Research Article

Increasing trend of Carbapenem resistant Gram negative bacteria in an Intensive care unit

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Abstract

Background: Ventilator-associated pneumonia (VAP) has been associated with morbidity, mortality, prolonged hospitalization and extra health-care costs, requires rapid diagnosis and initiation of the appropriate antibiotic treatment.

Objective: To analyse the microbiological profile of ventilator associated pneumonia in a tertiary care hospital.

Material and Methods: This is a cross–sectional study conducted in Intensive Care Units with hundred VAP patients who satisfied the Clinical Pulmonary Infection Score (CPIS) >6.

Results: *Acinetobacter baumannii* (46.61%) was the most common isolate followed by *Klebsiella pneumoniae* (26.31%) and *Pseudomonas aeruginosa* (17.29%). Metallo-betalactamases was produced by 64.70% of non-fermenters and extended spectrum beta lactamases (ESBL) was produced by 54.28% of *Klebsiella pneumoniae*. AmpC β-lactamases were produced by 17.07% and 4.70% of the members of *Enterobacteriaceae* and non-fermenters, respectively.

Conclusion: Malpractice of antibiotics usage has led to emergence of new broad spectrum β-lactamase. The emergence of carbapenemase-producing multidrug resistant (MDR) gram-negative bacteria is major public health problem particularly in the hospital settings. Infections due to these organisms lead to life-threatening illness which is difficult to manage as there are limited treatment options. Prevention of VAP may be carried out by early isolation and decreasing the length of stay along with proper knowledge of the MDR organisms.

KEY WORDS: Ventilator associated pneumonia, multi-drug resistant, carbapenemases

Introduction

Health care-associated infections (HAI) or nosocomial infections represent a major health issues to mankind in terms of personal distress, economical loss, morbidity, and mortality.[1,2] Among all HAI, pneumonia is assumed to be one of the leading causes of death.[3] The occurrence of pneumonia is more in the intensive care units (ICUs) chiefly because of utilization of invasive procedures such as mechanical ventilation.[4-6] Ventilator-associated pneumonia (VAP) is defined as pneumonia occurring more than 48 hrs after endotracheal intubation; initiation of mechanical ventilation (MV) including pneumonia developing even after extubation.[7] Ventilator-associated pneumonia indirectly influences the length of stay, cost of treatment, and mortality. Nearly 10%–20% patients on MV for longer than 48 hrs develop VAP.[8,9] VAP is less severe and is likely with a better prognosis and diagnosis during first 4 days, caused by antibiotic sensitive bacteria. Late onset VAP, which develops after 4 days after initiation of MV, is caused by multidrug resistant (MDR) pathogens and associated with increased mortality and morbidity.[10] The common pathogens causing VAP include aerobic Gram-negative rods such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Escherichia coli*.[9,11,12] VAP due to Methicillin resistant *Staphylococcus aureus* (MRSA) has been rapidly emerging.[9,12] Treatment of VAP is usually supportive, along with administration of proper antibiotics. The selection of proper

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antimicrobial agents, active against the VAP pathogens is an important determinant for reducing morbidity and mortality. Appropriate antimicrobial therapy, when initiated early, has shown to reduce mortality among critically ill patients with VAP. Late onset VAP is commonly associated with administration of inappropriate antibiotics and caused by MDR pathogens such as *Pseudomonas species* and *Acinetobacter* species. MDR pathogens are resistant to three or more antimicrobial classes. Drug resistance is due to production of extended-spectrum β-lactamases (ESBL), AmpC β-lactamase, or metallo-β-lactamase (MBL).[9,12] The Gram-positive cocci that are resistant to penicillin and at least two other antibiotic classes were defined as MDR pathogens.[18] There is an urgent need of local surveillance data at routine interval as the frequency of specific MDR pathogens causing VAP may vary by hospital, patients' population, type of ICU patients, exposure to antibiotics, and changes over time.[26]

Materials and Methods

Study Design and Settings

After getting approval from the Research and Ethical Committee a cross-sectional study was conducted in the tertiary care hospital, for a period of 24 months. The study was conducted in four ICUs and informed consent was obtained from each patient's next of kin. The study was conducted in Intensive Cardiac Care Unit (ICCU), Medical Intensive Care Unit (MICU), Respiratory Intensive Care Unit (RICU), and Surgical Intensive Care Unit (SICU). The patients were either admitted directly or transferred from other wards such as surgery, medicine, neurology, cardiology, obstetrics and gynecology and pulmonology. To prevent the transfer of organisms from one patient to another proper aseptic precaution were followed while handling each patient's samples.

Sample Size

One hundred patients who satisfied the Clinical Pulmonary Infection Score (CPIS) >6 were taken as a case of VAP and were included in this study.

Procedure for Data Collection

All patients were monitored at frequent intervals for the development of VAP using CPIS scoring. The medical history and data were recorded from their medical records and bedside charts.

Criteria for Diagnosis of VAP

The diagnosis of VAP was based on clinical and microbiological criteria.[9] Patients on mechanical ventilation for less than 48 hrs; patients in ICU and not receiving ventilator support and have developed pneumonia; patients diagnosed to have lower respiratory tract infections such as pulmonary tuberculosis, chronic obstructive pulmonary disease, acute respiratory distress syndrome, bronchial asthma on admission were excluded. Patients who satisfied the CPIS >6[11,13,14] and quantitative culture of endotracheal aspirate with growth thresholds greater than equal to 10^6 cfu/mL[15] were included in the study. Based on these criteria, 100 patients were diagnosed with VAP and included in the study [Table 1].

Microbiological Techniques

Endotracheal aspirate (ETA) samples were collected with proper aseptic precautions and sent immediately to microbiology laboratory for processing and were identified based on standard microbiological techniques.[15] Following that Gram stained findings were considered for interpretation of culture report; polymorphonuclear neutrophils >10 per high power field and >1 bacteria per oil immersion field and presence of intracellular bacteria.[19] Ziehl–Neelsen stained preparations were also observed to detect possible co-existence of pulmonary tuberculosis. Antibiotic susceptibility testing of these bacterial isolates were carried out by employing Kirby- Bauer disk diffusion method on Muller Hinton agar (MHA) plate according to CLSI guidelines 2013.[16] All the discs were procured commercially from Hi-Media Laboratories Limited except Meropenem disc which was procured from BD Company. The quality control for all the disc (both Hi-media & BD disc) were carried out using *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 25923 and disc potency were checked. Antibiotics were used as per CLSI guidelines and, the diameter of the zone of inhibition was measured and interpretation of susceptibility was carried out. 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.[16] Amp C β-lactamases detection was done by Amp C disk method. Flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disk is indicative of positive result.[17] MBL detection was carried out by imipenem-EDTA combined disk method, modified Hodge test (MHT) and double disc synergy test (DDST). An increase in zone size of at least 7 mm around the meropenem–EDTA disc compared to meropenem without EDTA was recorded as an MBL-producing strain by combine disk method. The presence of clover leaf type of indentation at the intersection of the test organism and ATCC *E. coli* 25922, within the zone of inhibition of meropenem susceptibility disc was interpreted as positive MHT result as per CLSI guideline 2013.[16] Enhancement of zone of inhibition in the area between Meropenem and EDTA disc in comparison with the zone of inhibition on the far side of the drug (meropenem) was interpreted as a positive result by DDST.[27] Among the gram-negative bacilli (GNB) isolated from VAP, those producing ESBL or Amp-C β-lactamases or MBL, and/or resistant to three or more antimicrobial classes were defined as MDR pathogens. The Gram-positive cocci, resistant to penicillin and at least two other antibiotic classes were defined as MDR pathogens.[18] MRSA detection was carried out by cefoxitin disk diffusion method. If the inhibition

Table 1: Clinical pulmonary infection score (CPIS)[11,13,14]

Fever (ºC)	≥38.4 and ≤39	Point 1
	<36 or >39	Point 2
Total leukocyte count (TLC)	4000–11000	Point 0
	<4000 or >11000	Point 1
	>500 band forms	Point 1 (additional)
Oxygenation (mmHg) PaO_2/Fio_2	>240 or ARDS	Point 0
	<240 or no evidence of ARDS	Point 2
Chest radiograph	No infiltrate	Point 0
	Diffuse or patchy	Point 1
	Localized infiltrate	Point 2
Progression of infiltrate	No progression	Point 0
	Progression (no ARDS/CHF)	Point 2
Semiquantitative culture	No growth	Point 0
	Moderate or heavy growth	Point 1
Gram stain	Same morphology	Point 1 (additional)

Maximum score 12. CPIS >6 is suggestive for VAP

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.[16]

Result

During the 24-month study period, a total of 989 patients who were on mechanical ventilation in the ICCU, MICU, RICU, SICU were prospectively reviewed. Among them only 669 patients were ventilated for more than 48 hrs. Only 100 patients who satisfied the criteria as described were part of the study among them, 77 (77%) were male and 23 (23%) were female in our study. Age-wise distributions of clinically suspected VAP cases were studied and it was found that 50 patients (50%) belonged to the age group 21–40 years, followed by 25 patients (25%) in 41–60 years age group and 23 patients (23%) in more than 60 years age group. In this study total 133 organisms were isolated from culture of endotracheal aspirates.

Microbial Pattern in VAP Cases

The isolation was monomicrobial in 65 cases (65%) and polymicrobial in 35 cases (35%). Among 133 organisms, 126 (94.74%) were GNB and 7 (5.26%) were Gram-positive cocci among them most common isolate was *Acinetobacter species* 62 isolates (46.61%) followed by *K. pneumoniae* 29 isolates (26.31%), and *P. aeruginosa* 23 isolates (17.29%).

Early-onset VAP pathogens versus Late-onset VAP pathogens

Total 25 organisms (18.79%) isolated in early onset VAP which includes *K. pneumoniae, P. aeruginosa, A. baumannii*, *Escherichia coli*, Citrobacter species, *Serratia marcesence,* and *Methicillin sensitive Staphylococcus aureus* (MSSA). Total 108 organisms (81.21%) isolated in late onset VAP in highest order are *A. baumannii, K. pneumoniae, P. aeruginosa, Klebsiella oxytoca*, and Methicillin resistant *Staphylococcus aureus* (MRSA).

Comparison of Bacterial Patterns of VAP in all Four ICUs

Non-fermenters (64%) were the most predominant pathogens causing VAP in the ICUs. Common pathogens isolated in ICUs are *A. baumannii* (46.61%), *K. pneumoniae* (26.31%), followed by *P. aeruginosa* (17.29%). VAP episodes due to Gram-positive bacteria (5.26%) were relatively less common in the ICUs [Table 2].

Detection of ESBL, AmpC β-lactamase, and metallo-betalactamase

Metallo-betalactamases was produced by 64.70% of non-fermenters and ESBL was produced by 54.28% of *K. pneumoniae*. AmpC β-lactamases were produced by 17.07% and 4.70% of the members of *Enterobacteriaceae* and non-fermenters, respectively [Table 3]. Ninty-two (69.17%) of the 133 VAP pathogens in our study were MDR. These MDR pathogens included Gram-negative bacteria producing ESBL, AmpC β-lactamases, MBL, and Gram-positive organism showing resistance to cefoxitin, tetracycline, clindamycin, and erythromycin.

Antibiotic Resistance Pattern

The antibiotic resistance pattern of the various etiological agents of VAP is summarized in Table 4. *Acinetobacter baumannii* and *K. pneumoniae* are resistant to most of the

Table 2: Etiological agents of VAP in different ICUs

Organisms	No of isolates	ICCU	MICU	RICU	SICU
Gramnegative non-fermenters					
Acinetobacter baumannii	62	2	4	19	37
Pseudomonas aeruginosa	23	1	2	07	13
Gram-negative fermenters					
Serratia marcesence	01	-	01	-	-
Citrobacter species	02	-	-	02	-
Klebsiella oxytoca	02	-	01	01	-
Escherichia coli	01	-	-	01	-
Klebsiella pneumoniae	35	01	06	11	17
Gram-positive isolates					
MSSA	05	01	01	01	02
MRSA	02	-	-	01	01

Table 3: ESBL, AmpC β–lactamase, and MBL production among the VAP pathogen

Organisms	No of isolates	ESBL	AmpC β-lactamase	MBL
Acinetobacter baumannii	62	-	02	49
Pseudomonas aeruginosa	23	01	02	06
Serratia marcesence	01	-	-	-
Citrobacter species	02	01	-	-
Klebsiella oxytoca	02	-	-	01
Escherichia coli	01	-	-	-
Klebsiella pneumoniae	35	19	07	10
MSSA	05	-	-	-
MRSA	02	-	-	--

antibiotics. Of the 126 Gram-negative bacterial isolates, 80 isolates (63.49%) showed resistance to carbapenem group of drugs (meropenem and imipenem). Among them maximum resistance was shown by *A. baumannii* (90.32%), followed by *K. pneumoniae* (45.71%), *P. aeruginosa* (26.08%). Susceptibility pattern of GNB to carbapenems are shown in the Table 5.

Discussion

VAP is an important nosocomial infection among ICU patients; MDR pathogens such as *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* were the common organisms causing VAP. In our study 65% of monomicrobial flora was noted as compared to 35% of polymicrobial flora which is contradictory to study by Pawer et al.; 48% patients were infected with monomicrobial infection and 52% patients had polymicrobial infection.[22] In this study, *A. baumannii* (46.61%) was the most common isolate followed by *K. pneumoniae* (26.31%) and *P. aeruginosa* (17.29%). Singhal et al. had reported *Acinetobacter* species (44.80%) as the most common organism followed by *P. aeruginosa* (40.1%) and *K. pneumoniae* in 5.7% cases in their study.[23] In our study, 19% VAP cases were "Early onset" and 81% were categorized as "Late onset" which was in concordance with the result obtained by Mukopadhyay et al.[24] who found 38% early onset VAP and 62% late onset VAP whereas in other study by Gadani et al.[25] 30% were early onset VAP and 70% were late onset VAP. GNB such as *Pseudomonas* spp. and *Acinetobacter* spp. are associated with late-onset VAP as it was observed by other workers.[20,21] Late-onset VAP was associated with higher rates of infection with MDR *A. baumannii*, *K. pneumoniae*, and *P. aeroginosa* but in early onset VAP these were sensitive to most of the antibiotics. We also observed that non-fermenters (64%) were the most predominant pathogens causing VAP in the ICUs.

Table 4: Antibiotic resistance patterns of isolates

		Antibiotics resistance pattern of gram-negative bacteria (non-fermenters)													
Organisms	**No of isolates**	AMP	CIP	CTR	CAZ	GEN	COT	PIT	MRP	IMP	AZ	TOB	CFM	CL	AK
A. baumannii	62	62	57	58	58	56	55	56	56	56	13	56	56	00	56
P. aeruginosa	23	-	-	08	06	07	23	08	06	06	03	05	07	00	05

		Antibiotics resistance pattern of Gram-negative bacteria (Fermenters) in numbers														
		AMP	CIP	CTR	CAZ	GEN	COT	PIT	MRP	IMP	AZ	TOB	CFM	CL	AK	TGC
K. pneumoniae	35	35	29	30	30	27	27	26	16	16	05	18	29	00	18	00
Citrobacter spp	02	02	01	01	01	01	01	01	01	01	00	01	01	00	01	00
K. oxytoca	02	02	01	01	01	02	02	01	01	01	00	01	01	00	01	00
E. coli	01	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
S. marcesence	01	01	00	01	01	01	00	00	00	00	00	00	01	01	00	00

	Antibiotics resistance pattern of Gram-positive bacteria in numbers							
		CFT	CIP	CD	ERY	LZ	P	TET	COT
M.S.S.A	05	00	03	00	00	00	05	00	00
M.R.S.A.	02	02	01	02	02	00	02	01	01

AMP, Ampicillin; CIP, Ciprofloxacin; CFT, cefoxitin; CTR, Ceftriaxone; CAZ, Ceftazidime; GEN, Gentamicin; COT, Cotrimoxazole; PIT, Piperacillin Tazobactam; P, penicilin; CFM, Cefepime; MRP, Meropenem; TGC, Tegicycline; AZ, Azoteronam; TOB, Tobramycin; AK, Amikacin; CL, Colistin; IMP, Imipenem; CD, clindamycin; ERY, erythromycin; LZ, Linezolid; TET, Tetracyclin.

Table 5: Susceptibility pattern of Gram-negative bacilli to carbapenems

Organism	Total number of isolates	Carbapenem resistance (%)	Carbapenem sensitive (%)
Acinetobacter baumannii	62	56 (90.32%)	06 (9.68%)
Pseudomonas aeruginosa	23	06 (26%)	17 (74%)
Serratia marcesence	01	00 (00%)	01 (100%)
Citrobacter species	02	01 (50%)	01 (50%)
Klebsiella oxytoca	02	01 (50%)	01 (50%)
Escherichia coli	01	00 (00%)	01 (100%)
Klebsiella pneumoniae	35	16 (45.7%)	19 (54.3%)

VAP episodes due to Gram-positive bacteria (5.26%) were relatively less common in the ICUs. The knowledge of this difference in pathogens causing VAP in different ICU settings will guide the administration of appropriate empirical antibiotics for treatment of the infection. In a study at a tertiary care referral hospital in India, *Pseudomonas* spp. and *Acinetobacter* spp. were reported the common agents causing late-onset VAP, whereas the members of Enterobacteriaceae and *Acinetobacter* spp. were observed causing early-onset VAP.[9] In our study *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa* were reported to be the most common causes of late-onset VAP, whereas member of Enterobacteriaceae, non-fermenters, and MSSA were common agents causing early-onset VAP. However, the aetiological agents may vary according to the patients, health-care settings, and countries.[26]

Conclusion

VAP is common among ventilated patients basically caused by MDR pathogens. An early isolation followed by prevention of prolonged antibiotic therapy may lead to reduce mortality; associated with late-onset VAP. A detailed multicenter study on VAP is required to determine for proper understanding of it. Also knowledge of the susceptibility pattern of the local pathogens should guide the choice of antibiotics, in addition

to the likelihood of organisms, as there is an increasing prevalence of MDR pathogens in late-onset VAP.

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