Original article

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A Three-Year Analysis of The Microbiological Characteristics of Pathogens Associated with Ventilator-Associated Pneumonia at the Clinic Of Anesthesia, Resuscitation and Intensive Therapy, University Clinical Center Niš

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Ventilator-associated pneumonia (VAP) remains one of the most common and severe infections in intensive care units. The aim of this stady was to analyze the microbiological profile and temporal distribution of isolates from endotracheal aspirates (ETA) between 2022 and 2024.
This retrospective study included all positive ETA samples processed at the University Clinical Center Niš. Annual distribution and frequency of main clinical pathogens were evaluated. A total of 281 microorganisms were isolated. *Acinetobacter spp.* was the most frequent pathogen (n=119) but showed a decreasing trend (51 → 40 → 28). *Klebsiella spp.* demonstrated a clear increase in frequency, particularly in 2024 (35 isolates). Considerable yearly variation in pathogen distribution was observed. A marked reduction in *Acinetobacter spp.* isolation and simultaneous rise in *Klebsiella spp.* highlight changing epidemiological patterns with important implications for empirical therapy in ICU patients.

Keywords: *ETA, VAP, Acinetobacter, Klebsiella, ICU infections.*

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Trogodišnja analiza mikrobioloških karakteristika uzročnika ventilator–asocirane pneumonije na Klinici za anesteziju i intenzivnu terapiju Univerzitetskog kliničkog centra Niš

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Ventilator-asocirana pneumonija (VAP) predstavlja jednu od najčešćih i najtežih infekcija u jedinicama intenzivne nege. Cilj je analizirati mikrobiološki profil, distribuciju i dinamiku izolata iz endotrahealnih aspirata (ETA) tokom perioda 2022–2024. Ova retrospektivna studija obuhvatila je sve pozitivne ETA uzorke obrađene u laboratoriji UKC Niš. Analizirani su broj i udeo izolata po godinama, kao i pripadnost glavnim kliničkim patogenima. Ukupno je izolovano 281 mikroorganizama. Najzastupljeniji patogen bio je *Acinetobacter spp.* (n=119), sa opadajućim trendom tokom godina (51 → 40 → 28). *Klebsiella spp.* je pokazala porast broja izolata, naročito tokom 2024. godine (35 izolata). Rezultati pokazuju značajne razlike u distribuciji patogena kroz posmatrani period.Uočen je izražen pad izolata *Acinetobacter spp.*, uz istovremeni porast *Klebsiella spp.* kao vodećeg uzročnika teških respiratornih infekcija, što ima važne implikacije za empirijsku terapiju u intenzivnoj nezi.

Ključne reči: *ETA, ventilator-asocirana pneumonija, Acinetobacter, Klebsiella, distribucija patogena.*

INTRODUCTION

Ventilator-associated pneumonia (VAP) is one of the most common and severe nosocomial infections among patients receiving invasive mechanical ventilation. According to European and American studies, VAP occurs in 10–30% of mechanically ventilated patients and significantly contributes to increased morbidity, mortality, and length of stay in intensive care units (ICUs) (1–3). The diagnosis of VAP is based on a combination of clinical findings, radiological features, and microbiological confirmation of lower respiratory tract infection.

The microbiological profile of VAP is predominantly characterized by Gram-negative bacteria, most commonly *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Among Gram-positive pathogens, *Staphylococcus aureus*, particularly methicillin-resistant strains (MRSA), represents the most important etiological agent (4–6). Local epidemiology and antimicrobial resistance patterns vary considerably between institutions and regions, highlighting the importance of continuous microbiological surveillance and adjustment of empirical therapy.

Endotracheal aspirate (ETA) sampling is a practical, minimally invasive, and widely used method for obtaining lower respiratory tract specimens in patients with suspected VAP. Although bronchoalveolar lavage (BAL) is methodologically superior, ETA is most commonly used in routine clinical practice and epidemiological studies due to its simplicity and good diagnostic correlation (7,8).

Monitoring etiological agents and their resistance patterns over extended periods is crucial for optimizing therapy and reducing the burden of VAP. Therefore, local studies analyzing pathogen distribution and resistance trends in intensive care settings are essential.

AIM

The aim of this study was to analyze the microbiological characteristics of pathogens causing ventilator-associated pneumonia by reviewing the distribution of the most common bacterial isolates obtained from endotracheal aspirates at the Clinic of Anesthesiology and Intensive Care, University Clinical Center (UCC) Niš, during the period from 2022 to 2024.

MATERIALS AND METHODS

This retrospective study included patients treated at the Clinic of Anesthesiology and Intensive Care, UCC Niš, in whom ventilator-associated pneumonia was clinically suspected between January 2022 and December 2024. All endotracheal aspirate (ETA) samples obtained from patients receiving invasive mechanical ventilation and submitted for microbiological analysis due to suspected VAP were included, in accordance with established diagnostic criteria (clinical, laboratory, and radiological indicators of lower respiratory tract infection).

ETA samples were processed at the Center for Microbiology, Institute of Public Health Niš, following standard microbiological protocols. Cultures were grown on selective and non-selective media and incubated for 24–48 hours at appropriate temperatures. For each culture, the isolated microorganism was recorded. Only samples with significant growth of pathogenic microorganisms according to laboratory criteria for ETA were analyzed.

Data were collected from electronic laboratory records and analyzed using descriptive statistical methods, including frequencies and percentages. Results are presented in tables and figures.

RESULTS

During the period from 2022 to 2024, a total of 287 endotracheal aspirate samples from mechanically ventilated patients treated at the Clinic of Anesthesiology and Intensive Care, UCC Niš, were analyzed. Annual distribution showed that 103 samples were processed in 2022, 90 in 2023, and 94 in 2024.

Year 2022

In 2022, Gram-negative bacteria predominated. *Acinetobacter* spp. was the most frequently isolated pathogen, accounting for 51 isolates (49.5%). Other isolates included *Pseudomonas aeruginosa* – 12 (11.7%), *Klebsiella* spp. – 9 (8.7%), *Proteus mirabilis* – 9 (8.7%), *Serratia* spp. – 10 (9.7%), *Klebsiella pneumoniae* – 2 (1.9%), *Enterococcus faecalis* – 2 (1.9%), *Enterobacter* spp. – 1 (1.0%), *Staphylococcus aureus* – 1 (1.0%), while other microorganisms accounted for 6 isolates (5.8%).

Year 2023

In 2023, a slightly lower number of ETA samples was recorded, with a similar distribution of isolates. The most frequent pathogens were *Acinetobacter* spp. – 40 (44.4%), *Pseudomonas aeruginosa* – 13 (14.4%), *Klebsiella* spp. – 10 (11.1%), *Proteus mirabilis* – 4 (4.4%), *Escherichia coli* – 4 (4.4%), *Klebsiella pneumoniae* – 4 (4.4%), *Serratia* spp. – 3 (3.3%), *Staphylococcus aureus* – 2 (2.2%), *Enterococcus* spp. – 1 (1.1%), *Corynebacterium* spp. – 1 (1.1%), and other microorganisms – 8 (8.8%).

Year 2024

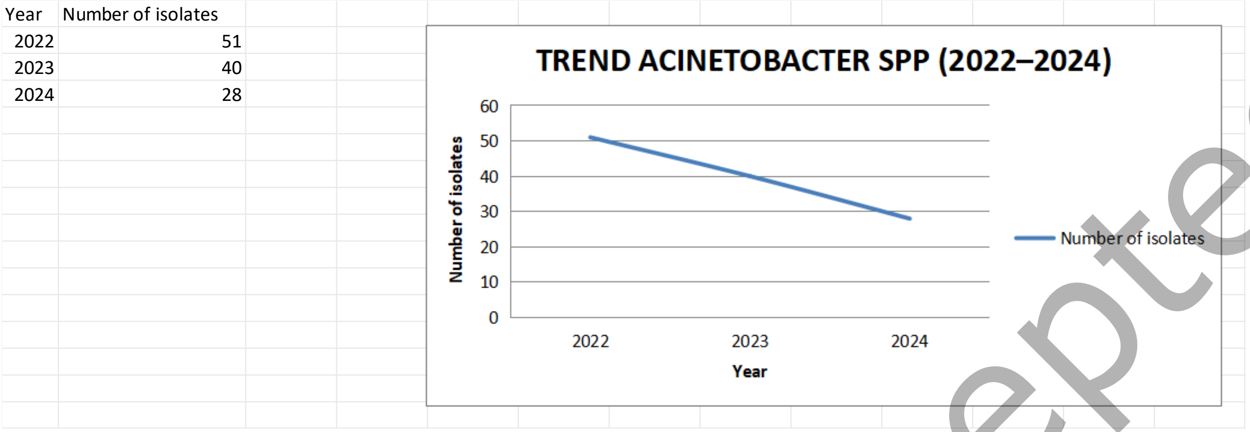
In 2024, a total of 94 ETA samples were analyzed. The most frequently isolated bacteria were *Acinetobacter* spp. – 28 (29.8%), *Klebsiella* spp. – 25 (26.6%), *Klebsiella pneumoniae* – 10 (10.6%), *Pseudomonas aeruginosa* – 11 (11.7%), *Proteus mirabilis* – 9 (9.6%), *Escherichia coli* – 2 (2.1%), *Enterococcus faecium* – 1 (1.1%), and other microorganisms – 8 (8.5%).

Overall Trends (2022–2024)

Across the entire three-year period, *Acinetobacter* spp. was the most prevalent VAP pathogen (Figure 1), accounting for 119 isolates (41.5% of all ETA samples). This was followed by *Klebsiella* spp. and *Klebsiella pneumoniae* with approximately 50 isolates in total, and *Pseudomonas aeruginosa* with 36 isolates. Other bacteria were isolated considerably less frequently. The dominance of *Acinetobacter* spp., together with the consistently high prevalence of *Pseudomonas aeruginosa* and Enterobacterales, highlights the ongoing importance of these pathogens in the etiology of VAP at UCC Niš over the observed period (Table 1).

Table 1. Distribution of pathogens by year

Pathogen	2022	2023	2024
Acinetobacter spp	51	40	28
Klebsiella spp+pneumoniae	11	14	35
Pseudomonas aeruginosa	12	13	11
Proteus mirabilis	9	4	9
Serratia spp	10	3	0
E.coli	0	4	2
Staph aureus	1	2	0
Enterococcus spp	2	1	1
Other	6	8	8



Year	Number of isolates
2022	51
2023	40
2024	28

Figure 1. Trend Acinetobacter spp.

DISCUSSION

This three-year retrospective study analyzed the microbiological characteristics of pathogens causing ventilator-associated pneumonia in ICU patients. The results demonstrate a clear predominance of Gram-negative bacteria throughout the study period, with *Acinetobacter* spp. being the most frequently isolated pathogen in all three years. Its prevalence ranged from 40% in 2023 to 49.5% in 2022, which is consistent with reports from other tertiary care centers in the region, where *Acinetobacter* represents a leading cause of VAP and a major challenge due to high levels of antimicrobial resistance (9–12).

The second most common isolates were *Klebsiella* spp. and *Pseudomonas aeruginosa*, which together accounted for a substantial proportion of the etiological spectrum. Notably, the proportion of *Klebsiella* spp. increased during the study period, from 9.7% in 2022 to 26.6% in 2024. This increase may reflect changes in colonization patterns, modifications of therapeutic protocols, or the spread of resistant clones, emphasizing the need for enhanced epidemiological surveillance. Similar increasing trends of *Klebsiella* spp. in VAP have been reported in several European studies (13,14).

Pseudomonas aeruginosa maintained a relatively stable prevalence (11–14% annually), which is expected given its propensity to colonize invasive medical devices and its ability to form biofilms. Comparable rates have been reported in other countries (15,16).

Enterobacterales such as *Proteus mirabilis*, *Escherichia coli*, *Enterobacter* spp., and *Serratia* spp. were present in smaller but consistent proportions. Their frequency varied throughout the observation period without a clear increasing or decreasing trend, likely reflecting the heterogeneous etiology of VAP and the influence of individual patient factors and duration of mechanical ventilation.

Regarding Gram-positive pathogens, *Staphylococcus aureus* was rarely isolated (1–2%), which is lower than in many international reports where *S. aureus*, particularly MRSA, accounts for 10–20% of VAP cases (17–19). This finding may be explained by local epidemiological characteristics and lower MRSA circulation within this ICU. The occasional isolation of *Enterococcus* spp. most likely represents respiratory tract colonization, as these organisms are less commonly true causes of pneumonia.

Overall, the VAP pathogen profile observed in this study corresponds to patterns typical of high-burden ICUs, where multidrug-resistant Gram-negative bacteria predominate. The consistently high prevalence of *Acinetobacter* spp. represents a significant therapeutic and epidemiological challenge. These findings underscore the importance of continuous microbiological surveillance, adjustment of empirical therapy based on local resistance data, and strict implementation of preventive measures to reduce the incidence of VAP.

CONCLUSION

During the three-year period analyzed in this study, Gram-negative bacteria predominated as causative agents of ventilator-associated pneumonia, with *Acinetobacter* spp. being the most frequently isolated pathogen in all three years. *Pseudomonas aeruginosa* and *Klebsiella* spp. also showed significant and stable prevalence, whereas Gram-positive pathogens were rarely isolated. An increased proportion of *Klebsiella* spp. and *Klebsiella pneumoniae* was observed in 2024, suggesting possible changes in local epidemiology and colonization patterns within the ICU.

These results confirm that the etiological spectrum of VAP remains largely dominated by highly resistant Gram-negative pathogens, which has important clinical and therapeutic implications. Reliance on local microbiological data is essential for rational selection of empirical therapy and improvement of antimicrobial stewardship. Continuous monitoring of microbiological trends, together with the implementation of preventive measures, remains fundamental for reducing VAP incidence and improving outcomes in intensive care patients.

REFERENCE

1.Kalil AC, Metersky ML, Klompas M, et al. Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 clinical practice guidelines by the Infectious Diseases Society of America and the American Thoracic Society. **Clin Infect Dis.** 2016;63(5):e61–e111.

2.Koulenti D, Tsigou E, Rello J. Nosocomial pneumonia in 27 ICUs in Europe: perspectives from the EU-VAP/CAP study. **Eur J Clin Microbiol Infect Dis.** 2017;36(11):1999–2006.

3.Magill SS, O'Leary E, Janelle SJ, et al. Changes in prevalence of health care–associated infections in U.S. hospitals. **N Engl J Med.** 2018;379:1732–44.

4.Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. **N Engl J Med.** 2010;362(19):1804–13.

5.Torres A, Niederman MS, Chastre J, et al. International ERS/ESICM/ESCMID/ALAT guidelines for management of hospital-acquired pneumonia and ventilator-associated pneumonia. **Eur Respir J.** 2017;50(3):1700582.

6.Kadri SS, Adjemian J, Lai YL, et al. Difficult-to-treat resistance in gram-negative bacteremia at 173 US hospitals. **Clin Infect Dis.** 2018;67(12):1803–14.

7.Luyt CE, Hékimian G, Koulenti D, Chastre J. Microbiological diagnosis of ventilator-associated pneumonia: invasive versus non-invasive sampling techniques. **Crit Care.** 2014;18(3):148.

8.Fagon JY, Chastre J, Wolff M, et al. Invasive and noninvasive strategies for management of suspected ventilator-associated pneumonia. **Ann Intern Med.** 2000;132(8):621–30.

9.Kalil AC, et al. Management of adults with hospital-acquired and ventilator-associated pneumonia. **Clin Infect Dis.** 2016;63:e61-111.

10.Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. **N Engl J Med.** 2010;362:1804-13.

11.Garnacho-Montero J, et al. Acinetobacter baumannii ventilator-associated pneumonia: epidemiology and outcome. **Clin Microbiol Infect.** 2015;21:527-33.

12.Munoz-Price LS, Weinstein RA. Acinetobacter infection. **N Engl J Med.** 2008;358:1271-81.

13.Logre E, et al. Klebsiella pneumoniae in ICU-acquired infections. **Intensive Care Med.** 2014;40:1392-403.

14.Martin A, et al. Trends in gram-negative resistance in ventilator-associated pneumonia. **J Hosp Infect.** 2020;105:663-71.

15.Teerawattanapong N, et al. Pseudomonas aeruginosa infections in ventilated patients. **Infect Drug Resist.** 2018;11:1037-46.

16.Ferrer M, et al. Microbiology of VAP: a European perspective. **Intensive Care Med.** 2010;36:752-61.

17.Torres A, et al. Staphylococcus aureus ventilator-associated pneumonia. **Clin Infect Dis.** 2010;51:S262-70.

18.Koulenti D, et al. Global epidemiology of VAP. **Eur Respir Rev.** 2017;26:160064.

19.Vincent JL, et al. International study of prevalence of infection in ICU patients. **JAMA**. 2009;302:2323-9.