



Original Article

## Biofilm Formation and Antimicrobial Resistance in Acinetobacter Species Causing Ventilator-Associated Pneumonia: A Prospective Observational Study

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### ABSTRACT

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**Background:** Ventilator-associated pneumonia (VAP) remains a significant cause of morbidity and mortality in critically ill patients, frequently associated with multidrug-resistant organisms such as *Acinetobacter* species. Biofilm formation further enhances persistence and resistance to antimicrobial therapy.

**Objective:** To characterize *Acinetobacter* species isolated from VAP, evaluate their antimicrobial resistance profile, and determine the association between biofilm formation and resistance.

**Methods:** A prospective observational study was conducted over one year among 108 mechanically ventilated patients with clinical suspicion of VAP. Endotracheal aspirates were processed using standard microbiological techniques. Identification and antimicrobial susceptibility testing were performed using the VITEK 2 Compact system, and results were interpreted according to CLSI 2023 guidelines. Biofilm formation was assessed using Tube Method and Congo Red Agar. Statistical analysis was performed using chi-square test.

**Results:** *Acinetobacter* spp. were isolated in 11.1% of cases (12/108), with *Acinetobacter baumannii* accounting for 75% of isolates. A high level of antimicrobial resistance was observed, with meropenem resistance of 100%, imipenem resistance of 83.3%, and amikacin resistance of 91.7%. Biofilm formation was detected in 83.3% of isolates by Tube Method and 75% by Congo Red Agar. Biofilm-producing isolates demonstrated significantly higher resistance compared to non-producers ( $p < 0.05$ ). Tigecycline exhibited comparatively lower resistance rates.

**Conclusion:** Multidrug-resistant, biofilm-forming *Acinetobacter* spp. are prominent pathogens in VAP. The significant association between biofilm formation and antimicrobial resistance underscores the need for strict infection control measures and rational antibiotic use.

**Keywords:** Ventilator-associated pneumonia; *Acinetobacter baumannii*; Biofilm; Antimicrobial resistance; ICU.

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### INTRODUCTION

Ventilator-associated pneumonia (VAP) is one of the most common healthcare-associated infections in intensive care units and contributes significantly to patient morbidity, mortality, and healthcare expenditure (Kollef, 1993; Chastre & Fagon, 2002; Hunter, 2012). The incidence of VAP is particularly high among patients requiring prolonged mechanical ventilation and invasive support.

Among the etiological agents, *Acinetobacter* species—especially *Acinetobacter baumannii*—have emerged as clinically important pathogens due to their remarkable ability to survive in hospital environments and develop resistance to multiple antimicrobial agents (Peleg et al., 2008; Fournier & Richet, 2006). The growing burden of antimicrobial resistance, particularly in low- and middle-income countries, particularly high in developing countries including India (Taneja & Sharma, 2008) further complicates treatment outcomes (Gandra et al., 2017; Indian Council of Medical Research, 2022). Biofilm formation is a key virulence factor that facilitates bacterial persistence and resistance. Biofilms formed on endotracheal tubes serve as reservoirs for infection and significantly reduce antibiotic penetration, leading to treatment

failure (Donlan, 2002; Costerton et al., 1999; Hall-Stoodley et al., 2004). Furthermore, biofilm-associated bacteria exhibit enhanced resistance mechanisms compared to planktonic forms (Mah & O'Toole, 2001).

Despite increasing recognition of these factors, there remains a paucity of data correlating biofilm formation with antimicrobial resistance in VAP. Therefore, the present study was undertaken to address this gap.

## MATERIALS AND METHODS

This prospective observational study was conducted in the intensive care units of a tertiary care teaching hospital over a period of one year.

A total of 108 patients receiving mechanical ventilation for more than 48 hours and clinically suspected of VAP were included in the study. Patients with pre-existing pneumonia or those who developed pneumonia within 48 hours of intubation were excluded.

Endotracheal aspirates were collected under strict aseptic precautions and processed using standard microbiological techniques. Identification and antimicrobial susceptibility testing were performed using the VITEK 2 Compact system. Antimicrobial susceptibility results were interpreted based on minimum inhibitory concentration (MIC) values in accordance with CLSI 2023 guidelines (Clinical and Laboratory Standards Institute, 2023).

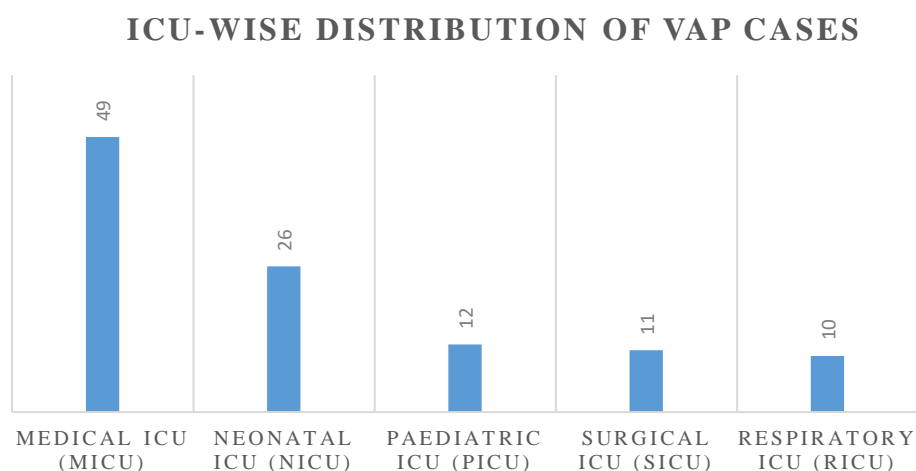
Biofilm formation was assessed using the Tube Method and Congo Red Agar technique (Donlan, 2002; Hall-Stoodley et al., 2004).

Statistical analysis was performed using chi-square test to determine the association between biofilm formation and antimicrobial resistance. A p-value <0.05 was considered statistically significant.

## RESULTS (STRENGTHENED)

**A total of 108 patients were included in the study, with a predominance of male patients.**

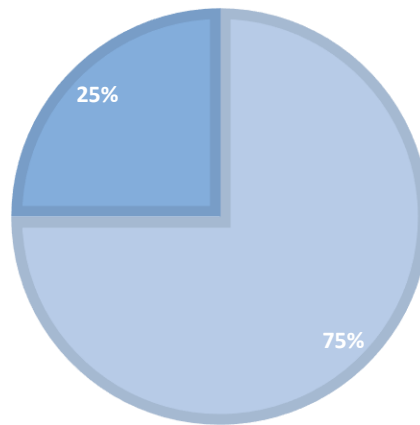
*Acinetobacter* spp. were isolated in 12 cases, accounting for 11.1% of VAP cases. Among these isolates, *Acinetobacter baumannii* was the predominant species, constituting 75% of isolates, while non-baumannii species accounted for the remaining proportion.



**Figure 1 ICU-wise distribution of ventilator-associated pneumonia cases**

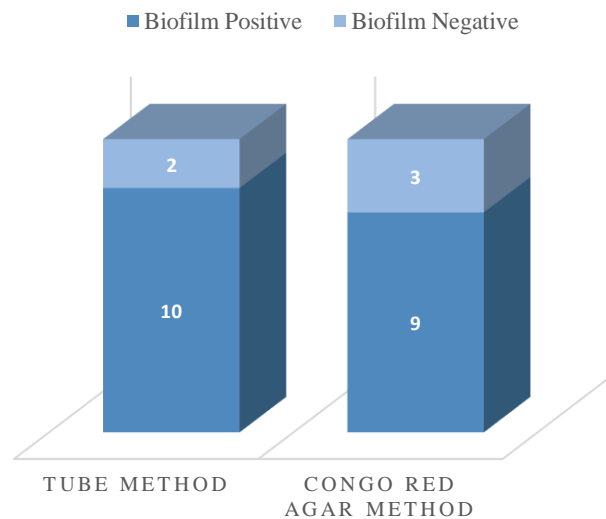
The highest proportion of VAP cases was observed in the Medical ICU, followed by other intensive care units, indicating a higher burden of infection among critically ill adult patients.

■ Acinetobacter baumannii ■ Acinetobacter junii



**Figure 2 Distribution of Acinetobacter species isolated from VAP**

The predominance of *A. baumannii* among isolates highlights its significant role as a nosocomial pathogen in ventilated patients.



**Figure 3 Detection of biofilm formation by Tube Method and Congo Red Agar**

Biofilm formation was detected in 83.3% of isolates by Tube Method and 75% by Congo Red Agar, demonstrating a high prevalence of biofilm-producing strains.

**Table 1 Antimicrobial resistance pattern of Acinetobacter isolates**

Antibiotic	Total Tested	Resistant	Resistance (%)
Meropenem	10	10	100.0
Imipenem	12	10	83.3
Amikacin	12	11	91.7
Tigecycline	7	2	28.6
Colistin	2	1	50.0

A high level of antimicrobial resistance was observed across multiple antibiotic classes. Resistance to meropenem was 100%, while imipenem resistance was 83.3%, indicating widespread carbapenem resistance. Aminoglycoside resistance was also high, with 91.7% resistance to amikacin. In contrast, tigecycline demonstrated comparatively lower resistance rates, suggesting limited therapeutic options.

**Table 2 Comparison of antimicrobial resistance between biofilm-producing and non-biofilm-producing isolates**

Antibiotic	Biofilm Producers (n=10)	Non-Producers (n=2)
Meropenem	90% (9/10)	50% (1/2)
Amikacin	80% (8/10)	50% (1/2)

Biofilm-producing isolates demonstrated significantly higher resistance to multiple antibiotics compared to non-biofilm producers. This association was found to be statistically significant ( $p < 0.05$ ), indicating a strong relationship between biofilm formation and antimicrobial resistance.

## DISCUSSION

The present study underscores the significant role of *Acinetobacter* spp., particularly *A. baumannii*, in ventilator-associated pneumonia. The predominance of *A. baumannii* observed in this study is consistent with previous reports highlighting its importance as a nosocomial pathogen (Peleg et al., 2008; Fournier & Richet, 2006).

The high level of carbapenem resistance observed is indicative of the growing challenge of multidrug resistance in critical care settings. Similar trends have been reported globally, attributed to mechanisms such as carbapenemase production and efflux pumps (Nordmann et al., 2011; Poirel & Nordmann, 2006). Similar trends have been reported in ICU-based surveillance studies (Rosenthal et al., 2020).

Biofilm formation was found to be highly prevalent among isolates and showed a significant association with antimicrobial resistance. Biofilms act as a protective barrier, reducing antibiotic penetration and enhancing bacterial survival (Hall-Stoodley et al., 2004; Mah & O'Toole, 2001).

These findings are in agreement with global surveillance reports emphasizing the rising burden of antimicrobial resistance and the need for effective infection control measures (World Health Organization, 2022; Centers for Disease Control and Prevention, 2019). Clinical guidelines also highlight the importance of targeted therapy and antimicrobial stewardship in managing VAP (Kalil et al., 2016).

## LIMITATIONS

- Limited number of *Acinetobacter* isolates
- Single-centre study
- Restricted antimicrobial panel

## CONCLUSION

The study demonstrates that multidrug-resistant, biofilm-forming *Acinetobacter* spp. are major contributors to ventilator-associated pneumonia. The significant association between biofilm formation and antimicrobial resistance highlights the need for stringent infection control practices and rational antibiotic usage. These findings contribute valuable insights into the management of VAP in critical care settings.

## Funding:

The authors received no financial support for this research.

## Conflict of Interest:

The authors declare that there is no conflict of interest

## Ethical Approval:

This study was approved by the Institutional Ethics Committee of Ballari Medical College & Research Centre, Ballari, Karnataka, India (Reg. No. EC/NEW/INST/2022/3017, NECRBHR, Department of Health Research (DHR)). Informed consent was obtained as per institutional guidelines.

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