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ORIGINAL ARTICLE

Ventilator associated pneumonia: bacterial agents and antibiogram at a Tertiary Care Centre in Kathmandu

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Abstract

The most common nosocomial infection seen in patients under mechanical ventilation is ventilator-associated pneumonia (VAP). This study is conducted to study rate of VAP, bacterial agents, and their antibiogram. This was a hospital based, observational cross-sectional study of all the patients who were mechanically ventilated in the intensive care unit at Nepal Medical College Teaching Hospital, Kathmandu during a period of one year. Endotracheal aspirates were processed for bacterial isolation and identification and their antibiotic susceptibility test. Significant bacterial growth was considered on the basis of significant gram stain and semi-quantitative culture obtained by endotracheal sampling.

Significant bacterial growth was found in 48(57.8%) endotracheal aspirates (n = 83) of which 4(8.3%) were from cases of VAP and 44(91.7%) from Ventilator associated condition (VAC).VAP rate among patients was 4.8%. Among four VAP cases, two endotracheal aspirates grew Klebsiella pneumoniae, one grew Acinetobacter calcoaceticus baumannii complex, and one grew both Pseudomonas aeruginosa and K. pneumoniae. All isolates of K. pneumoniae were MDR and ESBL producers where two of them were AmpC β-lactamase and MBL producers. The P. aeruginosa isolated was MDR and produced AmpC beta lactamase, MBL and ESBL. The isolated Acinetobacter calcoaceticus baumannii complex was also MDR. VAP is a commonly encountered complication in mechanically ventilated patients. The MDR pathogens associated with VAP and VAC call for special attention to care for the ventilated and need for strict adherence to infection control practices including VAP bundle care.

Keywords: VAP; VAC; MDR; ESBL; MBL; AmpC β lactamase

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The most common nosocomial infection seen in patients under mechanical ventilation is ventilator-associated pneumonia (VAP). Following endotracheal intubation, VAP usually occurs after 48–72 h (1). Centers for Disease Control and Prevention (CDC) established a surveillance definition, ventilator-associated events (VAEs). VAE consists of ventilator-associated conditions (VAC), infection-related ventilator-associated complications (IVAC, a subset of VAC with infectious signs), and possible VAP (IVAC with microbiological evidence of pneumonia) (2). The new surveillance criteria for possible or probable VAP as defined by CDC is patient has a baseline period of stability or improvement on the ventilator, defined by ≥2 calendar days of stable or decreasing daily minimum FiO, or PEEP values, (the baseline period is defined as the 2 calendar days immediately preceding the first day of increased daily minimum PEEP or FiO₂ is said to have VAC (3). On or after calendar day

3 of mechanical ventilation and within 2 calendar days before or after the onset of worsening oxygenation, the patient meets both of the following criteria: 1) Temperature >38°C or <36°C, OR white blood cell count $\geq 12,000 \text{ cells/mm}^3 \text{ or } \leq 4,000 \text{ cells/mm}^3 \text{ AND } 2)$ A new antimicrobial agent(s) is started and is continued for ≥4 qualifying antimicrobial days classify the patient as an IVAC. Possible ventilator-associated pneumonia (PVAP): On or after calendar day 3 of mechanical ventilation and within 2 calendar days before or after the onset of worsening oxygenation, one of the following criteria is met: 1).

Criterion I: Positive culture of one of the following specimens, meeting quantitative or semi-quantitative thresholds as outlined in protocol, without requirement for purulent respiratory secretions (3):

Endotracheal aspirate (ETA), ≥ 10⁵ CFU/mL or corresponding semi-quantitative result

- Bronchoalveolar lavage (BAL), ≥ 10⁴ CFU/mL or corresponding semi-quantitative result
- Lung tissue, ≥ 10⁴ CFU/g or corresponding semi-quantitative result
- Protected specimen brush, ≥10³ CFU/mL or corresponding semi-quantitative result
- 2) Criterion II: Purulent respiratory secretions (defined as secretions from the lungs, bronchi, or trachea that contain ≥25 neutrophils and ≤10 squamous epithelial cells per low power field PLUS organism identified from one of the following specimens such as sputum, ETA, BAL, lung tissue, protected specimen brush (3).
- 3) Criterion III: One of the following positive tests: a. organism identified from pleural fluid, b. lung histopathology, defined as: (1) abscess formation or foci of consolidation with intense neutrophil accumulation in bronchioles and alveoli; (2) evidence of lung parenchyma invasion by fungi (hyphae, pseudohyphae, or yeast forms); (3) evidence of infection with the viral pathogens (3).

There are very few reports from Nepal on this, so this study was carried out to determine the baseline prevalence and to detect bacterial etiological agents with their anti-biogram in cases of VAP and VAC.

Materials and methods

This was a hospital based, observational cross–sectional study of all the patients admitted in general intensive care unit (ICU) and Paediatiric ICU (PICU) of Nepal Medical College Teaching Hospital (NMCTH) during January 2020 to December 2021, who were mechanically ventilated without a prior diagnosis of pneumonia. Data analysis was done using MS Excel and were presented as frequency and percentage. Prevalence rate of VAP is reported with 95% CI.

ETAs were obtained from patients on mechanical ventilation with assistance from nurse in the ICU. The samples were processed within 1 h (4). Semiquantitative culture of the ETA was done using a standardized wire loop of 1.2 mm diameter (1 µL volume), and inoculated on blood agar and MacConkey agar. The plates were incubated aerobically overnight at 37°C (5). A colony forming unit (CFU) count of 105/mL (i.e. 100 colonies) or 104/mL (i.e. 10 colonies), respectively, was considered significant (6). The identification of bacterial isolate was carried out using standard bacteriological procedures including Gram stain, colony morphology on blood agar, Mac-Conkey agar, chocolate agar and biochemical tests according to standard microbiological technique (7). Antimicrobial susceptibility test (AST) was performed for all the isolates using Kirby-Bauer's disc diffusion method (8).

ESBL screening was performed by using ceftazidime (CAZ) (30 µg) and cefotaxime (CTX) (30 µg) disk. When

the zone of inhibition was <22 mm for CAZ and <27 mm for CTX, the isolate was considered as ESBL producer as recommended by CLSI. Confirmation of ESBL production was done by the Combination Disk method in which CAZ and CTX(9) alone and in combination with clavulanic acid (CA) (10 μ g) was used. When the zone diameter of either CAZ or CTX (or both) in the presence of CA is >5 mm larger than the zone diameter of respective agent alone, the isolate was considered as ESBL producer (10).

Metallo-β-lactamases (MBL) production was detected by the imipenem-EDTA disc method. Two imipenem discs were placed on the surface of agar plate at a distance of 25mm from center to center and EDTA (750 μ g) was added to one of them. After 24 h of incubation at 37°C, if the zone of inhibition of imipenem-EDTA disc is \geq 7 mm than the disc with imipenem alone, the isolate was considered as MBL producer (11).

For screening AmpC β -lactamases susceptibility to cefoxitin (30 μ g) was tested and organisms resistant to this antibiotic (showing the zone of inhibition of diameter <18 mm) were screened as potential AmpC producers and underwent further confirmatory tests (12). The screened isolates were further tested for confirmation by combination disk test with 3-Aminophenyl boronic acid (APBA) (13).

Results

A total of 83 ETAs were processed for aerobic bacterial isolation. Out of these, significant bacterial growth was found in 48 (57.8%) aspirates. Among 48 (57.8%) aspirates with significant bacterial growth, 4 (8.3%) were from cases of VAP and 44 (91.7%) were from VAC (Table 1).

Of the total of 83 patients who were mechanically ventilated, the age ranged from 2 to 79 years. There were more male patients than female patients (68 versus 15) (Table 2).

Among the four cases of VAP, all four bacterial isolates were Gram-negative bacilli. Antibiogram of bacteria isolated from VAP is shown in Tables 3 and 4.

Among the 18 bacterial isolates from VAC, 44.4% isolates were of *Klebsiella pneumoniae* followed by

Table 1. Culture result of endotracheal aspirate cultures (n = 83)

Culture result	Number	Percent
No growth	35	42.2
Growth	48	57.8
Total	83	100
Growth with VAC and VAP/IVAC	18	37.5
Growth without VAC/IVAC	30	62.5
Total	48	57.8

VAC: ventilator associated condition; VAP: ventilator-associated pneumonia; IVAC: infection-related ventilator-associated complications.

Table 2. Age and gender wise distribution of mechanically ventilated patient

Age group (years)	Male	Female	Total
0–10	3	0	3
11–20	3	0	3
21-30	13	0	13
31-40	10	7	17
41–50	10	0	10
51–60	12	4	16
61–70	8	3	11
71–80	9	1	10
Total	68	15	83

Table 3. Number and types of bacterial isolates from VAP (n = 4)

Isolates	Number	Percent
Klebsiella pneumoniae	2	50
A. calcoaceticus baumanii	1	25
Pseudomonas aeruginosa+ Klebsiella	1	25
pneumoniae		
Total	4	100

VAP: ventilator-associated pneumonia.

Table 4. Antibiogram of bacteria isolated from VAP

Antibiogram	Klebsiella pneumoniae (n = 2)	A. calcoaceticus baumanii complex (n = 1)	Pseudomonas aeruginosa (n = 1)	
	Resistant	Resistant Resistant		
Ceftazidime		I	I	
Cefotaxime	2	1	-	
Ceftriaxone	2	1	-	
Gentamicin	I	0	0	
Tobramycin	0	0	0	
Ciprofloxacin	I	1	1	
Levofloxacin	I	1	1	
Trimethoprim/ sulfamethoxazole	1	1		
Piperacillin-tazobactam	2	1	I	
Imipenem	2	1	1	

VAP: ventilator-associated pneumonia.

A. calcoaceticus baumanii complex (27.7%), Pseudomonas aeruginosa (22.2%) and Escherichia coli (5.5%). Antibiogram of bacteria isolated from VAC-is shown in Table 5.

Rate of VAP was 4.8%. The prevalence of MDR, ESBL, MBL, AmpC-β-lactamase producing bacteria in VAP is shown in Table 6.

The prevalence of MDR, ESBL, MBL, AmpC-βlactamase producing bacteria in VAC is shown in Table 7.

Discussion

This study was conducted among the ICU patients who were mechanically ventilated at NMCTH. Kathmandu from January 2020 to December 2021 to study the rate of ventilator associated pneumonia, the causative bacterial agents, and their antibiogram and determine the prevalence of MDR, ESBL, MBL, AmpC-β-lactamase producing bacteria in VAP. A total of 83 ETA were collected from the mechanically ventilated patients admitted in ICU and processed in the bacteriology laboratory. In this study, the incidence of VAP was found to be 4.8% that was similar to findings of Chouhdari et al. which was (8%) performed at Loghman Hakim Hospital, Tehran, Iran, in the year 2017 (14). A higher incidence of VAP was found in a study done by Mathai et al. (38%) from Christian Medical College, Ludhiana, Punjab, India, in the year 2016 (15). Our estimation was low as compared with other studies. Two main reasons for this low rate can be: 1. compliance with prevention strategies such as basic practices to prevent VAP including: decrease duration of MV and length of ICU stay, avoidance of intubation if possible, minimize sedation, maintain and improve physical conditioning, elevate the head of the bed and maintain ventilator circuits and 2. Although the sample size was sufficient for assessing VAP, the study was conducted during the COVID 19 pandemic presented clinical challenges including restricted access to ICU patients and limited follow up data collection. As a result the outcomes of patients with VAP could not be assessed.

The highest prevalence of mechanically ventilated patients in our study were found to be patients from age groups 31–40 years (n = 17, 20.5%) followed by 51–60 years (n = 16,19.3%). Shrestha DK et al. reported that incidence of VAP was highest among patients aged between 15 and 25 years of age (25.7%) at Tribhuvan University Teaching Hospital, Institute of Medicine, Nepal (16). This study also revealed that the incidence of VAP was more among males (75%).

In our study the most common pathogens responsible for VAP were found to be gram-negative bacteria. The organisms isolated from VAP in patients in our hospital were K. pneumoniae, and A. calcoaceticus baumanii complex. Similar study done by Mishra DR. et al showed most frequent organisms were K. pneumoniae, Acinetobacter spp and P. aeruginosa (17). This study helped us in the early diagnosis of VAP by clinical suspicion combined with bedside examination, radiographic examination, and microbiologic analysis of respiratory secretions and to determine the baseline incidence of VAP. VAP caused by bacterial pathogens that normally colonize the oropharynx and gastrointestinal tract, or

Table 5. Antibiogram of bacteria isolated from VAC

Antibiogram	Klebsiella pneumoniae (n = 8)	Klebsiella pneumoniae A. calcoaceticus baumanii $(n = 8)$ complex $(n = 5)$		Escherichia coli (n = 1)	
	R	R	R	R	
Ceftazidime		5	4	I	
Cefotaxime	8	5	-	1	
Ceftriaxone	8	5	-	1	
Gentamicin	5	0	0	0	
Tobramycin	5	0	0	0	
Ciprofloxacin	5	1	1	0	
Levofloxacin	5	1	1	0	
Trimethoprim/sulfamethoxazole	5	1	-	1	
Piperacillin-tazobactam	5	5	4	0	
Imipenem	5	3	4	0	

VAC: ventilator associated condition.

Table 6. The prevalence of multiple drug resistant, extended spectrum beta-lactamase, metallo- β lactamases, AmpC- β -lactamase producing bacteria in VAP

Organism	Multiple enzyme producer	AmpC β lactamase detection	MBL	MDR	ESBL
Klebsiella pnemoniae ($n = 3$)	2	2	2	3	3
A. calcoaceticus baumanii complex $(n = 1)$	0	0	0	1	0
Pseudomonas aeruginosa $(n = 1)$	1	I	1	1	1

VAP: ventilator-associated pneumonia.

Table 7. The prevalence of multi-drug resistant, extended spectrum beta-lactamase, metallo-β- lactamases, AmpC-β-lactamase producing bacteria in VAC

Organism	Multiple enzyme producer	AmpC β lactamase producer	MBL producer	MDR	ESBL producer
Klebsiella pnemoniae ($n = 8$)	3	3	3	8	5
A. calcoaceticus baumanii complex $(n = 5)$	2	3	2	5	3
Pseudomonas aeruginosa $(n = 4)$	4	4	4	4	4
Escherichia coli $(n = 1)$	0	0	0	0	0

VAC: ventilator associated condition.

that are acquired through transmission by health-care workers from environmental surfaces or from other patients. Mechanically ventilated patients are unconscious therefore there is no clearance of the secretion in the oropharynx, leading to increased rate of microbial colonization. These colonized microbial pathogens in the accumulated secretion pass along the endotracheal tube, reaching the distal airway resulting in higher of colonization in lower respiratory tract, leading to VAP.

In our study the resistance was seen to most of the commonly used antimicrobials in all types of VAP isolates. Majority of bacterial isolates demonstrated resistance to

commonly prescribed antibiotics in the ICU setting. MDR was found to be prevalent in all types of bacterial isolates from VAP and VAC. Similar finding was reported in a study by Adhikari K. et al. at Kathmandu Model Hospital (18). Due to the increasing incidence of MDR organisms in ICUs, an early and correct diagnosis of VAP is essential for optimal antibiotic treatment. Isolation of the causative organism from ET secretions and its culture sensitivity is crucial in the management of VAP. The development of antibiotic resistance is associated with high morbidity and mortality, particularly in the ICU setting (19).

ESBL producing isolates (75%) were also found among VAP in this study. All isolates of K. pneumoniae were ESBL producers. Similar finding was reported from a study conducted at tertiary care hospital, Manipal, India by Dey et al. where 100% of K. pneumoniae from VAP were ESBL producers (18).

Single isolate (n = 1, 100%) *P. aeruginosa* and two out of three (n = 2, 66.66%) K. pnuemoniae isolates were found to be MBL producer whereas none of the A. calcoaceticus baumanii complex produced MBL.

In this study, EDTA-IPM combined disk method was used to detect MBL. This is a simple test that can be used in any tertiary health care. Recently emergence of MBL enzymes in gram-negative bacilli is alarming and reflects the excessive use of carbapenem.

Single isolate (n = 1, 100%) of *P. aeruginosa* and two out of three (n = 2, 66.66%) Klebsiella pnuemoniae isolates were found to be Amp C β-lactamase producers, whereas single isolate of A. calcoaceticus baumanii complex was not found to be Amp C β-lactamase producer.

In this study the highest rates of resistance of P. aeruginosa, Klebsiella pnuemoniae and A. calcoaceticus baumanii complex isolates were against ceftazidime and ceftriaxone. Screening of cefoxitin resistance during routine sensitivity tests can aid in early detection of AmpC β -lactamase producers and use of effective antibiotic therapy. From this study, we came to know that resistant bacteria are common in our ICU. It is wise to control this situation by rational use of antibiotics, identifying the pathogen, choosing correct antibiotics, practicing antimicrobial stewardship. In this way, we will be able to maintain or prolong the efficacy of existing drug.

The incidence of VAP can be prevented by strict hand hygiene before patient contacts, adopting careful intubation techniques, oral intubation, avoidance of unplanned extubations and reintubations, maintaining adequate endotracheal cuff pressure, appropriate use of analgesia and sedation and early use of physical therapy and mobilization.

The four most common colonizing organism isolated from VAC from the patient on mechanical ventilated were K. pnemoniae, A. calcoaceticus baumanii complex, P. aeruginosa, E. coli. Among VAP positive patients prior colonization was observed with the same organism that were isolated from VAC suggesting role of ETA culture in aiding the diagnosis of VAP.

Conclusion and recommendations

Conclusion

VAP is a commonly encountered complication in mechanically ventilated patients. The overall incidence of VAP in our ICUs was low.

- Multidrug resistant Gram-negative bacteria were the major bacteria causing VAP.
- Klebsiella pneumoniae and A. calcoaceticus baumanii complex were commonest organisms causing VAP in our setting.
- Multiple enzymes producing isolates were Klebsiella pnemoniae, P. aeruginosa
- More attention should be paid to planning and providing appropriate and regular training programs for all Medical professionals to update the information and follow the clinical guidelines and necessary facilities must be provided with high-quality services in the ICU to improve health care quality, and to reduce the rate of VAP.
- Need for strict adherence to infection control practices including VAP bundle care

Recommendations

- Most commonly K. pneumoniae followed by A. calcoaceticus baumanii complex, P. aeruginosa, and E. coli were observed in VAC and VAP infections in ICU. To prevent their dissemination, hospital policies for housekeeping, isolation and disinfection should address this issue.
- Isolates from ICU, which are MDR, ESBL, AmpC β lactamase-, MBL-producers, warrants careful laboratory practice to detect these resistant bacteria for the timely management and infection control measures.
- Surveillance of VAE must be done regularly

Limitation

This was a hospital-based cross-sectional study conducted for 1 year during the period of COVID 19 pandemic and the outcome of the patients with VAP could not be assessed.

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Funding and conflict of interest

None to declare.

Ethical consideration

Ethical clearance was obtained from the institutional review committee of NMCTH.

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