coli.* Indiscriminate antibiotic usage is the major contributing factor for the development of MDR. It has been suggested that MDR organisms or *Pseudomonas species* are high risk organisms or patients who develop VAP due to these organisms are at high risk for poor clinical outcome.

The present study was carried out between Jan 2006 - Dec 2010 to detect the bacterial pathogens and to know their susceptibility pattern among clinically suspected VAP cases admitted in ICU of different hospitals in an urban town in Karnataka.

2. Material and Methods

Adult patients admitted to ICU with history of organo-phosphorus poisoning, road traffic accident, snake bite, chronic obstructive pulmonary disease, sepsis formed the study population.

Selection of Patients

Population selected for the study were patients on mechanical ventilation for more than 48hrs fulfilling any two of the clinical criteria for ventilator associated pneumonia such as new and persistent pulmonary infiltrate on the chest radiograph and any of the following [6]. 1. Fever ≥ 38°C or hypothermia ≤ 36°C. 2). WBC count ≥10000 mm^3 or ≤4000 mm^3. 3). Purulent tracheal secretion.

Collection of the Endotracheal Aspirate (EA): [7]

EA were collected from patients who fulfilled the above criteria using a Ramsom's mucous extractor and transported to the laboratory for further processing. Endotracheal Aspirate (EA) was collected at the time of admission, 48 hrs, 72 hrs and at the end of 96hrs. Further samples were collected after 8days depending on the duration of MV. Gram stain of the cytospin specimen showing ≥25 polymorphonuclear cells/low power field and bacteria/ oil immersion field was considered as diagnostic. Quantitative cultures (QEA) of the specimen were done on MacConkey agar, Blood agar and the organisms were identified by standard biochemical tests. Quantitative cultures showing 10^5 cfu/ml were considered diagnostic. Isolates identified were subjected to antibiotic susceptibility testing on Mueller Hinton Agar by Kirby Bauer disc diffusion method using a panel of antibiotics as per CLSI guidelines. Gram negative bacilli resistant to 3rd generation cephalosporins were tested for production of extended spectrum β lactamase (ESBL) and AmpC β lactamase. Metallo β lactamase (MBL) were detected among isolates resistant to carbapenems. *Staphylococcus aureus* resistant to cefoxitin were tested for MRSA. Descriptive statistics were used such as percentages to analyse the data obtained from the study.

3. Results:

A total of 806 patients were put on mechanical ventilator in different health care centres during Jan 2006 - Dec 2010.The baseline study of the samples did not show evidence of any infection. Three hundred and twenty eight patients were weaned from ventilator support at the end of 48 hrs of MV. Samples collected after 48hrs, 72hrs and 96hrs showed the presence of significant number of PMN cells and one or more bacteria. QEA yielded ≥ 10^5 cfu/ml in 478 cases (59.30%).

Gram negative bacilli were isolated in 351 cases (73.43%) as shown in Table I. *Acinetobacter baumannii* was the major pathogen isolated 106 (22.17%), followed by *Pseudomonas aerugionsa* 91 (19.66%) , *Klebsiella pneumoniae* 87 (24.78%), *Escherichia coli* 57 (16.23%), *Proteus species* 4 (1.13%) and *Citrobacter species* 2 (0.5%). Among the gram positive cocci isolated 127 (26.56%) *Staphylococcus aureus* was isolated in 54 cases (11.44%) as shown in Table 2.

The infection was polymicrobial in 97 cases (20.29%). The *antibiogram* (Table 3) of the gram negative bacilli showed the *Acinetobacter baumannii* (46.22%) and *Pseudomonas aeruginosa* (18.68%) were multidrug resistant. *Acinetobacter baumannii* showed highest resistance to ampicillin (100%) followed by cefotaxime (88.6%), ceftazidime (78%), amikacin (48%), imipenem (42.4%) and meropenem (42.4%). *Pseudomonas aeruginosa* also showed highest resistance to ampicillin (100%) followed by 47.2% to cefotaxime and ceftazidime and 18.6% to imipenem and meropenem, Among the gram positive cocci *Staphylococcus* aureus was the commonest isolate of which 10 were resistant to cefoxitin. Ten of these were MRSA (11.44%) as shown in Table 4.

Among the isolates 32.18% of *Klebsiella pneumoniae* 66.66% of *Escherichia coli* were ESBL producers. Fourteen *Acinetobacter baumannii* (26.41%) and 10 Pseudomonas *aeruginosa* (10.98%) were MBL producers. Two *Pseudomonas aeruginosa* (2.19%) and one *Acinetobacter baumannii* (0.9%) were Amp C β lactamase producers. All the MBL strains were sensitive to polymyxin B, colistin and piperacillin/ tazobactam. Amp C β lactamase producers were sensitive to imipenem, meropenem and piperacillin/tazobactam.

All strains of *Klebsiella pneumoniae* and *Escherichia coli* including ESBL producers were sensitive to imipenem, meropenem and piperacillin- tazobactam, of the 54 *Staphylococcus aureus*

isolated 10 were MRSA (18.15%). All the MRSA strains were resistant to penicillin and erythromycin, while 100% sensitivity was seen for vancomycin and linezolid.

Table 1: Gram Negative bacteria isolated

Name of the isolate	Number
Acinetobacter baunannii	106
Pseudomonas aeruginosa	91
Klebsiella pneumonia	87
Escherichia coli	57
Proteus sps	08
Citrobacter sps	02
Total	**351**

Table 2: Gram positive isolates

Name of the isolate	Number
Staphylococcus aureus	54
Coagulase negative Staphylococcus aureus	40
α-Haemolytic Streptococcus	33
Total	127

Table 3: Antibiogram of the isolates

ANTIBIOTIC	Acinetobacter baunannii		Pseudomonas aeruginosa		Klebsiella pneumonia		Escherichia coli		Proteus sps		Citrobacter sps	
	S	R	S	R	S	R	S	R	S	R	S	R
AMPICILLIN	0	106	0	91	2	85	5	52	0	8	0	2
GENTAMYCIN	11	95	31	60	50	37	39	18	7	1	2	0
AMIKACIN	55	51	51	40	64	23	38	19	7	1	2	0
CIPROFLOXACIN	21	85	33	58	38	49	27	30	6	2	2	0
OFLOXACIN	22	84	34	57	38	49	27	30	6	2	2	0
CHLORAMPHENICOL	36	70	31	60	53	34	34	23	6	2	2	0
PIPERACILLIN-TAZOBACTUM	68	38	42	49	66	21	21	36	8	0	2	0
CEFTAZIDIME	23	83	48	43	42	45	22	35	8	0	2	0
IMIPENEM	61	45	74	17	74	13	57	0	8	0	2	0
MEROPENEM	61	45	74	17	51	36	57	0	8	0	2	0
CEFOTAXIME	12	94	48	43	42	45	13	44	7	1	2	0
CEFEPIME	21	85	48	43	42	45	19	38	7	1	2	0
TETRACYCLINE	47	59	55	36	50	38	30	27	7	1	2	0

a.S – Sensitive, b. R – Resistant

Table 4: Antibiogram of Gram Positive Isolates

ANTIBIOTIC	Staphylococcus aureus		CONS		α-haemolytic Streptococci	
	S	R	S	R	S	R
Penicillin	0	54	30	3	33	33
Ampicillin	20	34	28	5	30	30
Amoxy/clav	20	34	28	5	33	33
Erythromycin	15	39	30	3	33	33
Ciprofloxacin	15	39	33	0	33	33
Cephalaxin	10	44	33	0	33	33
Cefotaxime	10	44	33	0	-	-
Linezolid	54	0	33	0	-	-
Cefoxitin	44	10	33	0	-	-

a.CONS-Coagulase negative Staphylococcus,

4. Discussion

The present study aimed at detecting the incidence of VAP in the ICU of different health care centres of urban city in Karnataka. This study demonstrates that VAP as an important nosocomial infection among patients receiving MV in the ICU's of our city. VAP is a form of hospital acquired pneumonia is a serious infection with a high mortality rate and in the literature, the overall incidence of VAP in ICU's ranges from 10-70% [3].

The incidence of VAP was 59.30%.and the rate of VAP was 210/1000 ventilator days (21%). Such high rate of VAP has been reported from few other centers in India by Rakshit et al and Mukhopadhyay et al [8, 9]. The high incidence of VAP and MDR in our study could be attributed to lack of barrier nursing and over use of second line drugs.

The pathogens which are responsible for VAP vary depending on the duration of mechanical ventilation, prior antibiotic exposure and length of hospital stay. Gram negative bacilli have been found to be major pathogens of VAP (73.43%) [1]. *Acinetobacter baumannii* (22.17%) followed by *Pseudomonas aeruginosa* (19.66%), *Klebsiella pneumoniae*(18.20%), *Escherichia coli* (11.92%) and *Staphylococcus aureus* (11.29). Similar pattern of bacterial isolation has been reported by Dey et al [7].

Acinetobacter baumannii (22.17%) was the common organism isolated followed by *Pseudomonas aeruginosa* in our study. Earlier studies have shown that *Pseudomonas aeruginosa* was the most common organism [8]. In the present study, *Acinetobacter baumannii* was found to be the most common organism causing VAP, followed by *Pseudomonas aeruginosa.* Although *Acinetobacter species* is less virulent than *Pseudomonas species,* they are becoming more and more resistant to commonly used antibiotics [8, 11, 12].

There is high antibiotic resistance in gram negative pathogens which are isolated from ICUs that are resistant to ceftazidime, cefotaxime, ciprofloxavin, gentamicin and amikacin. Resistance to carbepenems is on a rise all over the world due to the production of metallo β Lactamase [12]. Recent studies have shown the increasing incidence of multidrug resistant pathogens among patients with VAP [8, 9]. A study by Dey et al showed the increased incidence of MDR organisms in the ICU, an early and correct diagnosis of VAP is a challenge for optimal treatment. The emergence of MDR pathogens can be prevented by adopting an institutional antibiotic policy and dose de-esculation regimens [10, 11].

ESBL and Amp C β lactamases are of increasing clinical concern. ESBLs are most commonly produced by Klebsiella species and Escherichia coli but may also occur in other gram negative bacilli. They are typically plasmid mediated clavulanate susceptible enzymes that hydrolyze penicillins, extended spectrum cephalosporins (cefotaxime, ceftriaxone, ceftazidime, cefepime and others) and aztreonam. Amp C β lactamases are cepholosporinases that are poorly inhibited by clavulanic acid. They can by differentiated from other ESBLs by their ability to hydrolyze cephamycins (cefoxitin, cefotetan) as well as extended spectrum cephalosporins. In our study ESBL producers were common among Enterobacteriaceae. Escherichia coli 38/66 (57.57%) and Klebsiella pneumoniae 28/66 (42.42%) similar results have been reported by Dey et al [10].

Non-lactose fermenting isolates resistant to carbapenems were screened for the production of MBL by modified hodge test as per CSLI guidelines. Fourteen MBL were detected among Acinetobacter baumannii and 10 were MBL producers among Pseudomonas eruginosa isolates. Presently there is concern about the acquisition of plasmid mediated metallo β lactamases active against carbapenems and antipsuedomonal penicillins and cephalosporins [10]. These isolates were resistant to imipenem. Combination therapy was used to treat these patients. Prolonged use of carbapenems predisposes to VAP by panresistant Acinetobacter baumannii and Pseudomonas aeruginosa. Potential MDR was a threat to our ICUs and hospital settings and maximum number of MDR were obtained from patients with history of previous antibiotic exposure, a longer duration of mechanical ventilation and co-morbid factors.

5. Conclusion:

VAP is increasingly associated with MDR pathogens. Production of ESBL, AmpC ß lactamase, MBL and MRSA were responsible for multidrug resistance of these pathogens. Local epidemiology data like this should be collected at all centres, as it would help guiding the initial empirical antibiotic therapy and reduce morbidity mortality and cost of hospitalization. Colistin and piperacillin/tazobactam may be used for successful treatment of multidrug resistant *Acinetobacter species* and *Pseudomonas species* as they showed good in vitro activity against MDR pathogens.

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