e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2026; 18(6); 464-468

Original Research Article

Quantitative Endotracheal Aspirate–Based Surveillance of Ventilator Associated Pneumonia in Adult ICU Patient – Risk Factors and Bacterial Etiology

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Received: 14-05-2026 / Revised: 10-06-2026 / Accepted: 18-06-2026
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Conflict of interest: Nil

Abstract:

Background: Ventilator-associated pneumonia (VAP) is a common nosocomial infection in mechanically ventilated patients and is associated with increased morbidity, mortality, and length of ICU stay. Accurate diagnosis and knowledge of local bacterial etiology are essential for appropriate management.

Objectives: To monitor adult mechanically ventilated ICU patients for the development of VAP using clinical and radiological criteria, to analyse the associated risk factors, and to identify bacterial etiological agents by quantitative processing of endotracheal aspirates.

Methods: This prospective study included adult ICU patients on mechanical ventilation for more than 48 hours at Rangaraya Medical College, Kakinada, from December 2017 to August 2019. VAP was diagnosed using clinical pulmonary infection score, chest radiographic findings, and clinical criteria. Endotracheal aspirates were processed quantitatively for bacterial isolation and identification. Clinical variables were analysed as possible risk factors.

Results: Of 100 ventilated patients, 33 were confirmed to have VAP. Although 75 endotracheal aspirates were culture positive, only 33 yielded significant pathogens, while 42 represented colonisers. Prior antibiotic use, stress ulcer prophylaxis, nasogastric tube placement, and reintubation were common associated factors. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were the most frequent isolates.

Conclusion: VAP constituted a significant problem in ventilated ICU patients, with gram-negative bacteria predominating. Quantitative endotracheal aspirate culture was useful for confirming infection and differentiating colonisation.

Keywords: Ventilator-Associated Pneumonia, Endotracheal Aspirate, Quantitative Culture, Intensive Care Unit, Bacterial Profile.

DOI: 10.25258/ijcpr.18.6.79

Introduction

Ventilator-associated pneumonia (VAP) remains one of the most important healthcare-associated infections in mechanically ventilated adults and continues to contribute substantially to prolonged ventilation, longer ICU stay, antimicrobial exposure, and adverse outcomes [1]. Recent evidence shows that VAP develops through a complex interaction of host vulnerability, endotracheal-tube biofilm formation, microaspiration, and changing ICU microbiology, while its diagnosis still relies on a combination of clinical suspicion, radiological evidence, and microbiological confirmation [1]. Systematic review data also indicate that the occurrence of VAP is influenced by several patient- and care-related risk factors, underscoring the need for active surveillance in ventilated adults [2]. Quantitative processing of endotracheal aspirates is particularly relevant because it helps distinguish true infection from airway colonization and allows identification of the bacterial etiological agents responsible for VAP, thereby supporting rational antimicrobial therapy [3]. In addition, recent microbiological studies emphasize that the bacteriological spectrum and resistance profile of VAP vary across centres, making local data essential for guiding empirical treatment and infection-control strategies [4]. The aim of the present study was to monitor mechanically ventilated adult ICU patients for the development of VAP using clinical and radiological criteria, analyse the associated risk factors, and quantitatively process endotracheal aspirates to identify the bacterial etiological profile of VAP in a tertiary care hospital.

Methods

This prospective hospital-based study was conducted in the departments of Microbiology Rangaraya Medical College, Kakinada, from December 2017 to August 2019. Adult ICU patients who had been on mechanical ventilation for >48 hours and developed clinical features suggestive of pneumonia were included. Ethical approval was obtained from the Institutional Ethics Committee, and consent was obtained from each patient's attendant or legally acceptable representative. All microbiological findings were communicated promptly to the treating physicians to aid in patient management. Patients <18 years and those who had been mechanically ventilated for <48 hours were excluded. VAP was suspected in patients who developed a new and persistent pulmonary infiltrate on chest radiograph after 48 hours of mechanical ventilation along with at least two of the following: fever ≥38°C or hypothermia ≤36°C, leukocytosis ≥10,000/mm³ or leukopenia ≤4,000/mm³, and purulent tracheal secretions [1, 5].

Detailed clinical and demographic data were collected using a structured proforma; age, sex, address, date of admission, duration of ICU stay, duration of mechanical ventilation, and level of consciousness. Data regarding the presence of nasogastric tube, intravenous sedation, prior antibiotic therapy, impaired consciousness, tracheostomy, reintubation, stress ulcer prophylaxis, emergency intubation, and associated underlying diseases were also documented. Patients were followed clinically from the time of inclusion until microbiological confirmation and relevant risk-factor assessment were completed. The diagnosis of VAP was thus based on a combination of clinical findings, radiological evidence, and microbiological examination. This combined approach helped distinguish true infection from non-infectious pulmonary infiltrates and ventilator-related colonization, which is particularly important in critically ill patients receiving prolonged ventilatory support [1, 5].

Endotracheal aspirates (ETAs) were collected under strict aseptic precautions as per the institutional protocol. The sample was transported immediately and processed. Respiratory samples were mechanically liquefied and homogenized by vortexing with sterile glass beads for 1 minute and then centrifuged at 3,000 rpm for 10 minutes. The processed specimen was subjected to Gram staining and quantitative culture using standard culture techniques. Bacterial isolates were identified by colony morphology, Gram reaction, and standard biochemical tests. Quantitative culture helped differentiate significant infection from colonization, and isolates considered etiologically significant were further analysed. Antimicrobial susceptibility testing of bacterial isolates was performed as per prevailing laboratory guidelines.

Data were entered in Microsoft Excel and analysed using SPSS version 20. Descriptive statistics were used to summarise demographic, clinical, radiological, and microbiological variables as frequencies, percentages, mean, and standard deviation. Association between VAP and categorical risk factors was assessed using the chi-square test or Fisher's exact test whenever appropriate. A p value of <0.05 was considered statistically significant.

Results

Total 100 members were included. As shown in Table 1, VAP was confirmed in 33 members, 67% were non-VAP. Prior antibiotic use was the most common risk factor among confirmed VAP cases, being present in 11 (33.3%), followed by stress ulcer prophylaxis in 8 (24.2%), nasogastric tube placement and reintubation in 5 patients each (15.2%), impaired consciousness in 3 (9.1%), and intravenous sedation in 1 (3.0%) (Table 2). Tracheostomy was not observed among confirmed VAP cases. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were the predominant pathogens, each accounting 9 each (27.3%), followed by *Acinetobacter* species (6; 18.2%), *Escherichia coli* (4; 12.1%), methicillin-sensitive *Staphylococcus aureus* (MSSA) (3; 9.1%) and *Enterobacter* species (2; 6.1%) (Table 3). Late-onset VAP was more common than early-onset VAP, occurring in 20 cases (60.6%) compared to 13 cases (39.4%). Among confirmed VAP patients, 26 (78.8%) were discharged, whereas 7 patients (21.2%) died.

Table 1: Frequency of VAP and diagnostic yield

Parameter	Number	%
Study members	100	100
Confirmed VAP cases (CPIS >6)	33	33
Non-VAP cases	67	67
Culture-positive ETA samples	75	75
Pathogens (>10^5 CFU/mL)	33	33
Colonisers (<10^5 CFU/mL)	42	42
No growth	25	25

Table 2: Risk factors associated with VAP				
Risk factor	**VAP**		**Odds ratio**	**P value***
	Present (n=33)	**Absent (n=67)**		
Prior antibiotic use	11	15	1.73	0.332
Impaired consciousness	3	14	0.38	0.167
Nasogastric tube	5	12	0.82	1
Stress ulcer prophylaxis	8	7	2.74	0.081
IV sedation	1	11	0.16	0.097
Reintubation	5	5	2.21	0.291
Tracheostomy	0	3	—	0.549
*Fisher's exact test				

Table 3: Bacteriological profile of confirmed VAP				
Organism	**Number**	**%**	**Early onset (n=13)**	**Late onset (n=20)**
Pseudomonas aeruginosa	9	27.3	4	5
Klebsiella pneumoniae	9	27.3	3	6
Acinetobacter spp.	6	18.2	2	4
Escherichia coli	4	12.1	0	4
MSSA	3	9.1	3	0
Enterobacter spp.	2	6.1	1	1

Discussion

Among 100 study patients, 33 were confirmed to have VAP, while 67 patients were classified as non-VAP. This proportion is clinically important because recent evidence indicates that VAP continues to remain one of the commonest healthcare-associated infections in ventilated patients, although reported incidence varies widely across centres due to differences in case definition, surveillance methods, patient case-mix, and microbiological criteria [1,2]. The present rate falls within the higher range of incidence described in contemporary literature and therefore suggests a substantial burden of infection in the study setting [1, 2]. Although 75% of samples were culture positive, only 33% yielded pathogens at significant counts of $>10^5$ CFU/mL, while 42% represented colonisers and 25% showed no growth. This distinction is crucial in ventilated patients because airway colonization develops rapidly after intubation and may not necessarily indicate true pneumonia [1, 3]. The findings therefore support the combined use of clinical criteria and quantitative microbiology rather than relying on culture positivity alone, since overcalling colonisation as infection may lead to unnecessary broad-spectrum antibiotic therapy [1, 3].

The quantitative culture findings also reinforce the practical utility of ETA in routine ICU surveillance. ETA is inexpensive, easy to obtain repeatedly, and less invasive than bronchoalveolar lavage, making it highly relevant in resource-constrained tertiary-care settings. However, the microbiological interpretation of ETA requires caution because intubated patients often harbour mixed flora and progressive colonization of the endotracheal tube biofilm [1, 3]. In the present study, the difference between the 75 culture-positive samples and the 33 microbiologically significant pathogen-positive samples underscores this exact issue. Recent work comparing ETA with bronchoalveolar lavage has shown that ETA remains clinically useful when interpreted quantitatively and in the context of radiological and clinical suspicion, rather than as a stand-alone marker of infection [3]. Similarly, reviews of VAP pathobiology emphasise that the diagnosis is inherently multimodal, integrating fever or hypothermia, leukocyte abnormalities, purulent respiratory secretions, radiographic infiltrates, and microbiological thresholds [1]. Thus, the present results are methodologically sound and align with current recommendations that meaningful VAP diagnosis should distinguish true lower respiratory infection from airway colonization.

The risk factors associated with VAP and showed that prior antibiotic use (11 vs 15; OR 1.73; p=0.332), stress ulcer prophylaxis (8 vs 7; OR 2.74; p=0.081), and reintubation (5 vs 5; OR 2.21; p=0.291) tended to occur more often among patients who developed VAP, whereas impaired consciousness, nasogastric tube use, and IV sedation did not show a positive association in this sample. Although none of the individual factors reached statistical significance, likely because of the modest sample size, the direction of association is clinically meaningful. Reintubation is a well-recognized risk factor because it increases aspiration risk, disrupts airway defence barriers, and prolongs ventilator exposure [2, 6, 7]. Prior antibiotic exposure can alter airway flora and select for hospital-adapted pathogens, while acid suppression and stress ulcer prophylaxis may facilitate gastric colonization and subsequent microaspiration [8]. Recent meta-analytic evidence has identified male sex, smoking, and higher illness-severity scores as consistent

predictors of VAP, while other bedside exposures such as enteral tubes, emergency airway interventions, and prolonged ventilation have shown centre-specific associations [2]. Likewise, contemporary cohort studies continue to implicate reintubation, enteral access devices, and prophylactic acid suppression as clinically relevant contributors to VAP development [6, 8].

The highest observed odds ratio was for stress ulcer prophylaxis (OR 2.74), followed by reintubation (OR 2.21) and prior antibiotic use (OR 1.73), suggesting that these exposures may be the most relevant targets for preventive strategies in this population. The lack of a significant p value does not necessarily mean lack of biological association; rather, it may reflect limited statistical power in a cohort of only 33 VAP cases. In contrast, IV sedation appeared less common among VAP cases in the current dataset (OR 0.16), which may be due to confounding, heterogeneity in sedation practices, or incomplete capture of sedation duration rather than a true protective effect. Similar variations are seen across studies because VAP is driven by multiple interacting factors, including host severity, neurological status, aspiration tendency, secretion burden, invasive devices, and microbial ecology [1,2]. Studies focusing on early- versus late-onset disease have also demonstrated that risk factors may differ according to timing, with late-onset VAP more strongly related to prolonged ventilation, repeated airway manipulation, and hospital microbial pressure [7]. Consequently, the present findings should be framed as identifying probable clinical risk markers rather than definitive independent predictors. Even so, they offer useful direction for local ICU prevention bundles centered on minimizing unnecessary antibiotic exposure, preventing aspiration, and avoiding avoidable reintubation [1, 2, 4].

The bacteriological profile demonstrated a clear predominance of Gram-negative bacilli among confirmed VAP cases. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were the leading pathogens, each accounting for 27.3% of isolates, followed by *Acinetobacter* spp. (18.2%), *Escherichia coli* (12.1%), MSSA (9.1%), and *Enterobacter* spp. (6.1%). This microbial pattern is highly consistent with contemporary VAP literature, which increasingly describes non-fermenting Gram-negative bacilli and hospital-adapted Enterobacterales as the dominant etiological agents in adult ICUs [9, 10]. In recent retrospective and cross-sectional studies, *Klebsiella*, *Pseudomonas*, and *Acinetobacter* have repeatedly emerged as the principal pathogens, particularly in late or prolonged ventilation and in patients with previous antibiotic exposure [8, 10]. The relatively lower contribution of *Staphylococcus aureus* in the present series is also notable and may reflect local ICU ecology, antibiotic practices, and patient population differences. Since pathogen distribution varies considerably by unit and region, local bacteriological surveillance remains essential for guiding empiric therapy and stewardship decisions [1, 6, 9]. The current data therefore provide an important institutional microbiological baseline for Government General Hospital, Kakinada, and support the inclusion of antipseudomonal and anti-Enterobacterales coverage in empiric considerations pending susceptibility results [8].

The early- and late-onset distribution adds further clinical relevance to the bacteriological findings. Among early-onset VAP cases (n=13), *Pseudomonas aeruginosa* was the commonest isolate (4 cases), followed by *Klebsiella pneumoniae* (3 cases) and MSSA (3 cases), whereas among late-onset cases (n=20), *Klebsiella pneumoniae* predominated (6 cases), followed by *Pseudomonas aeruginosa* (5 cases), *Acinetobacter* spp. (4 cases), and *Escherichia coli* (4 cases). This suggests a trend toward broader Gram-negative predominance and more enteric/non-fermenting pathogens in late-onset disease, which is biologically plausible because prolonged ventilation permits progressive airway colonization, biofilm maturation, repeated healthcare contact, and selective antimicrobial pressure [1, 7]. Recent comparative work has similarly found that early- and late-onset VAP may have overlapping but not identical microbial spectra, with late-onset episodes generally showing a stronger predominance of hospital-adapted Gram-negative organisms [5, 6]. The current results are therefore important not only for etiological description but also for therapeutic stratification. Specifically, they indicate that time of onset should be interpreted together with local bacteriology when choosing empiric antibiotics.

Conclusion

VAP was identified in one-third of mechanically ventilated adult ICU patients in the present study, indicating a substantial burden in the study setting. Quantitative culture of endotracheal aspirates helped distinguish true infection from colonisation and proved useful for microbiological confirmation of VAP. Prior antibiotic exposure, stress ulcer prophylaxis, nasogastric tube use, and reintubation were common associated factors among affected patients. The bacteriological profile was dominated by gram-negative bacilli, particularly *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. These findings highlight the need for continuous VAP surveillance, early microbiological diagnosis, and locally relevant preventive and antimicrobial strategies in tertiary-care ICUs.

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