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BETA-LACTAM RESISTANCE OF GRAM-NEGATIVE BACILLI ISOLATED FROM CLINICAL BIOSUBSTRATES

313.02 – MICROBIOLOGY, MEDICAL VIROLOGY

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CONTENTS

INTRODUCTION

Relevance of the research

Antimicrobial resistance (AMR) has emerged as one of the most critical global public health threats of the 21st century. Beyond its devastating impact on health, AMR places a significant social and economic burden on healthcare systems, driving up medical costs and contributing to treatment failures, which can sometimes be fatal [1,2,3,4,5].

In this context, Gram-negative bacilli (GNB) are of particular concern due to their significant ability to rapidly develop and spread multiple resistance mechanisms [6]. The situation is especially alarming in infections caused by multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) bacteria, for which therapeutic options are extremely limited or, in some cases, completely unavailable [7,8]. Their high capacity to adapt to antibiotic pressure makes these pathogens a major challenge in medical practice, particularly in the context of healthcare-associated infections [6,9,10].

The relevance of this topic is strongly supported by recent reports from the World Health Organization (WHO), the Centers for Disease Control and Prevention (CDC) [11,12,13], and the European Centre for Disease Prevention and Control (ECDC) [14,15]. These reports classify carbapenem- and third-generation cephalosporin-resistant GNB — including *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* — as a critical priority for the development of new antibiotics. They also note that 10 of the 18 major health threats linked to antibiotic-resistant microorganisms are caused by GNB [16,17,18].

Non-fermenting GNB are among the main causes of invasive infections, accounting for up to 75% of cases, including both healthcare-associated and community-acquired infections. Alarmingly, these pathogens are also responsible for up to 42% of infection-related deaths [6,11].

In the Republic of Moldova, as in many other resource-limited countries, the problem is further worsened by the irrational and excessive use of antimicrobial agents, the lack of comprehensive antimicrobial stewardship programmes, and insufficient microbiological surveillance capacity [19]. Local studies reveal a worrying trend of increasing prevalence of extended-spectrum β-lactamase (ESBL)-producing *Enterobacterales* and carbapenem-resistant strains. Among the priority pathogens identified are carbapenemase-producing *K. pneumoniae* (KPC), metallo-β-lactamase (MBL) and oxacillinase (OXA) producers, *P. aeruginosa*, *A. baumannii*, and third-generation cephalosporin-resistant GNB, including ESBL-producing *E. coli* [19,20,21].

At present, the capacity of microbiology laboratories in the country to detect ESBLs and carbapenemases remains limited. This is largely due to the wide variety of these enzymes and the absence of a clearly defined diagnostic algorithm. Consequently, some AMR-related mechanisms may go undetected, increasing the risk of underdiagnosis [21,22,23].

This situation turns AMR from a theoretical threat into a pressing clinical and epidemiological reality. Patients with infections caused by resistant GNB undergo longer hospital stays, require more expensive treatments, and experience significantly higher mortality rates. At the same time, healthcare systems come under growing pressure, and the risk of returning to a so-called “post-antibiotic era,” where common infections may once again become life-threatening, is becoming increasingly real [16,24,25].

Therefore, an in-depth investigation of antimicrobial resistance in GNB is highly relevant today, highlighting the need to develop a standardized algorithm for detecting resistance mechanisms, as well as effective national policies for surveillance, prevention, and control. These

measures should be aligned with the principles of the "One Health" approach and international recommendations.

High-quality research, data, and analysis are essential for developing new measures to combat AMR and for supporting policymakers in improving actions in this area.

The research purpose was to assess resistance profiles to β-lactams and determine the phylogenetic groups of priority GNB, with the development of a standardized methodological algorithm for detecting resistance mechanisms, and to propose effective measures for the prevention and control of antimicrobial resistance.

Research objectives are as following: to determine the antimicrobial resistance profiles of GNB isolated from various clinical biosubstrates; to perform molecular description of ESBLs and carbapenemases associated with GNB; to conduct a comparative analysis of different microbiological techniques for identifying beta-lactam-resistant GNB, with the development of a standardized methodological algorithm; and to propose effective measures for the prevention and control of infections caused by MDR GNB.

Research hypothesis. The microbiological and epidemiological characteristics of antimicrobial resistance in GNB, shaped by the variability of resistance mechanisms and the effectiveness of surveillance and control strategies, play a main role in reducing the socioeconomic burden of infections caused by resistant bacteria and in strengthening efforts to combat AMR.

Scientific novelty and originality of the results. In the Republic of Moldova, a comprehensive study of priority GNB was conducted using contemporary phenotypic methods and molecular biology techniques, which enabled the identification and characterization of resistance patterns and the detection of the main resistance mechanisms of these pathogens, as well as the assessment of the position of these GNB within global phylogenetic trees. These data are extremely important for forecasting the evolution of resistance of these organisms at the regional level.

The study results served as the basis for developing a standardized algorithm for detecting antimicrobial resistance mechanisms, which will be implemented in microbiology laboratories within the national AMR surveillance network.

Findings that contributed to solving the scientific problem. This study identified the microbiological range of priority GNB, as well as the genotypic and phenotypic diversity of antimicrobial-resistant strains, including: the prevalence of ESBL- and carbapenemase-producing GNB strains in Moldova; the range of resistance enzymes in GNB; the predominant sequence type among circulating GNB strains in the country. A standardized algorithm for detecting AMR markers was developed, along with evidence-based recommendations for improving AMR surveillance and control.

Practical implementation of the results. Based on the research findings, a standardized algorithm for detecting antimicrobial resistance mechanisms in priority GNB was developed and scientifically justified.

To improve the quality of microbiological investigations, two practical guides were developed for medical personnel involved in the collection, transport, and processing of blood, cerebrospinal fluid (CSF), and urine samples for bacteriological testing.

Within the study, the guide *"Detection of Antimicrobial Resistance Mechanisms, Interpretation and Clinical Application of the Results"* was developed and approved by Order No. 1239 of 29.12.2023, as well as the methodological instruction *"Detection of Antimicrobial Resistance Mechanisms"*, approved at the meeting of the Quality Management Council of 'Nicolae

Testemițanu' State University of Medicine and Pharmacy, minutes No. 2 of 29.11.2023. These educational materials were incorporated into the study programs for lectures and practical sessions for students, residents, and physicians, and were implemented in practice in the laboratories of public health centers.

The brochure *"Method for raising awareness in children about the prevention of antimicrobial resistance"*, developed during the study, is a valuable resource for introducing children to the world of microorganisms — both friendly and harmful — as well as to protective measures aimed at preventing infections caused by pathogenic microbes (Annex 7). This work was presented at the *International Exhibition of Innovation and Technology Transfer EXCELLENT IDEA – 2023, 2nd Edition, Chișinău*, under the title *"Method for Raising the Degree of Awareness in Children About the Prevention of Antimicrobial Resistance"*, where it was awarded a silver medal.

The leaflets, posters, and interactive games developed based on this brochure were presented at the event "European Researchers' Night".

Research results approval. The research methodology and study design were reviewed and approved during the meeting of the Research Ethics Committee of Nicolae Testemițanu USMF, with a favourable opinion issued for the doctoral research project titled "Beta-lactam Resistance in Gram-Negative Bacilli Isolated from Clinical Biosubstrates", meeting minutes No. 1 of January 10, 2022.

The thesis topic was discussed and approved within the primary unit at the joint meeting of the doctoral leadership, the members of the supervisory committee, and the staff of the Scientific Laboratory for Antimicrobial Resistance Surveillance, the Microbiology Laboratory, and the Department of Epidemiological Surveillance of Healthcare-Associated Infections and Antimicrobial Resistance (excerpt from the minutes No. 1 of the joint meeting of 11.02.2022), as well as at the specialized Scientific Seminar 313: Immunology, Microbiology, Virology, specialties 321.09 Infectious, Tropical and Parasitic Diseases, and 313.02 Medical Microbiology, Medical Virology (excerpt from the minutes No. 1 of 27.01.2023)

The PhD thesis results. The research findings are summarised in 28 scientific publications, including four as first author and two as sole author. These comprise: four articles published in SCOPUS-indexed journals, six articles in national scientific journals, one article in an international journal, two abstracts presented at international scientific forums, eight abstracts at national forums, one guide, one methodological guideline, and one educational brochure. The results were also shared through eight active presentations at scientific events. As part of this work, four innovation certificates were obtained.

PhD thesis volume and structure. The thesis is presented across 65 pages of core text and includes the following sections: title page, table of contents, lists of abbreviations, tables, and figures, introduction, four chapters, general conclusions, recommendations, 165 bibliographic references, and ninet annexes. The visual material comprises 14 tables and 31 figures.

Keywords: *antimicrobial resistance, MDR Gram-negative bacilli, resistance mechanisms, extended-spectrum β-lactamases, carbapenemases.*

PHD THESIS CONTENT

1. THE EVOLUTION OF ANTIMICROBIAL RESISTANCE IN GRAM-NEGATIVE BACTERIA

This chapter presents a comprehensive overview of the most relevant findings from the scientific literature over the past decade concerning the evolution of antimicrobial resistance in GNB. It describes the theoretical foundations underlying the development of major resistance mechanisms commonly found in GNB worldwide, alongside national and international research efforts in the field of microbiological diagnosis of AMR and the epidemiological trends associated with this phenomenon. The chapter also highlights the critical role of GNB in infectious diseases and emphasises the clinical significance of these pathogens. Particular attention is given to the main resistance mechanisms identified in GNB, as well as to modern laboratory techniques used for their detection, outlining both their strengths and limitations. The chapter concludes by presenting the rationale that led to the initiation of this research.

2. STUDY MATERIALS AND METHODS

2.1. General characteristics of the study

A comprehensive study was carried out on GNB strains collected between 2020 and 2023. The research was conducted at the Microbiology Laboratory of the National Agency for Public Health, in collaboration with the Genomic Epidemiology Centre of the Technical University of Denmark (Collaboration Agreement No. 1 of 24.04.2023). Laboratory investigations were performed *in vitro* on suspected GNB strains submitted to the National Reference Laboratory for confirmation of resistance mechanisms. These strains were referred by all laboratories within the National AMR Epidemiological Surveillance System, which includes 24 laboratories — ten regional laboratories of the National Agency for Public Health, ten laboratories from public healthcare institutions, and four private laboratories.

The study material included GNB strains isolated from blood, CSF, and urine samples.

To meet the research objectives and fulfil the overall purpose of the study, investigations were conducted in several stages, as described below.

The first stage of the study consisted of analyzing 480 relevant bibliographic sources on AMR in GNB, identified through the MEDLINE, PubMed, HINARI platforms and web interfaces, with particular emphasis on national literature addressing resistance detection methods and the epidemiological situation in the country.

In the second stage, isolates resistant to at least one beta-lactam were selected, the appropriate methodology was established, microbiological investigations and statistical analysis were conducted, and ultimately five conclusions and a set of recommendations were formulated, confirming the achievement of the objectives and the attainment of the proposed goal.

In *the third stage*, the research findings were shared through multiple means, including publications within national and international scientific journals, abstracts, and active participation with presentations or posters at both national and international scientific events. The results also led to the development of a practical guide, a methodological handbook, and an educational brochure.

2.2. Research methods

The study of beta-lactam-resistant GNB was carried out as a comprehensive investigation. To achieve its objectives, a combination of the following methods was used, namely descriptive and analytical methods, applied to synthesise and summarise data from the scientific literature;

analytical methods, used to assess and compare different techniques for detecting resistance mechanisms; microbiological methods, employed to identify resistance mechanisms in the studied strains; epidemiological methods, used to determine the prevalence of phylogenetic groups of GNB and the types of resistance enzymes found in these pathogens; statistical methods, used in processing, analysing, and interpreting the research data.

Microbiological Method

The detection and characterization of beta-lactam resistance in GNB strains were carried out using classical microbiological and contemporary molecular–genetic methods, including culture purity verification and strain reidentification, antimicrobial susceptibility testing by the Kirby–Bauer method and the Vitek-2 Compact system with extended antimicrobial panels according to EUCAST standards, MIC determination, and internal quality control using the *E. coli* ATCC 25922 reference strain.

The methods for testing resistance mechanisms included the screening and phenotypic confirmation of AmpC-, ESBL-, and carbapenemase-producing strains using disc-diffusion, chromogenic, colorimetric, immunochromatographic, and gradient methods in accordance with EUCAST standards, as well as molecular biology techniques such as PCR and bacterial genome sequencing following the manufacturers' instructions. The results were validated using reference strains for internal quality control.

Bioinformatic analysis of the sequenced bacterial genomes was performed using the services of the Centre for Genomic Epidemiology. ResFinder v4.6.0 was used to identify antimicrobial resistance genes and their genomic positions, PlasmidFinder v2.1 for plasmid detection, and MLST v2.0 for sequence typing. Phylogenetic trees were constructed with CSI Phylogeny 1.4, visualized with iTOL, and reference genomes included *K. pneumoniae* ST395, *E. coli* ST131 and *A. baumannii* ST2063. Hypervirulent strains were identified using Kleborate via PathogenWatch.

2.3. Mathematical and statistical data processing

The data were automatically processed using RStudio (version 2024.09.1+394) and Python (version 3.12.3), open-source tools that enabled a rigorous, reproducible, and transparent analysis, with source code available upon request. For categorical variables, absolute and relative frequencies with 95% confidence intervals were calculated and visualized using bar charts, while hypothesis testing was performed with Pearson's Chi-square test with Monte Carlo simulation (100,000 random samples); all analyses were interpreted using a significance threshold of 0.05, consistent with the statistical and clinical relevance of the observed differences.

The limitations of the study. For *P. aeruginosa*, insufficient genomic coverage prevented the construction of phylogenetic trees and their subsequent analysis, thereby limiting the assessment of strain circulation and their placement in global phylogenetic trees.

Interpretation of the results was further hindered by the uneven distribution of isolates across species and geographical regions, variations in laboratory procedures, and the use of phenotypic methods for ESBL detection in non-sequenced isolates, which may miss certain resistance mechanisms. Moreover, the limited number of laboratories participating at the beginning of the surveillance program and the absence of data from animal, food, or environmental sources restricted the assessment of the evolutionary and seasonal dynamics of resistance and the analysis of intersectoral transmission within a One Health framework.

3. DISTRIBUTION OF GRAM-NEGATIVE BACILLI AND THEIR ANTIMICROBIAL RESISTANCE PROFILES

3.1. Diversity and prevalence of Gram-negative bacilli species isolated from clinical biosubstrates

An analysis of the diversity of GNB species isolated from clinical samples collected by laboratories within the Antimicrobial Resistance Surveillance System between 2020 and 2023 revealed that *K. pneumoniae* was the most commonly isolated species, accounting for 47.1% (95% CI, 45.2–49.5) of cases. This was followed by *E. coli* at 42.9% (95% CI, 41.0–45.3), *A. baumannii* at 7.4% (95% CI, 5.3–9.6), and *P. aeruginosa*, which was found in 2.7% (95% CI, 0.1–4.4) of infection cases.

For *E. coli* and *K. pneumoniae,* the highest isolation rates came from the intensive care unit, accounting for 41.3% (95% CI, 39.5–43.1) of strains. This was followed by internal medicine wards at 18.2% (95% CI, 16.8–19.6), urology at 13.4% (95% CI, 12.1–14.7), and surgery at 6.3% (95% CI, 5.4–7.2).

P. aeruginosa and *A. baumannii* strains were predominantly isolated from ICU patients, making up 76.5% (95% CI, 67.3–85.8) of samples, with smaller proportions found in the pediatric ICU at 9.9% (95% CI, 3.4–16.4) and surgery at 6.2% (95% CI, 0.9–11.4).

3.2. Antimicrobial resistance profiles of the isolates studied

Analysis of the *E. coli* strains revealed that out of 1,306 tested isolates, 57.0% (95% CI, 48.3–66.5) were resistant to penicillins, 61.8% (95% CI, 49.3–68.5) to cephalosporins, and only 2.1% (95% CI, 0.1–4.5) to carbapenems. Resistance was also found in 72.1% (95% CI, 64.3–81.5) of strains against fluoroquinolones, 32.2% (95% CI, 24.8–40.2) against aminoglycosides, and 3.0% (95% CI, 1.3–5.1) against colistin.

Among the MDR strains, 42.1% (95% CI, 36.3–49.7) of *E. coli* showed resistance to three or more classes of antimicrobials. Notably, 1.4% (95% CI, 0.2–2.1) exhibited extensive resistance, being resistant to all tested antibiotics except colistin, and 0.3% (95% CI, 0.0–1.9) were pan-resistant, showing resistance to every antibiotic tested.

The majority of *K. pneumoniae* strains—93.9% (95% CI, 85.3–98.9)—were resistant to cephalosporins and fluoroquinolones; 81.5% (95% CI, 76.1–89.6) to aminoglycosides; 51.1% (95% CI, 43.8–58.6) to carbapenems; and 15.0% (95% CI, 8.7–23.4) to colistin (figure 1).

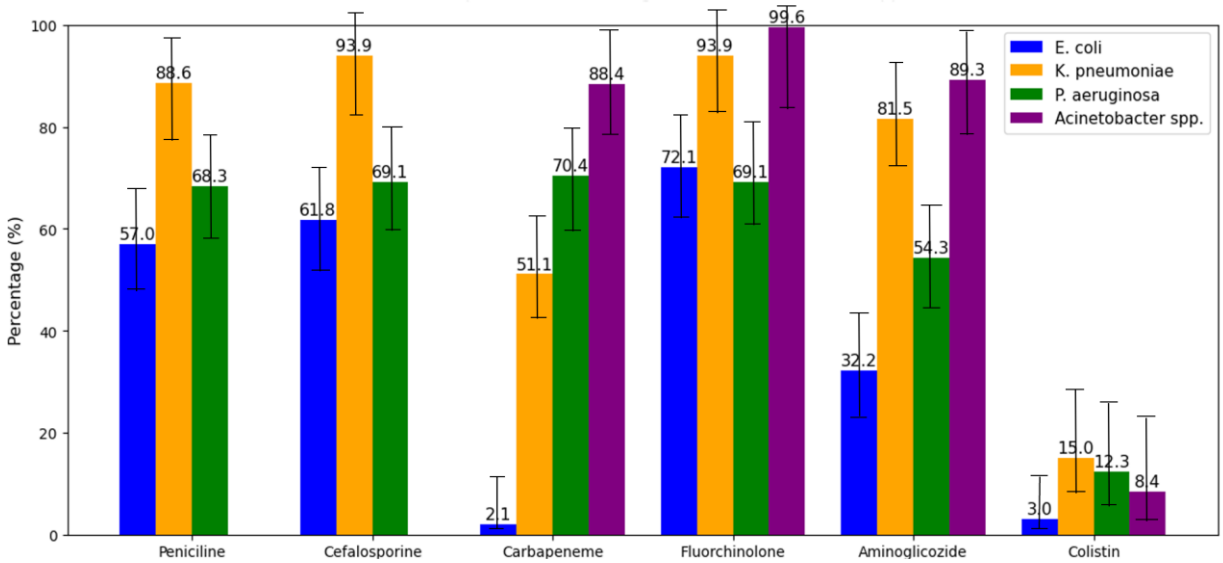

Figure 1. **Resistance of studied GNB Species to selected antimicrobial groups**

More than 70.0% (95% CI, 63.4–76.9) of *K. pneumoniae* strains were MDR, exhibiting combined resistance to penicillins, cephalosporins, aminoglycosides, and fluoroquinolones. XDR strains made up 47.0% (95% CI, 40.3–55.6) of the *K. pneumoniae* isolates, while PDR strains—resistant to all tested antimicrobials—were found in 13.7% (95% CI, 6.2–20.3) of these isolates.

P. aeruginosa showed resistance rates of 70.4% (95% CI, 63.8–77.6) to carbapenems, 69.1% (95% CI, 61.3–75.4) to cephalosporins, the same percentage to fluoroquinolones, 68.3% (95% CI, 58.3–76.5) to penicillins, 54.3% (95% CI, 46.4–62.2) to aminoglycosides, and 12.3% (95% CI, 8.7–18.1) to colistin.

Among *P. aeruginosa* isolates, 66.7% (95% CI, 58.8–76.5) were MDR, 49.4% (95% CI, 38.3–56.5) were XDR, and 12.3% (95% CI, 8.8–16.3) were PDR.

A. baumannii is becoming increasingly resistant to multiple groups of antimicrobials, leaving almost no effective treatment options for the severe infections it usually causes. In this study, 99.6% (95% CI, 95.4–100) of the *A. baumannii* strains were resistant to fluoroquinolones, 89.3% (95% CI, 83.6–92.3) to aminoglycosides, and 88.4% (95% CI, 84.7–96.5) showed resistance to last-resort antibiotics. In our study, 8.4% (95% CI, 2.3–12.8) of isolates were resistant to colistin. Nearly all strains were MDR (99.1%, 95% CI, 98.8–99.9), with 8.4% (95% CI, 1.5–14.6) classified as PDR.

Analysis of resistance mechanisms found in Gram-negative bacilli by various methods

AST was used as a screening method to identify the production of extended-spectrum beta-lactamases (ESBLs) and carbapenemases. Antibiogram analysis helped find strains suspected of producing resistance enzymes. During this process, some strains were found to have developed two or more resistance mechanisms simultaneously.

Using the DDST method, resistance to penicillins was confirmed in only 2.7% (95% CI, 1.5–3.8) of the *E. coli* isolates suspected of producing AmpC β-lactamase. Of the 1,271 *K. pneumoniae* strains resistant to penicillins and suspected of AmpC production, only 2.2% (95% CI, 1.4–3.0) were confirmed by DDST.

Isolates suspected of ESBL production were tested using three phenotypic methods. With the cultural method, 90.1% (95% CI, 88.0–92.0) of the *E. coli* isolates formed pink or violet colonies on chromogenic medium, thus confirming ESBL production. Among the suspected *K. pneumoniae* colonies, 45.1% (95% CI, 39.0–44.0) proved to be ESBL producers, forming blue colonies on this medium.

DDST confirmed ESBL production in 90.0% (95% CI, 88.0–92.0) of *E. coli* strains and in 36.6% (95% CI, 34.0–39.0) of *K. pneumoniae* strains suspected of producing ESBL enzymes.

The confirmation of carbapenemase-producing strains was performed using the PCR method. In *E. coli*, the dominant carbapenemase gene was oxa-48, detected in 34.9% (95% CI, 29.5–38.8) of isolates suspected of producing carbapenemases. This was followed by *blaNDM* at 23.3% (95% CI, 20.4–26.5), *blaKPC* at 14.0% (95% CI, 10.6–17.0), *blaVIM* at 12.8% (95% CI, 8.3–15.7), and *blaIMP* at 10.5% (95% CI, 5.8–13.2) among the suspected carbapenemase-producing strains.

In *K. pneumoniae*, OXA-48 was also the most commonly detected enzyme, present in 64.8% (95% CI, 61.1–66.5) of carbapenemase-suspected isolates. This was followed by NDM at 44.0% (95% CI, 39.9–49.5), KPC at 15.4% (95% CI, 10.3–19.4), IMP at 2.6% (95% CI, 0.8–4.5), and VIM at 2.5% (95% CI, 0.6–5.7).

The prevalence of resistance genes in *P. aeruginosa* isolates differed from that seen in *E. coli* and *K. pneumoniae.* The *bla*NDM gene was the most common, found in 42.0% of suspected

carbapenemase-producing strains (95% CI, 38.3–48.4), followed by *bla*OXA-48 at 37.0% (95% CI, 32.5–41.6), *bla*KPC at 23.5% (95% CI, 18.1–26.2), and *bla*VIM at 16.0% (95% CI, 11.3–21.6). The *bla*IMP gene was not detected in any of the *P. aeruginosa* strains tested by PCR (figure 2).

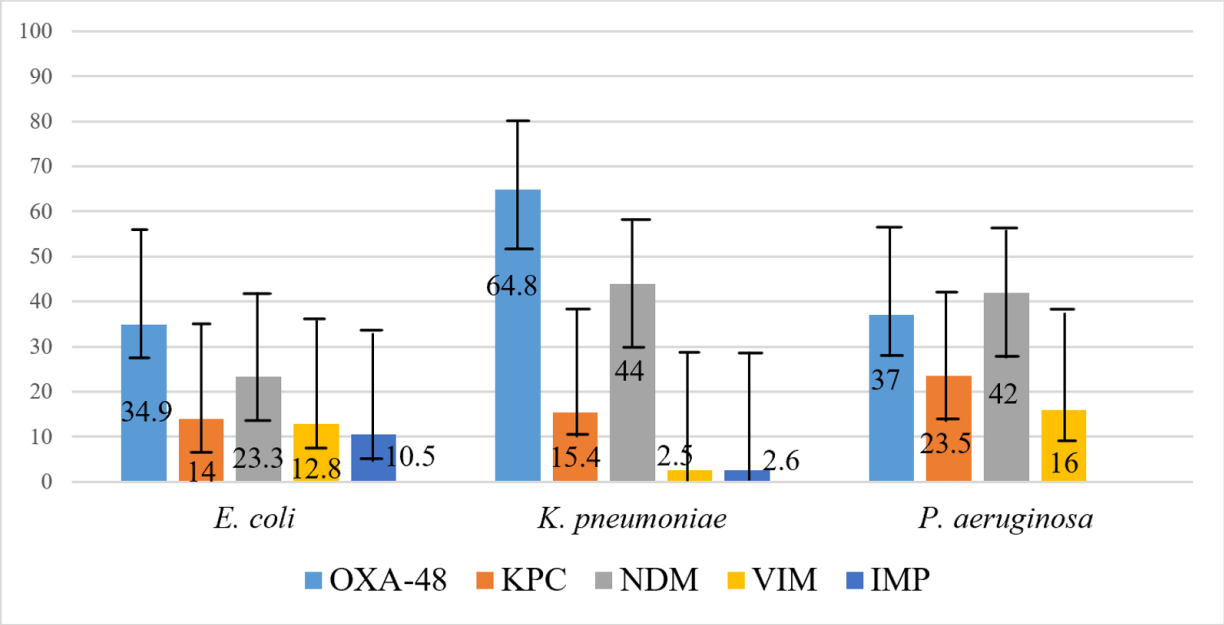

Figure 2**. Spectrum of resistance enzymes found by PCR in isolates of *E. coli, K. pneumoniae,* and *P. aeruginosa***

A. baumannii shows a unique resistance enzyme profile compared to the other species studied. Among the resistance genes identified in the *A. baumannii* strains were *bla*OXA-23, *bla*OXA-40, and *bla*OXA-58. Out of 214 isolates suspected of producing carbapenemases, 84.6% (95% CI, 82.7–86.5) were confirmed. The most common carbapenemase was OXA-23, found in 43.5% (95% CI, 38.9–46.5) of the suspected strains, followed closely by OXA-40 producers at 41.1% (95% CI, 37.8–44.9), and OXA-58 producers at 7.5% (95% CI, 5.4–9.6).

3.3. Sensitivity and specificity of tests used to detect antimicrobial resistance mechanisms

The tests used to identify antimicrobial resistance mechanisms in GNB included in this study were evaluated based on their sensitivity and specificity.

To detect ESBL production in *E. coli* and *K. pneumoniae*, phenotypic methods such as DDST, combined disc tests, CHROMagar™ ESBL chromogenic media, and the E-Test were employed. The performance of these ESBL detection methods was assessed using the E-Test as the gold standard, due to its higher sensitivity, reliability, and suitability.

Among the three phenotypic tests, the combined disc test showed the highest sensitivity and specificity. However, when comparing the combined disc test to the other methods in terms of overall accuracy, no statistically significant difference was observed ($p = 0.207$).

For carbapenemase detection, the immunochromatographic test demonstrated the highest sensitivity and specificity among the phenotypic tests. It showed a sensitivity of 86.2% (95% CI, 84.0–88.2) and a specificity of 91.7% (95% CI, 88.7–93.9), significantly outperforming both the CarbaNP test ($p < 0.001$) and the MAST CarbaPACE test ($p < 0.001$).

The detailed performance results of the immunochromatographic test in identifying specific resistance enzymes are presented in Table 1.

The immunochromatographic test detected 82.1% (95% CI, 80.0–85.5) of OXA-48-producing strains, 91.4% (95% CI, 83.9–95.6) of OXA-23, 36.4% (95% CI, 29.8–43.5) of KPC,

55.9% (95% CI, 51.7–60.2) of NDM, 41.7% (95% CI, 28.8–55.7) of VIM, and 8.6% (95% CI, 3.0–22.4) of IMP producers.

Table 1. **Performance of the immunochromatographic test for identifying resistance enzyme types**

Results	**Positive / Total tested**	TP	TN	FP	FN	Sensitivity (95% CI)	Specificity (95% CI)
OXA-48	593/722	593	457	85	122	82.1% (80.0-85.5)	84.3% (81.0-87.1)
OXA-23	85/93	85	110	11	8	91.4% (83.9-95,6)	90.0% (83.5-94.2)
KPC	68/187	68	1041	18	119	36.4% (29.8-43.5)	98.3% (97.3-98.9)
NDM	279/499	279	733	29	220	55.9% (51.5-60.2)	96.2% (94.6-97.3)
VIM	20/48	20	1132	8	28	41.7% (28.8-55.7)	99.3% (98.6-99.6)
IMP	3/35	3	1124	26	32	8.6% (3.0-22.4)	97.7% (96.7-98.5)

TP – true positives; TN – true negatives; FP – false positives; FN – false negatives; DDST – double-disc synergy test.

The test showed high sensitivity and specificity for detecting OXA-23 and OXA-48 enzymes. For the other enzymes, it demonstrated high specificity but lower sensitivity, struggling particularly with detecting KPC, VIM, and IMP producers. Moderate agreement was observed between this test and PCR results for NDM detection.

4. GENOTYPES AND PHYLOGENETIC GROUPS OF MULTIDRUG-RESISTANT GRAM-NEGATIVE BACILLI

4.1. Diversity of resistance genes and MLST profiles of antimicrobial-resistant Gram-negative bacilli

The strains sequenced in this study were selected based on the ECDC Study Protocol for Genomic Surveillance of Carbapenem- and/or Colistin-Resistant Enterobacteriaceae in the EU, version 2.0p, 2018. The protocol specifies the collection of the first 10 consecutive, non-duplicate bacterial isolates from clinical samples obtained for diagnostic purposes (blood, CSF, urine, sputum, and wound secretions). For each set of 10 resistant isolates, one strain of the same species but susceptible to carbapenems was subsequently selected [26].

At the regional level, the strains were tested for antimicrobial resistance and then sent for confirmation to the National Reference Laboratory for AMR within the National Agency for Public Health. After confirming antimicrobial resistance and the corresponding resistance mechanisms, the strains, accompanied by metadata (clinical, epidemiological, and microbiological data), were sent to the Center for Genomic Epidemiology at the Technical University of Denmark for sequencing and analysis

Klebsiella pneumoniae

For the phylogenetic analysis of *K. pneumoniae*, 99 strains isolated from blood and CSF were examined.

Using bioinformatics tools, the phylogenetic analysis revealed that all strains belonged to 16 different sequence types (STs) and shared familiar allelic profiles listed in the *Center for Genomic Epidemiology's MLST* database.

Bioinformatic analysis showed that sequence type ST395 was the most common, found in 55 *K. pneumoniae* strains, followed by ST377 in 12 strains, ST23 in 5 strains, ST11 in 4 strains, and ST1026 in 4 strains. Additionally, unique isolates with sequence types ST14, ST15, ST25, ST37, ST101, ST147, ST370, ST380, ST405, ST1037, and ST6381 were identified.

Importantly, the ST23 genes associated with the hypervirulence of *K. pneumoniae* strains isolated from blood were detected in five isolates. Beta-lactamases including CTX-M (blaCTX-M-55), OXA (blaOXA-1, blaOXA-48), TEM (blaTEM-1B), SHV (blaSHV-45), and LAP (blaLAP-2) were also found in these hypervirulent strains.

Using whole-genome sequencing data, a phylogenetic tree was constructed for *K. pneumoniae* ST395, the most common sequence type found in the country (Figure 3).

The analysis revealed genetic diversity among the studied *K. pneumoniae* ST395 strains, with several strains showing closely related nucleotide sequences.

The sequencing results also demonstrated epidemiological links between the isolates. All ST395 strains were grouped into two clusters: Cluster I included 32 isolates with similar genomic structures, while Cluster II comprised 23 isolates spread across several subclusters.

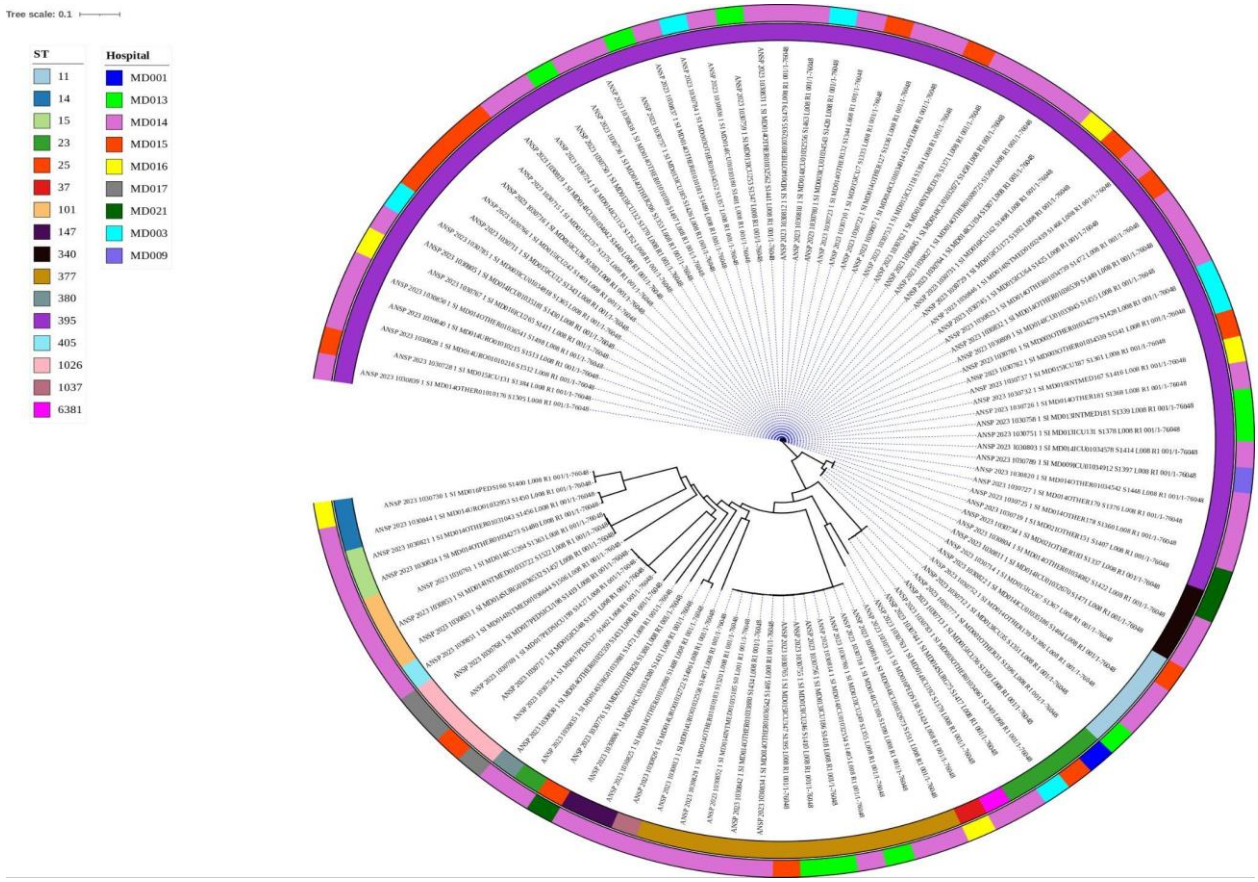

Figure 3. **Phylogenetic analysis of *K. pneumoniae* ST395 (iTOL)**

The analysis of the emergence of isolates belonging to Cluster I across different hospitals in the country showed that the first invasive isolates were identified at the beginning of the study in a republican-level hospital (MD015A), in the intensive care unit. Subsequently, ST395 spread further, and in addition to the aforementioned institution, *K. pneumoniae* ST395 strains belonging to the same

cluster were identified in a tertiary-level hospital (MD014A), both in the intensive care unit and in other therapeutic wards. Over the course of the study, such isolates were also detected in the intensive care units of two additional secondary-level institutions (MD013A and MD016A). In the final year of the study, multiple strains from Cluster I (ST395) were isolated from blood and urine in municipal medical institutions (MD013A, MD016A, MD014A, and MD015A), as well as in a district-level institution.

The first invasive isolate among the 23 *K. pneumoniae* ST395 strains belonging to Cluster II was identified in a tertiary-level medical institution (MD021A). Invasive isolates were also reported by the medical institutions MD014A, MD015A, and MD016A.

Subsequently, strains from the second cluster were recorded in institution MD013A, in the intensive care and internal medicine wards, as well as in the intensive care units of the medical institutions MD014A and MD015A.

During the study period, the number of isolates within this cluster increased, reaching 11 isolates identified in MD013A (intensive care and urology wards), MD016A (internal medicine ward), MD014A (intensive care unit), and MD015A (intensive care and urology wards). Two urine isolates were identified in outpatient facilities, demonstrating the spread of this strain to other healthcare institutions across the country.

One invasive *K. pneumoniae* ST395 strain was not assigned to any cluster; it was isolated in a tertiary-level hospital (MD014A), in a therapeutic-profile ward.

Escherichia coli

Among the 17 sequenced *E. coli* isolates, nine different sequence types (STs) were identified, showing varied frequencies, predominance, and a high level of allelic diversity. This genetic diversity likely reflects the species' extensive genetic variability and the ability of virulence and antibiotic resistance genes to be transferred horizontally within bacterial populations that colonize the human body.

All *E. coli* isolates were grouped into nine distinct STs based on MLST analysis of their genome sequences (Figure 4).

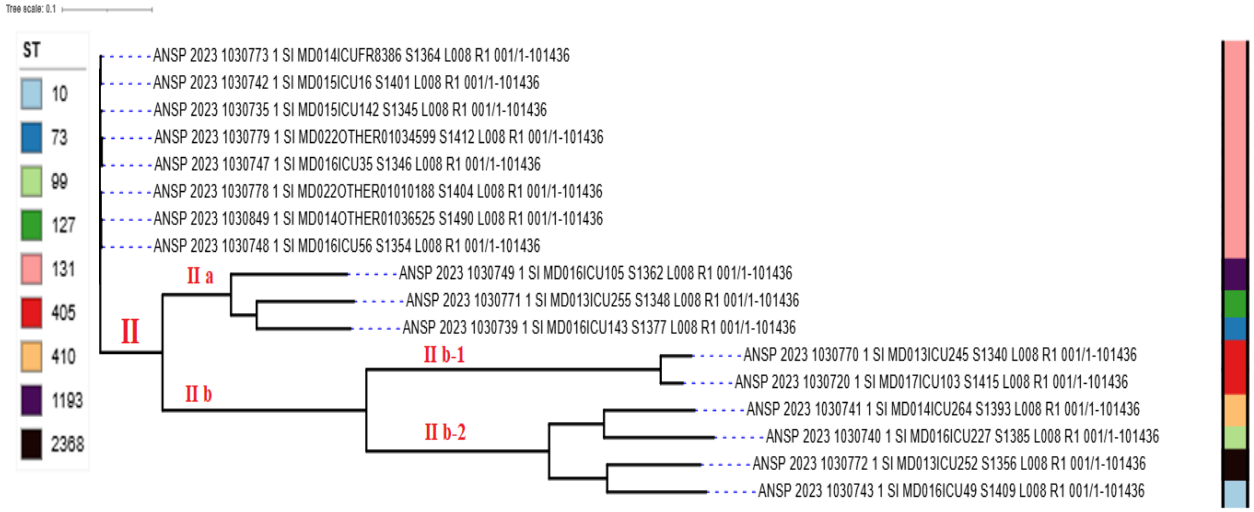

Figure 4. **Phylogenetic tree of *E. coli* strains based on MLST-assembled nucleotide sequences (MLST, iTOL)**

The most common and dominant sequence types (ST) among the *E. coli* isolates were ST131 (n = 9) and ST405 (n = 2). All other STs appeared in just one isolate each. ST131 was the most frequently found type in Moldova, accounting for 50% of all *E. coli* isolates analyzed, reflecting its genotypic dominance. This likely reflects the widespread endemic presence of this ST among strains isolated from hospitalized patients.

The phylogenetic analysis of the *E. coli* sequence types identified two main groups: Cluster I, which consists solely of *E. coli* ST131, and Cluster II, which includes all the other STs. Cluster II is further divided into two subgroups: Ia, containing three STs (ST1193, ST127, ST73), and Ib, which splits into two more subgroups—IIb-1 (ST405) and IIb-2 (ST410, ST99, ST2368, ST10).

Using the whole-genome sequencing data, a phylogenetic tree was built for Cluster I, focusing on *E. coli* ST131, the most prevalent sequence type found in the country (Figure 5).

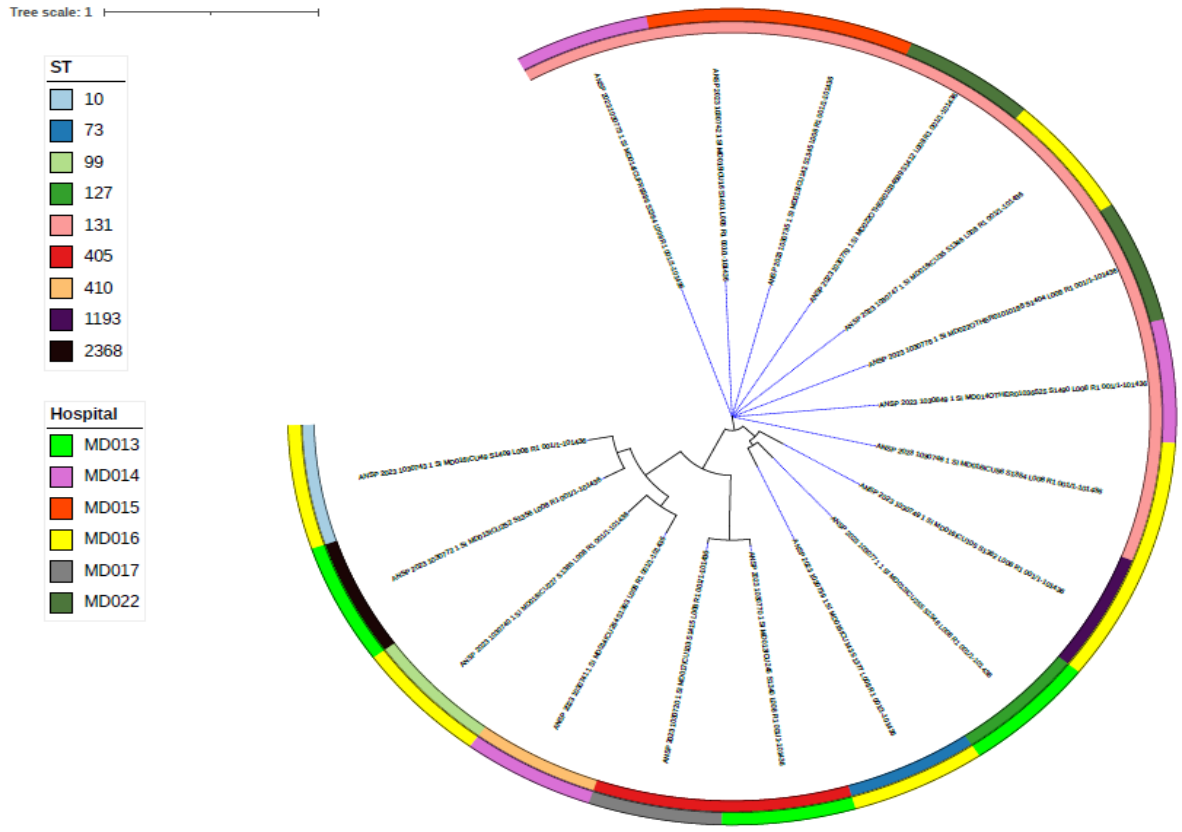

Figure 5. **Phylogenetic analysis of *E. coli* isolates based on whole-genome multilocus sequence typing (MLST, iTOL)**

Phylogenetic analysis of *E. coli* isolates enabled tracking of strain dissemination across medical institutions in the country. The first *E. coli* ST131 isolate was detected at IMSP MD014A in the intensive care unit, and subsequently two additional ST131 strains were isolated in another tertiary-level hospital (MD015A). Later, such isolates were identified in the intensive care units of three other medical institutions nationwide: MD014A – one strain, MD022A – two strains, and MD016A – two strains.

The *E. coli* isolates were typed using whole-genome sequencing, with a focus on detecting resistance genes.

All isolates were analyzed for antimicrobial resistance using bioinformatic methods across the antibiotic classes studied: aminoglycosides, beta-lactams, macrolides, sulfonamides, tetracyclines, and trimethoprim (Figure 6). In the heatmap, red indicates the presence of resistance genes, while green indicates their absence.

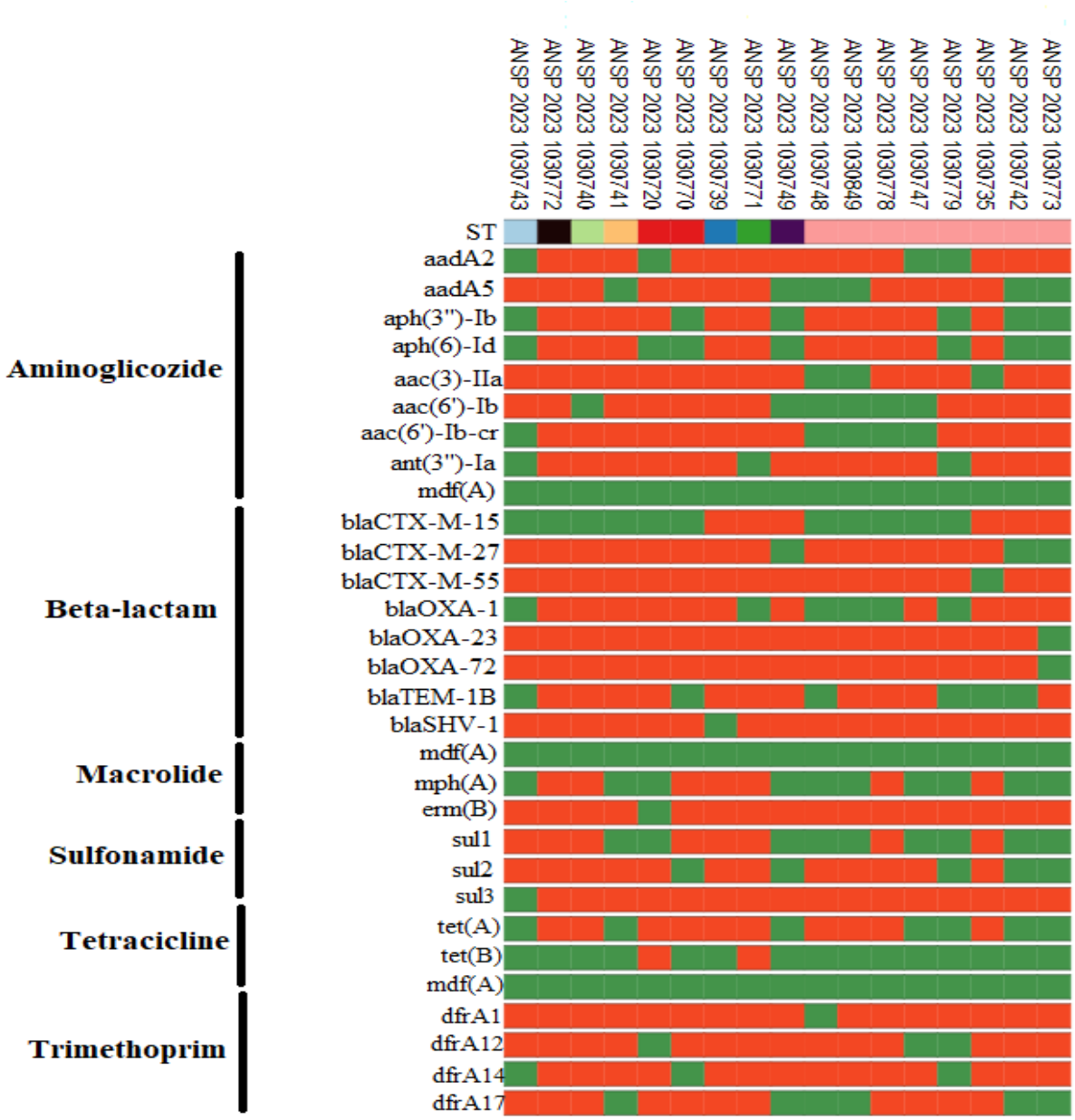

Figure 6. **Typing of *E. coli* isolates and bioinformatic genome analysis (ResFinder, iTOL)**

Analysis of the genome sequences revealed 30 acquired antimicrobial resistance genes spanning six different classes.

Within the CTX-M family, the *bla*CTX-M-15 gene was detected in six isolates, *bla*CTX-M-27 in 14 isolates, and *bla*CTX-M-55 was present in nearly all isolates (six in total).

The blaOXA-1 gene appeared in 11 *E. coli* genomes, while *bla*OXA-23 and *bla*OXA-72 were found in most of the sequenced isolates (16 samples), with only one isolate lacking these genes.

Among the β-lactamase genes, *bla*TEM-1B was identified in 11 isolates.

A total of nine aminoglycoside resistance genes were detected: *aadA2* in 13 isolates; *aadA5, aph(3'')-Ib*, and *aac(6')-Ib* each in 11 isolates; *aac(6')-Ib-cr* in 12; *aph(6)-Id* in ten; and both *aph(3')-IIa* and *ant(3')-Ia* in 14 isolates each.

The macrolide resistance gene *erm(B*) was found in almost all analyzed genomes, while the *mdf(A*) gene was not detected in any isolate.

The sulfonamide resistance gene *sul1* appeared in the genomes of eight *E. coli* strains, *sul2* in twelve strains, and *sul3* in 16 strains.

Among the four genes linked to trimethoprim resistance, *dfrA1* was present in 16 isolates, *dfrA12* and *dfrA14* each in 14 isolates, and *dfrA17* in 11 isolates (Figure 6).

Acinetobacter baumannii

Whole-genome sequencing of *A. baumannii* offers deeper insight into the evolutionary relationships within groups of isolates, their virulence potential, and antibiotic resistance profiles. Like the previously mentioned isolates, based on single-nucleotide polymorphism genomic analysis of *A. baumannii* strains helps establish correlations between isolates and trace epidemiological connections.

To assess the whole-genome sequencing data from *A. baumannii* strains isolated from patient clinical samples, a phylogenetic tree was built using 27 isolates based on single-nucleotide polymorphism analysis (Figure 7).

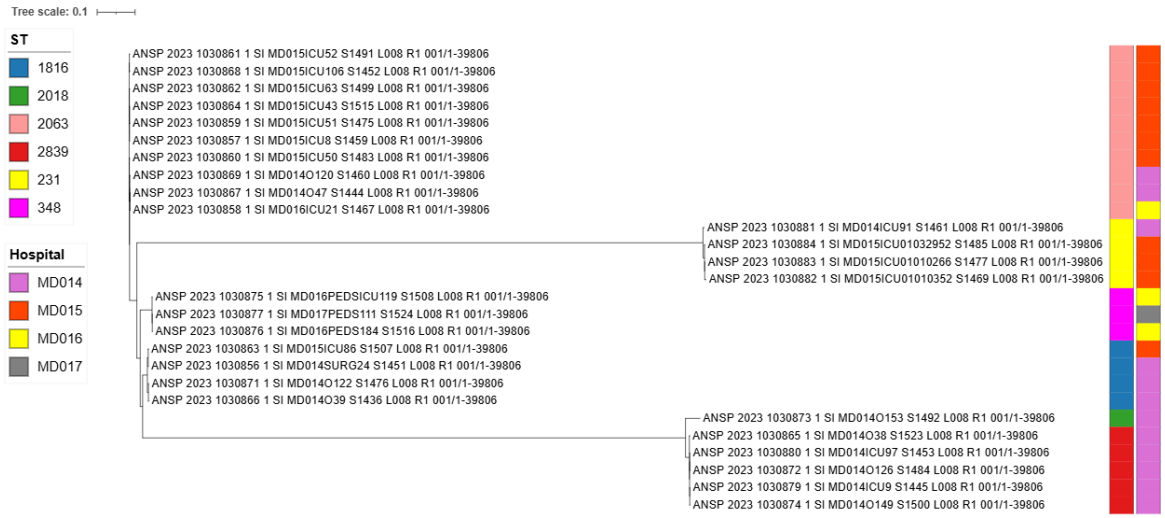

Figure 7. **Phylogenetic tree of *A. baumannii* strains based on SNP analysis and assembled according to MLST profiles (MLST, iTOL).**

All sequenced and analyzed *A. baumannii* isolates were grouped into six clusters according to their sequence types, with each cluster containing between one and ten isolates. MLST phylogenetic analysis showed that sequence type ST2063 was the most common, identified in ten isolates from clinical samples, followed by ST2839 in five isolates, and ST231 and ST1816, each found in four isolates.

It was observed that the first isolates of *A. baumannii* ST2063 and *A. baumannii* ST2839 initially appeared in two medical institutions: MD015A and MD014A. By the end of the study, analysis of the phylogenetic tree revealed that *A. baumannii* belonging to the six STs had been isolated from four medical institutions: MD014A – 8 strains (ST2839 – 5 strains, ST1816 – 4 strains, ST2018 – 1 strain, ST231 – 1 strain, ST2063 – 2 strains), MD015A – 11 strains (ST2063 – 7 strains, ST231 – 3 strains, ST1816 – 1 strain), MD016A – 3 strains (ST348 – 2 strains, ST2063 – 1 strain), MD017A – 1 strain, A. baumannii ST348.

4.2. Development of a standardized algorithm for detecting antimicrobial resistance mechanisms

Evaluating the accuracy of the tests used to identify ESBLs and carbapenemases — based on their sensitivity and specificity — helped identify the most reliable methods. These findings

were included in the algorithm developed based on the results (Figure 8), who will guide clinicians in making informed treatment decisions.

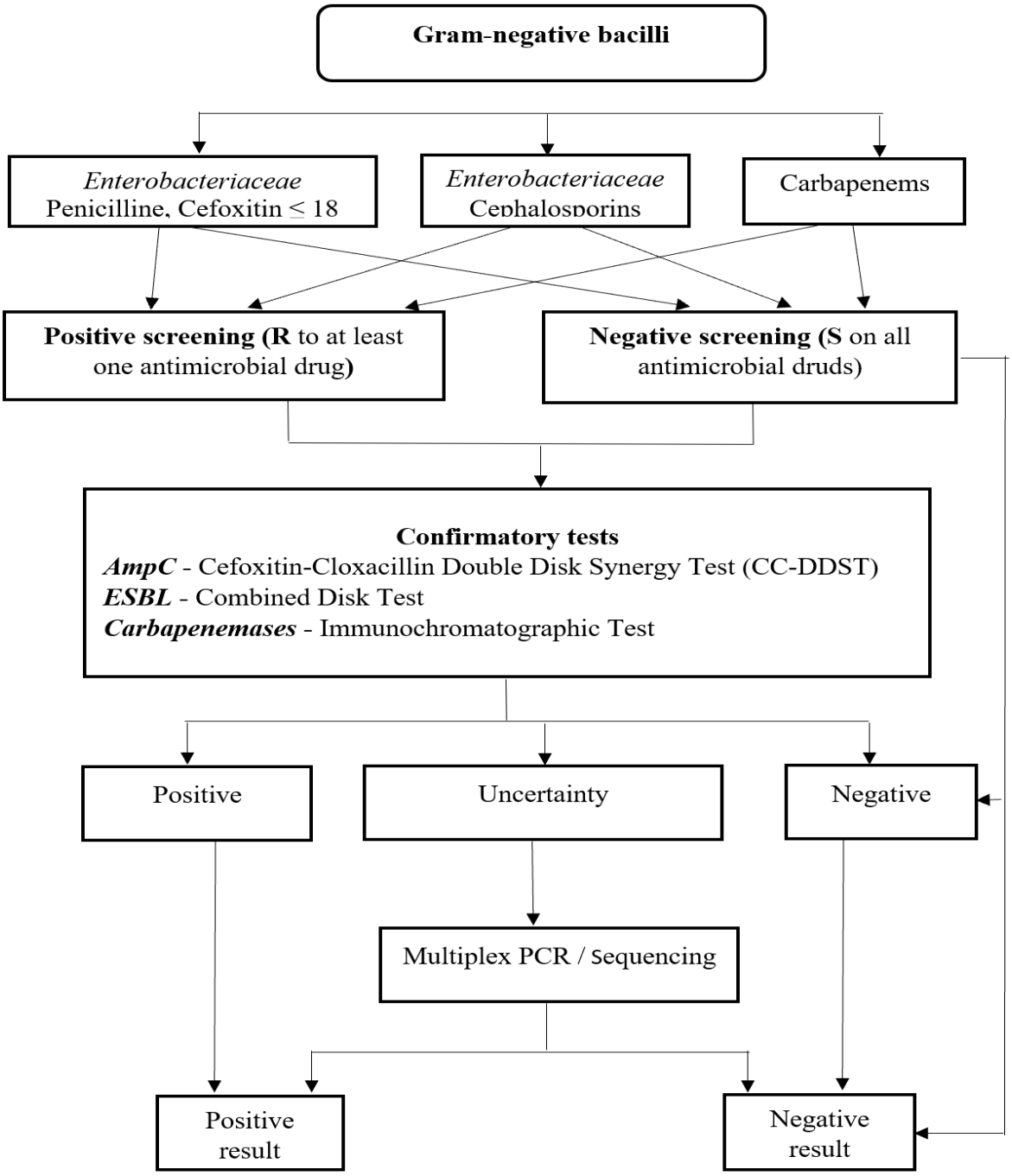

Figure 8. **Algorithm for detecting antimicrobial resistance mechanisms in GNB**

For detecting ESBL, the combined disc test showed the highest sensitivity and specificity among the three phenotypic methods used.

This test also offers the advantage of being able to process many strains at once, while remaining easy to use and cost-effective. The disadvantage of this test is the prolonged time required to obtain results, ranging from 24 to 48 hours, compared with only a few minutes for carbapenemase detection tests.

Of the screening tests evaluated for carbapenemase-producing strains, the immunochromatographic RESIST-5 OKNVI test stood out for its excellent sensitivity and specificity. It also delivers results quickly—often within ten minutes—and, as a commercial test, it is reasonably priced and accessible.

Although PCR is considered the gold standard for detecting AMR, its widespread use in microbiology labs across the country is limited. The technique requires costly equipment and reagents, as well as highly trained, specialized staff, which restricts its broader implementation.

GENERAL CONCLUSIONS

1. Evaluation of resistance patterns among the GNB included in the study (n = 3003) revealed high and very high levels of resistance to the antimicrobials used. *A. baumannii* proved to be resistant to virtually all antimicrobial classes, including 88.4% (95% CI, 85.6–93.9) to carbapenems and over 90% to other drug classes. *P. aeruginosa* showed carbapenem resistance in 70.4% (95% CI, 63.6–79.5) of isolates, while *K. pneumoniae* exhibited resistance to cephalosporins in 93.9% (95% CI, 85.6–93.9) of cases and to penicillins in 88.6% (95% CI, 85.9–94.2). Statistically significantly higher resistance levels were observed in isolates from blood and CFS compared with urine isolates, particularly those collected from patients in intensive care and surgical wards, compared with other hospital departments.
2. Of the 3003 GNB strains investigated, at least one antimicrobial resistance mechanism was detected in 87.2% (95% CI, 81.4–92.8). ESBL production was confirmed in 90.3% (95% CI, 88.0–92.0) of the suspected *E. coli* isolates, while carbapenemase production was most frequently detected in *K. pneumoniae*—96.9% (95% CI, 95.0–98.0) of the suspected strains—with OXA-48 being the predominant enzyme in both species. *P. aeruginosa* most frequently produced the NDM beta-lactamase, whereas *A. baumannii* predominantly produced OXA-23. Statistically significantly higher resistance was detected in *K. pneumoniae* and *P. aeruginosa* strains producing two or three types of carbapenemases.
3. Phylogenetic tree analysis of the sequenced strains highlighted the main clusters with the most frequent sequence types (STs) among species circulating nationwide: ST131 in 47.1% of *E. coli* strains, ST395 in 56.6% of *K. pneumoniae* strains, and ST2063 in 37.0% of *A. baumannii* strains. Tracking the spread of antimicrobial resistance showed that initially eight strains with the aforementioned sequence types were detected in four medical institutions, whereas after three years, 26 strains were identified across different wards in 14 medical institutions nationwide.
4. The assessment of phenotypic tests for detecting resistance mechanisms revealed that the combined disc test had the highest sensitivity and specificity for ESBL detection (99.0% and 98.9%, respectively), with no statistically significant difference compared to other tests performed ($p > 0.001$). For carbapenemase detection, the immunochromatographic method showed a sensitivity of 86.2% and specificity of 91.7%, which was significantly better than the other methods used ($p < 0.001$).
5. By comparing these phenotypic methods to molecular biology techniques (PCR, sequencing) as the gold standard, a standardized algorithm was developed to select the most effective methods based on cost-efficiency, time, and the resources available in laboratories across the country.
6. Monitoring the GNB resistance profiles associated to infectious diseases is crucial for tracking the spread of AMR and identifying new treatment options. It also underlines the implementation of national strategies to curb this issue, highlighting the urgent need for a comprehensive and effective system of monitoring and control.

RECOMMENDATIONS

1. To encourage close collaboration between clinicians and laboratory experts to select appropriate tests and accurately interpret results, ensuring effective guidance for antimicrobial treatment.
2. Tp regularly update and adapt national clinical guidelines and protocols, while improving strategies to control AMR.
3. To implement the newly developed algorithm for detecting resistance mechanisms in GNB, based on research findings, to enhance the quality and reliability of AMR surveillance data.
4. To closely monitor hypervirulent strains and investigate healthcare-associated infection outbreaks to identify sources and prevent their spread within medical facilities.
5. To implement antimicrobial stewardship programs across all healthcare institutions nationwide.
6. To use the research findings to reinforce the National Program for AMR Surveillance and Control (2019–2028), including systematic evaluation of key progress indicators.

SUGGESTIONS FOR FUTURE RESEARCH

1. To investigate genetic factors driving healthcare-associated infections using microbiological methods.
2. To analyze the economic impact of antimicrobial-resistant GNB infections in Moldova and future outlook.
3. To evaluate antimicrobial consumption in medical institutions in relation to the rise of MDR organisms.
4. To conduct whole-genome sequencing and phylogenetic studies of GNB (such as *Salmonella* spp. and *E. coli*) involved in acute diarrheal diseases and outbreak dynamics.
5. To assess antimicrobial use in healthcare settings and its correlation with the emergence of resistance mechanisms in circulating microbes.
6. To identify microbial virulence factors and forecast the evolution of AMR.

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- **Abstracts/summaries in proceedings of international scientific conferences:**

1. **Anton M**. Analiza filogenetică a tulpinilor de *Escherichia coli* și *Klebsiella pneumoniae* izolate din sânge. În: Patrimoniul cultural de ieri – implicaţii în dezvoltarea societăţii durabile de mâine. Supliment al revistei științifice „*Authentication and Conservation of Cultural Heritage. Research and Technique*", Chișinău. Iași-Chișinău-Lviv: 11-12 februarie 2025, Ediția 11, p. 364. ISSN 2558 – 894X.
2. **Anton M**. Evaluarea rezistenței *Pseudomonas aeruginosa* și *Acinetobacter baumannii* la diferite clase de antibiotice în ultimii 10 ani. În: Patrimoniul cultural de ieri –

implicaţii în dezvoltarea societăţii durabile de mâine. Supliment al revistei ştiinţifice „*Authentication and Conservation of Cultural Heritage. Research and Technique*", Chişinău. Iași-Chișinău-Lviv: 11-12 februarie 2025, Ediția 11, p. 470. ISSN 2558 – 894X.

- **Abstracts/ summaries in proceedings of national scientific conferences with international participation**

3. Burduniuc O., **Bivol M.**, Brinza O., Craciun O., Balan G. Emergence of carbapenem-resistant enterobacteriaceae: overview of a major public health challenge. *One Health & Risk Management (Materials of the National Scientific Conference with international participation „One health" approach in a changing world*), 2021, 2(4S), p. 29. Available at: https://journal.ohrm.bba.md/index.php/journal-ohrm-bba-md/article/view/193
4. **Anton M**., Mihalachi N., Burduniuc O. Analysis of antimicrobial resistance in clinical strains of *Klebsiella pneumoniae*, In: *One Health & Risk Management (Materialele Conferinței Naționale cu participare internațională „O singură sănătate – realizări și provocări"),* 2023, nr.2(S_Rez), supl. nr. 1, p. 12. Available at: https://journal.ohrm.bba.md/index.php/journal-ohrm-bba-md/article/view/591
5. **Anton M**., Burduniuc O., Neronova N., Balan G. Antimicrobial resistance analysis of clinical Escherichia coli. *One Health & Risk Management (Materialele Conferinței Naționale cu participare internațională „O singură sănătate – realizări și provocări")*, 2023, p. 49. Available at: https://journal.ohrm.bba.md/index.php/journal-ohrm-bba-md/article/view/503
6. Grumeza M., **Anton M**., Burduniuc A. The role of the microbiological laboratory in diagnosing the resistance of microorganisms to antimicrobials: literature review. In: *One Health and Risk Management (Materialele Conferinței Naționale cu participare internațională „Abordarea O singură sănătate – realizări și provocări")* 2023, nr. 2(S_Rez), supl. nr. 1, p. 16. ISSN 2587-3458. https://ibn.idsi.md/ro/vizualizare_articol/191974 7.4.

- **Abstracts/summaries in proceedings of national scientific conferences**

7. **Anton (Bivol) M**., Tapu L., Burac O., Lozneanu I., Burduniuc O. Antimicrobial resistance of gram-negative bacilli isolated from invasive infections. In: *Revista de Ştiinţe ale Sănătăţii din Moldova (Culegere de rezumate ale Conferinței Științifice Anuale „Cercetarea în biomedicină și sănătate: Calitate, excelență și performanță")*, 2022, nr. 3 An.1(29), p. 126. ISSN 2345-1467. https://ibn.idsi.md/vizualizare_articol/168324
8. **Anton M**., Mihalachi N., Bălan G. Caracterizarea genetică a tulpinilor de *Acinetobacter baumannii* multirezistente la antimicrobiene. În: *One Health and Risk Management (Materialele Conferinței Științifico-practice Naționale „Fiecare doză de vaccin contează")*, Ediție specială, 2023, nr. 1(S), p.58. ISSN 2587-3458. https://ibn.idsi.md/ro/vizualizare_articol/183534/datacite
9. Bunescu I., Holban T, Burduniuc O, **Anton M**., Sinițîna I. Clinical- evolutionary and diagnostic particularities in septicemia. În: *Moldovan Journal of Health Sciences, Culegere de rezumate ale Conferinței Științifice Anuale „Cercetarea în biomedicină și sănătate: Calitate, excelență și performanță"*, 19-21 octombrie 2022, Anexa 1, p.130,

ISSN 2345-1467. Disponibil la https://repository.usmf.md/handle/20.500.12710/24579

- **Active participation with presentations and posters at scientific events:**

✓ International

1. **Anton M**. Analiza filogenetică a tulpinilor de *Escherichia coli* și *Klebsiella pneumoniae* izolate din sânge. *Conferința științifică internațională „Patrimoniul cultural de ieri – implicații în dezvoltarea societății durabile de mâine”. Iași-Chișinău-Lviv*, Chișinău 11-12 februarie 2025 (sectiunea 14, pag. 25)
2. **Anton M.,** Perjeru M., Lozneanu I., Țapu L., Croitoru C., Bălan G., Burduniuc O. Method for raising the degree of awareness in children about the prevention of antimicrobial resistance. *International exhibition of innovation and technology transfer EXCELLENT IDEA – 2nd edition*. Chisinau, 18 septembrie 2023.

✓ National

3. **Anton M**. Rezistența la antimicrobiene a bacililor Gram negativi izolați din infecții invazive. *Conferința științifică anuală „Cercetarea în biomedicină și sănătate: calitate, excelență și performanță*. Chișinău, 19-21 octombrie 2022.
4. **Anton M**. Antimicrobiene: clasificare, mecanisme de acțiune. Rezistența microorganismelor la antimicrobiene (RAM). *Workshop medical: Programele de stewardship antimicrobian – elemente esențiale în prevenirea rezistenței la antimicrobiene, Conferința științifică anuală „Cercetarea în biomedicină și sănătate: calitate, excelență și performanță.* Chișinău 19-21 octombrie 2022.
5. **Anton M**. Analiza rezistenței antimicrobiene a tulpinilor clinice de *Escherichia coli*. *Săptămâna medicală balcanică, ediția a XXXVII-a „Perspective ale medicinei balcanice în era post COVID-19”.* Chișinău 7-9 iunie 2023.
6. **Anton M**. Importanța testării microbiologică a hemoculturilor. Infecțiile invazive cu bacili gramnegativi rezistenți la antimicrobiene. *Conferința națională cu participare internațională „Actualități în pediatrie și impactul imunizării asupra morbidității și mortalității copiilor în Republica Moldova.* Chișinău, 22-23 septembrie 2023.
7. **Anton M**. Analiza rezistenței patogenilor gram-negativi non-fermentativi de importanță clinică. *Conferința științifică anuală „Cercetare în biomedicină și Sănătate: Calitate, excelență și performanță”*. Chișinău, 18-20 octombrie 2023.
8. **Anton M.** Sistemul de Supraveghere Epidemiologică a rezistenței microorganismelor la antimicrobiene în Republica Moldova. *Conferința națională cu participare internațională „Abordarea O Singură Sănătate – realizări și provocări”.* Chișinău, 23-24 noiembrie 2023.
9. **Anton M**. Analysis of antimicrobial resistance in clinical strains of Klebsiella pneumoniae. *Conferința națională cu participare internațională „Abordarea O Singură Sănătate – realizări și provocări”.* Chișinău, 23-24 noiembrie 2023.
10. **Anton M**. Sistemul de supraveghere epidemiologică a rezistenței microorganismelor la antimicrobiene în Republica Moldova. *Conferința națională „Sănătatea și fenomenul rezistenței la antimicrobiene în țările cu venituri mici și medii din Europa de Est”*. Chisinău, 27 ianuarie 2024.

11. **Anton M**., Perșeru M., Lozneanu I., Colac S. Metodă de creștere a gradului de conștientizare la copii cu privire la prevenirea rezistentei la antimicrobiene. *Nopatea tinerilor cercetători*. Chișinău, 29 septembrie 2023.

ADNOTARE

La tema tezei de doctor în științe medicale a doctorandei Anton Maria: „Rezistența la beta-lactamine a bacililor Gram-negativi izolați din biosubstrate clinice".

Specialitatea 313.02 – Microbiologie, virusologie medicală.

Actualiatate. Rezistența BGN la antimicrobiene reprezintă una dintre cele mai stringente probleme de sănătate publică la nivel global. În ultimele două decenii, patogenii *E. coli, K. pneumoniae, P. aeruginosa* și *A. baumannii* au dezvoltat mecanisme complexe de rezistență, incluzând producerea de beta-lactamaze cu spectru extins (ESBL) și carbapenemaze.

Scopul lucrării: Evaluarea profilurilor de rezistență la β-lactamine și stabilirea grupurilor filogenetice ale BGN prioritari cu elaborarea algoritmului metodologic standardizat de detectare a mecanismelor de rezistență.

Obiectivele lucrării: determinarea profilurilor de rezistență la antimicrobiene ale BGN izolați din diferite biosubstraturi clinice; caracterizarea moleculară a BLSE și a carbapenemazelor aferente BGN; analiza comparativă a diferitor tehnici microbiologice de identificare a BGN rezistenți la beta-lactamine cu dezvoltarea unui algoritm metodologic standardizat; propunerea de măsuri eficiente de prevenire și control a infecțiilor cauzate de BGN multirezistenți.

Noutatea şi originalitatea ştiinţifică: S-a realizat un studiu complex prin utilizarea motodelor de biologie moleculară, care a permis aprecierea şi evaluarea poziţiei BGN circulanți pe teritoriul țării în arborii filogenetici globali, lucru important pentru argumentarea tendinței evolutive a rezistenței BGN și argumentarea terapiei empirice la pacienții cu astfel de infecții.

Rezultatele obținute au stat la baza elaborarării unui algoritm standardizat care urmează a fi implelentat în laboratoarele microbiologice din cadrul retelei de supraveghere a RAM.

Rezultate obținute: a fost identificat spectrul microbiologic și diversitatea genotipică a BGN prioritari, inclusiv prevalența tulpinilor de BGN producătoare de BLSE și de carbapenemaze; spectrul de enzime la BGN; tipul de secvență predominant pe teritoriul țării. A fost elaborat un algoritm standardizat de determinare a markerilor rezistenței și propuse de măsuri îmbunătățire pentru supravegherea și controlul RAM bazate pe dovezi

Semnificaţia teoretică: Studiul realizat va aduce un aport semnificativ la actualizarea și metodologiei de determinare a mecanismelor de rezistență la preparatele antimicrobiene.

Valoarea aplicativă: Rezultatele au fost incluse în programele de învățământ pentru studenţi, rezidenţi şi medici. De asemenea au fost elaborate materiale utile, inclusiv ghiduri pentru îndrumarea medicilor în activitatea profesională şi pliante informative pentru conștientizarea populaţiei despre problema RAM.

Implementarea rezultatelor științifice: elaborarea algoritmului de determinare a mecanismelor de rezistență la antimicrobiene ale BGN; elaborarea a 3 ghiduri pentru personal medical și implementarea lor in diferite instituții medicale și o indicație metodică inclusă în programul de instruire pentru studenți, rezidenți și în cadrul cursurilor de specializare.

Structura tezei: introducere, patru capitole, concluzii generale şi recomandări, bibliografie (165) titluri, 9 anexe, 65 pagini de text de bază, 14 tabele şi 31 figuri. Rezultatele sunt publicate în 28 lucrări științifice.

Cuvinte-cheie: bacili Gram-negativi, rezistența la antimicrobiene, mecanisme de rezistență, beta-lactamaze cu spectru extins, carbapenemaze.

ANNOTATION

For the doctoral thesis in medical sciences of the PhD candidate Anton Maria: "Beta-actam Resistance of Gram-negative Bacilli Isolated from Clinical Biosubstrates."

Specialty 313.02 – Microbiology, Medical Virology.

Relevance. Antimicrobial resistance among Gram-negative bacilli (GNB) is one of the most pressing global public health challenges. Over the past two decades, pathogens such as E. coli, K. pneumoniae, P. aeruginosa, and A. baumannii have developed complex resistance mechanisms, including the production of extended-spectrum beta-lactamases (ESBLs) and carbapenemases.

Aim: To evaluate the β-lactam resistance profiles and establish the phylogenetic groups of priority GNB, with the development of a standardized methodological algorithm for detecting resistance mechanisms.

Objectives: determination of antimicrobial resistance profiles of GNB isolated from various clinical biosubstrates; molecular characterization of ESBLs and carbapenemases in GNB; comparative analysis of different microbiological techniques used to identify beta-lactam–resistant GNB and development of a standardized methodological algorithm; proposal of effective measures for the prevention and control of infections caused by multidrug-resistant GNB.

Scientific novelty and originality: A comprehensive study was carried out using molecular biology methods, allowing the assessment and positioning of circulating GNB in the country within global phylogenetic trees—an important aspect for understanding the evolutionary trends of GNB resistance and justifying empirical therapy in patients with such infections.

The obtained results formed the basis for developing a standardized algorithm to be implemented in microbiology laboratories within the national AMR surveillance network.

Results obtained: The microbiological spectrum and genotypic diversity of priority GNB were identified, including the prevalence of ESBL- and carbapenemase-producing strains; the spectrum of resistance enzymes; and the predominant sequence type circulating in the country. A standardized algorithm for determining resistance markers was developed, and evidence-based improvements for AMR surveillance and control were proposed.

Theoretical significance: The study provides a significant contribution to updating and improving the methodology for determining antimicrobial resistance mechanisms.

Practical value: The results have been included in educational programs for students, residents, and physicians. Useful materials were developed, including guidelines for assisting healthcare professionals in their practice and informational leaflets to raise public awareness about AMR.

Implementation of scientific results: Development of an algorithm for determining antimicrobial resistance mechanisms in GNB; elaboration of three guidelines for healthcare personnel and their implementation in various medical institutions; and preparation of a methodological instruction included in training programs for students, residents, and specialization courses.

Structure of the thesis: Introduction, four chapters, general conclusions and recommendations, bibliography (165 titles), 9 appendices, 65 pages of main text, 14 tables, and 31 figures. The results are published in 28 scientific works.

Keywords: Gram-negative bacilli, antimicrobial resistance, resistance mechanisms, extended-spectrum beta-lactamases, carbapenemases.