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Research Article

Antimicrobial resistance in bacteria causing ventilator-associated pneumonia in a tertiary care hospital: one year prospective study

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ABSTRACT

Background: Ventilator-associated pneumonia (VAP) is the most common infection diagnosed in intensive care units (ICUs). The causative organisms of VAP vary among different populations and are increasingly associated with resistance against various antimicrobial agents. Objective of current study was to determine the bacteriological etiology of VAP, antimicrobial susceptibility pattern of the isolates and detect the presence of extended-spectrum β-lactamases (ESBL), metallo β-lactamases (MBL) and AmpC β-lactamases in multidrug resistant isolates causing VAP in the medical ICU.
Methods: A prospective study was carried out over a year to know the various etiological agents of VAP and their drug susceptibility patterns. ESBL, MBL and AmpC β-lactamases were detected in various isolates by combination disk method, imipenem-EDTA combined disk method and AmpC disk method respectively.
Results: The majority of bacterial isolates causing VAP were found to be gram negative bacilli. Acinetobacter spp accounted for 34.28% of VAP cases followed by Pseudomonas aeruginosa which was responsible for 25.71% cases. Other gram negative bacilli isolated were Klebsiella pneumoniae, Citrobacter freundii, Enterobacter spp, and Escherichia coli. Out of the total 70 isolates, 67 (95.7%) were multidrug resistant and not even a single isolate was sensitive to all the drugs tested.
Conclusions: Most of the pathogens causing VAP in our institute were multidrug resistant and in many isolates this resistance was due to production of ESBL, MBL, and AmpC β-latamases. Polymixin-B and colistin were found to be highly effective against multidrug resistant Acinetobacter spp and P. aeruginosa.

Keywords: Ventilator-associated pneumonia, Intensive care unit, ESBL, MBL, AmpC β-lactamases

INTRODUCTION

Ventilator associated pneumonia (VAP) is the most common infection diagnosed in intensive care units (ICUs). VAP is defined as pneumonia that occurs 48 hours or more after endotracheal intubation or tracheostomy, caused by infectious agents not present or incubating at the time mechanical ventilation was started.[1] It can be of two types. Early-onset VAP, defined as occurring within the first 4 days of mechanical ventilation, usually carries a better prognosis, and is more likely to be caused by antibiotic sensitive bacteria. Late onset VAP occuring 5 days or more after mechanical ventilation is more likely to be caused by multidrug resistant (MDR) pathogens, and is associated with increased patient mortality and morbidity.[2] The specific microbial causes of VAP are many and varied. Gram negative bacteria, including Pseudomonas aeruginosa, Acinetobacter spp and enteric gram negative rods are implicated in 55-85% of VAP cases. High rates of

Haemophilus influenzae, Streptococcus pneumoniae, Methicillin sensitive Staphylococcus aureus (MSSA), or susceptible Enterobactericeae were constantly found in early onset VAP, whereas P. aeruginosa, Acinetobacter spp, Methicillin resistant Staphylococcus aureus (MRSA), and multi drug resistant gram negative bacteria were significantly more frequent in late onset VAP.[3] Prompt usage of appropriate antibiotics is essential to optimize the outcome of VAP. Unfortunately, antimicrobial resistance has escalated dramatically within the past decade and has created obstacles to effective antibiotic choices. In critically ill patients requiring prolong mechanical ventilation in ICUs, P. aeruginosa and Acinetobacter spp, which are resistant to many antibiotics, account for 30-40% of VAP.[4] Appropriate choice of antibiotics requires awareness of relevant pathogens, antimicrobial resistance patterns, and the host and demographic factors that may lead to infection and/or evolution of antibiotic resistance.

The aim of our study was to determine the bacteriological etiology of VAP and the antimicrobial susceptibility pattern of the isolates. We also aimed to detect the presence of extended-spectrum β-lactamases (ESBL), metallo β-lactamases (MBL) and AmpC β-lactamases in multidrug resistant isolates causing VAP in the ICU.

METHODS

A prospective study was conducted in Department of Microbiology in association with the multidisciplinary ICU of the Pulmonary and Critical Care Medicine Department of our institute for a period of one year extending from June 2009 to May 2010. VAP rate was defined as the number of ventilator-associated pneumonias per 1,000 ventilator days.[5] Patients who received mechanical ventilation for more than 48 hours were included in the study. Modified clinical pulmonary infection score (CPIS) was followed as a screening method to clinically diagnose VAP. The diagnosis of VAP was based on clinical and microbiological criteria. A clinical suspicion of VAP was made in patients with modified CPIS score >6.[6]

The diagnosis was confirmed when significant growth was obtained in the culture of the samples. Endotracheal aspirates (ETA) and bronchoalveolar lavage (BAL) samples of the patients were collected and sent immediately to the laboratory for microbiological processing. Gram staining was done after making smears of the samples and the samples were then inoculated on blood agar, MacConkey agar and chocolate agar. Semi-quantitative cultures were done.[7] The MacConkey plates were incubated at 37^0C while blood agar and chocolate agar were incubated at 37^0C in presence of 5-10% carbon dioxide. Growth $>10^5$ CFU/ml was taken as the cutoff threshold for endotracheal aspirates while growth $>10^4$ CFU/ml was taken as cutoff for BAL.[8,9] Samples showing growth less than these thresholds were assumed to be due to colonization or contamination. In case of significant growth, isolate was identified using standard microbiological techniques.[10] Antibiotic testing was done by Kirby Bauer disk diffusion method for each isolate.[11] An isolate was considered as MDR, if it was resistant to at least three classes of antimicrobial agents. ESBL was detected by combination disk method. Organism was considered to be ESBL producer if there was ≥5 mm increase in zone diameter of ceftazidime-clavulanate disk as compared to zone diameter of disk containing ceftazidime alone.[12] Amp C β-lactamases detection was done by Amp C disk method. A positive test appeared as flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disk.[13] MBL detection was done by imipenem-EDTA combined disk method. If the increase in inhibition zone with the imipenem and EDTA disk was ≥7 mm than the imipenem disk alone, it was considered as MBL positive.[14] MRSA detection was done by cefoxitin disk diffusion method. If the inhibition zone around the cefoxitin disk was >22 mm then the isolate was considered MSSA and if the zone was <21 mm then it was considered as MRSA.[12]

Statistical analysis

The statistical analysis was performed using standard tests. Fisher's exact test was applied when two or more set of variables were compared. P value less than 0.05 was considered statistically significant.

RESULTS

A total of 105 patients who were on mechanical ventilation for more than 48 hours were included in the study. Sixty patients fulfilled the clinical and microbiological criteria for the diagnosis of VAP. The incidence of VAP in our study was 57.14% and the incidence density of VAP was 31.7 per 1000 ventilator days. Out of the 60 cases 21 (35%) were categorized under early onset group and 39 (65%) under the late onset group. In relation to gender the incidence of VAP was more among males (65%) than females (35%) and in different age groups the incidence of VAP was highest in patients more than 55 years of age (73.68%).

The majority i.e. 95.7% of bacterial isolates causing VAP were found to be gram negative bacilli. Acinetobacter spp accounted for a maximum 34.28% of VAP cases followed by P. aeruginosa which was responsible for 25.71% cases. Other gram negative bacilli isolated were Klebsiella pneumoniae, Citrobacter freundii, Enterobacter spp, and Escherichia coli (Table 1). Out of the total 70 isolates, only 3 isolates were gram positive bacteria. Among them 2 isolates were of Staphylococcus aureus and 1 was Enterococcus spp. Among the total 60 episodes of VAP reported, 10 episodes of VAP were polymicrobial and 50 episodes were monomicrobial. In the monomicrobial episodes gram negative isolates accounted for 96% (48/50) and even in polymicrobial episodes of VAP gram negative bacilli were predominant accounting for 90% of etiological agents. Among gram

positive bacteria S. aureus accounted for 9.52% of early onset VAP. Majority of late onset VAP episodes were also caused by gram negative bacteria, maximum by A. baumannii followed by P. aeruginosa, K. pneumoniae, C. freundii, and Enterobacter spp.

Out of the two isolates of S. aureus, one was MRSA which was resistant to cephalexin, doxycycline and ciprofloxacin. The single isolate of Enterococcus spp was also found to be resistant to vancomycin, gatifloxacin, pristinamycin (Table 2). Gram negative bacteria were also found to be highly resistant to various drugs such as co-trimoxazole, doxycycline, amikacin, ciprofloxacin, ceftazidime, aztreonam, meropenem, piperacillin/ tazobactam. Colistin, polymixin-B and cefoperazone / sulbactam combination were found to be quite effective (Table 3).

Out of the total 70 isolates, 67 (95.7%) were multidrug resistant and not even a single isolate was sensitive to all the drugs tested. Some of this rsistance can be attributed to the presence of various degradative enzymes like ESBLs, AmpC β-lactamase and MBLs within these pathogens. Out of the total 24 isolates of Acinetobacter spp, 12 (50%) isolates produced AmpC β-lactamase. In P. aeruginosa, it was seen to be produced by 22.2% isolates, in K. pneumoniae by 26.67% isolates, and in C. freundii, it was seen to be produced by 66.67% isolates. The ESBL production was highest in case of E. coli (100%).It was also produced by 66.67% of Enterobacter spp isolates, 16.67% of C. freundii and 13.34% of K. pneumoniae isolates. The MBL production was maximum in case of P. aeruginosa (27.18%). In case of Acinetobacter spp, it was 20.83% and in K. pneumoniae only one isolate produced MBL (Table 4).

Table 1: Distribution of organisms isolated from samples in VAP patients.

Bacterial isolates		Number	Percentage
Gram positive bacteria	Staphylococcus aureus	2	2.85
	Enterococcus spp	1	1.43
Gram negative bacteria	Acinetobacter baumannii	23	32.86
	Acinetobacter lwoffii	1	1.42
	Pseudomonas aeruginosa	18	25.71
	Klebsiella pneumoniae	15	21.43
	Citrobacter freundii	6	8.58
	Enterobacter spp.	3	4.28
	Escherichia coli	1	1.43
Total		70	100

Table 2: Antibiotic susceptibility pattern of gram positive bacteria isolated from VAP patients.

No. (%) of resistant strains													
Bacterial isolates	**No. of isolates**	**Ce**	**E**	**Cd**	**G**	**Cf**	**Va**	**Do**	**Lz**	**Cp**	**Gf**	**Ac**	**Pm**
Staphylococcus aureus	2	1 (50)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	1 (50)	0 (0)	2 (100)	0 (0)	0 (0)	1 (50)
Enterococcus spp	1	-	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)

Cefoxitin (Ce), Erythromicin (E), Clindamycin (Cd), Gentamicin (G), Ciprofloxacin (Cf), Vancomycin (Va), Doxycycline (Do), Linezolide (Lz), Cephalexin (Cp), Gatifloxacin (Gf), Amoxyclav (Ac), and Pristinamycin (Pm).

Table 3: Antibiotic susceptibility pattern of gram negative bacteria other than pseudomonas aeruginosa isolated from VAP patients.

No. (%) of strains resistant												
Bacterial isolates	**No of isolates**	**Co**	**Do**	**Ak**	**Cf**	**Ca**	**Ao**	**Mr**	**Pt**	**Pb**	**Cl**	**Cfs**	**Tc**
Acinetobacter baumannii	23	22 (95.6)	19 (82.6)	19 (82.6)	20 (87)	21 (91.3)	23 (100)	6 (26)	18 (78)	0 (0)	0 (0)	2 (8.7)	6 (26)
Klebsiella pneumoniae	15	10 (66.6)	8 (53.3)	8 (53.3)	10 (66.6)	14 (93.3)	14 (93.3)	3 (20)	2 (13.3)	0 (0)	0 (0)	0 (0)	0 (0)
Citrobacter freundii	6	5 (83.3)	3 (50)	3 (50)	4 (66.7)	5 (83.3)	6 (100)	0 (0)	2 (33.3)	0 (0)	0 (0)	0 (0)	2 (33.3)
Enterobacter spp	3	3 (100)	3 (100)	2 (66.7)	3 (100)	2 (66.7)	3 (100)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)	1 (33.3)
Escherichia coli	1	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Acinetobacter lwoffii	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Co-trimoxazole (Co), Doxycycline (Do), Amikacin (Ak), Ciprofloxacin (Cf), Ceftazidime (Ca), Aztreonam (Ao), Meropenem (Mr), Piperacillin/Tazobactam (Pt), Polymixin B (Pb), Colistin (Cl), Cefoperazone/ Sulbactam (Cfs), and Ticarcillin/clavuanalate (Tc)

Table 4: Distribution of AmpC, ESBL, and MBL in bacterial isolates from VAP patients.

Bacterial isolates	AmpC producer (%)	ESBL producer (%)	MBL producer (%)
Acinetobacter spp	50	0	20.8
Pseudomonas aeruginosa	22.2	0	27.2
Klebsiella pneumoniae	26.7	13.3	6.7
Citrobacter freundii	66.7	16.7	0
Enterobacter species	33.3	66.7	0
Escherichia coli	0	100	0

DISCUSSION

VAP is the most frequent ICU acquired infection occurring in 10% to 65% of the ventilated patients.[15] Diagnosing VAP requires a high clinical suspicion combined with bed side examination, radiographic examination, and microbiological analysis of respiratory secretions. Aggressive surveillance is vital in understanding local factors leading to VAP and the microbiologic milieu of a given unit. Judicious antibiotic usage is essential as resistant organisms continue to plague ICUs and critically ill patients.

In our study the incidence of VAP was 57.14%. This figure is at the higher end of the range of 15-58% as reported by other investigators. The incidence density of VAP in our study was 31.7 per 1000 ventilator days which were high but compatible with ICUs in developing countries. The higher incidence of VAP in our study can be attributed to the fact that the total number of cases in the study and the study duration was less as compared to other studies showing fewer incidences. One more reason for this high incidence can be the lack of adequate nursing staff (which should ideally be 1:1 as compared to 4:1 in our institute) which may have adversely affected the quality of care given to the patients.

The development and spread of antibiotic resistant bacteria is common in ICUs mainly because of heavy use of antibiotics. Multiple antibiotic resistance to useful antibiotics, including the penicillins, cephalosporins, aminoglycosides, and fluoroquinolones, has gradually increased among a number of gram negative pathogens, especially P. aeruginosa, Acinetobacter spp. K. pneumoniae and Enterobacter spp.[16]

On performing the antimicrobial susceptibility testing of gram positive bacteria and studying their antibiotic susceptibility pattern, it was observed that vancomycin and linezolide were the most effective antibiotics for S. aureus. In our study the incidence of MRSA was 50% i.e. one of the two isolates of S. aureus was MRSA. Linezolide, doxycyline and ciprofloxacin were found to be effective against Enteroccus spp. This resistance pattern of gram positive bacteria isolated from patients of VAP was in accordance with other studies.[17]

When considering gram negative bacteria Acinetobacter spp was the most common isolate and its resistance pattern showed that majority of isolates were multi-drug resistant. Polymixin-B and colistin were found to be highly effective. All strains of P. aeruginosa were also uniformly sensitive to polymixin-B and colistin but 27.8% strains showed resistance to meropenem. In other gram negative bacilli also resistance to multiple drugs was seen.

An important group of resistant VAP pathogens are carbapenem-resistant gram negative bacteria, production of MBL being one of their major defence mechanisms. MBL producing P. aeruginosa, Acinetobacter spp and other gram negative bacilli have been isolated from patients of VAP.[18] In our study, five isolates of P. aeruginosa (27.18%), one isolate of Klebsiella spp (6.67%), and five isolates of Acinetobacter spp (20.83%) were metallo β- lactamases enzyme producing strains, detected by imipenem-EDTA disk method.

ESBLs are most commonly produced by Klebsiella spp and E. coli but may also occur in other gram-negative bacteria. They are typically plasmid-mediated clavulanate susceptible enzymes that hydrolyze penicillins, expanded-spectrum cephalosporins (cefotaxime, ceftriaxone, ceftazidime, cefepime and others) and aztreonam. AmpC β-lactamases are cephalosporinases that are poorly inhibited by clavulanic acid. They can be differentiated from other ESBLs by their ability to hydrolyze cephamycins (Cefoxitin, Cefotetan) as well as other extended-spectrum cephalosporins.[19] In our study ESBL production was seen in members of family Enterobacteriaceae and AmpC β-lactamases were detected in Acinetobacter spp and P. aeruginosa also. These findings are similar to the studies done by previous authors.[20]

In conclusion, most of the pathogens causing VAP in our institute were multidrug resistant and in many isolates this resistance was due to production of ESBL, MBL, and AmpC β-latamases. Polymixin B and colistin were found to be highly effective against multidrug resistant Acinetobacter spp and P. aeruginosa. This study provided information regarding the pathogens causing VAP and their drug susceptibility patterns, which can be of great assistance to the physicians for the prophylaxis and treatment of VAP patients. The relative prevalence of individual pathogens varies substantially between different geographic regions, different institutions and even different units in the same hospital. Local microbiology and antibiotic susceptibility data is essential for making informed antibiotic treatment choices.

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