BACTERIOLOGICAL PROFILE OF VENTILATOR ASSOCIATED PNEUMONIA IN A TERTIARY CARE CENTRE OF DAKSHINA KANNADA DIST.

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ABSTRACT

BACKGROUND

Mechanical ventilation can increase the risk of VAP 7 - 21 times and 10% - 25% of patients undergoing mechanical ventilation develop pneumonia. The aetiological agents of VAP vary with different patient populations and types of ICUs. Prompt administration of appropriate antibiotics seems to be the only intervention that alters outcome once the diagnosis is established.

MATERIALS AND METHODS

This is a descriptive study with seventy-four non-repetitive endotracheal aspirate from patients who were on mechanical ventilation for more than 48 hrs. and in whom ventilator associated pneumonia was suspected using modified CPIS score and quantitative culture was confirmed using modified two-fold dilution scheme. Kirby-Bauer disk diffusion method was adopted for antimicrobial susceptibility testing.

RESULTS

The most common isolate was pseudomonas aeruginosa (27%) followed by Acinetobacter spp. (20.3%), Klebsiella spp. (16.2%) and E. coli (12%). Among gram positive bacteria in our study, only Staphylococcus aureus (9.5%) was isolated. Amikacin and levofloxacin were found to be better against non-fermenters; 10% were carbapenemase producers, MDR isolates were observed in enterobacteriaceae members. MRSA was found in 28.6%.

CONCLUSION

In conclusion, the incidence of VAP and the prevalence of multidrug resistant microorganisms were quite high in our ICU setup. A local surveillance program at each centre is essential, as the knowledge of local resistant patterns is vital for selecting the appropriate agents for treating infections.

KEYWORDS

VAP, Gram Negative Isolates, MDR Pathogens, MRSA.

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BACKGROUND

Ventilator-associated pneumonia (VAP) is defined as pneumonia occurring more than 48 hours after the initiation of endotracheal intubation and mechanical ventilation (MV) including pneumonia developing even after extubation.¹ Mechanical ventilation can increase the risk of VAP 7 - 21 times and 10% - 25% of patients undergoing mechanical ventilation develop pneumonia.² It results in high mortality and morbidity, prolonged lengths of hospitalisation and also increased cost of hospitalisation. The mortality rates for VAP range from 20% to 75% according to the study population.³

Accurate data on aetiological agents and the epidemiology of VAP are limited by the lack of a 'gold standard' for diagnosis. However, regardless of the diagnostic technique used, there have been dramatic variations in the distribution of pathogens and drug resistance patterns. Because early and

appropriate antimicrobial therapy is an important goal in the setting of life-threatening infections, taking into account factors that modulate bacterial ecology and the susceptibility of causative organisms is crucial for optimal management.

Prompt administration of appropriate antibiotics seems to be the only intervention that alters outcome once the diagnosis is established.¹ Microorganism responsible for VAP may differ according to the population of patients in the ICU, the duration of hospital and ICU stays, and the specific diagnostic methods used. The high rate of respiratory infections due to gram negative bacilli in this setting has been documented. Resistance rate of gram negative bacilli to penicillins, cephalosporins, Fluoroquinolones and aminoglycosides varies between hospitals, but is generally increasing.²

A number of studies from India have investigated the causative organisms of VAP. Pseudomonas spp., Acinetobacter spp., Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus were identified as the common VAP pathogens with varying prevalence. Upto 40% of these infections can be polymicrobial. Pseudomonas spp., Acinetobacter spp. and even Enterobacteriaceae are quite often multidrug-resistant due to production of extended spectrum beta (β)-lactamases (ESBL), AmpC β -lactamases (AmpC) or metallo- β -lactamases (MBL). The aetiological agents of VAP vary with different patient populations and types of ICUs. Therefore, the local microbial flora causing VAP

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needs to be studied in each setting to guide more effective and rational utilisation of antimicrobial agents.⁴

MATERIALS AND METHODS

This descriptive study with seventy-four non-repetitive endotracheal secretion samples received between April 2016 and April 2017 were processed in Microbiology laboratory, Yenepoya Medical College Hospital Laboratory. According to standardised procedures, we abstracted the following information prospectively from each patient for more than 48 hours under mechanical ventilation: demographic data (age, sex), cause of admission, comorbidities (COPD, CHF and Renal failure) and length of ICU stay period, duration of ventilation therapy, prior antibiotics and occurrence of VAP.

Exclusion criteria were: 1) Pneumonia being the cause of admission, 2) Intubation without mechanical ventilation in ICU, 3) Patients who were suffering from chest trauma.

A clinical criterion for diagnosis of VAP was made by modified clinical pulmonary infection score (CPIS).⁵

CPIS Points	0	1	2
Temperature (°C)	≥ 36.5 and ≤ 38.4	≥ 38.5 and ≤ 38.9	≥ 39 and ≤ 36
Leucocyte count (per mm ³)	4000-11000	< 4000 or > 11000	< 4000 or > 11000 + band forms ≥ 500
Tracheal secretions	Rare	Abundant	Abundant + Purulent
PaO ₂ / FiO ₂ mm Hg	> 240 or ARDS		
Chest radiograph	No infiltrate	Diffuse infiltrate	≤ 240 and no ARDS Localised infiltrate
Culture of tracheal aspirate	Light growth or no growth	Moderate or heavy growth of pathogenic bacteria	Moderate or heavy growth of pathogenic bacteria and presence of the same bacteria in Gram stain

Table 1. Modified CPIS Score for Diagnosis of VAP

Establishment of aetiologic diagnosis required isolation of bacteria in significant quantity from a sample of lower respiratory tract secretions (BAL > 10⁴ cfu/mL). Only the first episode of documented VAP was taken into account. Acquired specimens by endotracheal aspiration were transferred to the microbiological laboratory immediately. By using a modified two-fold dilution scheme, we determined the quantitative culture in cfu/mL. The media and incubation conditions were appropriate for the cultivation of the most probable respiratory pathogens. Antimicrobial susceptibility of the clinical isolates to routinely used antibiotics was determined by Kirby-Bauer disk diffusion method.^{6,7}

The organisms isolated by quantitative culture of the endotracheal aspirate (EA) from VAP patients were identified based on standard microbiological techniques. The susceptibility of the clinical isolates to some routinely used antibiotics was determined by the Kirby-Bauer disk diffusion method. Ampicillin (AMP), Amoxy-clav (AMC), piperacillin (PI), ciprofloxacin (CIP), cefepime (CPM), cefotaxime (CTX), ceftazidime (CAZ), levofloxacin (LE), gentamicin (G),

amikacin (AK), tetracycline (T), Cotrimoxazole (COT), chloramphenicol (C), piperacillin-tazobactam (PTZ) and meropenem (MR) were tested for Enterobacteriaceae. Similar drugs along with tobramycin (TOB), tigecycline (TGC), colistin (CL) and piperacillin-tazobactam (PTZ) were tested for non-fermenters. Ampicillin, tetracycline (T), erythromycin (E), clindamycin (CD), ciprofloxacin, gentamicin linezolid (LZ), teicoplanin (TEI) and vancomycin (VA) were tested for *S. aureus*. Methicillin resistance in *S. aureus* was determined using Cefoxitin (CX) disc 30 µg.⁷

Combination disk method using both ceftazidime, alone and in combination with clavulanic acid, was performed for detection of extended spectrum-lactamase (ESBL) among the members of Enterobacteriaceae. Five mm or more increase in zone of inhibition for ceftazidime-clavulanic acid disk compared to the ceftazidime disk was taken as confirmatory evidence of ESBL production.^{8,9}

RESULTS

Out of 74 non-repetitive isolates, the most common isolate was *Pseudomonas aeruginosa* (27%) followed by *Acinetobacter* spp. (20.3%), *Klebsiella* spp. (16.2%) and *E. coli* (12%). Among gram positive bacteria in our study, only *Staphylococcus aureus* (9.5%) was isolated.

Isolate	Number	% Isolation
<i>Pseudomonas aeruginosa</i>	20	27
<i>Acinetobacter</i> spp.	15	20.3
<i>E. coli</i>	9	12
<i>Klebsiella</i> spp.	12	16.2
<i>Citrobacter</i> spp.	1	1.4
<i>Enterobacter</i> spp.	3	4
<i>P. vulgaris</i>	1	1.4
<i>Staphylococcus aureus</i>	7	9.5
<i>S. maltophilia</i>	3	4
<i>Burkholderia</i>	1	1.4
<i>Sphingomonas</i>	1	1.4
<i>Flavobacter meningosepticum</i>	1	1.4
Total	74	

Table 2. Distribution of Isolates from Endotracheal Aspirates

Figure 1. Prevalence of Isolates in CT Culture

Isolate	AMP	AMC	PI	CPM	CAZ	CTX	CIP	LE	G	AK	T	COT	C	I	MR	PTZ
E. coli	89	44	67	78	67	78	78	11	67	44	44	33	11	33	11	67
Klebsiella	100	58	50	75	75	83	67	17	67	5	33	42	42	50	25	58
Proteus	-	0	100	0	0	100	100	0	100	0	-	100	100	0	100	100
Citrobacter	-	100	0	0	0	0	0	0	100	0	-	0	0	50	0	0
Enterobacter	100	100	0	0	0	34	67	0	34	0	100	0	0	50	0	0

Table 3. Percentage resistance among Enterobacteriaceae

Among enterobacteriaceae high resistance was seen for β -lactams including cephalosporins for E. coli and Klebsiella (70% and 73% respectively), whereas Citrobacter and Enterobacter showed good sensitivity (60% and 80%), but Proteus spp. showed varied resistance to these drugs. Ciprofloxacin resistance (64%) was much higher in enterobacteriaceae members, while good sensitivity was observed with levofloxacin (94%). Among aminoglycosides only 10% resistance was noted for amikacin, while 74% of isolates showed Gentamicin resistance. Meropenem showed a better sensitivity (78%) pattern as compared with imipenem (64%) for enterobacteriaceae members.

Isolate	PI	CPM	CAZ	G	AK	TOB	CIP	LE	COT	C	T	I	MR	PTZ	PB	TGC	CL
Pseudomonas	20	20	30	30	20	0	15	0	40	-	40	25	10	15	5	5	5
Acinetobacter	40	67	73	67	60	0	67	0	47	40	40	53	40	53	0	0	0
NFGNB	67	67	83	33	50	0	50	17	50	67	17	83	17	83	0	0	17

Table 4. Percentage resistance among Non-Fermenters

Among the antibiotics used, pseudomonas isolates had a good sensitivity to β -lactams (PI- 80%, CPM- 80% and CAZ- 70%), among aminoglycosides and quinolones, resistance to Gentamicin, amikacin and ciprofloxacin was low (30%, 20% and 15%), while all isolates were sensitive to tobramycin and levofloxacin; 10% of the pseudomonas isolated were potential carbapenemase producers.

Acinetobacter spp. and other non-fermenters had high resistance to routinely used antibiotics like B-lactams (66.6%) and aminoglycosides (52%). Among quinolones, sensitivity for levofloxacin (82%) was better than ciprofloxacin (42%).

All non-fermenters showed good sensitivity to higher antibiotics like tobramycin (100%), polymyxin B (98%), colistin (96%) and tigecycline (98%).

(28.6%) and erythromycin (42.9%); however, high resistance was seen in ampicillin (71.4%) and gentamicin (57.1%).

DISCUSSION

VAP is an important nosocomial infection among critically ill patients receiving mechanical ventilation. The clinical diagnosis of VAP can be made using the modified CPIS score. Quantitative cultures of the lower respiratory secretions obtained by endotracheal aspiration (EA) or bronchoscopic techniques such as bronchoalveolar lavage (BAL) are essential for deciding appropriate therapy for the VAP patients. In recent years, various investigators are recommending quantitative analysis of EA as a simple and useful tool for the diagnosis of VAP.

The majority of bacterial isolates in our study were Pseudomonas aeruginosa (27%) followed by Acinetobacter spp. (20%) and Klebsiella spp. (16%). These findings are in concordance with other researchers Mahshid Talebi Taher et al,² found Pseudomonas aeruginosa and Klebsiella pneumonia (50% each), RM Saldanha Dominic et al¹ in their study found a similar order of prevalence. Pseudomonas accounted for 41.14% followed by Klebsiella species (15.43%) and Acinetobacter species (10.28%). On the other hand, Ranjit S and Bhattarai B¹⁰ found a higher incidence of Acinetobacter spp. (34%) followed by Pseudomonas spp. (19%) and Klebsiella spp. (12%).

In our study pseudomonas isolates had a good sensitivity to β -lactams (PI- 80%, CPM- 80% and CAZ- 70%) and resistance to Gentamicin, amikacin and ciprofloxacin was low (30%, 20% and 15%). These findings are in line with the results by RM Saldanha Dominic et al,¹ who found Pseudomonas to be most sensitive to Amikacin (52.78%) and Ceftazidime (47.22%); 10% of the pseudomonas isolated were potential carbapenemase producers. Kotgire Santosh A and Tankhiwale Nilima¹¹ in their study showed that 7 of 20 isolates were carbapenemase producers Acinetobacter spp. and other non-fermenters had high resistance to routinely used antibiotics like B-lactams (66.6%), aminoglycosides (52%) and also imipenem (53%), meropenem (40%) resistance was moderately high in our study. All non-fermenters showed good sensitivity to higher antibiotics like

Figure 2. % Resistance in Staphylococcus aureus

Out of seven Staphylococcus aureus isolates, MRSA was found in 28.6%. All isolates were sensitive to linezolid, teicoplanin and vancomycin. Moderate degree of resistance was found for amoxy-clav (28.6%), ceftazidime (42.9%), clindamycin (42.9%), ciprofloxacin (42.9%), cotrimoxazole

tobramycin (100%), polymyxin B (98%), colistin (96%), tigecycline (98%). In a study by RM Saldanha Dominic et al,¹ *Acinetobacter* was found to be most sensitive to chloramphenicol (50%) and Amikacin (38.89%). Kotgire Santosh A and Tankhiwale Nilima¹¹ in their study showed a better sensitivity for *Acinetobacter* spp. Of the 5 *Acinetobacter* spp., 2 (40%) were AmpC β -lactamase producers and they were sensitive to imipenem and meropenem, while no M β L producers were seen. The remaining isolates were sensitive to aminoglycosides, ciprofloxacin and b-lactams.

We found a high resistance for β -lactams (70%) including cephalosporins (73%), Ciprofloxacin (64%), Gentamicin (74%) for *E. coli* and *Klebsiella*, while good sensitivity was observed with levofloxacin (94%) and amikacin (90%). RM Saldanha Dominic et al¹ showed that *Klebsiella* was most sensitive to Amikacin (66.67%) and Ciprofloxacin (59.26%). *E. coli* was most sensitive to Amikacin and Ciprofloxacin (50% each). Kotgire Santosh A and Tankhiwale Nilima¹¹ on the other hand found that all the strains of *E. coli* and *K. pneumoniae* were sensitive to imipenem, meropenem and piperacillin tazobactam, while the remaining enterobacteriaceae isolates were sensitive to gentamicin, amikacin, ciprofloxacin and ceftazidime. Also, MRSA prevalence in our study was found in 28.6% isolates. The MRSA prevalence is diverse in various studies; RM Saldanha Dominic et al¹ (69.5 %), Ranjit S and Bhattarai B¹⁰ (0%), Kotgire Santosh A and Tankhiwale Nilima¹¹ (53.3%).

CONCLUSION

The commonest organism, which was isolated from the ET aspirate cultures were non-fermenting gram-negative bacilli as seen in various studies done in India. Multidrug resistant organisms are increasing. Requirement of mechanical ventilation for more than four days increases the risk. These patients need special attention towards preventive measures. The overall outcome of VAPs could improve with the antimicrobial policies of individual centres. So, in conclusion, the potential MDR in ICU and hospital settings emphasises the judicious use of antimicrobial therapy, so as to decrease the incidence of VAP and the overall morbidity, mortality and the longer stay of patients in the hospital.

REFERENCES

- [1] Dominic RMS, Prashanth HV, Shenoy S, et al. A clinico-microbiological study of ventilator-associated pneumonia in a tertiary care hospital. *Int J Biol Med Res* 2012;3(2):1651-4.
- [2] Taher MT, Mousavi SAJ, Pour HM. Ventilator associated pneumonia: microbiology and identification of antimicrobial resistance pattern by disk-diffusion and E. test methods. *Clinical Infectious Diseases* 2008;3(1):13-8.
- [3] Alp E, Voss A. Ventilator associated pneumonia and infection control. *Ann Clin Microbiol Antimicrob* 2006;5:7.
- [4] Joseph NM, Sistla S, Dutta TK, et al. Ventilator-associated pneumonia in a tertiary care hospital in India: role of multi-drug resistant pathogens. *J Infect Dev Ctries* 2010;4(4):218-25.
- [5] Pugin J, Auckenthaler R, Mili N, et al. Diagnosis of ventilator-associated pneumonia by bacteriologic analysis of bronchoscopic and nonbronchoscopic "blind" bronchoalveolar lavage fluid. *Am Rev Respir Dis* 1991;143(5 Pt 1):1121-9.
- [6] Forbes BA, Sahm DF, Weissfeld AS. *Bailey & Scott's diagnostic microbiology*. 12th edn. Mosby Elsevier, China. 2007.
- [7] Winn WC, Allen SD, Janda WM, et al. *Koneman's color Atlas and Textbook of Diagnostic Microbiology*. 6th edn. Philadelphia. Lippincott Williams & Wilkins, 2006:1443-71.
- [8] Miles RS, Amyes SGB. Laboratory control of antimicrobial therapy. In: Mackie, McCartney (eds). *Practical medical microbiology*. 14th edn. Elsevier publishers New Delhi, 2006:151-77.
- [9] Performance standards for antimicrobial susceptibility testing. 27th edn. M100, 2017.
- [10] Ranjit S, Bhattarai B. Incidence and risk factors for ventilator-associated pneumonia in Kathmandu university hospital. *Kathmandu University Medical Journal* 2011;9(1):28-31.
- [11] Santosh AK, Nilima T. Study of multidrug resistant (MDR) isolates in patients with ventilator associated pneumonia in a rural hospital. *Journal of Clinical and Diagnostic Research* 2011;5(7):1363-6.