Original Article

Ventilator-associated pneumonia in a tertiary care hospital in India: role of multi-drug resistant pathogens

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Abstract

Background: Ventilator-Associated Pneumonia (VAP) is the most frequent intensive-care-unit (ICU)-acquired infection. The aetiology of VAP varies with different patient populations and types of ICUs.

Methodology: A prospective study was performed over a period of 15 months in a tertiary care hospital to determine the various aetiological agents causing VAP and the prevalence of multidrug resistant (MDR) pathogens. Combination disk method, Modified Hodge test, EDTA disk synergy (EDS) test and AmpC disk test were performed for the detection of extended spectrum beta-lactamases (ESBL), carbapenemases, metallo-beta-lactamases (MBL) and AmpC β-lactamases respectively.

Results: *Enterobacteriaceae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Candida* spp. were more common in early-onset VAP, while non-fermenters (*Pseudomonas* spp. and *Acinetobacter* spp.) were significantly associated with late-onset VAP (*P* value 0.0267, Chi-square value 4.91). Thirty-seven (78.7%) of the 47 VAP pathogens were multidrug resistant. ESBL was produced by 50% and 67% of *Escherichia coli* and *Klebsiella pneumoniae* respectively. MBL was produced by 20% of *P. aeruginosa.* AmpC beta-lactamases were produced by 33.3% and 60.7% of the *Enterobacteriaceae* and non-fermenters respectively. Of the *S. aureus* isolates, 43% were methicillin resistant. Prior antibiotic therapy and hospitalization of five days or more were independent risk factors for VAP by MDR pathogens.

Conclusions: VAP is increasingly associated with MDR pathogens. Production of ESBL, AmpC beta-lactamases and metallo beta-lactamases were responsible for the multi-drug resistance of these pathogens. Increasing prevalence of MDR pathogens in patients with late-onset VAP indicate that appropriate broad-spectrum antibiotics should be used to treat them.

Key words: ventilator-associated pneumonia; extended spectrum beta-lactamase; intensive care unit; metallo-beta-lactamase; ampC-beta-lactamase

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Introduction

Ventilator-associated pneumonia (VAP) is defined as pneumonia occurring more than 48 hours after endotracheal intubation and initiation of mechanical ventilation (MV) including pneumonia developing even after extubation [1]. VAP is the most frequent intensive-care-unit (ICU)-acquired infection, occurring in 9 to 24% of patients intubated for longer than 48 hours [2,3].

Early-onset VAP, which occurs during the first four days of MV, usually is less severe, associated with a better prognosis, and is more 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].

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 [5-7]. Up to 40% of these infections can be polymicrobial [3,8]. *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) [9,10]. The aetiological agents of VAP vary with different patient populations and types of ICUs [1,4]. Therefore, the local microbial flora causing VAP needs to be studied in each setting to guide more effective and

rational utilization of antimicrobial agents. To better understand the aetiology of VAP in India, this study was conducted in two different ICUs in our tertiary care hospital.

The objectives of this study were to determine the prevalence and risk factors of MDR pathogens among our VAP patients and to determine their antibiotic susceptibility pattern as well as detect the presence of ESBL, AmpC β-lactamases, carbapenemases and metallobetalactamases in these VAP pathogens.

Materials and methods

Study Design

This prospective observational cohort study was conducted in two intensive care units (ICU) of a tertiary university hospital in India from October 2006 to December 2007. This study was approved by the Research and Ethical committees of Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER) and informed consent was obtained from each patient's next of kin.

Setting

The study was conducted in the Medicine Intensive Care Unit (MICU) and Critical Care Unit (CCU) of Jawaharlal Institute of Post-Graduate Medical Education and Research (JIPMER). This is an 860-bed tertiary care hospital and Institution of National Importance in India. The departments of Microbiology, Medicine and Anesthesiology and Critical Care were involved in this study. Each ICU is comprised of 8 well-spaced beds and patients were either admitted directly to the ICU or transferred from other wards, namely medicine, surgery, obstetrics, neurology and cardiology wards. Post-operative patients requiring ventilation were admitted in the CCU, while the patients with medical conditions necessitating ventilation were admitted in the MICU. Three nurses are posted in an ICU with a nurse patient ratio of 1: 2.7.

Subject and sample size

During the 15-month study period, a total of 538 patients who were intubated and on mechanical ventilation in the CCU and MICU were prospectively reviewed. Among them only 206 patients who were ventilated for more than 48 hours were eligible for inclusion in the study. Six of these 206 patients were assessed to have developed pneumonia within 48 hours of mechanical ventilation and were excluded. The remaining 200 patients were included in the study.

Procedure for data collection

All patients included in the study were monitored at frequent intervals (every three days) for the development of VAP using clinical and microbiological criteria until either discharge or death. The clinical parameters were recorded from their medical records and bedside charts. Details of antibiotic therapy, surgery, use of steroids, duration of hospitalization, presence of neurological disorders, and impairment of consciousness were also noted.

Criteria for diagnosis of VAP

The diagnosis of VAP was based on clinical and microbiological criteria [3]. A clinical suspicion of VAP was made in patients with a Modified Clinical Pulmonary Infection Score (CPIS) > 6 [11]; the diagnosis was confirmed by performing a quantitative culture of the endotracheal aspirate and observing $\geq 10^5$ cfu/ ml [12-14]. Based on these criteria, 36 of 200 enrolled patients were diagnosed with VAP.

Microbiological techniques

The organisms isolated by quantitative culture of the endotracheal aspirate (EA) from VAP patients were identified based on standard microbiological techniques [15]. The susceptibility of the clinical isolates to some routinely used antibiotics was determined by the Kirby-Bauer disk diffusion method [16]. Ampicillin, ciprofloxacin, ceftriaxone, ceftazidime, gentamicin, amikacin, and meropenem were tested for *Enterobacteriaceae*. Amikacin, gentamicin, ceftazidime, ciprofloxacin, meropenem, gatifloxacin, colistin, piperacillin- tazobactam and ticarcillin were tested for *Pseudomonas* spp. and *Acinetobacter* species. Penicillin, tetracycline, erythromycin, ciprofloxacin, gentamicin and vancomycin were tested for *S. aureus*. Susceptibility of *S. aureus* to oxacillin was determined using **o**xacillin-salt screen agar containing 6µg/ ml oxacillin and 4% NaCl [17]. High level gentamicin, ampicillin, tetracycline and vancomycin were tested for *Enterococcus* spp. Tetracycline, erythromycin, oxacillin, ciprofloxacin and cephalexin were tested for *Streptococcus pneumoniae*. Ampicillin, tetracycline, erythromycin and trimethoprim-sulfamethoxazole were tested for *Haemophilus influenzae*.

Combination disk method using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid, was performed for detection of extended spectrum β-lactamase (ESBL) among the members of *Enterobacteriaceae* [18]. Five mm or more increase in zone of inhibition for either cefotaxime-clavulanic acid or ceftazidime-clavulanic acid disk compared to the cefotaxime or ceftazidime disk respectively was taken as confirmatory evidence of ESBL production. Amp C disk test was performed for detection of AmpC β-lactamase [19]. A flattening or indentation of the cefoxitin inhibition zone in the vicinity of the disk with test strain was interpreted as positive for the production of AmpC β-lactamase. An undistorted zone was considered as negative. Modified Hodge test was carried out for detection of carbapenemase as described previously [20]. The presence of a cloverleaf-shaped zone of inhibition due to carbapenemase production by the test strain was considered positive. EDTA disk synergy test (EDS) was done using both meropenem and ceftazidime for detection of metallo-β-lactamases (MBL) [20]. The presence of an expanded growth inhibition zone between meropenem or ceftazidime and EDTA was interpreted as positive for MBL production. VAP pathogens, such as *Pseudomonas* spp., *Acinetobacter* spp., and enteric Gram-negative bacilli expressing ESBL, AmpC β-lactamases or MBL, MRSA and multidrug-resistant *S. pneumoniae* (resistant to penicillin and at least two other antibiotic classes) were defined as "multi-drug resistant" (MDR) pathogens [4,21].

Statistical analysis

Data entry and analysis were done using SPSS for Windows Version SPSS 16.0 (SPSS Inc, Chicago, Illinois). Means and standard deviations (SD) were calculated as required for numerical variables.

The chi-square test or Fisher's exact test was used to compare two groups. Univariate analysis was used to compare the variables for the outcome groups of interest. We confirmed the results of these tests with logistic regression analysis. This was necessary to avoid producing spuriously significant results with multiple comparisons. Results of the logistic regression analyses are reported as adjusted odd ratios with their 95% confidence intervals. All *P* values < 0.05 were considered statistically significant.

Results

The demographic data of the 36 patients diagnosed with VAP have been described in detail in our previously published article [3].

Microbial Patterns

The most common causative agents of early-onset VAP are members of *Enterobacteriaceae* (25%) and *Acinetobacter* spp. (25%). Methicillin sensitive *S. aureus* (13%) was the most common Gram-positive bacteria associated with early-onset VAP (Table 1). *Pseudomonas* spp. (39%) and *Acinetobacter* spp. (32%) were the most common pathogens causing late-onset VAP. Fifty percent of the *S. aureus* associated with late-onset VAP were MRSA (Table 2).

Early-onset VAP pathogens versus late-onset VAP pathogens

Enterobacteriaceae, H. influenzae, S. aureus, S. pneumoniae, Candida spp. were more common in early-onset VAP, while non-fermenters were significantly associated with late-onset VAP (*P* value 0.0267, Chi-square value 4.91) (Tables 1 and 2). The antibiotic resistance pattern of the various etiological agents of early-onset VAP and late-onset VAP are summarized in Tables 1 and 2. None of the *Acinetobacter* spp. causing early-onset VAP were colistin resistant, while 20% resistance to colistin was observed among *Acinetobacter* spp. associated with late-onset VAP.

Comparison of bacterial patterns of VAP in MICU and CCU

Non-fermenters (77.8%) were the most predominant pathogens causing VAP in the CCU, while in the MICU along with non-fermenters (48.3%), members of *Enterobacteriaceae* (24.1%) and Gram-positive bacteria (24.1%) commonly caused VAP. VAP episodes due to Gram-positive bacteria (5.6%) were relatively less common in the CCU (Figure 1).

Detection of ESBL, AmpC β-lactamase and Metallobetalactamase

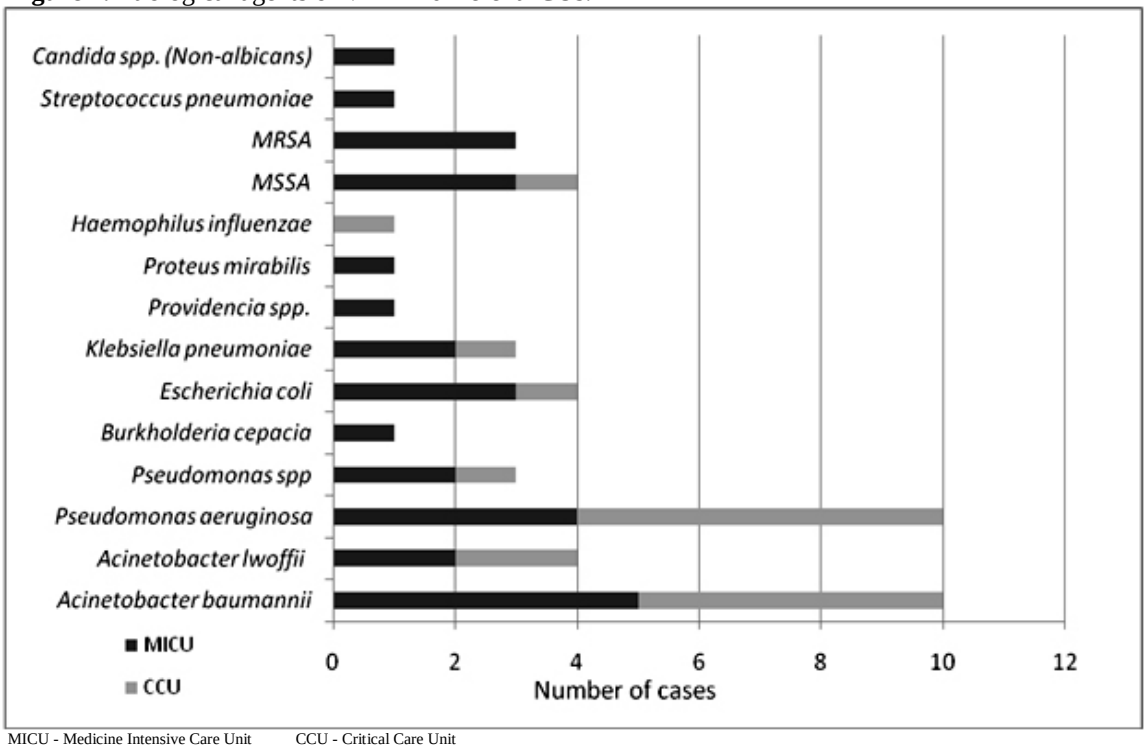
ESBL was produced by 50% and 67% of *E. coli* and *K. pneumoniae* respectively. Only two of the ten *P. aeruginosa* isolates were tested for metallobetalactamases and both were positive, while

Table 1. Etiological agents of early-onset VAP.

Etiological agent (no. of isolates)	Antibiotic resistance pattern (%)										
	AMP	**TIC**	**PTZ**	**GEN**	**AMK**	**CIP**	**GAT**	**CTR**	**CAZ**	**MEM**	**CL**
Gram negative bacteria											
Non-fermenters											
Acinetobacter baumannii (3)	-	100	33	100	100	100	100	-	100	100	0
Acinetobacter lwoffii (1)	-	100	0	100	100	100	100	-	100	100	0
Pseudomonas aeruginosa (1)	-	100	0	100	100	100	100	-	100	100	100
Burkholderia cepacia (1)	-	100	0	100	100	0	0	-	0	0	100
Enterobacteriaceae											
Escherichia coli (2)	100	-	-	100	0	100	-	100	100	0	-
Klebsiella pneumoniae (1)	-	-	-	100	0	100	-	100	100	0	-
Providencia spp. (1)	100	-	-	100	0	100	-	100	100	0	-
	PEN	**AMP**	**OXA**	**CFL**	**TET**	**ERY**	**CIP**	**GEN**	**VAN**	**SXT**	-
Other Gram negative bacteria											
Haemophilus influenzae (1)	-	0	-	-	0	0	-	-	-	0	-
Gram positive bacteria											
MSSA (2)	50	-	0	-	0	0	0	0	0	-	-
MRSA (1)	100	-	100	-	0	100	0	0	0	-	-
Streptococcus pneumoniae (1)	100	-	-	0	100	100	0	-	-	-	-
Fungi											
Candida spp. (1)	-	-	-	-	-	-	-	-	-	-	-

VAP – Ventilator-associated pneumonia AMP – Ampicillin, TIC – Ticarcillin, PTZ – Piperacillin-tazobactam, GEN – Gentamicin, AMK – Amikacin, CIP – Ciprofloxacin, GAT – Gatifloxacin, CTR – Ceftriaxone, CAZ – Ceftazidime, MEM – Meropenem, CL – Colistin, PEN – Penicillin, OXA – Oxacillin, CFL – Cephalexin, TET – Tetracycline, ERY – Erythromycin, VAN – Vancomycin, SXT – Trimethoprim-sulfamethoxazole MSSA - Methicillin sensitive *Staphylococcus aureus*MRSA - Methicillin resistant *Staphylococcus aureus*

Figure 1. Etiological agents of VAP in different ICUs.



MICU - Medicine Intensive Care Unit CCU - Critical Care Unit

Table 2. Etiological agents of late-onset VAP.

Etiological agent (no. of isolates)	Antibiotic resistance pattern (%)										
	AMP	**TIC**	**PTZ**	**GEN**	**AMK**	**CIP**	**GAT**	**CTR**	**CAZ**	**MEM**	**CL**
Gram negative bacteria											
Non-fermenters											
Acinetobacter baumannii (7)	-	71	43	100	86	100	100	-	100	57	14
Acinetobacter lwoffii (3)	-	67	33	100	100	100	100	-	100	67	33
Pseudomonas aeruginosa (9)	-	22	22	89	67	78	56	-	67	22	78
Pseudomonas spp. (3)	-	100	33	100	67	67	67	-	67	33	67
Enterobacteriaceae											
Escherichia coli (2)	100	-	-	100	0	100	-	100	100	0	-
Klebsiella pneumoniae (2)	-	-	-	100	0	100	-	100	100	0	-
Proteus mirabilis (1)	100	-	-	100	100	100	-	100	100	0	-
	PEN	**AMP**	**OXA**	**CFL**	**TET**	**ERY**	**CIP**	**GEN**	**VAN**	-	-
Gram positive bacteria											
MSSA (2)	100	-	0	-	0	0	0	0	0	-	-
MRSA (2)	100	-	100	-	100	100	100	100	0	-	-

VAP – Ventilator-associated pneumonia AMP – Ampicillin, TIC – Ticarcillin, PTZ – Piperacillin-tazobactam, GEN – Gentamicin, AMK – Amikacin, CIP – Ciprofloxacin, GAT – Gatifloxacin, CTR – Ceftriaxone, CAZ – Ceftazidime, MEM – Meropenem, CL – Colistin, PEN – Penicillin, OXA – Oxacillin, CFL – Cephalexin, TET – Tetracycline, ERY – Erythromycin, VAN – Vancomycin
MSSA - Methicillin sensitive *Staphylococcus aureus* MRSA - Methicillin resistant *Staphylococcus aureus*

AmpC β-lactamases were produced by 33.3% and 60.7% of the members of *Enterobacteriaceae* and non-fermenters respectively (Table 3).

MDR pathogens

Thirty-seven (78.7%) of the 47 VAP pathogens in our study were multi-drug resistant (MDR). These MDR pathogens included Gram-negative bacteria (non-fermenters and members of *Enterobacteriaceae*) producing ESBL, AmpC β-lactamases or MBL, MRSA and *S. pneumoniae* showing resistance to oxacillin, tetracycline and erythromycin.

Risk Factors

Administration of prior antibiotic therapy was a significant risk factor for VAP caused by MDR pathogens (RR, 2.18; 95% CI, 0.74 to 6.42; P = 0.0404). Table 4 shows that current hospitalization of five days or more was also a significant risk factor for VAP caused by MDR pathogens (RR, 1.79; 95% CI, 0.88 to 3.61; P = 0.0301). Multivariate logistic regression analysis confirmed that prior antibiotic therapy and current hospitalization of five days or more were independent predictors of VAP caused by MDR pathogens (Table 5).

Discussion

VAP is an important nosocomial infection among ICU patients receiving MV. Multidrug resistant pathogens such as *P. aeruginosa*, *A. baumannii* and *S. aureus* (42.9% of them being MRSA) were the common organisms causing VAP. This highlights the need for treatment of the VAP cases with second-line antibiotics effective against these MDR pathogens. This finding also emphasises the need for stringent preventive measures for VAP, as the treatment of an established VAP becomes very expensive [22]. Non-fermenters such as *Pseudomonas* spp. and *Acinetobacter* spp. were significantly associated with late-onset VAP as it was observed by other workers [23,24]. But in our study even in patients with early-onset VAP, *Acinetobacter* spp. was the most common pathogen because most of them had risk factors for MDR pathogens.

Late-onset VAP was associated with higher rates of infection with MRSA and colistin resistant MDR *Acinetobacter* spp., but the resistance of the non-fermenters to the other antibiotics was almost the same in both early- and late-onset VAP. Many of the early-onset VAP cases had the risk factors such as prior antibiotic therapy and current hospitalization for five days or more for infection with MDR pathogens. That could be the reason for the almost similar susceptibility pattern of the isolates from late-onset and early-onset VAP. Even the American Thoracic Society guidelines supports the same reasoning by suggesting that patients with early-onset VAP who have received prior antibiotics or who have had prior

Table 3. ESBL, AmpC β - lactamase and MBL production among the VAP pathogens.

Bacteria (no. of isolates)	ESBL	AmpC β-lactamase	MBL[a] (no. of isolates tested)
Non-fermenters			
Pseudomonas aeruginosa (10)	-	4	2 (2)
Pseudomonas spp. (3)	-	2	0 (1)
Burkholderia cepacia (1)	-	1	NS
Acinetobacter baumannii (10)	-	7	0 (8)
Acinetobacter lwoffii (4)	-	3	0 (2)
Enterobacteriaceae			
Escherichia coli (4)	2	1	NS
Klebsiella pneumoniae (3)	2	0	NS
Providencia spp. (1)	0	1	NS
Proteus mirabilis (1)	0	1	NS

[a] Only meropenem resistant strains were screened for MBL production; NS – The isolates were not screened for MBL production as they were sensitive to meropenem. ESBL – Extended spectrum β-lactamase MBL – Metallo-β-lactamase

Table 4. Univariate analysis of the risk factors for VAP by MDR pathogens.

S. No.	Risk factor	Non-MDR (n = 7) (%)	MDR (n = 29) (%)	Relative risk (95% confidence limits)	*P* value
1.	Hospitalization of 5 d or more	3 (42.9)	25 (86.2)	1.79 (0.88 to 3.61)	0.0301
2.	Prior antibiotic therapy	4 (57.1)	27 (93.1)	2.18 (0.74 to 6.42)	0.0404
3.	Impaired consciousness	0 (0)	8 (27.6)	1.33 (1.08 to 1.65)	0.3093
4.	Neurological disorders	3 (42.9)	8 (27.6)	0.87 (0.58 to 1.29)	0.6499
5.	Surgery	1 (14.3)	4 (13.8)	0.99 (0.62 to 1.59)	1.0000
6.	Steroid therapy	0 (0)	8 (27.6)	1.33 (1.08 to 1.65)	0.3093

MDR – Multi-drug resistant

Table 5. Logistic regression analysis of the risk factors for VAP by MDR pathogens.

	P value	Adjusted Odds ratio	95% confidence interval	
			Lower	Upper
Prior antibiotic therapy	.019	25.428	1.688	382.935
Hospitalization of 5 d or more	.019	18.688	1.616	216.153

VAP – Ventilator-associated pneumonia MDR – Multi-drug resistant

hospitalization within the past 90 days are at greater risk for colonization and infection with MDR pathogens and should be treated similarly to patients with late-onset VAP [4].

We also observed that non-fermenters (77.8%) were the most predominant pathogens causing VAP in the CCU, while in the MICU along with non-fermenters (48.3%), members of *Enterobacteriaceae* (24.1%) and Gram-positive bacteria (24.1%) were also commonly causing VAP. VAP episodes due to Gram-positive bacteria (5.6%) were relatively less common in the CCU. 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.

We observed that colistin is highly active against *Acinetobacter* spp., while piperacillin-tazobactam has good activity against *Pseudomonas* spp. But as we have studied only a small number of isolates, these findings need to be further confirmed by larger clinical trials, as they may have a major impact on the treatment of these VAP pathogens. AmpC β-

lactamase was produced by most of the non-fermenters, especially *Acinetobacter* spp., while MBL was produced only by *P. aeruginosa* consistent with other studies [25,26]. Similarly ESBL and AmpC β-lactamases were produced by a large proportion of the *Enterobacteriaceae*.

In the present study, we found that prior antibiotic therapy and current hospitalization of five days or more were independent predictors of VAP caused by MDR pathogens by multivariate logistic regression. This emphasizes the need for judicious selection of patients for antibiotic therapy. The prophylactic use of antibiotics is therefore not recommended, and exposure to antibiotics is a significant risk factor for colonization and infection with nosocomial multidrug-resistant pathogens as observed by other authors [1,27,28]. The rational use of appropriate antibiotics may reduce patient colonization and subsequent VAP with MDR pathogens. Similarly, unnecessary prolonged hospitalization of the patients should be avoided as far as possible. But it may not be feasible in most situations as the patients' condition may demand prolonged hospital stay. However, the knowledge of this risk factor should suggest the possibility of infection due to MDR pathogens in patients developing VAP after hospitalization for five days or more.

As the study was conducted in a resource-limited setting, only small number of patients with VAP in a single center were studied, which could be considered a limitation of our study. In addition, we recognize that the findings of this study may not necessarily reflect the situations in other similar centers in India. Hence, we suggest further multi-centered studies with larger patient numbers to confirm our findings, in particular the high incidence of MDR pathogens.

To conclude, VAP is increasingly associated with MDR pathogens. Production of ESBL, AmpC β-lactamases and metallo β-lactamases were responsible for the multi-drug resistance of these pathogens. Knowledge of the susceptibility pattern of the local pathogens should guide the choice of antibiotics, in addition to the likelihood of organisms (early- or late-onset VAP). As there was an increasing prevalence of MDR pathogens in late-onset VAP, appropriate antibiotics should be used to treat them. Patients with early-onset VAP who have received prior antibiotics or who were hospitalized earlier should also be treated similarly to those with late-onset VAP, as they are at higher risk for infection with MDR pathogens. Colistin and piperacillin-tazobactam may be used for successful treatment of multi-drug resistant *Acinetobacter* spp. and *Pseudomonas* spp. respectively as they showed good *in vitro* activity against these MDR pathogens.

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