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Original article

COMPREHENSIVE ANALYSIS OF MICROBIOLOGICAL SURVEILLANCE AT THE CLINIC FOR ANESTHESIA AND INTENSIVE THERAPY IN NIS: A THREE YEAR FOLLOW UP

(Three-Year Microbiological Report from the Clinic for Anesthesia in Nis)

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Summary

Introduction*: Intensive care units (ICUs) represent essential components of modern hospital systems dedicated to the management of critically ill patients. Frequent use of broad-spectrum antibiotics in ICUs and prolonged hospitalization contribute to the emergence and spread of multidrug-resistant microorganisms.* ***Aim****: Presenting the results of microbiological sampling at the Clinic for Anesthesia and Intensive therapy, UCC Nis, from 2022-2024, and to emphasize the importance of microbiological analyses in the timely identification of pathogens and the rational use of antibiotics.* ***Methods****: A prospective cross-section study was conducted at the Clinic for Anesthesiology and Intensive Therapy, UCC Nis, from 2022 to 2024. Samples were taken from blood, urine, aspirates from the endotracheal tube and oral cavity, drainage contents, intraoperatively obtained abdominal cavity contents, pleural punctures, and swabs (wound, anal region, groin, oral cavity, and axilla). After sampling, the samples were sent for microbiological analysis, and all positive results were recorded.* ***Results****: In 2022, the highest number of positive results were recorded in blood cultures, wound swabs, and endotracheal aspirates. The most commonly isolated bacteria were Acinetobacter sp. (21.93%), Klebsiella sp. (18.36%) and Pseudomonas aeruginosa (7.63%). In 2023, the majority of bacteria were isolated from blood, groin swabs, and wound swabs. Dominant bacteria included Klebsiella sp. (25%), Acinetobacter sp. (17.14%), and Enterococcus faecium (10.69%). In 2024, the most dominant samples came from blood, groin swabs, and oral cavity swabs, with the most frequently isolated bacteria being Klebsiella sp. (20.15%), Enterococcus faecium (19.26%), and Acinetobacter sp. (14.41%).* ***Conclusion****: Microbiological analyses and surveillance of antimicrobial resistance patterns are the most important issues for optimizing therapeutic outcome and prevention of the spread of resistant pathogens. Our results revealed the emergence of extensively drug-resistant Klebsiella spp. isolates, as well as the rising number of Enterococcus faecium isolated in various types of samples. Acinetobacter sp. remained the predominant pathogen isolated from endotracheal aspirates.*

Key words: *intensive care unit; antimicrobial resistance; healthcare-associated infections; antibiotic stewardship*

Introduction

Intensive Care Units (ICUs) are specialized hospital departments dedicated to the management of patients with critical and life-threatening conditions that necessitate continuous monitoring and advanced therapeutic interventions. These settings are often characterized by the frequent administration of broad-spectrum antibiotics, prolonged hospital stays, and the use of multiple invasive procedures, all of which contribute to an increased risk of healthcare-associated infections (HAIs). Consequently, microbiological analyses play an essential role in the daily operations of intensive care units.[1]

Microbiological diagnostics encompass the examination of diverse clinical specimens, including blood, urine, wound swabs, tracheal aspirates, and others, with the objective of accurately identifying

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causative pathogens. Timely detection and precise identification of microorganisms enable the implementation of targeted antimicrobial therapy, thereby improving treatment outcomes, preventing unnecessary antibiotic use, and reducing the emergence of antimicrobial resistance.

The importance of microbiological analyses in ICUs extends beyond individual patient level. It also encompasses epidemiological surveillance within the department, providing valuable data for monitoring infection trends, identifying potential sources of outbreaks, and guiding infection control measures. The methodology, accuracy, and efficiency of microbiological laboratory services represent a critical component of multidisciplinary teamwork responsible for the care of critically ill patients, ultimately contributing to the improvement of healthcare quality and patient safety.[2]

Integration of microbiological diagnosis into antimicrobial stewardship programs is crucial in ICUs. Timely culture results enabled targeted therapy and antibiotic de-escalation, reducing unnecessary broad-spectrum antibiotic use and selective pressure for antimicrobial resistance. Consequently, microbiological data serve as a cornerstone for individualized patient management and sustainable infection control practices in critical care settings.

Aim of the study

The aim of this study is to present the results of microbiological sampling conducted in the Intensive Care Unit of the Clinic for Anesthesiology and Intensive Therapy, University Clinical Center Nis, during the period 2022–2024. Special emphasis is placed on the identification and frequency of bacterial species isolated from various clinical specimens. The analysis of the obtained data aimed to determine the most prevalent bacterial species in this hospital environment, as well as potential patterns of their distribution in relation to sample type and patients' clinical status. In this regard, the study seeks to contribute to a better understanding of the microbiological profile of the ICU and to underscore the importance of regular microbiological monitoring for the improvement of infection prevention and control measures in healthcare settings.

Materials and methods

A prospective cross-sectional study was conducted at the Clinic for Anesthesiology and Intensive Care, University Clinical Center Nis, from January 2022 to December 2024. This study did not require approval from an ethics committee, as it was conducted exclusively using publicly available data sets, did not involve direct interaction with patients, and there was no risk of harm to the individuals included in the study.

The analysis included all patients hospitalized in the Intensive Care Unit (ICU). Collection of swab specimens for microbiological culture represents standard of care, given that it is routinely performed in our clinical practice. Collected samples comprised blood, urine, endotracheal aspirate, oral aspirate, drain content, intraoperatively collected abdominal fluid, pleural punctuate, wound swab, rectal swab, groin swab, oral cavity swab, axillary swab, tracheostomy tube swab, stool, and central venous catheter (CVC) tip. Samples were obtained under aseptic conditions. Inappropriately collected or contained samples were excluded from analysis.

Microbiological testing was performed according to current protocols in the Microbiology Laboratory of the Clinic for Pulmonary Diseases, University Clinical Center Nis, and to the Center for Microbiology of the Institute of Public Health Nis. Cultivation, bacterial isolation, and identification were carried out using conventional microbiological methods. Antibiotic susceptibility testing was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Data were collected for the years 2022, 2023, and 2024, followed by analyses for each individual year and for entire study period. Comparative analyses were conducted to assess the distribution of bacterial isolates by sample type, identification of the most prevalent bacteria species, and evaluation of antibiotic susceptibility patterns. In some cases, different bacteria were isolated from multiple materials or subsequent samples from the same patient. Data are presented as absolute and relative numbers for comparison purposes.

Results

During the three-year study period, a total of 3,771 samples tested positive: 1,035 in 2022, 1,272 in 2023, and 1,464 in 2024.

Of the 1,035 positive findings in 2022, the majority were obtained from blood cultures (N = 204), wound swabs (N = 125), endotracheal aspirates (N = 99), rectal swabs (N = 81), groin swabs (N = 76), oral cavity swabs (N = 64), and urine samples (N = 63). The distribution of positive findings by sample type is shown in **Figure 1**.

The most frequently isolated species were *Staphylococcus epidermidis* from blood (6.38%), *Acinetobacter sp.* from wound swabs (4.73%), and *Acinetobacter sp.* from endotracheal aspirates (4.93%). The prevalence of materials with isolated bacteria is presented in **Table 1**. The most frequently isolated bacteria in 2022 were: *Acinetobacter sp.* (21.93%), *Klebsiella sp.* (18.36%), *Pseudomonas aeruginosa* (7.63%), *Enterococcus faecium* (7.54%), *Staphylococcus epidermidis* (6.76%), *Escherichia coli* (6.57%), *Proteus mirabilis* (4.83%), *Enterococcus faecalis* (4.44%), *Klebsiella pneumoniae* (3.19%), and *Enterobacter sp.* (2.61%).

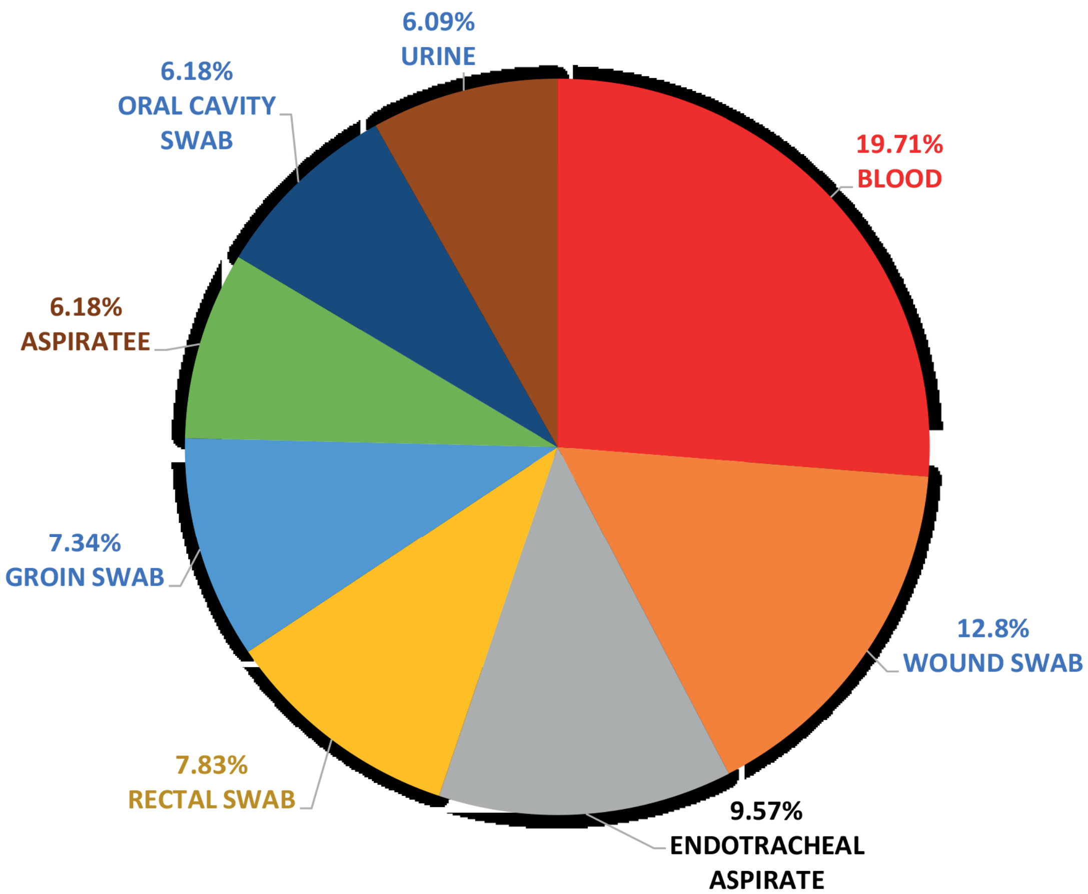

Figure 1. The distribution of positive findings by sample type for 2022.

Among the 1,272 positive samples in 2023, bacteria were most frequently isolated from blood (N = 218), groin swabs (N = 161), wound swabs (N = 136), oral cavity swabs (N = 121), urine (N = 111), rectal swabs (N = 104), endotracheal aspirates (N = 90), and axillary swabs (N = 82). The distribution of positive findings by material type is shown in **Figure 2**. The most commonly isolated species were *Staphylococcus epidermidis* from blood (5.7%), *Klebsiella sp.* from groin swabs (4.40%), and *Acinetobacter sp.* from wound swabs (2.99%). The distribution of positive findings with isolated bacteria is presented in **Table 2**. The most frequently isolated bacteria in 2023 were: *Klebsiella sp.* (25.00%), *Acinetobacter sp.* (17.14%), *Enterococcus faecium* (10.69%), *Pseudomonas aeruginosa* (6.60%), *Staphylococcus epidermidis* (6.13%), *Escherichia coli* (6.05%), *Proteus mirabilis* (5.27%), *Klebsiella pneumoniae* (3.62%), *Enterococcus faecalis* (3.54%), and *Staphylococcus aureus* (2.83%).

Table 1. The prevalence of materials with isolated bacteria for 2022.

			Acinetobacter sp.		Klebsiella sp.		Pseudomonas aeruginosa		Enterococcus faecium		Staphylococcus epidermidis	
Sample	N	%	N	%	N	%	N	%	N	%	N	%
Blood	204	19.71%	14	1.35%	8	0.77%	2	0.19%	10	0.97%	66	6.38%
Wound swab	125	12.08%	49	4.73%	17	1.64%	11	1.06%	1	0.10%	1	0.10%
Endotracheal aspirate	99	9.57%	51	4.93%	9	0.87%	12	1.16%	0	0.00%	0	0.00%
Rectal swab	81	7.83%	1	0.10%	23	2.22%	6	0.58%	16	1.55%	1	0.10%
Groin swab	76	7.34%	4	0.39%	19	1.84%	5	0.48%	22	2.13%	0	0.00%
Aspirate	64	6.18%	21	2.03%	13	1.26%	15	1.45%		0.00%	0	0.00%
Oral cavity swab	64	6.18%	9	0.87%	33	3.19%	5	0.48%	5	0.48%	0	0.00%
Urine	63	6.09%	8	0.77%	32	3.09%	7	0.68%	1	0.10%	0	0.00%
Drain content	43	4.15%	16	1.55%	2	0.19%	5	0.48%	1	0.10%	1	0.10%
Axillary swab	41	3.96%	6	0.58%	12	1.16%		0.00%	14	1.35%	0	0.00%
Abdominal fluid	34	3.29%	4	0.39%	3	0.29%	1	0.10%	1	0.10%	0	0.00%
Stool	13	1.26%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
CVC tip	12	1.16%	5	0.48%	1	0.10%	2	0.19%	1	0.10%	0	0.00%
Pleural punctate	10	0.97%	3	0.29%	0	0.00%	1	0.10%	1	0.10%	0	0.00%
Tracheostomy tube swab	10	0.97%	4	0.39%	2	0.19%	1	0.10%	0	0.00%	0	0.00%
Other	96	9.28%	32	3.09%	16	1.56%	6	0.58%	5	0.49%	1	0.09%
Total	1035	100.00%	227	21.93%	190	18.36%	79	7.63%	78	7.54%	70	6.76%

In 2024, among the 1,464 positive findings, the most frequent materials were blood (N = 232), groin swabs (N = 179), oral cavity swabs (N = 166), wound swabs (N = 132), rectal swabs (N = 122), axillary swabs (N = 108), urine (N = 106), and endotracheal aspirates (N = 94). The distribution of positive findings by sample type is shown in **Figure 3**. The most frequently isolated bacteria were *Staphylococcus epidermidis* from blood (4.58%), and *Enterococcus faecium* from groin swabs (5.60%) and oral cavity swabs (3.48%). The distribution of positive findings by sample type with isolated bacteria is presented in **Table 3**. The most frequently isolated bacteria in 2024 were: *Klebsiella sp.* (20.15%), *Enterococcus faecium* (19.26%), *Acinetobacter sp.* (14.41%), *Pseudomonas aeruginosa* (7.99%), *Escherichia coli* (7.17%), *Staphylococcus epidermidis* (4.99%), *Proteus mirabilis* (3.96%), *Enterococcus faecalis* (2.94%), *Enterobacter sp.* (2.94%), and *Staphylococcus sp.* (2.19%).

Across all three observed years, the highest number of positive samples was obtained from blood (N = 654 in total), distributed as follows: 2022 – N = 204, 2023 – N = 218, 2024 – N = 232. This was followed by groin swabs (N = 416 in total: 76 in 2022, 161 in 2023, and 179 in 2024), and wound swabs (N = 393 in total: 125 in 2022, 136 in 2023, and 132 in 2024). **Figure 4** shows the distribution of findings by sample type for all three years combined.

Among the isolated bacteria, *Klebsiella sp.* was the most prevalent (N = 803 in total: 190 in 2022, 318 in 2023, and 295 in 2024), followed by *Acinetobacter sp.* (N = 656 in total: 227 in 2022, 218 in 2023, and 211 in 2024), and *Enterococcus faecium* (N = 496 in total: 78 in 2022, 136 in 2023, and 282 in 2024). The distribution of bacterial isolates by material type for each year is shown in **Figure 5**.

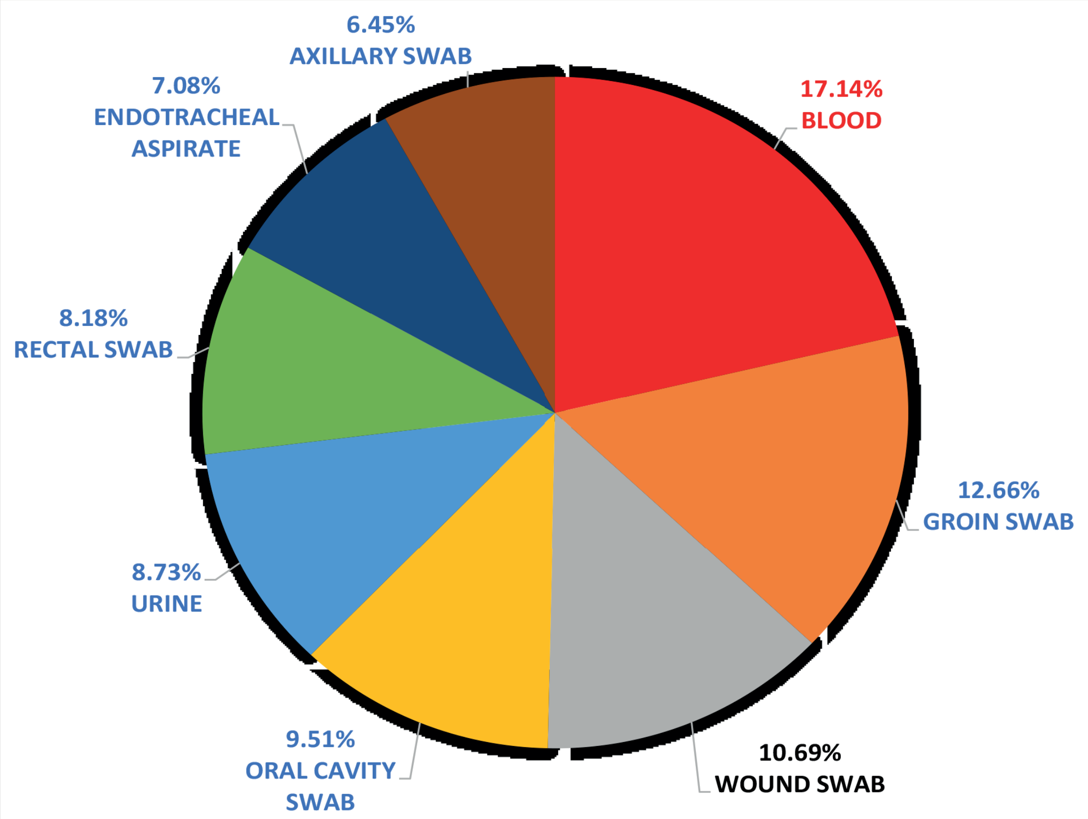

Figure 2. The distribution of positive findings by sample type for 2023.

Table 2. The prevalence of materials with isolated bacteria for 2023.

			Klebsiella sp.		*Acinetobacter sp.*		*Enterococcus faecium*		*Pseudomonas aeruginosa*		*Staphylococcus epidermidis*	
Sample	N	%	N	%	N	%	N	%	N	%	N	%
Blood	218	17.14%	7	0.55%	4	0.31%	7	0.55%	3	0.24%	73	5.74%
Groin swab	161	12.66%	56	4.40%	11	0.86%	49	3.85%	9	0.71%	0	0.00%
Wound swab	136	10.69%	22	1.73%	38	2.99%	6	0.47%	20	1.57%	0	0.00%
Oral cavity swab	121	9.51%	52	4.09%	19	1.49%	19	1.49%	3	0.24%	0	0.00%
Urine	111	8.73%	61	4.80%	10	0.79%	3	0.24%	3	0.24%	0	0.00%
Rectal swab	104	8.18%	38	2.99%	7	0.55%	18	1.42%	3	0.24%	0	0.00%
Endotracheal aspirate	90	7.08%	10	0.79%	40	3.14%	0	0.00%	13	1.02%	0	0.00%
Axillary swab	82	6.45%	29	2.28%	13	1.02%	26	2.04%	3	0.24%	0	0.00%
Aspirate	60	4.72%	12	0.94%	28	2.20%	0	0.00%	9	0.71%	0	0.00%
Drain content	37	2.91%	8	0.63%	13	1.02%	0	0.00%	1	0.08%	1	0.08%
Nose swab	24	1.89%	6	0.47%	5	0.39%	3	0.24%	1	0.08%	0	0.00%
Stool	15	1.18%	0	0.00%		0.00%	0	0.00%	0	0.00%	0	0.00%
Abdominal fluid	11	0.86%	0	0.00%	3	0.24%	1	0.08%	0	0.00%	0	0.00%
CVC tip	9	0.71%	1	0.08%	1	0.08%	0	0.00%	3	0.24%	3	0.24%
Pleural punctate	7	0.55%	0	0.00%	4	0.31%	0	0.00%	0	0.00%	0	0.00%
Other	86	6.76%	16	1.26%	22	1.73%	4	0.31%	13	1.02%	1	0.08%
Total	1272	100.00%	318	25.00%	218	17.14%	136	10.69%	84	6.60%	78	6.14%

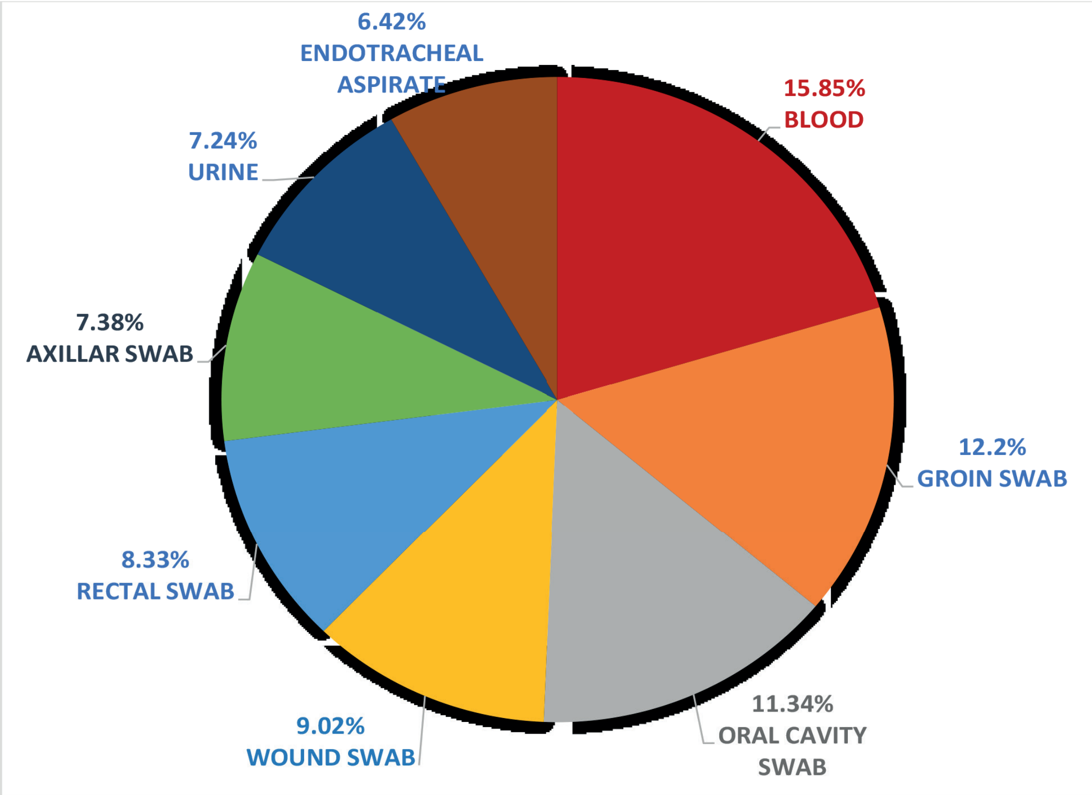

Figure 3. The distribution of positive findings by sample type for 2024.

Table 3. The prevalence of materials with isolated bacteria for 2024.

			Klebsiella sp.		Enterococcus faecium		Acinetobacter sp.		Pseudomonas aeruginosa		Escerichia coli		Staphylococcus epidermidis	
Sample	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Blood	232	15.85%	3	0.20%	13	0.89%	6	0.41%	6	0.41%	9	0.61%	67	4.58%
Groin swab	179	12.23%	30	2.05%	82	5.60%	18	1.23%	9	0.61%	18	1.23%	0	0.00%
Oral cavity swab	166	11.34%	48	3.28%	51	3.48%	22	1.50%	13	0/89%	17	1/16%	0	0.00%
Wound swab	132	9.02%	24	1.64%	7	0.48%	25	1.71%	33	2.25%	5	0.34%	0	0.00
Rectal swab	122	8.33%	16	1.09%	52	3.55%	7	0.48%	4	0.27%	27	1.84%	0	0.00
Axillary swab	108	7.38%	19	1.30%	48	3.28%	20	1.37%	4	0.27%	3	0.20%	3	0.20%
Urine	106	7.24%	43	2.94%	2	0.14%	13	0.89%	3	0.20%	13	0.89%	0	0.00
Endotracheal aspirate	94	6.42%	25	1.71%	1	0.07%	28	1.91%	11	0.75%	2	0.14%	0	0.00
Nose swab	63	4.30%	12	0.82%	14	0.96%	17	1.16%	5	0.34%	3	0.20%	3	0.20%
Aspirate	56	3.83%	12	0.82%		0.00%	22	1.50%	8	0.55%	2	0.14%	0	0.00
S Drain content	43	2.94%	18	1.23%	3	0.20%	5	0.34%	4	0.27%	1	0.07%	0	0.00
Abdominal fluid	15	1.02%	2	0.14%	3	0.20%		0.00%	2	0.14%	3	0.20%	0	0.00
CVC tip	15	1.02%	6	0.41%		0.00%	2	0.14%	1	0.07%		0.00%	0	0.00
Drain swab	11	0.75%	2	0.14%		0.00%	2	0.14%	1	0.07%	1	0.07%	0	0.00
Broncho-aspirate	10	0.68%	5	0.34%		0.00%	2	0.14%		0.00%		0.00%	0	0.00
Other	112	7.65%	30	2.05%	6	0.41%	22	1.50%	13	0.89%	1	0.07%	0	0.00
Total	1464	100.00%	295	20.15%	282	19.26%	211	14.41%	117	7.99%	105	7.17%	73	4.99%

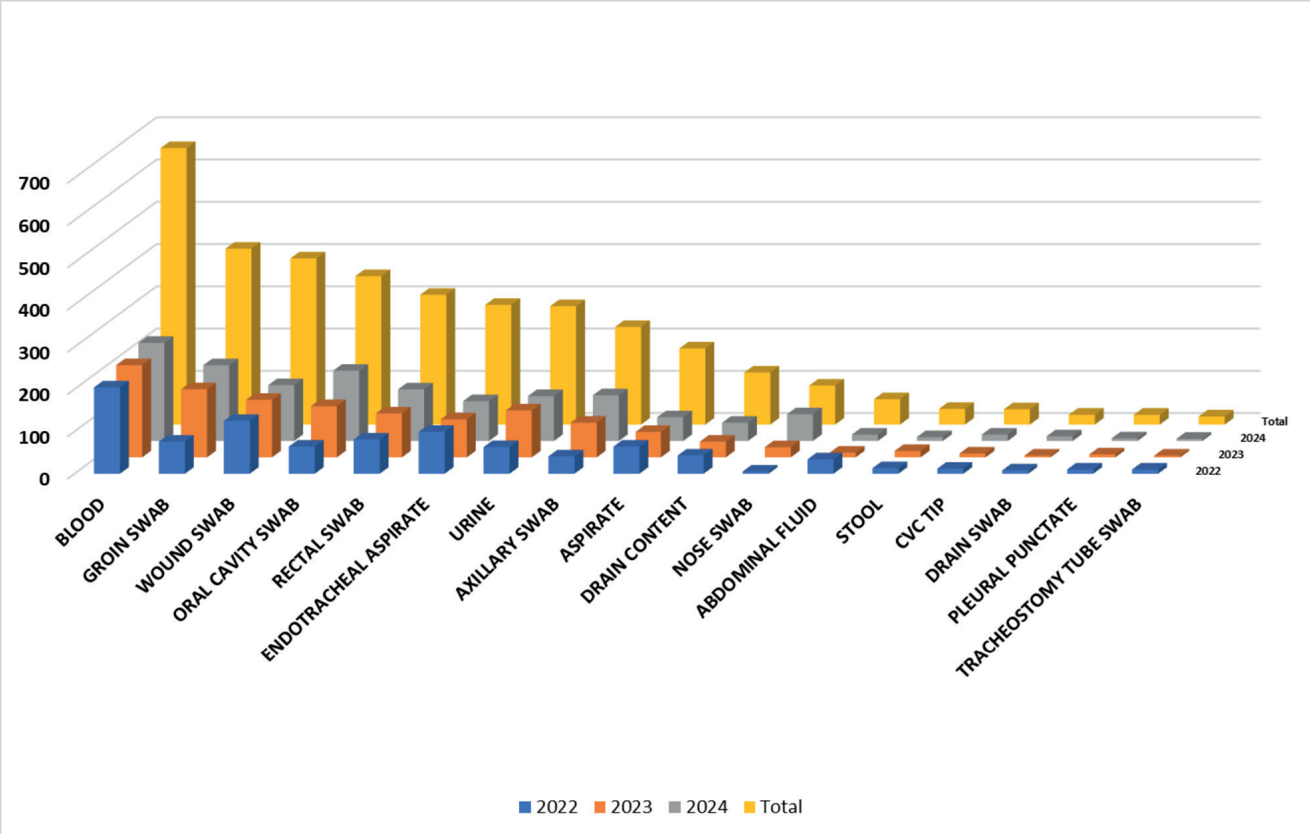

Figure 4. The distribution of findings by sample type for 2022-2024.

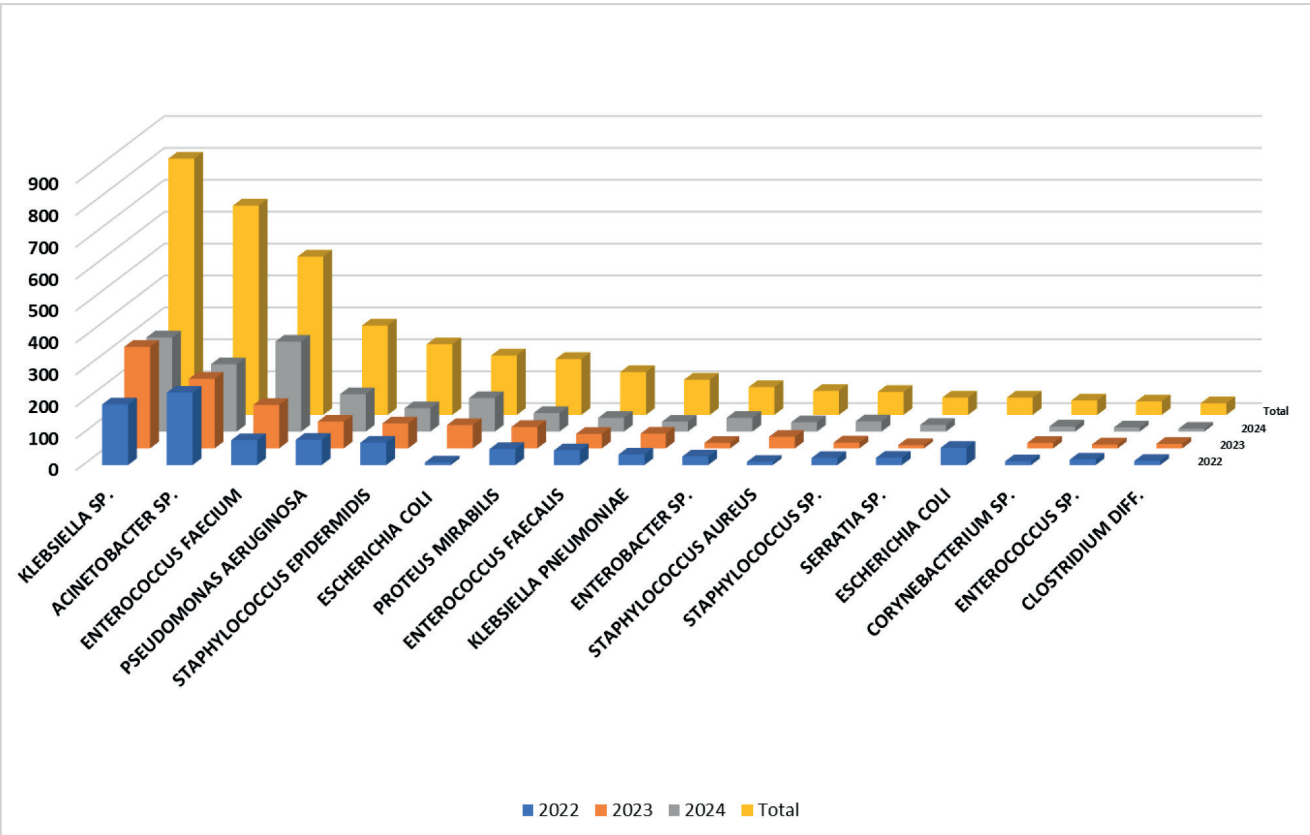

Figure 5. The distribution of bacterial isolates by material type for 2022-2024.

Discussion

Healthcare-associated infections (HAI) continue to pose a significant public health threat in Europe. According to data from the European Centre for disease Prevention and Control (ECDC) and the World Health Organization (WHO) Regional Office for Europe, the prevalence of HAI in 2011/12 was approximately 3.2 million cases, while annual mortality from antibiotic-resistant infections caused by antibiotic-resistant pathogens in the EU, Ireland and Norway. The prevalence of HAIs is notably lower in primary hospitals (4.8%) and higher in tertiary care institutions (7.2%). [3,4] Approximately 70% of hospital infections are attributed to resistant microorganisms, including carbapenem-resistant *Enterobacteriaceae* (CRE), while it is estimated that up to 20% of these infections could be preventable. [3,4] The most commonly reported HAIs are urinary tract infections (UTIs, 34.3%), respiratory tract infections (RTIs, 27.3%, with 55.2% being lower RTIs other than pneumonia), and skin infections (23.9%, of which 68.4% were cellulitis, soft tissue, or wound infections). [3]

In Europe, antimicrobial resistance (AMR) surveillance is primarily established through the ECDC and European Antimicrobial Resistance Surveillance Network (EARS-Net), with active participation from national networks and institutions.

Major Pathogens and Resistance Patterns

According to ECDC the predominant causative agents of HAIs are: *Escherichia coli* (32.6%), *SARS-CoV-2* (14.1%), *Klebsiella pneumoniae* (9.8%), *Staphylococcus aureus* (7.9%), *Proteus mirabilis* (6.8%), *Pseudomonas aeruginosa* (5.2%), *Enterococcus faecalis* (3.3%), *Enterobacter cloacae* (1.6%), *Providencia species* (1.6%), *Clostridioides difficile* (1.4%), and *Klebsiella species,* not specified (1.4%). (3,4) In recent years, the emergence of carbapenem-resistant *A. baumannii* (CRAB), carbapenem-resistant *Enterobacteriaceae* (CRE), vancomycin-resistant *enterococci* (VRE), and methicillin-resistant *S. aureus* (MRSA) has become an increasingly serious concern.

Data from Serbia

Our study showed that the most common samples collected over three years were blood cultures (643; 17.3%), wound swabs (393; 10.4%), urine cultures (280; 7.42%), and endotracheal aspirate (248; 6.6%). The most common isolates from blood cultures throughout the study period were Gram-positive cocci (*S. epidermidis*), while A*cinetobacter spp.* was the most common isolate from wound swabs and endotracheal aspirates. In urine cultures, as expected, Gram-negative bacteria from the *Enterobacteriaceae* family, primarily *E. coli* and *Klebsiella spp*. were the most frequent. The number of samples has increased steadily over the years due to a higher frequency of sampling.

In Serbia, comparative data on HAIs between general wards and ICUs from 2017 shows a prevalence of 4.2% in general wards and 15.9% in ICUs. A study from Belgrade from 2019/2021 indicated an increased ICU prevalence of 32.7%. [5] Bacterial resistance to carbapenems and other last-resort antibiotics remains high, exceeding 50%, except for tigecycline, colistin, and linezolid. [6]

According to the National Antimicrobial Resistance Control Program in Serbia, ICUs infections are dominated by bloodstream infections, ventilator-associated pneumonia (VAP), and catheter-associated urinary tract infections. The greatest concern lies in the increasing number of *Acinetobacter spp.* isolates resistant to multiple antibiotic classes, along with rising use of reserve agents such as colistin and tigecycline. [5,6] Despite limited recent ICU-specific data from Nis, available evidence suggests high antibiotic consumption and a correlation between antibiotic use and bacterial resistance, highlighting the urgent need for antimicrobial stewardship programs, particularly in ICUs.

ECDC data from 2019 indicate that 95.8% of ICU pneumonia cases were ventilator–associated (VAP). The same source reports that among ICU patients hospitalized for more than two days, the rates were approximately 8% for pneumonia, 6% for bloodstream infections, and 3% for urinary tract infections 3%. [3] Another European study has reported VAP rates ranging from 9% to 27%. [7] Mortality associated with VAP is substantial, with reports suggesting mortality rates between 15-50%, with higher rates observed among surgical ICU patients. [8,9] Pathogens responsible for VAP are typically Gram-negative organisms, with *Klebsiella pneumoniae* (27.8%), *Acinetobacter baumannii* (20.6%), *Pseudomonas aeruginosa* (15.4%), and *Escherichia coli* (9.6%) being the most frequently isolated pathogens [10] which corresponds with our results.

A study from England [11] monitoring ICU bloodstream infections from 2017 to 2023 found a decline in *S. aureus* isolates 810.8% vs.16.8% in 2004), an increase in *Enterococcus spp.* (17.8% vs 9.8%), and *Escherichia coli* (10.7% vs.3.7%), while *Pseudomonas aeruginosa* and *Candida spp.* frequencies remained similar. In our study, bloodstream infections during all three years were caused by Gram-positive bacteria - *S. epidermidis.* During the COVID-19 pandemic, there was a notable rise in bloodstream infections caused by *Klebsiella spp*., likely due to altered infection prevention and control measures during the pandemic.

According to Liao et al. [12] among 584 blood samples, the most common isolates were Gram-negative bacteria - *Klebsiella spp. (*14%), *Escherichia coli* (14%), *Enterococcus spp.* (11%), *Acinetobacter spp.* (10%), and *Enterobacter spp.* (9%).

Regarding urinary tract infections, Nouri et al. [13] reported *Escherichia coli* (59.6%), *Klebsiella pneumoniae* (9.9%), *Pseudomonas aeruginosa* (5.09%), *Enterobacter sp.* (2.2%), and *Klebsiella oxytoca* (1.2%) as the most common causative agents. These findings are in agreement with the data obtained in our study.

Antibiotic Use and Resistance Trends

Empirical antibiotic therapy, present in approximately 50% of cases, is a key driver of resistance. The most commonly used antibiotics are carbapenems, third- and fourth-generation cephalosporins, piperacillin-tazobactam, fluoroquinolones, glycopeptides, and polymyxins. [14] Southern and Eastern European countries generally exhibit higher resistance rates compared to Northern and Western Europe. *Klebsiella pneumoniae* resistance to carbapenems increased by 50% in 2023 compared to 2019, while *Acinetobacter baumannii* resistance reached up 85% in some countries. [3]

High AMR levels in ICUs necessitate particularly cautious selection of empirical antibiotic therapy for patients on mechanical ventilation or invasive catheters, taking into account local resistance data and institutional guidelines. Effective antimicrobial stewardship programs should include timely de-escalation of therapy following microbiological results, discontinuation of unnecessary antibiotics or invasive lines, and continuous monitoring of antimicrobial use.

Only two countries, Estonia and Italy, have available data on mortality associated with HAIs, encompassing a total of 402 patients, of whom 42.8% survived, 5.7% died as a direct result of infection, 11.2% deaths were unrelated to infection, 23.9% were possibly infection-related, and for 16.4% the relationship was uncertain. [3]

The COVID-19 pandemic further exacerbated the situation; during 2020/21 period, a significant increase in resistance among ICU isolates was recorded compared to the pre-pandemic period, attributed to excessive empirical antibiotic use and prolonged mechanical ventilation. Resistance to imipenem, meropenem, and ciprofloxacin was markedly higher among COVID-19 patients. [15]

The emergence of carbapenem-resistant *Acinetobacter baumannii* (CRAB) is particularly alarming issue, as it represents a critical challenge in treating hospital-acquired infections worldwide. CRAB has become endemic in several regions, including countries with advanced infection control systems such as Greece, where nearly complete resistance to carbapenems has been observed, often accompanied by resistance to colistin and ampicillin/sulbactam. Infections with CRAB are associated with high morbidity and mortality.

Another worrying development is the spread of strains producing metallo-β-lactamases, particularly NDM (New Delhi metallo-β-lactamase), which further limits therapeutic options. Clinical isolates of *Acinetobacter* in Greece exhibit near-complete resistance to carbapenems and are often multidrug-resistant, including resistance to last-line agents such as colistin and ampicillin/sulbactam. Treatment of CRAB infections remains highly challenging and typically relies on combination regimens (e.g., cefiderocol or sulbactam/durlobactam combinations), though the availability of these agents remains limited. Despite new therapeutic approaches, *A. baumannii* infections remain associated with high mortality and prolonged ICU stays, underscoring the need for resistance monitoring, rational antibiotic use, and the development of new antimicrobials. (16) Pan-resistance of *A. baumannii* isolates to carbapenems and aminoglycosides is linked to genomic plasticity enabling overexpression of efflux pumps. (17) The increasing resistance of *A. baumannii* to carbapenems calls for a more rationalized approach to their use and the introduction of molecular diagnostic tools (PCR or bla-OXA51) to differentiate colonization from infection. [16]

Phenotypic carbapenem resistance in *Enterobacterales* (carbapenem-resistant *Enterobacterales* – CRE) is most often due to β-lactamase production that hydrolyzes carbapenems and structural mutations affecting outer membrane permeability. Key mechanisms include extended-spectrum β-lactamases (ESBLs) and AmpC cephalosporinases, which may be chromosomally encoded or plasmid-mediated. Increased production of AmpC and ESBL enzymes, combined with poring loss or modification, leads to antibiotic accumulation in the periplasmic space and reduced efficiency. [18]

Mortality associated with CRE infections is high, reaching 40-50%, and continues to rise according to national reports. [19,20] Risk factors for CRE colonization ad infection include recent (within 30 days) use of carbapenems, vancomycin, or metronidazole, organ or stem-cell transplantation, mechanical ventilation, and fecal incontinence. [21] In regions where *K. pneumoniae* resistance to carbapenems exceeds 20%, ceftazidime-avibactam and meropenem-vaborbactam should be prioritized in therapy. [22] Resistance of *E. coli* to carbapenems remains low (< 1.3%) due to a lower likelihood of carbapenemase production compared to *Klebsiella* and *Acinetobacter* species; however, increasing resistance to cephalosporins indicates enhanced ESBL production. [23] The observed reduction in *E. coli* susceptibility to ciprofloxacin and *K. pneumoniae* to levofloxacin likely reflects reduced empirical use of these agents as part of antibiotic stewardship initiatives. [24]

Diagnostic and Preventive Strategies

The standard diagnostic procedure for suspected HAIs includes Gram staining (results within 24 hours), culture-based identification (results within 48 hours), and a complete antibiogram available after approximately 72 hours. [25]

Improved infection control remains crucial in preventing HAIs—this includes hand hygiene, aseptic technique during invasive procedures, proper isolation, surveillance, and education. Robust infection surveillance systems provide essential data for developing and continuously updating antibiotic therapy guidelines, antimicrobial stewardship programs, and infection control policies.

Conclusion

High levels of antimicrobial resistance in the ICU highlight the need for continuous surveillance and strict infection control. The predominance of multi-resistant pathogens underscores the importance of timely microbiological diagnostics and effective antimicrobial stewardship. A coordinated surveillance and stewardship approach is essential to optimize therapy, limit resistance, and improve outcomes in critically ill patients. Collaboration between public health institutions, academic centers, and the pharmaceutical industry could significantly enhance information exchange, foster the development of new antimicrobial agents, and strengthen the global response to the challenge of microbial resistance.

The surveillance conducted in our ICU encompassed a mixed one that admits surgical, medical and trauma critically ill patients. Our trend during a three-year follow-up period revealed some unexpected, yet concerning findings. Of particular note was the emergence of extensively drug-resistant *Klebsiella spp.* isolates among the analyzed samples, as well as the rising number of *Enterococcus faecium* isolates in various types of samples. Despite these alarming findings, *Acinetobacter sp.* remained the predominant pathogen isolated from endotracheal aspirates throughout the entire study period.

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