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PATHOGENIC AGENTS OF ACUTE DIARRHEAL DISEASES - MORPHO-CULTURAL PECULIARITIES, IDENTIFICATION METHODS, ANTIBIOTIC RESISTANCE AND THE SPREAD DYNAMICS IN CHISINAU

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CONTENT

CONCEPTUAL GUIDELINES OF RESEARCH……. 4
THESIS CONTENT……. 7
INTRODUCTION. 7
1. PATHOGENIC AGENTS OF ACUTE DIARRHEAL DISEASES: DIVERSITY, MICROBIOLOGICAL IDENTIFICATION AND ANTIMICROBIAL RESISTANCE. 7
2. MATERIALS AND METHODS……. 8
3.ETIOLOGICAL STRUCTURE OF PATHOGENIC AGENTS THAT CAUSE ACUTE DIARRHEAL DISEASES. 9
3.1. Verification of the performance of the automated method for identification of microorganisms and antimicrobial susceptibility testing …… 9
3.2. The spectrum and share of pathogens involved in the etiology of ADD..... 10
3.3. Morphocultural characteristics of pathogens that cause ADD……..... 13
3.4. The dynamics of the spread of ADD in PHMI TMA Buiucani, Chisinau municipality……... 14
4. PATHOGENIC AGENTS INVOLVED IN ACUTE DIARRHEA DISEASES AND THEIR SENSITIVITY TO ANTIBIOTICS. 21
4.1. Pathogenic and conditionally pathogenic agents causing acute diarrheal diseases and their sensitivity to antimicrobials..... 21
4.2. Conditionally pathogenic agents involved in the etiology of enterocolitis and their sensitivity to antimicrobials..... 22
4.3. Comparative analysis of the antibiotic resistance profile of enterobacteria involved in the etiology of acute diarrheal diseases ……..…. 23
GENERAL CONCLUSIONS. 27
RECOMMENDATIONS FOR IMPLEMENTATION. 28
RECOMMENDATIONS FOR CONTINUING RESEARCH ..…… 29
SELECTIVE BIBLIOGRAPHY. 29
PUBLICATIONS ON THE TOPIC OF THE THESIS. 31
ANNOTATION (in Romanian). 33
ANNOTATION (in English). 34
ANNOTATION (in Russian). 35

ABBREVIATION LIST

ADD – Acute diarrheal diseases

MIC – minimum inhibitory concentration

EUCAST – European Committee on Antimicrobial Susceptibility Testing

PHMI TMA Buiucani – Public Health Medical Institution, Territorial Medical Association Buiucani

WHO – World Health Organization

CFU/g – Colony-forming units/gram

UNICEF – United Nations International Children's Emergency Fund

CONCEPTUAL GUIDELINES OF RESEARCH

The actuality of the topic. Acute diarrheal diseases (ADD) are a growing public health problem, with a significant impact on both the health status of the population and the socio-economic system. ADD is one of the main causes of morbidity and mortality worldwide, with a major impact especially in developing countries, and among children under 5 years of age. According to data from the World Health Organization (WHO) and UNICEF, approximately 2 billion cases of diarrheal diseases are recorded worldwide annually, responsible for 1.6–1.9 million deaths in children under 5 years of age, with a significant prevalence in developing countries [20, 22]. ADD have a diverse etiology in which bacteria play an important role, especially in regions with variable socio-economic and sanitary conditions, such as the Republic of Moldova [14]. In the Republic of Moldova, ADD remains a relevant public health problem, especially among children, which justifies the need for continued research and implementation of prevention strategies [12, 14].

Both globally and nationally, one of the greatest current threats to public health is antibiotic resistance. Pathogens have developed a wide range of defense mechanisms that allow them to survive the action of antibiotics. This phenomenon is a natural biological process that occurs during the use of antibiotics, but is accelerated by their incorrect or excessive use [15, 21]. In recent decades, the increase in antibiotic resistance has become a major problem, which requires rational use based on etiological diagnosis [24, 25].

In the Republic of Moldova, research on the role of pathogens in the etiology of acute diarrheal diseases and antimicrobial resistance profiles is still limited, and the available data come mainly from local studies, surveillance reports and investigations carried out on specific groups of microorganisms. Therefore, it is necessary to expand epidemiological and microbiological research at the national level for a more complete characterization of the etiological spectrum of acute diarrheal diseases and the trends in the evolution of antimicrobial resistance.

Description of the situation in the field of research and identification of research problems. In the Republic of Moldova, research on ADD is closely linked to the evolution of the public health system, socio-economic transformations and the progress of diagnostic and epidemiological surveillance methods. The analysis of this path highlights the transition from a predominantly descriptive and reactive approach to a modern one, based on prevention, continuous monitoring and multidisciplinary research. In the last decade, research on ADD has evolved significantly, integrating modern methods of microbiological diagnosis and advanced epidemiological analyses. Studies conducted within national institutions have highlighted the predominance of food and water transmission, the role of communities in the emergence of outbreaks and the important share of viral agents, especially rotavirus, in children [12].

Currently, in the Republic of Moldova, research is focused on an integrated approach to DID, including epidemiological, microbiological and environmental factors. The main directions are aimed at

developing digital surveillance systems, studying the impact of climate change on water-borne diseases, evaluating the effectiveness of public health interventions and promoting health education in the population. These approaches contribute to improving the capacity to prevent and control diarrheal diseases and adapting public health strategies to new global challenges. Studies conducted at the national level have highlighted that ADD outbreaks have an uneven territorial distribution, being more frequent during the warm period of the year and frequently associated with risk factors in collective institutions for children [12]. At the same time, the importance of strengthening epidemiological surveillance systems and using real-time data for monitoring and controlling communicable diseases is emphasized.

Current microbiological research aims not only to identify pathogens involved in the etiology of ADD, but also to monitor the phenomenon of antimicrobial resistance, considered a major challenge for public health. Antimicrobial resistance represents one of the most important threats to public health worldwide, being recognized by the World Health Organization as a major problem that compromises the effectiveness of the treatment of bacterial, viral, fungal and parasitic infections [23]. Globally, antimicrobial resistance was associated with approximately 4.95 million deaths in 2019, of which 1.27 million were directly attributed to infections caused by resistant microorganisms [24]. In 2024, the United Nations Member States adopted the first global targets for reducing mortality associated with bacterial antimicrobial resistance by 2030, highlighting the need to strengthen surveillance systems, rational use of antibiotics and implementation of the "One Health" approach [26]. In the Republic of Moldova, combating antimicrobial resistance is a priority of public health policies. National studies highlight the need for continuous monitoring of resistant bacterial strains and strengthening laboratory capacities for early detection of resistance mechanisms, especially in the case of enterobacteria and other pathogens involved in healthcare-associated infections [2, 3, 11]. Thus, both at the international and national levels, antimicrobial resistance is considered a complex challenge that requires coordinated measures for prevention, surveillance and prudent use of antimicrobials.

Scientific research methodology. The methodological and theoretical-scientific support was provided by the works of researchers in the field of microbiology, epidemiology and diagnosis of intestinal infections [1, 10, 13, 17, 16, 18, 19]. The research was carried out through an integrated microbiological and epidemiological approach, aimed at characterizing the pathogens involved in acute diarrheal diseases and assessing their sensitivity to antimicrobials. The etiological confirmation of the investigated cases was carried out by the coproculture method, according to the methodological indication "Microbiological diagnosis of intestinal infections", recommended by the Ministry of Health of the Republic of Moldova [10]. Data from the National Agency for Public Health Annual Reports for the period 2011–2024 were used to analyze and evaluate the multiannual dynamics of acute diarrheal diseases. Statistical processing, interpretation and graphical representation of the results were performed using contemporary data analysis

methods and specialized computer programs, which allowed the evaluation of the etiological structure, taxonomic diversity and current trends in antimicrobial sensitivity and resistance.

The aim of the thesis was to elucidate the taxonomic diversity and morpho-cultural particularities of pathogens of acute diarrheal diseases, implement microbiological identification methods, determine antibiotic sensitivity and resistance profiles and analyze the dynamics of their spread in the city of Chisinau.

To achieve this goal, the following objectives were set:

1. Determining the etiological structure and taxonomic diversity of pathogens involved in acute diarrheal diseases within TMA Buiucani, Chisinau municipality;

2. Characterizing the morpho-cultural peculiarities of the identified pathogens and highlighting their characteristics in relation to taxonomic affiliation;

3. Applying microbiological identification methods and evaluating their efficiency in identifying pathogens and determining antimicrobial susceptibility;

4. Analyzing the distribution and dynamics of pathogen circulation in relation to the study period, seasonality and age group structure of subjects with acute diarrheal diseases;

5. Determining antibiotic sensitivity and resistance profiles of isolated strains and highlighting current trends in antibiotic resistance.

Scientific novelty and originality. For the first time in Chisinau, based on microbiological monitoring carried out within the TMA Buiucani over an extended period (2011 – 2024), a complex characterization of pathogens involved in acute diarrheal diseases was carried out by integrating the analysis of the etiological structure, taxonomic diversity, morpho-cultural features and antimicrobial sensitivity profiles. The peculiarities of the circulation of pathogens were highlighted depending on the study period, seasonality and age group structure of patients, and trends in the change of the etiological structure and the spread of antibiotic resistance were identified. The study complements the existing data on the spectrum of bacterial agents involved in acute diarrheal diseases and provides an updated basis for interpreting their microbiological and epidemiological features in the conditions of Chisinau.

Scientific problem solved. The scientific problem regarding the characterization of the etiological structure and antimicrobial resistance of agents involved in acute diarrheal diseases within the TMA Buiucani was solved, by identifying the etiological spectrum, epidemiological particularities and circulation and resistance trends, providing scientific support for optimizing the surveillance, diagnosis and treatment of these infections.

Theoretical significance. New data were obtained, expanding and deepening knowledge on the etiological structure of acute diarrheal diseases, the taxonomic and morpho-cultural peculiarities of the pathogens involved, as well as the characteristics of their circulation and antimicrobial resistance in the conditions of the Chisinau municipality. The research results complement the existing data on the spectrum of etiological agents of ADD, their distribution by age and season, as well as current trends in antimicrobial

resistance, contributing to the development of the theoretical basis in the field of medical microbiology, epidemiology and infectology.

The applicative value of the work. Current data on the etiological structure of acute diarrheal diseases and antimicrobial susceptibility profiles of circulating pathogens within the TMA Buiucani, Chisinau municipality were provided. The results obtained can be used to optimize microbiological diagnosis, rational choice of antimicrobial therapy, monitoring antibiotic resistance and improving epidemiological surveillance and infection control measures. They can also serve as scientific support for the development of practical recommendations and strategies to prevent the spread of resistant bacterial strains.

Implementation of scientific results. The results of the study were implemented in practical activity as methodological support in the process of identification and confirmation of pathogens involved in acute diarrheal diseases within the Microbiological Laboratory Invitro Diagnostics SRL. The microbiological analyses from the Microbiological Laboratory of PHMI TMA Buiucani constituted the basic material for the development of the doctoral thesis in biological sciences, and the results obtained in the research are validated including through the National Epidemiological Surveillance System of antimicrobial resistance, of which the institution's laboratory is part, thus ensuring the relevance and credibility of the data. The research results are used and implemented in the teaching process, in the development of bachelor's and master's theses at educational institutions with a biological and ecological profile in the specialties: Biology, Molecular Biology, Applied Biological Sciences, Biology and Chemistry, Chemistry and Biology and Ecology.

Approval of scientific results. The thesis materials were discussed, approved and presented at 9 national and international scientific events.

Publications on the topic of the thesis. The results obtained from the research and the conclusions were presented in 14 scientific papers (12 of them without co-authors).

Key words: pathogens, acute diarrheal diseases, etiological structure, morpho-cultural features, spread dynamics, antimicrobial sensitivity.

THE CONTENT OF THE THESIS

The **Introduction** briefly presents the general characteristics of the work. The topicality and importance of the topic are argued, the purpose and objectives of the research are formulated, the research hypothesis is presented, the methodology used is described, and the novelty and scientific significance, the applied value of the obtained results, their approval and the structure of the thesis are highlighted.

1. PATHOGENIC AGENTS OF ACUTE DIARRHEAL DISEASES: DIVERSITY, MICROBIOLOGICAL IDENTIFICATION AND ANTIMICROBIAL RESISTANCE

Chapter 1 presents the theoretical foundation of research on acute diarrheal diseases (ADD), analyzing the evolution of knowledge about etiological agents, the development of microbiological identification methods and the problem of antimicrobial resistance. The main stages of research at the international level and in the Republic of Moldova are synthesized, highlighting the transition from classical microbiological methods to modern technologies of diagnosis and epidemiological surveillance. The taxonomic, biological and epidemiological characteristics of the main pathogens and opportunistic pathogens involved in ADD are described, *Salmonella* spp., *Shigella* spp., *Escherichia coli* with hemolytic properties, *Klebsiella* spp., *Proteus* spp., *Citrobacter* spp., *Enterobacter* spp., *Morganella* spp., *Providencia* spp., *Serratia* spp., *Hafnia* spp., *Pseudomonas aeruginosa*, *Aeromonas* spp., *Acinetobacter* spp. and *Acinetobacter* spp., and recent changes in taxonomic classification based on bacterial phylogenomics are analyzed. A separate section is dedicated to the evolution of antimicrobial therapy and the mechanisms of emergence and spread of bacterial resistance to antibiotics, with emphasis on the factors that favor the emergence of multiresistant strains and the need for continuous monitoring of antimicrobial susceptibility. The literature review highlights the importance of correct microbiological identification and surveillance of antimicrobial resistance for the selection of appropriate treatment, epidemiological control and reduction of the impact of acute diarrheal diseases on public health. Based on the study, the need for research was substantiated, the purpose and objectives of the thesis were formulated.

2. MATERIALS AND METHODS

The chapter presents the materials and methodology used to investigate the etiological agents of acute diarrheal diseases in the Microbiological Laboratory of PHMI TMA Buiucani, Chisinau municipality, during the years 2011–2024. The study was based on the analysis of 14335 clinical samples, from which 2976 bacterial strains were isolated, representing 14 taxa involved in the etiology of acute diarrheal diseases. Descriptive, microbiological, epidemiological and statistical methods were used to evaluate the etiological structure, taxonomic characteristics and antimicrobial susceptibility profiles. Identification of microorganisms and antibiotic susceptibility testing were performed by the classical microbiological method (2011–2018), based on cultivation, biochemical tests, serological confirmation and the Kirby–Bauer disk-diffusion method, and subsequently by the automated VITEK 2 Compact system (2019–2024), implemented and validated by comparing the results with the conventional method. The performance of the automated method was confirmed by the concordance of identification and antimicrobial susceptibility testing, without significant discrepancies. Statistical analysis included descriptive methods, correlational analyses, the χ^2 test and modern graphical representations, ensuring the rigorous assessment of the distribution of pathogens and the dynamics of antimicrobial resistance. The applied methodology constituted the experimental basis for achieving the research objectives and for the comparative interpretation of the obtained results.

3. ETIOLOGY OF PATHOGENS THAT CAUSE ACUTE DIARRHEAL DISEASES

3.1. Verification of the performance of the automated method for identification of microorganisms and antimicrobial susceptibility testing

In order to implement the VITEK 2 Compact automated system in the routine activity of the microbiological laboratory within PHMI TMA Buiucani, the performance of the automated method was verified by comparing the results obtained with those provided by the classical microbiological method, previously used as a reference method in the laboratory. The verification was carried out in 2019 and aimed to confirm the accuracy of the identification of microorganisms and the concordance of the results regarding antimicrobial sensitivity, under the specific working conditions of the laboratory. Identification by the classical microbiological method was based on the evaluation of morphological and cultural characteristics, supplemented by standardized biochemical tests (EnteroPluri-Test/API), and for *Salmonella* spp. and *Shigella* spp. the identification was additionally confirmed by agglutination with specific sera. In parallel, identification by the automated method was performed using the GN cards of the VITEK 2 Compact system, the results being automatically generated by comparing the biochemical profile of each strain with the international database integrated into the system. The results are presented in table 3.1.

Table 3.1. Identification agreement between the classical microbiological method and the VITEK 2 Compact system

Bacterial taxon	No. of strains	Concordance (%)
Shigella spp.	2	100
Salmonella spp.	4	100
Klebsiella spp.	47	100
Enterobacter spp.	17	100
Proteus spp.	17	100
Citrobacter spp.	7	100
E. coli hemolitică	10	100
Morganella spp.	3	100
P. aeruginosa	7	100
Total	**114**	**100**

The verification results revealed complete concordance of the identification of microorganisms, with no discrepancies between the classical microbiological method and the automated VITEK 2 Compact system, both at the genus and species level for all 114 investigated strains. Also, no significant differences were found in the interpretation of antimicrobial susceptibility between the two methods, the results falling within the acceptable limits provided by international recommendations on the verification of the performance of automated microbiological identification systems.

The results obtained confirm that the automated VITEK 2 Compact system ensures a correct and reproducible microbiological identification of the main etiological agents of acute diarrheal diseases and provides results comparable to those obtained by the classical microbiological method. The implementation

of this system allowed to reduce the time required for the identification of microorganisms and the determination of antimicrobial susceptibility, standardize the analytical process and reduce the influence of the human factor on the interpretation of the results, thus contributing to increasing the quality of microbiological diagnosis.

3.2. Spectrum and share of pathogens involved in the etiology of ADD

During the study period, from a total of 14335 clinical samples taken from patients with ADD, 2976 (20.8%) strains of enterobacteria were isolated and 14 pathogens involved in the etiology of ADD were identified: *Salmonella* spp. (serotypes *S.* Enteritidis and *S.* Typhimurium), *Shigella* spp. (species *S. flexneri* and *S. sonnei*), *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *Escherichia coli* with hemolytic properties, *Morganella* spp., *Serratia* spp., *Providencia* spp., *Hafnia* spp., *Pseudomonas aeruginosa*, *Aeromonas* spp. and *Acinetobacter* spp.

From an etiological point of view, *Klebsiella* spp. was predominant, representing 40.63% of the total isolated strains. This was followed by *Citrobacter* spp. – 12.77%, *Proteus* spp. – 12.63%, *Enterobacter* spp. – 10.11% and *E. coli* with hemolytic properties – 9.27%. Lower shares were recorded for *P. aeruginosa* – 6.05% and *Salmonella* spp. – 4.67%, while the other microorganisms were sporadically identified: *Serratia* spp. – 1.18%, *Providencia* spp. – 0.94%, *Morganella* spp. – 0.91%, *Shigella* spp. – 0.40%, *Aeromonas* spp. – 0.20%, *Hafnia* spp. – 0.17% and *Acinetobacter* spp. – 0.07% (fig. 3.1).

Analyzing the etiological structure of the isolated microorganisms, we mention a clear predominance of the *Klebsiella* genus, which had a share of over 40% of the total number of strains isolated in the study area. The results obtained confirm the major involvement of this genus in the etiology of ADD and deserve increased attention, and the presence of agents with low frequency, such as *Serratia* spp., *Providencia* spp., *Morganella* spp., *Shigella* spp., *Aeromonas* spp., *Hafnia* spp. and *Acinetobacter* spp. nevertheless confirms the existence of a varied etiology. Although the share of these microorganisms in the distribution of isolated strains is very low, their presence indicates a significant diversity of agents involved in ADD.

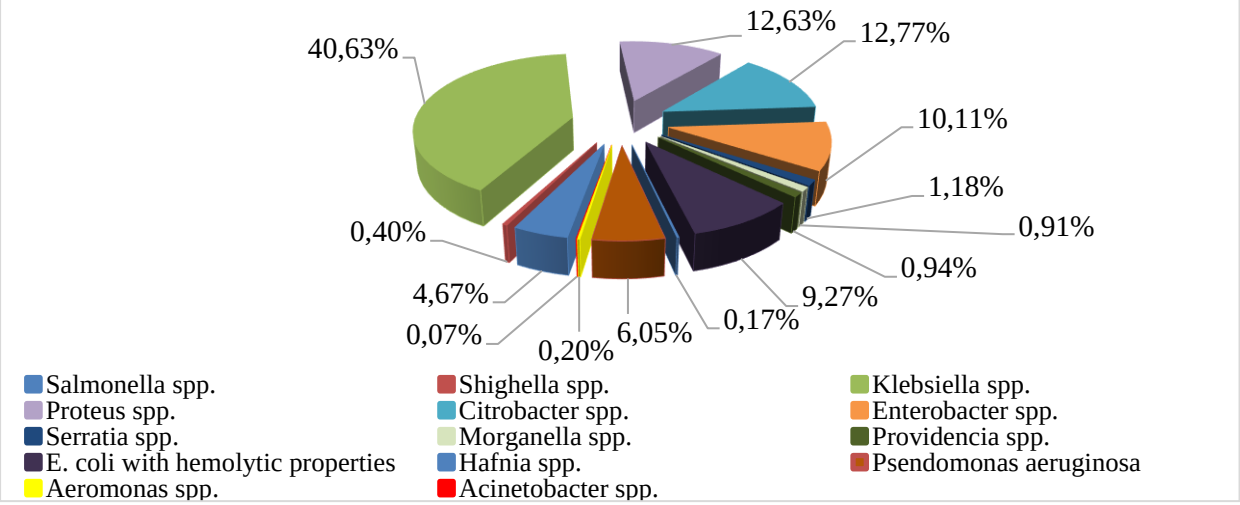


Figure 3.1. Spectrum and share (%) of pathogens isolated during the study period

Of the 14 isolated bacterial agents, two were identified as pathogens (*Salmonella* spp. and *Shigella* spp.), and the other 12 isolates were classified as conditionally pathogenic: *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *E. coli* with hemolytic properties, *Morganella* spp., *Serratia* spp., *Providencia* spp., *Hafnia* spp., *P. aeruginosa*, *Aeromonas* spp. and *Acinetobacter* spp. The strains of *Salmonella* spp. and *Shigella* spp. represented 5.07% (n=151) of the total number of isolated strains and 1.05% of the total number of investigated samples. Of these, the genus *Salmonella* was predominant, with a share of 92.05% (n=139), while *Shigella* spp. represented 7.95% (n=12). At the serotype level, *S.* Enteritidis was the most frequently identified (56.29%), followed by *S.* Typhimurium (35.77%), and *S. sonnei* with phases I (S) and II (R) and *S. flexneri* with serotypes 1–6 and variants X (6,7,8) and Y (3,4) were isolated in equal proportions, 3.97% each (fig. 3.2).

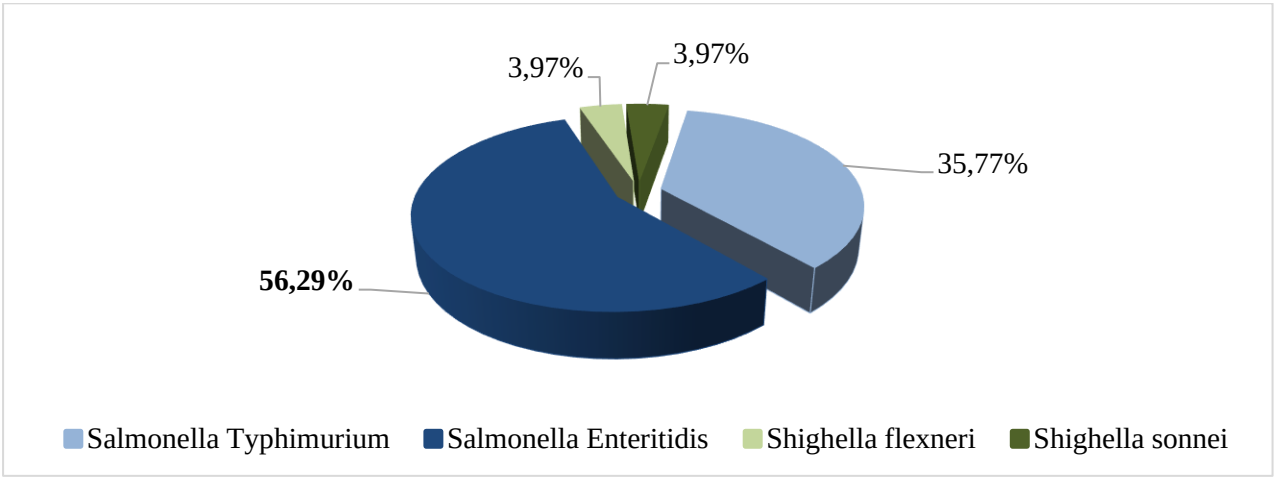


Figure 3.2. Etiological structure of the serotypes of pathogenic enterobacteria *Salmonella* spp. and *Shigella* spp. isolated during the study period

The analysis of the etiological structure highlighted the important role of the genera *Salmonella* and *Shigella* in the etiology of acute diarrheal diseases. Although they had a relatively low share in the total number of isolated strains, *Salmonella* spp. predominated, being mainly represented by the serotypes *S.* Enteritidis and *S.* Typhimurium. As for *Shigella* spp., the share of strains was much lower, and *S. sonnei* and *S. flexneri* recorded the same values. Bacteria of the genus *Shigella*, especially *S. flexneri* and *S. sonnei* are the cause of shigellosis – a form of acute bacterial diarrhea.

Conditionally pathogenic agents represented 94.93% (n=2825) of the total number of isolated strains and 19.71% of the total number of samples investigated in patients with ADD. The etiological spectrum was dominated by *Klebsiella* spp. (42.8%; n=1209) (fig. 3.3). The other conditionally pathogenic agents were identified with lower frequencies: *Citrobacter* spp. – 13.45%, *Proteus* spp. – 13.31%, *Enterobacter* spp. – 10.65%, *E. coli* with hemolytic properties – 9.77% and *P. aeruginosa* – 6.37% (fig. 3.3). The other genera had shares below 2%: *Serratia* spp. (1.24%), *Providencia* spp. (0.99%), *Morganella* spp. (0.96%), *Aeromonas* spp. (0.21%), *Hafnia* spp. (0.18%) and *Acinetobacter* spp. (0.07%).

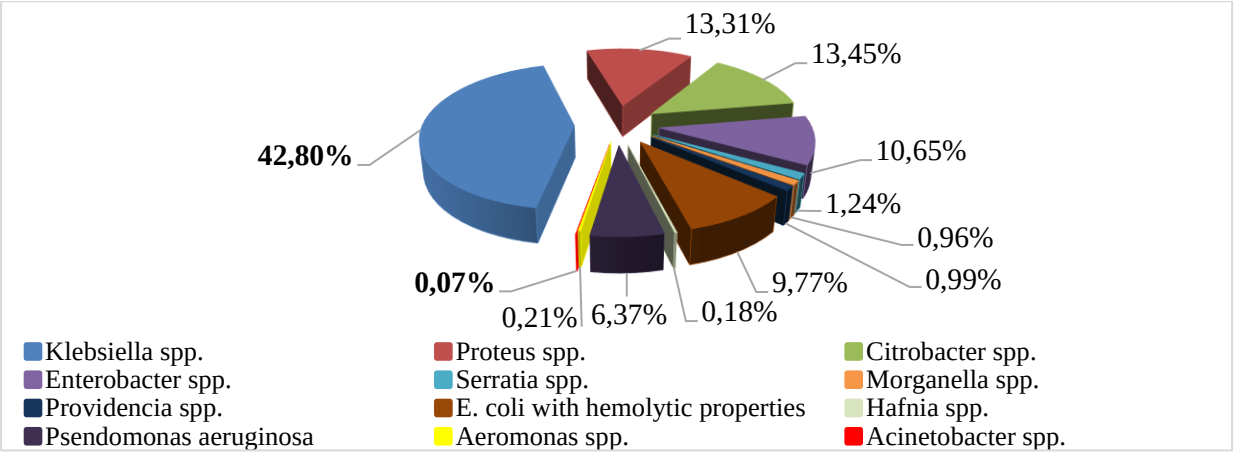


Figure 3.3. Etiological structure of opportunistic pathogens isolated during the study period

Among the opportunistic pathogens involved in the etiology of enterocolitis, *P. aeruginosa*, *Aeromonas* spp. and *Acinetobacter* spp. constituted 6.65% (n=188) of the total isolated strains. *P. aeruginosa* was the predominant species (95.7%; n=180), and *Aeromonas* spp. and *Acinetobacter* spp. were sporadically isolated, with shares of 3.19% and, respectively, 1.06% (fig. 3.4). These results highlight the fact that the opportunistic pathogens involved in the etiology of enterocolitis highlight the fact that the causative agents of enterocolitis, especially *P. aeruginosa*, although they had a relatively low share in the general structure of opportunistic pathogens, present a significant epidemiological importance due to their opportunistic pathogenic potential, their ability to adapt to various environments and the possibility of determining severe clinical forms, especially in vulnerable individuals.

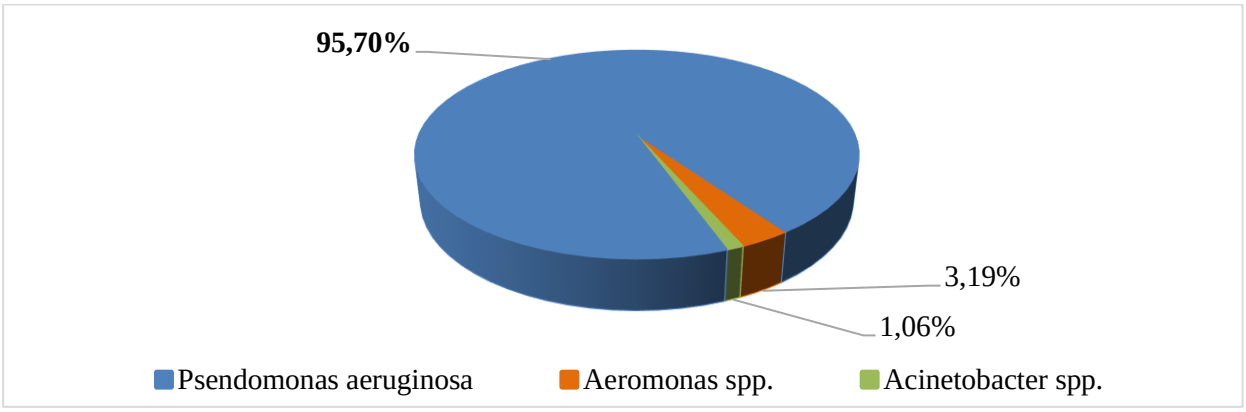


Figure 3.4. Share of opportunistic pathogens involved in the etiology of enterocolitis isolated during the study period

The analysis of the distribution of isolated strains according to age groups revealed the presence of etiological agents of ADD in both adults and children/adolescents aged between 0 and 17 years. The majority of strains were isolated from adult patients, constituting 67.98% of the total number of isolated strains, compared to 32.02% recorded in children/adolescents. The etiological spectrum of ADD was dominated, in both age groups, by the genus *Klebsiella*, with shares of 39.64% in children/adolescents and 36.39% in adults, indicating a relatively uniform distribution of this agent, with a slight predominance in the children/adolescent group (fig. 3.5).

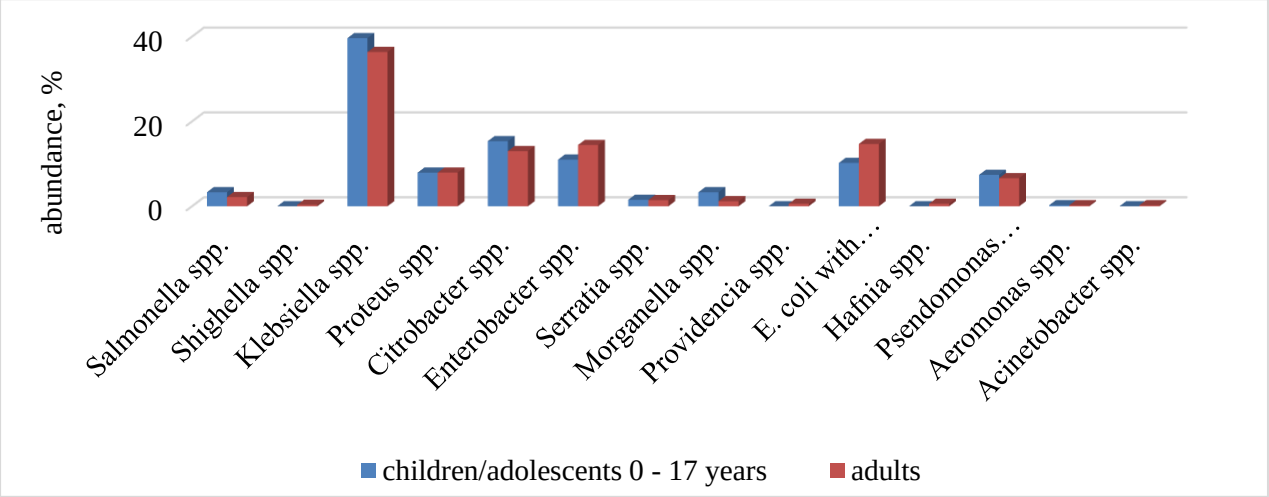


Figure 3.5. Etiological structure of isolated agents depending on age during the study period

Citrobacter spp. was identified more frequently in children/adolescents (15.35%) compared to adults (13.01%), while *Enterobacter* spp. had a higher proportion in adults (14.46%) compared to children/adolescents (11%). *E. coli* strains with hemolytic properties were more frequently isolated in adults (14.7%) compared to the pediatric group (10.23%). For *Proteus* spp. a similar distribution was observed between the two age groups (7.95% in adults and 7.93% in children/adolescents). In the case of *P. aeruginosa*, the proportion was slightly higher in children/adolescents (7.42%) compared to adults (6.63%).

The genus *Salmonella* was identified more frequently in children/adolescents (3.32%) compared to adults (2.17%), similar to *Morganella* spp. (3.32% vs. 1.2%). For *Aeromonas* spp. the distribution was comparable between groups (0.26% in children/adolescents and 0.24% in adults). The genera *Shigella*, *Providencia*, *Hafnia* and *Acinetobacter* were not isolated in children/adolescents, and in adults they showed low values.

The obtained results highlight significant variations in the incidence of ADD depending on age, emphasizing the need to adapt diagnostic and treatment strategies to the specific characteristics of each age group.

3.3. Morphological and cultural peculiarities of pathogens that cause ADD

The morphological and cultural characteristics of the bacterial agents involved in ADD were described according to standard criteria in the specialized literature [10], which include cell shape, Gram stain, mobility, colony appearance and behavior on selective media.

Following the analysis of the morphological and cultural characteristics, specific and relevant characters for the identification of the pathogens isolated during the study period were highlighted (tab. 3.2).

Table 3.2. Distinctive morphological and cultural features of pathogens isolated during the study period

Pathogens	Morphological peculiarities	Relevant cultural and biochemical features
Salmonella spp.	Gram-negative, motile bacilli, arranged singly or in pairs	On SS agar: colonies with black centers (H_2S production); on Kligler agar: H_2S production

Shigella spp.	Gram-negative, immobile bacilli	On Endo agar and SS agar: colorless, lactose-negative colonies, type "S/R"; on Kligler agar: gas-free glucose fermentation
Klebsiella spp.	Gram-negative, encapsulated, immobile bacilli	Large, mucoid colonies; on chromogenic agar: green colonies; on Kligler agar: lactose fermentation with gas
Enterobacter spp.	Gram-negative, motile, sometimes encapsulated bacilli	Less mucoid colonies; on Kligler agar: lactose fermentation with gas production
Proteus spp.	Gram-negative, motile bacilli	Liquid media become uniformly cloudy, a surface veil and characteristic odor appear; on MacConkey: colorless/slightly yellowish colonies; on Kligler agar: lactose negative, H_2S positive
Citrobacter spp.	Gram-negative, motile bacilli	On chromogenic agar: dark green colonies; on Kligler agar: slow fermentation and H_2S production
E. coli strains with hemolytic properties	Gram-negative, motile bacilli, sometimes encapsulated	On blood agar: β-hemolysis; on Kligler agar: fermentation of lactose, glucose and sucrose with production of acid and gas
Morganella spp.	Gram-negative, motile bacilli	On chromogenic agar: greenish-gray colonies; on Kligler agar: does not ferment lactose
Serratia spp.	Gram-negative, motile bacilli	On chromogenic agar: dark blue colonies; on Kligler agar: lactose negative
Providencia spp.	Gram-negative, motile bacilli	On chromogenic agar: colorless colonies; on Kligler agar: lactose negative
Hafnia spp.	Gram-negative, motile bacilli	On MacConkey: small, smooth, yellowish colonies; on Kligler agar: lactose negative
P. aeruginosa	Gram-negative, motile bacilli	On chromogenic agar: large, flat, fluorescent yellow-green colonies, characteristic odor; on Kligler agar: lactose negative
Aeromonas spp.	Gram-negative, motile bacilli	On blood agar: round, smooth colonies, with hemolysis
Acinetobacter spp.	Gram-negative, non-motile coccobacilli.	On MacConkey/Chromogen agar: smooth, opaque, lactose-negative colonies, without pigments

Thus, morpho-cultural examination represents an indispensable stage in the microbiological diagnostic algorithm, which, correlated with biochemical and clinical data, allows establishing a correct diagnosis, establishing an adequate treatment and applying effective measures for the prevention and control of acute diarrheal diseases. The use of these integrated approaches is widely supported in the specialized literature and medical microbiology textbooks, where detailed diagnostic schemes for important enteropathogenic agents (e.g. *Salmonella* spp., *Shigella* spp., *E. coli*, etc.) are described [13].

3.4. Dynamics of the spread of ADD in AMT Buiucani, Chisinau municipality

The analysis of the quantitative indices of the pathogens isolated during the study period highlighted the predominance of the genus *Klebsiella* spp. in the etiology of ADD in the municipality of Chisinau, PHMI TMA Buiucani (fig. 3.6). During the investigated period, the share of this genus was constantly high, ranging between 28.85% in 2023 and 52.23% in 2017, with a gradual decreasing trend in recent years.

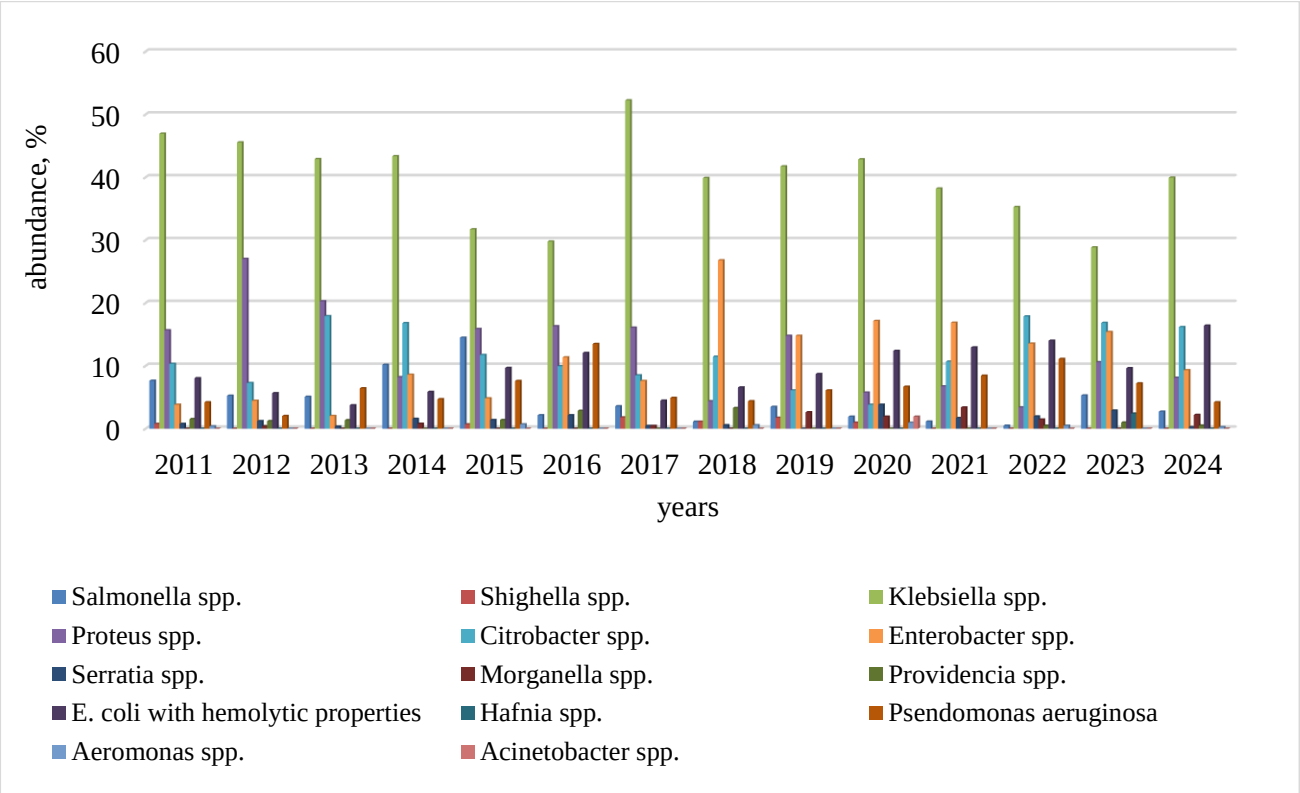


Figure 3.6. Share of quantitative indices of pathogens isolated during the study period

The proportion of other isolated pathogens varied during the study period, with most showing a general decreasing trend. The largest variations were recorded for *Salmonella* spp. (0.48%–14.48%), *Proteus* spp. (3.39%–27.02%), *Enterobacter* spp. (2.03%–26.78%) and *P. aeruginosa* (2.02%–13.48%). Some microorganisms have maintained high values in recent years, such as *Citrobacter* spp. (16.83% in 2023 and 16.17% in 2024) and *E. coli* with hemolytic properties (16.4% in 2024). *Hafnia* spp. and *Acinetobacter* spp. were identified only in 2023 and 2020, respectively.

Most pathogens were isolated annually, except for periods without identifications for *Shigella* spp., *Serratia* spp., *Morganella* spp., *Providencia* spp. and *Aeromonas* spp. *Salmonella* spp. was isolated every year, being associated with most cases of ADD: 100% in the years 2012–2014, 2016 and 2021–2024, and the lowest values were recorded in 2018 (50%). Infections caused by *Shigella* spp. were sporadic, being reported only in the years 2011, 2015 and 2017–2020, with shares ranging between 4.55% and 50%, and in the other years no cases were identified.

The analysis of the quantitative indices of the pathogens *Salmonella* spp. and *Shigella* spp., which had a share of 5.1% (n=151) of the total number of positive samples, highlights the predominance of *Salmonella* spp. throughout the study period. *Salmonella* spp. was isolated annually, with the maximum share (100%) in the years 2012–2014, 2016 and 2021–2024, and the lowest values in 2018 (50%) (fig. 3.7).

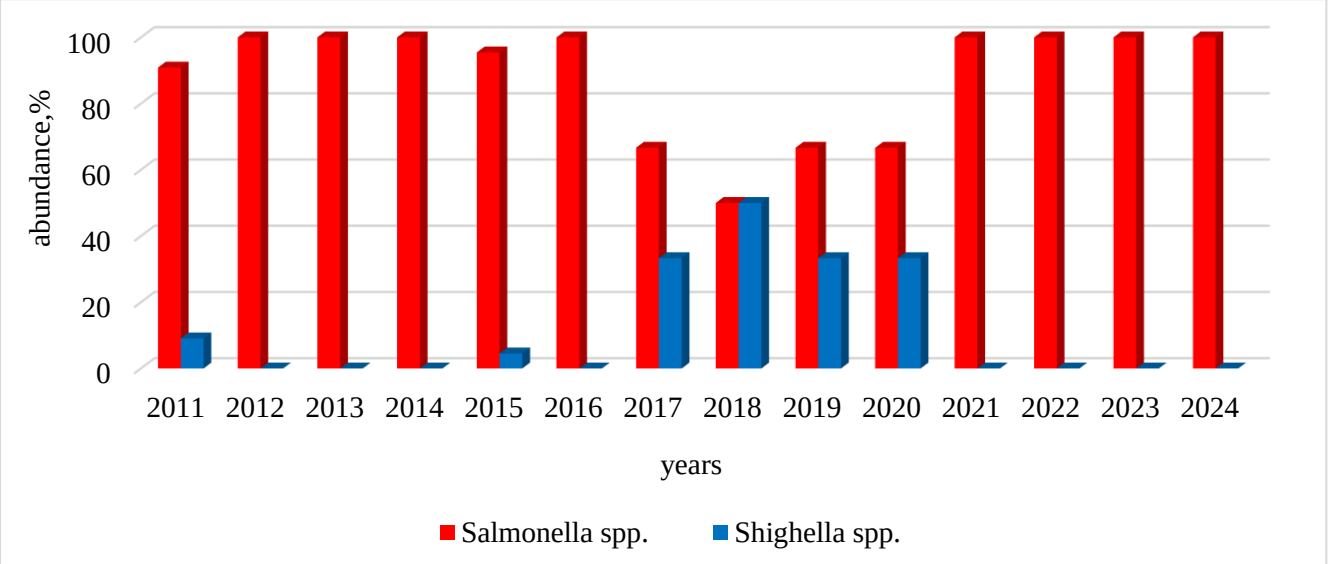


Figure 3.7. Share of quantitative indices of pathogenic enterobacteria *Salmonella* spp. and *Shigella* spp., isolated during the study period

ADD caused by *Shigella* spp. were sporadic, with variable distribution during the study period. The highest shares were recorded in 2018 (50%), 2017, 2019 and 2020 (33.33%), and in 2011 and 2015 the values were reduced (9.1% and 4.55%). In the periods 2012–2014, 2016 and 2021–2024, no cases caused by *Shigella* spp. were reported.

During the study period, the dominant serotypes of *Salmonella* spp. – *S.* Enteritidis and *S.* Typhimurium. *S.* Enteritidis was isolated every year, except for 2021, presenting a variation in share between 16.67% (in 2017) and 100% (in 2022). *S.* Typhimurium was isolated in the years 2011–2017, as well as in 2021, 2023 and 2024. Its share ranged from 18.18% (in 2024) to 100% (in 2021), indicating a significant, although less constant presence compared to *S.* Enteritidis (fig. 3.8).

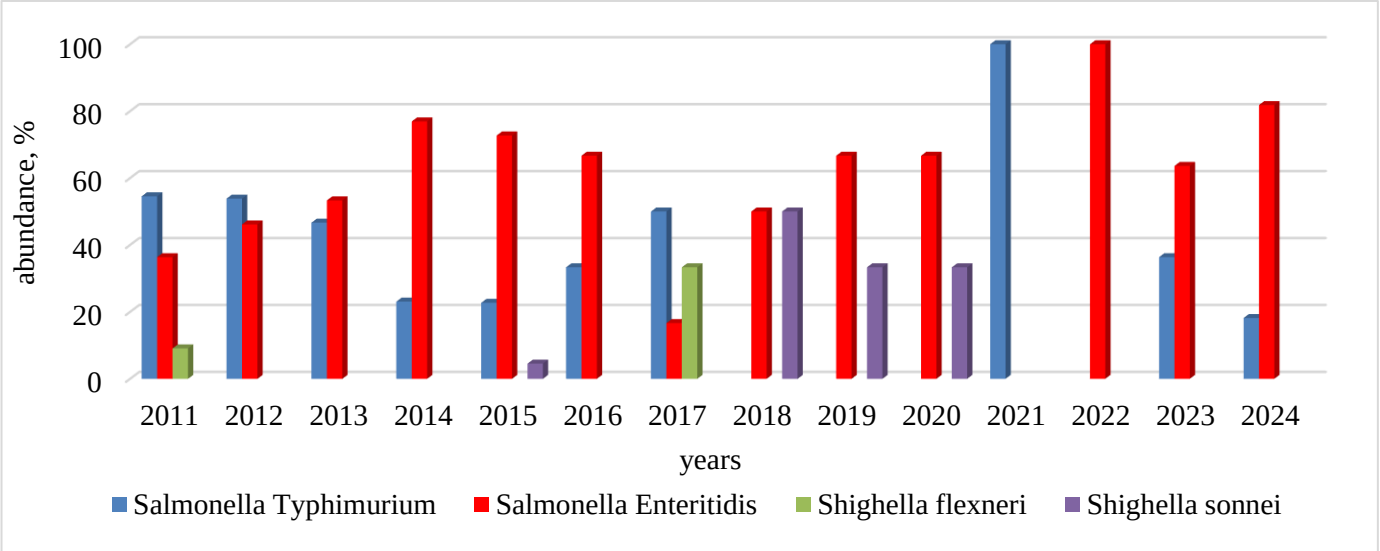


Figure 3.8. Share of quantitative indices of isolated serotypes during the study period

The analysis of the quantitative indices of the opportunistic pathogens *Klebsiella* spp., *Proteus* spp., *Citrobacter* spp., *Enterobacter* spp., *Serratia* spp., *Morganella* spp., *Providencia* spp., *E. coli* with

hemolytic properties, *Hafnia* spp., *P. aeruginosa*, *Aeromonas* spp. and *Acinetobacter* spp., which had a share of 94.9% (n=2825) of the total number of positive samples, shows that during the study period in TMA Buiucani most cases of ADD were caused by the *Klebsiella* genus (fig. 3.9). The values ranged between 30.4% in 2016 and 55.1% in 2017, remaining at a fairly high level throughout the study period, with a significant increase in 2024 (41%). *E. coli* with hemolytic properties showed an upward trend, reaching the maximum value in 2024 (16.8%). *Proteus* spp. recorded a general decrease, from maximum values in 2012 (28.5%) to lower values in recent years. *Citrobacter* spp. and *Enterobacter* spp. showed fluctuations, with increases in certain periods, followed by decreases. *P. aeruginosa*, *Serratia* spp., *Morganella* spp., *Providencia* spp., *Aeromonas* spp., *Acinetobacter* spp. and *Hafnia* spp. were isolated sporadically, with low weights, having a secondary epidemiological role.

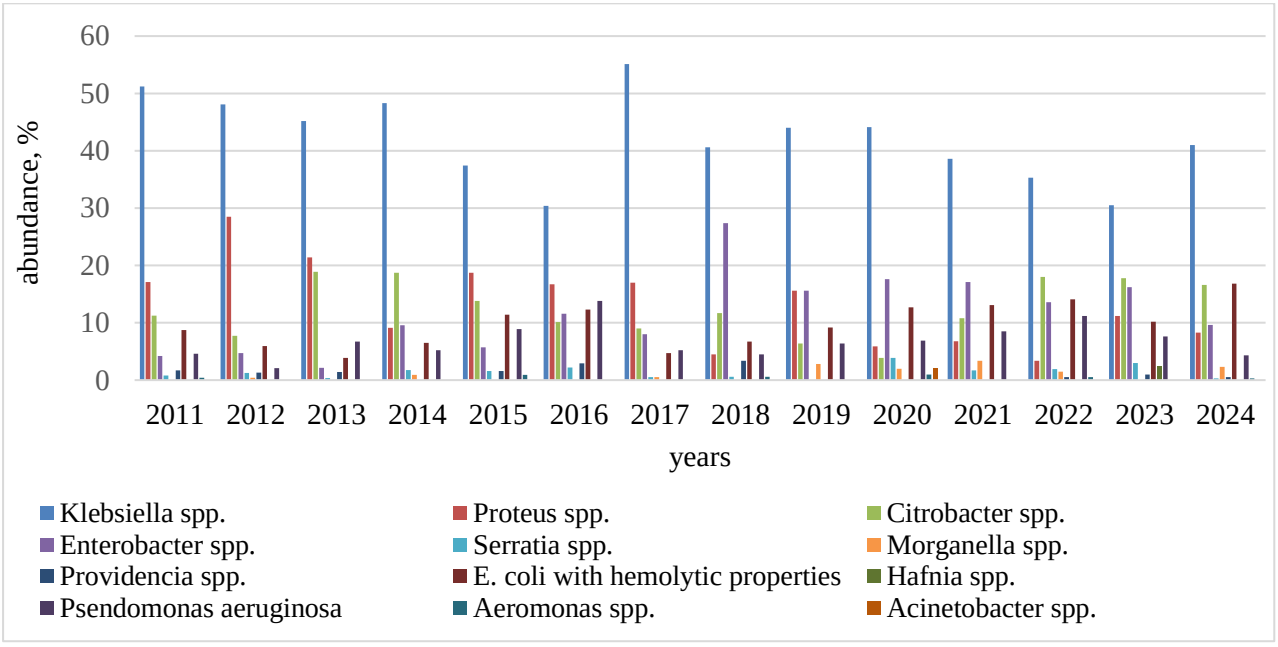


Figure 3.9. Share of quantitative indices of isolated serotypes during the study period

The results obtained demonstrate an etiological diversity of microorganisms involved in ADD during the period 2011–2024, with the predominance of opportunistic pathogens. At the same time, *Salmonella* spp. and *Shigella* spp. played an important role in the etiology of ADD, *Salmonella* spp. being the dominant pathogen, with constant isolations and values up to 100% in certain periods. The analysis of the distribution of microorganisms over time highlights the importance of monitoring their circulation for the evaluation of sources and transmission mechanisms in TMA Buiucani, mun. Chisinau.

The comparative analysis of the distribution of pathogens isolated during the period 2019 – 2024 by quarters highlights an increase in the number of strains and etiological diversity, more pronounced after 2021, with maximum values predominantly in the II and III quarters (fig. 3.10). The *Klebsiella* genus was the dominant pathogen throughout the analyzed period, and seasonal variations in isolations confirm the fluctuating nature of the circulation of microorganisms throughout the year.

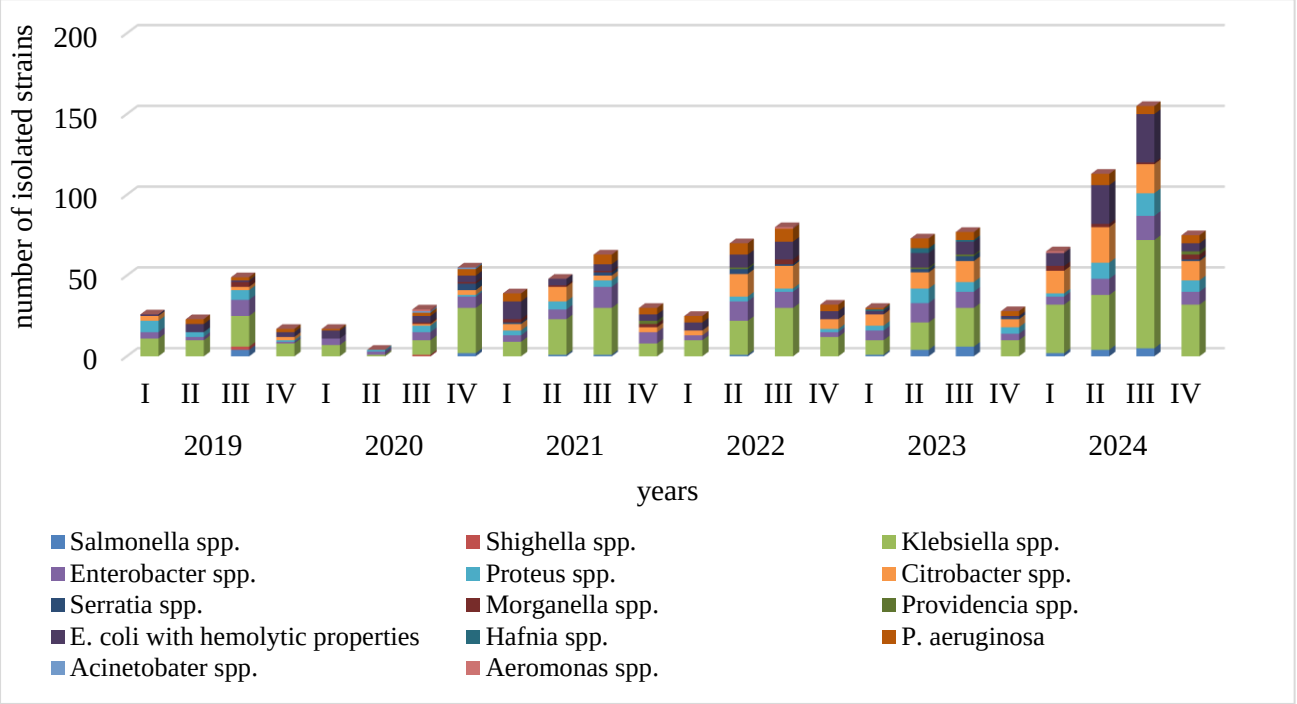


Figure 3.10. Distribution of isolated pathogens during the study period depending on the trimester

Compared to the period 2019–2020, the years 2021–2024 were characterized by a greater etiological diversity and an increased number of isolated strains, especially in the II and III trimesters. These results indicate the intensification of the circulation of pathogens and highlight the need for continuous epidemiological monitoring, especially in the warm season.

To identify population categories with increased vulnerability, the annual distribution of the share of ADD cases by age groups was analyzed - children/adolescents and adults. The analysis of the share of cases by age groups and by years is an essential element in understanding the epidemiological distribution of ADD and in substantiating public health interventions. At the same time, the analysis by years offers the possibility of highlighting temporal trends, possible increases or decreases in incidence and the impact of implemented prevention measures. The data obtained highlight important variations in the share of ADD cases depending on age groups and year. In the period 2019 – 2020, relatively low values are observed, with a predominance of children and adolescents in 2019 (15.09%), followed by a significant decrease in 2020 (7.67%), along with a slight increase among adults (9.04%) (fig. 3.11). Starting with 2021, an increase in the share of cases was observed in both age groups, more pronounced in adults. The trend was maintained in 2022–2024, with maximum values in 2024 (29.67% in children/adolescents and 35.18% in adults). The data indicate a general trend of increasing the share of cases over time, with fluctuations between age groups. Adults tend to show higher values in recent years, which may indicate increased exposure or a change in risk factors, while children and adolescents remain a consistently vulnerable category. These results highlight the need for differentiated prevention and control measures, specifically adapted to each age group.

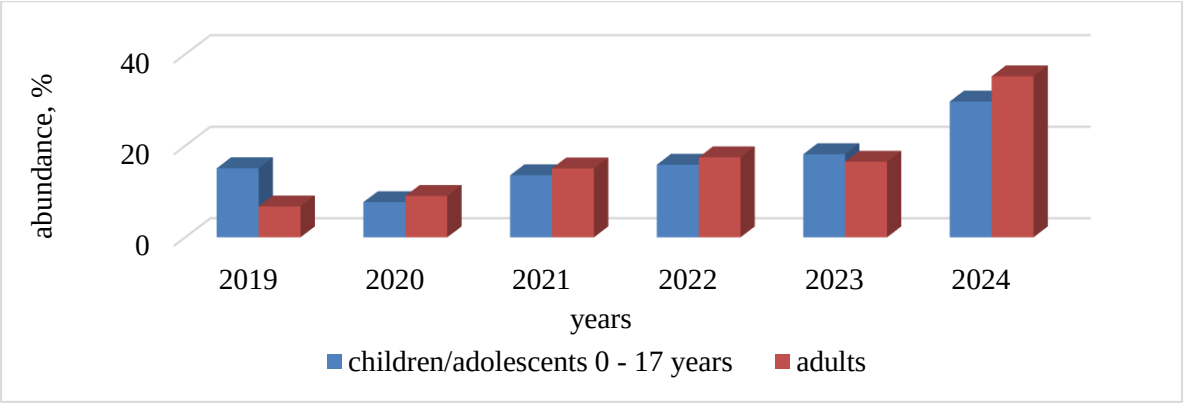


Figure 3.11. Annual distribution of the share of ADD cases by age groups

In order to highlight the most affected age groups and to identify the periods in which the number of cases recorded significant increases, an analysis of the distribution of strains by year, quarter and age group was performed (fig. 3.12). The analysis of the annual distribution of isolated strains by quarter and age group highlights a general increase in the number of isolated strains during the period 2019 – 2024, more pronounced in adults than in children/adolescents. After relatively low values in 2019 – 2020, the number of isolated strains is constantly increasing, reaching maximum levels in 2024. The highest number of isolated strains was recorded in adults in the third quarter of 2024 (120 strains), while in children/adolescents the maximum values ranged between 35 – 38 strains. In all periods analyzed, adults are the most affected category.

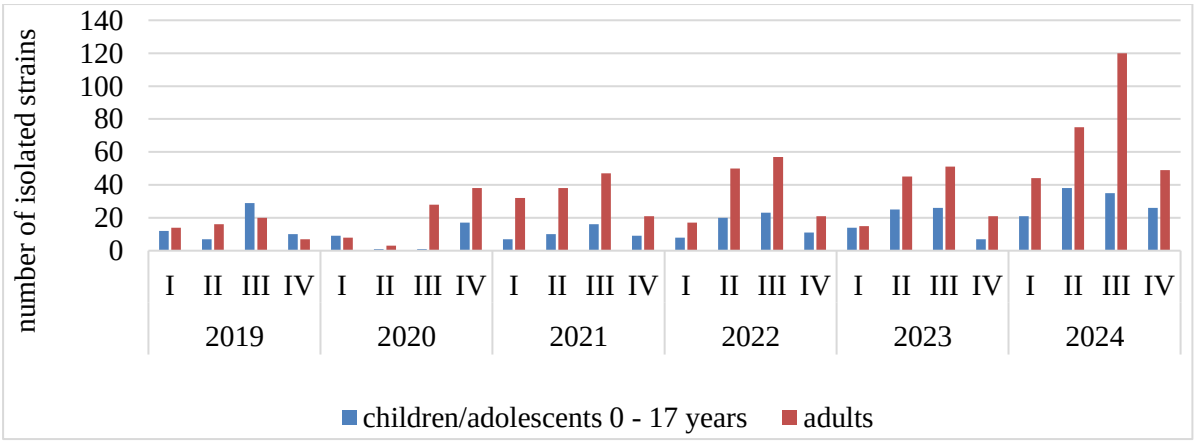


Figure 3.12. Annual distribution of isolated strains by quarter and age group

In order to obtain a more complete picture of the dynamics of pathogen circulation, identify periods of high risk and optimize prevention and control measures, the monthly distribution of samples received in the laboratory and positive strains isolated during the period 2011–2024 was analyzed (fig. 3.13). The analysis of the monthly distribution highlighted the seasonal nature of the circulation of ADD pathogens. The most positive samples and strains were recorded in the period April–November, with maximum values in July (506 samples and 148 isolated strains), and the lowest in December–March. A significant positive correlation was established between the number of samples received and isolated strains ($r=0.96$; $p \leq 0.000001$), confirming the intensification of pathogen circulation in the warm season. The analysis of the data obtained notes a predominance of cases in the warm season, possibly correlated with factors such

as high temperatures, hygiene conditions and food consumption, which favor the transmission of pathogens involved in ADD. This analysis contributes to a more rigorous assessment of the seasonal distribution and to the substantiation of appropriate surveillance and control measures.

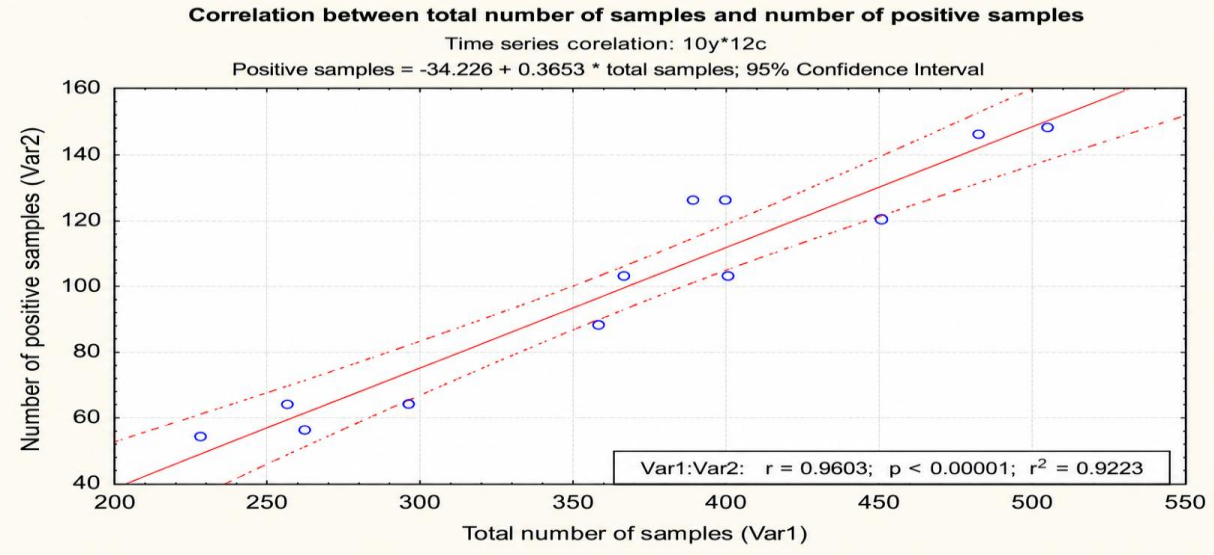


Figure 3.13. Correlation analysis between the number of positive samples and the total number of samples received monthly

An essential indicator for the correct assessment of the dynamics of pathogen circulation is the calculation of the share of positive strains out of the total number of samples received in the laboratory, by month. Analysis of the data obtained during the study period highlights seasonal variations independent of the volume of samples received in the laboratory (fig. 3.14).

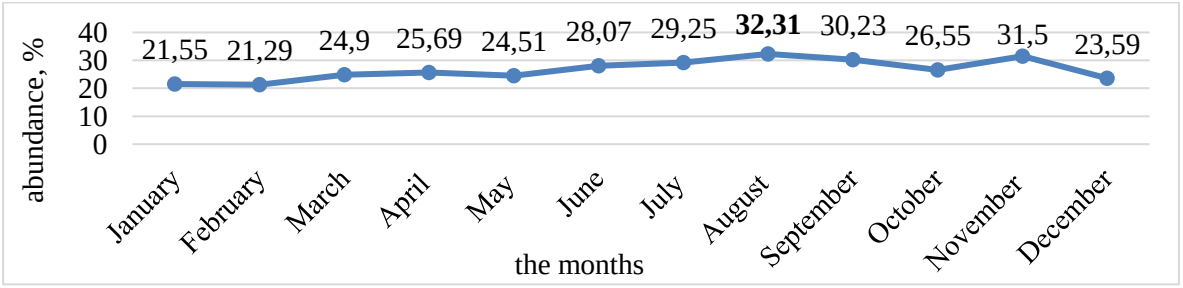


Figure 3.14. Monthly dynamics of the share of positive strains isolated during the study period

In the first months of the year (January–February), the positivity rate remained at low values (~21%). From March, a progressive increase was observed, with maximum values in August (32.31%), September (30.23%) and November (31.5%), followed by a decrease towards the end of the year (23.59% in December). The data indicate a seasonal intensification of the circulation of the pathogen during the warm period of the year. Thus, according to the data obtained, the increase in the share of positive strains in the summer months and in November suggests a real intensification of the circulation of the pathogen, and not just an effect of the increase in the number of samples analyzed.

The dynamics of the circulation of positive strains isolated during the study period also highlighted

annual variations in the circulation index, with a succession of periods in which the values were higher and periods of decrease, which indicates temporal changes in the intensity of the circulation of the analyzed etiological agents (fig. 3.15).

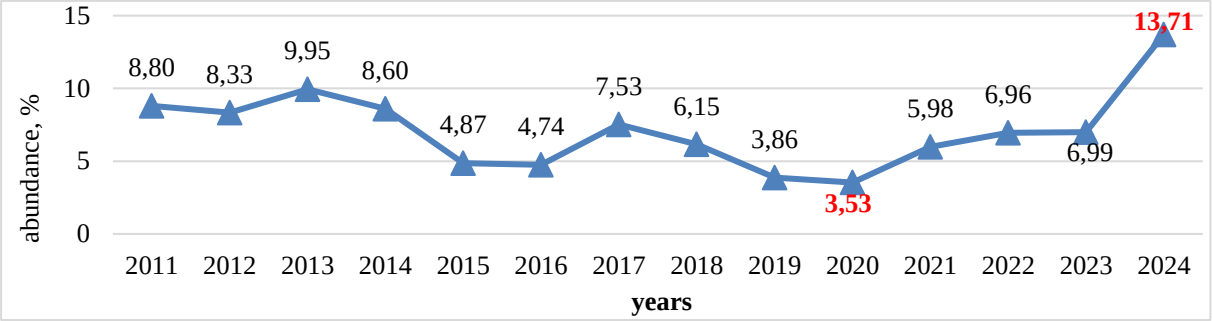


Figure 3.15. Dynamics of the circulation of etiological agents analyzed during the study period in PHMI TMA Buiucani

Most of the analyzed etiological agents were isolated annually, but the circulation index showed significant variations during the study period. In 2011–2014, high values were recorded (8.33–9.95%), followed by a decrease in 2015–2016 (4.87–4.74%). The period 2019–2020 was characterized by the lowest values (3.86% and 3.53%), probably due to the intensification of sanitary-epidemiological measures adopted and implemented by the authorities of the Republic of Moldova during this period in institutions and public transport, and compliance with personal hygiene rules is the surest condition for avoiding illness. Starting in 2021, the circulation index has progressively increased, reaching 13.71% in 2024. This trend can be associated with the relaxation of prevention measures, the resumption of collective activities, and the increase in the population's exposure to enteric agents through food, water, and interpersonal contact.

4. PATHOGENIC AGENTS INVOLVED IN ACUTE DIARRHEA DISEASES AND THEIR SENSITIVITY TO ANTIBIOTICS

4.1. Pathogenic and conditionally pathogenic agents causing acute diarrheal diseases and their sensitivity to antimicrobials

For pathogens (*Salmonella* spp. and *Shigella* spp.) and conditionally pathogenic agents (*Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *E. coli* with hemolytic properties, *Morganella* spp., *Serratia* spp., *Providencia* spp. and *Hafnia* spp.), involved in the etiology of ADD, antimicrobial susceptibility testing was performed during the period 2019–2024, to representative antibiotics, according to EUCAST recommendations: ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefotaxime, ceftazidime, cefepime, ertapenem, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, levofloxacin and trimethoprim/sulfamethoxazole [4-9]. Analysis of the antimicrobial resistance profile during the period 2019 – 2024 revealed important differences between pathogens and conditionally pathogenic agents (tab. 4.1). The highest resistance was recorded against penicillins, especially in *Shigella* spp. (100% to ampicillin), *Klebsiella* spp. (99.8%), *Citrobacter* spp. (98.2%),

Morganella spp. (95.7%) and *Enterobacter* spp. (95.1%). Resistance to amoxicillin/clavulanic acid also remained high for *Enterobacter* spp., *Citrobacter* spp., *Morganella* spp. and *Hafnia* spp. (80–95.7%).

Table 4.1. Pathogenic and conditionally pathogenic agents causing acute diarrheal diseases and their sensitivity to antimicrobials

Bacteria	Penicillins		β-lactams + inhibitor	β-lactams with extended spectrum			Carbapenems			Aminoglycosides		Fluoroquinolone		Antimetabolite
	AMP	AMC	TZP	CTX	CAZ	FEP	ERT	IMI	MEM	AMK	GEN	CIP	LEV	SXT
Salmonella spp.	32.3	19.4	0	0	0	0	0	6.5	0	70.9	70.9	6.5	6.5	3.3
Shigella spp.	100	66.6	0	0	0	0	0	0	0	0	0	0	0	100
Klebsiella spp.	99.8	26.9	9.9	15.8	13.8	7.2	2.8	2.2	2.8	0	0	6.9	5.9	21.9
Enterobacter spp.	95.1	92.6	2.5	9.8	5.5	5.5	3.7	0	0	0	0	4.9	4.9	7.4
Proteus spp.	42.3	19.6	11.3	19.6	14.4	10.3	10.3	4.1	2.1	2.1	2.1	6.2	6.2	8.2
Citrobacter spp.	98.2	92.3	1.2	17.9	2.4	0	0	0	0	0	0	5.9	5.9	7.1
Escherichia coli (hemolytic)	36.4	12.4	3.7	7.4	2.5	3.1	0	0	0	1.2	0	9.9	8.6	0
Morganella spp.	95.7	95.7	4.3	8.7	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3
Serratia spp.	55.6	38.9	0	5.6	0	0	0	0	0	0	0	5.6	5.6	5.6
Providencia spp.	60.0	20.0	0	0	0	0	0	0	0	0	0	0	0	0
Hafnia spp.	0	80.0	0	20.0	0	20.0	0	0	0	0	0	0	0	60.0

Resistance scale (%)

0 – 24.9 | 25 – 49.9 | 50 – 74.9 | 75 – 100

Resistence (%)

Antibiotic abbreviations

AMP – ampicillin
AMC – amoxicillin/clavulanic acid
TZP – piperacillin/tazobactam
CTX – cefotaxime
CAZ – ceftazidime
FEP – cefepime
ERT – ertapenem
IMI – imipenem
MEM – meropenem
AMK – amikacin
GEN – gentamicin
CIP – ciprofloxacin
LEV – levofloxacin
SXT – trimethoprim/sulfamethoxazole

Resistance to extended-spectrum β-lactams (piperacillin/tazobactam, third- and fourth-generation cephalosporins) and carbapenems was generally low, with most strains showing retained susceptibility. Exceptions were *Proteus* spp., which showed moderate levels of resistance to cephalosporins and ertapenem (10.3–19.6%), and *Hafnia* spp., with 20% resistance to cefotaxime and cefepime. Regarding aminoglycosides and fluoroquinolones, most enterobacteria showed low levels of resistance (<15%). An exception was *Salmonella* spp., which showed high resistance to amikacin and gentamicin (70.9%), but maintained high susceptibility to cephalosporins, carbapenems, and piperacillin/tazobactam. For trimethoprim/sulfamethoxazole, the highest levels of resistance were observed in *Shigella* spp. (100%), *Hafnia* spp. (60%), *Klebsiella* spp. (21.9%) and *Proteus* spp. (8.2%), while the other genera showed low values.

4.2. Conditionally pathogenic agents involved in the etiology of enterocolitis and their sensitivity to antimicrobials

For the opportunistic pathogens involved in the etiology of enterocolitis (*P. aeruginosa*, *Acinetobacter* spp. and *Aeromonas* spp.), antimicrobial susceptibility testing was performed during 2019–2024, to representative antibiotics, according to EUCAST recommendations: ticarcillin, ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, imipenem/relebactam, ceftazidime/avibactam and ceftolozone/tazobactam [4-9].

The analysis of the resistance profile for the three opportunistic pathogens involved in the etiology of enterocolitis investigated highlights the presence of variable levels of resistance to the tested antibiotics,

with the predominance of resistance to some antibiotics from the β-lactam class (tab. 4.2).

Table 4.2. Antimicrobial resistance profile (%) of opportunistic pathogens involved in the etiology of enterocolitis tested during the study period

No. (No.)	Antibiotic tested	Pseudomonas aeruginosa (R %)		Acinetobacter spp. (R %)		Aeromonas spp. (R %)	
		R %	No. of strains tested (N)	R %	No. of strains tested (N)	R %	No. of strains tested (N)
1	Ticarcillin	44,9	89	0	2	33,3	3
2	Ticarcillin/clavulanic acid	23.1	52	0	2	50.0	2
3	Piperacillin	17,3	52	0	2	0	2
4	Piperacillin/tazobactam	8.3	84	0	2	0	3
5	Ceftazidime	10,7	84	0	2	0	3
6	Cefepime	9,5	84	0	2	0	3
7	Aztreonam	14,3	84	0	2	0	3
8	Imipenem	1,2	84	0	2	0	3
9	Meropenem	0	84	0	2	0	3
10	Amikacin	0	84	0	2	0	3
11	Gentamicin	0	52	0	2	0	2
12	Ciprofloxacin	7,1	84	0	2	0	3
13	Tobramycin	1,2	84	0	2	0	3
14	Imipenem/relebactam	0	32	–	–	0	1
15	Ceftazidime/avibactam	18,7	32	–	–	0	1
16	Cefolozane/tazobactam	21,9	32	–	–	0	1

Resistance scale (%)

> 50	Very high resistance
30 – 49,9	High resistance
15 – 29.9	Moderate resistance
5 – 14,9	Low resistance
0 – 4.9	Very low resistance / Absent

Antibiotic abbreviations

Ticarcillin (TIC)
Ticarcillin/clavulanic acid (TIC/AC)
Piperacillin (PIP)
Piperacillin/tazobactam (PIP/TAZ)
Ceftazidime (CAZ)
Cefepime (FEP)
Aztreonam (ATM)
Imipenem (IMP)
Meropenem (MEM)
Amikacin (AMK)
Gentamicin (GEN)
Ciprofloxacin (CIP)
Tobramycin (TOB)
Imipenem/relebactam (IMP/REL)
Ceftazidime/avibactam (CAZ/AVI)
Cefolozane/tazobactam (ICT)

The analysis of the antimicrobial resistance profile of *P. aeruginosa*, *Acinetobacter* spp. and *Aeromonas* spp. during the study period highlights important differences between the investigated species. *P. aeruginosa* showed the highest levels of resistance, especially to ticarcillin (44.9%), ticarcillin/clavulanic acid (23.1%), ceftolozane/tazobactam (21.9%) and ceftazidime/avibactam (18.7%). Resistance to piperacillin (17.3%), aztreonam (14.3%) and ceftazidime (10.7%) was moderate, while resistance to carbapenems (imipenem – 1.2%, meropenem – 0%), aminoglycosides and fluoroquinolones remained low. *Acinetobacter* spp. was represented by a very small number of isolates (n=2), all of which were sensitive to the tested antibiotics, without the identification of resistant strains. For *Aeromonas* spp., the small number of strains investigated (n=1–3) revealed resistance only to ticarcillin/clavulanic acid (50%) and ticarcillin (33.3%), while sensitivity to the other antibiotics tested was preserved.

These results highlight the importance of performing an antibiogram to guide an effective and rational antibiotic therapy, as well as the need for prudent use of classical antibiotics, in order to prevent therapeutic failure and limit the development of antimicrobial resistance.

4.3. Comparative analysis of the antibiotic resistance profile of enterobacteria involved in the etiology of acute diarrheal diseases

The enterobacteria investigated using the same antimicrobial panel were included in the comparative analysis: *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *E. coli* with hemolytic properties, *Morganella* spp., *Serratia* spp., *Providencia* spp., *Aeromonas* spp., *Salmonella* spp. and *Shigella*

spp. *P. aeruginosa* strains were not included in this analysis, because the antimicrobial susceptibility assessment was performed using a specific antibiotic panel, different from the one applied to enterobacteria, in accordance with EUCAST recommendations [4-9]. *Hafnia* spp. and *Acinetobacter* spp. were also excluded from the comparative statistical analysis due to the low number of isolates, insufficient to obtain robust estimates.

For the comparative characterization of the antimicrobial resistance profile, the results of susceptibility (S), intermediate susceptibility (I) and resistance (R) were analyzed for each bacterial species during the period 2019–2024. Antibiotics were grouped into eight therapeutic classes, depending on pharmacological affiliation and mechanism of action, and for each class the average value of bacterial resistance was calculated, used in the comparative analysis of antibiotic resistance profiles.

The comparative analysis of resistance profiles highlights the existence of sharp differences both between the classes of antimicrobials and between the bacterial taxa investigated (fig. 4.1).

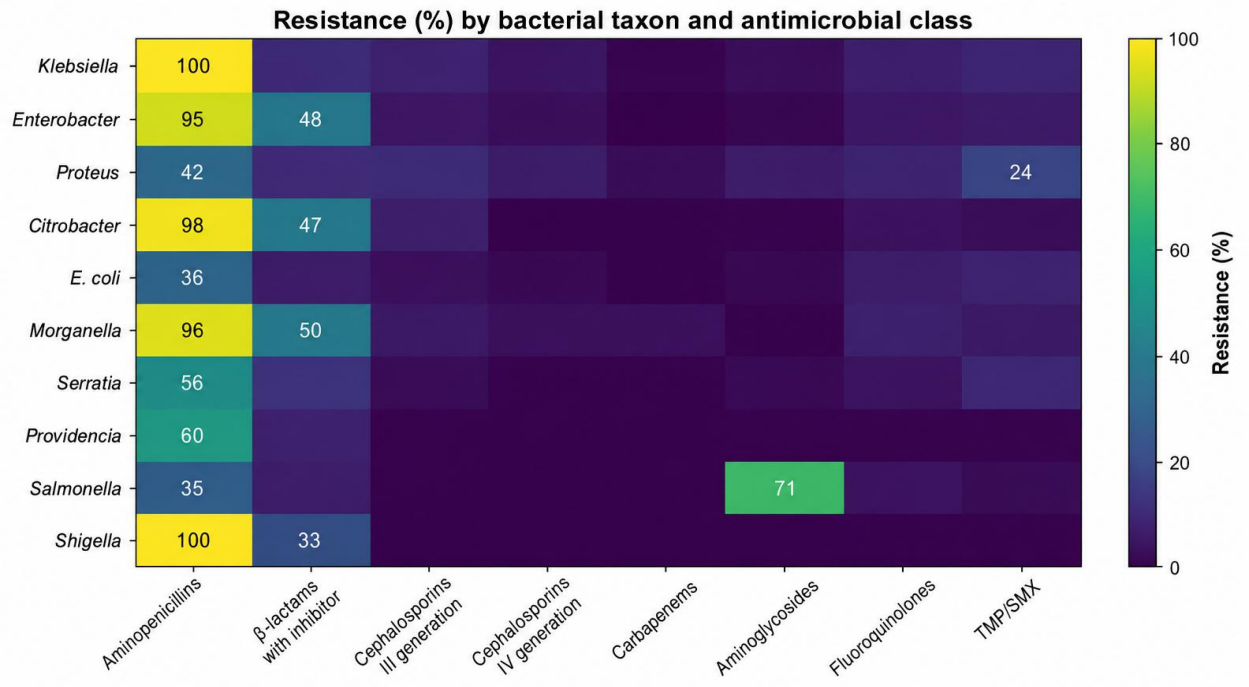


Figure 4.1. Heat map of the comparative resistance profile of enterobacteria to the main classes of antimicrobials. The classes of antimicrobials are presented in order of pharmacological classification

The most obvious pattern identified is represented by the very high resistance to aminopenicillins. For most of the investigated microorganisms, resistance to ampicillin exceeds 90%, reaching 100% in *Klebsiella* spp. and *Shigella* spp., approximately 98% in *Citrobacter* spp., 96% in *Morganella* spp. and 95% in *Enterobacter* spp. Significantly lower levels were recorded only for *E. coli* with hemolyzing properties, *Salmonella* spp. and *Proteus* spp., but even in these cases the resistance remains important (35-42%). These results confirm the sharp decrease in the therapeutic utility of aminopenicillins in enterobacterial infections treatment associated with ADD.

To assess the stability of the antibiotic resistance profile and identify possible temporal changes in

bacterial sensitivity, the dynamics of resistance of the investigated enterobacteria to the main classes of antimicrobials during the period 2019 – 2024 was analyzed (fig. 4.2). The comparative analysis allows the assessment of the persistence of bacterial resistance over time and the identification of therapeutic classes for which efficacy remained constant or, on the contrary, showed decreasing trends. The results highlight the existence of distinct evolutionary patterns for the different classes of antimicrobials (fig. 4.2). Aminopenicillins showed the highest levels of resistance throughout the study period, with low annual fluctuations and no change in the general trend. The stability of this profile suggests that resistance to this class is already well established in the investigated bacterial populations and represents a persistent characteristic of enterobacteria involved in the etiology of acute diarrheal diseases.

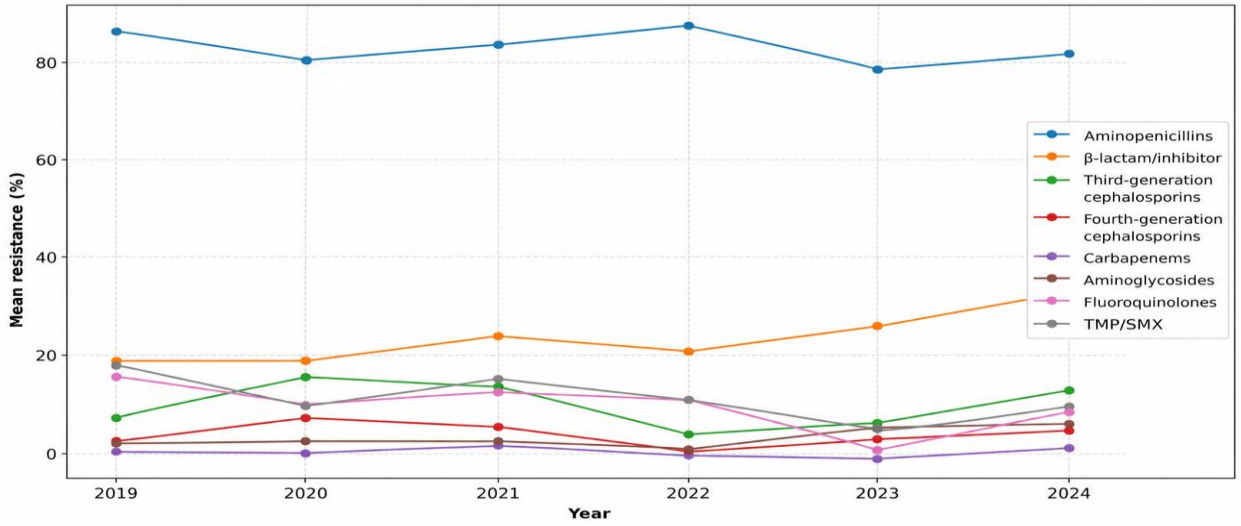


Figure 4.2. Comparative dynamics of enterobacterial resistance to the main classes of antimicrobials in the period 2019–2024

β-Lactam/β-Lactamase Inhibitor combinations showed a similar profile to aminopenicillins, but with lower levels of resistance. Although their efficacy remained superior, a significant increase in resistance was observed towards the end of the analyzed period ($p < 0.000001$), suggesting a gradual decrease in efficacy. Third- and fourth-generation cephalosporins maintained low and stable levels of resistance throughout the study period, confirming high therapeutic efficacy. Carbapenems showed the most favorable profile, with consistently very low resistance and no statistically significant variations. Aminoglycosides and fluoroquinolones recorded intermediate levels of resistance, with statistically significant annual variations ($p = 0.000665$ and $p = 0.000233$), but without important changes in efficacy. For trimethoprim/sulfamethoxazole (TMP/SMX) moderate variations were observed ($p = 0.0119$), without a clear trend of increase or decrease.

Overall, the hierarchy of antimicrobial classes by level of resistance remained stable during the period 2019–2024. Carbapenems and 4th generation cephalosporins remained the most effective, and aminopenicillins continued to show the highest levels of resistance.

To verify whether the differences observed between the resistance profiles of the investigated enterobacteria are statistically supported, the heterogeneity of resistance was assessed for each antibiotic

using the χ^2 test (tab. 4.3).

Table 4.3. Analysis of the heterogeneity of the resistance profile of the enterobacteria investigated for the antibiotics analyzed (χ^2 test)

Antibiotic	χ^2	df	p heterogeneity between taxa
Ampicillin	539.472	9	<0.001
Amoxicillin/clavulanic acid	490.959	9	<0.001
Piperacillin/tazobactam	32.042	9	<0.001
Cefotaxime	22.376	9	0.0078
Ceftazidime	44.462	9	<0.001
Cefepime	23.019	9	0.0062
Ertapenem	33.441	9	<0.001
Imipenem	17.331	9	0.0438
Meropenem	15.008	9	0.0907
Amikacin	408.817	9	<0.001
Gentamicin	239.292	9	<0.001
Ciprofloxacin	10.686	9	0.2978
Levofloxacin	11.645	9	0.2341
Trimethoprim/sulfamethoxazole	31.981	9	<0.001

The significant differences in the resistance profile between bacterial taxa highlight the fact that the choice of antimicrobial treatment cannot be based exclusively on general data on antibiotic resistance, but must be adapted to the particularities of each etiological agent. In this context, rapid identification of the microorganism involved and performing an antibiogram remain essential elements for the establishment of a targeted therapy, reducing the risk of therapeutic failure and limiting the selection of multiresistant strains.

For the comparative assessment of the efficacy of the investigated antimicrobials, the antibiotics were ordered according to the average level of bacterial resistance recorded for all the enterobacteria analyzed. The ranking was built based on the weighted average resistance value (%) calculated for each antibiotic, using the results obtained for all the 11 enterobacteria included in the comparative analysis. This approach allows an objective ranking of antimicrobials in relation to their antibacterial activity and the identification of antibiotics that maintain their microbiological efficacy, as well as those for which bacterial resistance is a major problem (fig. 4.3).

The analysis of the results highlights important differences between the antibiotics investigated. The lowest levels of resistance were recorded for meropenem, ertapenem and imipenem, followed by cefepime and the third-generation cephalosporins (cefotaxime and ceftazidime), which confirms their high microbiological efficacy. Amikacin, gentamicin, levofloxacin, ciprofloxacin and trimethoprim/sulfamethoxazole showed an intermediate level of resistance, maintaining satisfactory antibacterial activity. In contrast, amoxicillin/clavulanic acid and, in particular, ampicillin recorded the highest levels of resistance, indicating the reduced efficacy of aminopenicillins. Overall, carbapenems and cephalosporins remain the most active antibiotics against the enterobacteria investigated, while aminopenicillins show the most unfavorable resistance profile.

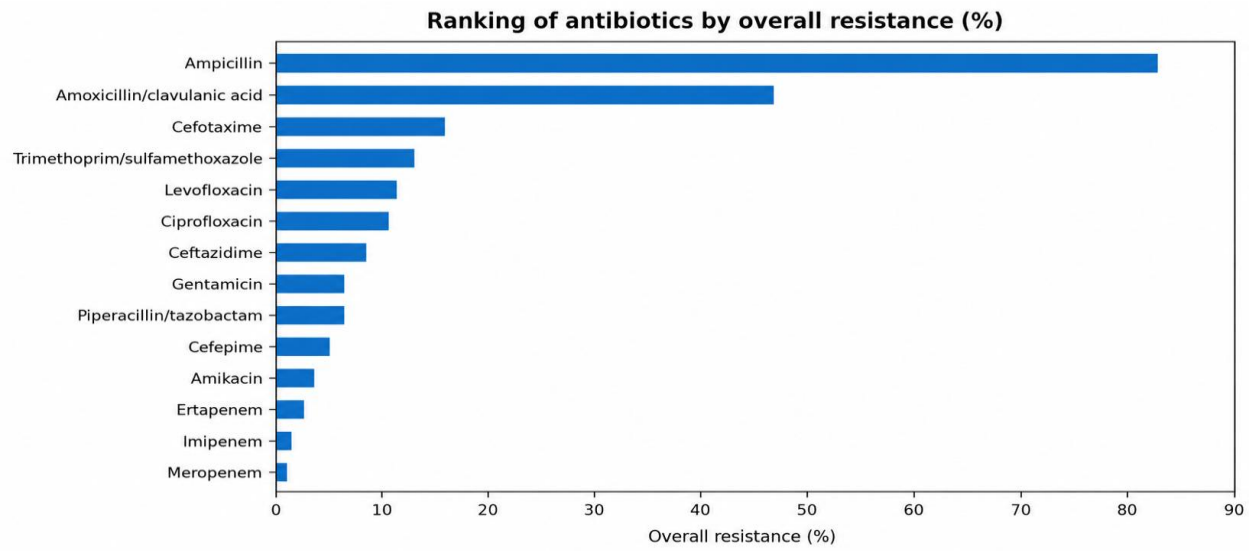


Figure 4.3. Comparative ranking of antibiotics according to the average level of bacterial resistance

The results demonstrate important differences in antimicrobial efficacy both between therapeutic classes and between bacterial taxa involved in the etiology of acute diarrheal diseases. High levels of resistance to aminopenicillins and, to a lesser extent, to β-lactam/β-lactamase inhibitor combinations limit their use in empirical therapy. In contrast, carbapenems and fourth-generation cephalosporins maintained high microbiological efficacy. The differences in resistance between bacterial taxa highlight the need to adapt treatment to the etiological agent. Rapid identification of the microorganism and performing an antibiogram remain essential for choosing a targeted therapy, reducing therapeutic failure and limiting the spread of multiresistant strains.

The relative stability of the comparative antimicrobial resistance profile observed during 2019–2024 demonstrates the effectiveness of continuous monitoring of bacterial susceptibility and emphasizes the need to maintain a permanent microbiological surveillance system. The data obtained can constitute a scientific basis for updating national empirical antibiotic therapy guidelines, optimizing antimicrobial stewardship policies, and strengthening measures to control the spread of bacterial resistance in the Republic of Moldova.

GENERAL CONCLUSIONS

The results obtained in correlation with the purpose and objectives formulated in the doctoral thesis "Pathogenic agents of acute diarrheal diseases – morpho-cultural particularities, identification methods, antibiotic resistance and spread dynamics in the municipality of Chisinau", allowed the formulation of the following general conclusions:

1. During the period 2011 - 2024, based on the investigation of 14,335 clinical samples collected from patients with acute diarrheal diseases within the PHMI TMA Buiucani, 2976 bacterial strains (20.8%) were isolated and 14 agents involved in the etiology of ADD were identified, which demonstrates the high taxonomic diversity and heterogeneous nature of the etiological structure of these infections.

2. It was established that the etiological structure of ADD is dominated by conditionally pathogenic microorganisms (94.9%), with the predominance of the genus *Klebsiella* spp. (40.63%), followed by *Citrobacter* spp. and *Proteus* spp., while the pathogens *Salmonella* spp. and *Shigella* spp. had a reduced share, highlighting the increasing role of opportunistic flora in the etiology of the investigated cases.

3. It was demonstrated that among the pathogens involved in ADD, the genus Salmonella predominates, represented especially by the serotypes *S.* Enteritidis and *S.* Typhimurium, while *Shigella* spp. presented sporadic distribution and limited serological spectrum, characterized by the predominance of the serotypes *S. sonnei* and *S. flexneri*.

4. The characterization of the morpho-cultural peculiarities of the identified bacterial agents was carried out and their value for microbiological differentiation and confirmation of the etiological diagnosis of acute diarrheal diseases was established.

5. The dynamic nature of the circulation of agents involved in ADD was highlighted, manifested by seasonal and annual variations in the etiological structure, with increased circulation in the warm quarters of the year and an increase in the number of isolated strains during the period 2021 – 2024, which indicates changes in the epidemiological process in the investigated population.

6. It was established that antimicrobial susceptibility profiles differ significantly between the investigated taxonomic groups, being characterized by increased levels of resistance to traditionally used antibiotics, especially ampicillin and amoxicillin/clavulanic acid, and by maintaining high sensitivity to carbapenems, aminoglycosides and other modern classes of antimicrobials.

7. The integrated comparative analysis of antibiotic resistance profiles revealed the existence of common patterns of susceptibility and resistance among the enterobacteria investigated, demonstrating that carbapenems and 4th generation cephalosporins maintained the highest antibacterial activity, while aminopenicillins and β-lactam/β-lactamase inhibitor combinations showed the highest levels of resistance.

8. The results obtained confirm the value of continuous microbiological monitoring and integrated assessment of antimicrobial susceptibility profiles for substantiating empirical and targeted antibiotic therapy, optimizing epidemiological surveillance and limiting the spread of resistant bacterial strains involved in the etiology of acute diarrheal diseases.

RECOMMENDATIONS FOR IMPLEMENTATION

1. It is recommended to use the data obtained on the etiological structure and dynamics of the circulation of agents involved in ADD to optimize the microbiological and epidemiological surveillance system at local and national levels.

2. It is recommended to capitalize on the sensitivity and antibiotic resistance profiles determined within the study for the periodic updating of local empirical antibiotic therapy protocols and for promoting the rational use of antimicrobials based on the antibiogram.

3. It is recommended to adapt the measures for the prevention, surveillance and control of ADD to the seasonal particularities highlighted in the study, by intensifying microbiological monitoring activities in the II and III trimesters, characterized by the most intense circulation of etiological agents.

4. It is recommended to implement and expand the use of automated methods for identification and testing of antimicrobial susceptibility in microbiological laboratories, in order to reduce diagnostic time and optimize therapeutic conduct.

5. It is recommended to use the research results in the development of continuous training programs for medical and laboratory personnel involved in microbiological diagnosis, epidemiological surveillance and management of acute diarrheal diseases.

RECOMMENDATIONS FOR CONTINUING RESEARCH

1. It is recommended to expand the microbiological monitoring of ADD at the national level, to assess regional variations in the etiological structure and antibiotic resistance profiles.

2. It is recommended to complete microbiological investigations by applying molecular methods (PCR, genomic sequencing and molecular typing), in order to identify the genetic mechanisms of antimicrobial resistance and the phylogenetic relationships between circulating strains.

3. It is recommended to deepen research on the role of conditionally pathogenic microorganisms in the etiology of ADD, with a focus on virulence factors, adaptation mechanisms and their epidemiological potential.

4. It is recommended to carry out longitudinal studies on the evolution of antibiotic resistance in enterobacteria and to assess the impact of policies for the prudent use of antimicrobials on the dynamics of bacterial resistance.

5. It is recommended to develop integrated models for comparative analysis of antibiotic resistance, based on synthetic indicators and multivariate statistical methods, to assess trends in the evolution of bacterial resistance and to substantiate microbiological surveillance strategies.

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ADNOTARE

Guțu Nadejda. „Agenții patogeni ai bolilor diareice acute – particularități morfo-culturale, metode de identificare, antibioticorezistența și dinamica răspândirii în municipiul Chișinău”, teză de doctor în științe biologice, Chișinău, 2026.

Teza constă din: introducere, 4 capitole, concluzii generale și recomandări, bibliografie din 226 titluri, 4 anexe, 132 pagini de text de bază, 44 figuri, 32 tabele. Rezultatele obținute au fost publicate în 14 lucrări științifice.

Cuvinte cheie: agenți patogeni, boli diareice acute, structura etiologică, particularități morfo-culturale, dinamica răspândirii, sensibilitate la antimicrobiene.

Domeniul de studiu: 163.04. Microbiologie

***Scopul lucrării*:** Elucidarea diversității taxonomice și a particularităților morfo-culturale ale agenților patogeni ai bolilor diareice acute, implementarea metodelor de identificare microbiologică, determinarea profilurilor de sensibilitate și rezistență la antibiotice și analiza dinamicii răspândirii lor în municipiul Chișinău.

***Obiectivele*:** Determinarea structurii etiologice și a diversității taxonomice a agenților patogeni implicați în bolile diareice acute în cadrul AMT Buiucani, municipiul Chișinău; Caracterizarea particularităților morfo-culturale ale agenților patogeni identificați și evidențierea caracteristicilor acestora în raport cu apartenența taxonomică; Aplicarea metodelor microbiologice de identificare și evaluarea eficienței acestora în identificarea agenților patogeni și determinarea sensibilității la antimicrobiene; Analiza distribuției și a dinamicii circulației agenților patogeni în raport cu perioada de studiu, sezonalitatea și structura pe grupe de vârstă a subiecților cu boli diareice acute; Determinarea profilurilor de sensibilitate și rezistență la antibiotice ale tulpinilor izolate și evidențierea tendințelor actuale ale antibioticorezistenței.

Noutatea și originalitatea științifică. Pentru prima dată în municipiul Chișinău, în baza monitorizării microbiologice realizate în cadrul AMT Buiucani pe o perioadă extinsă (2011–2024), a fost realizată caracterizarea complexă a agenților patogeni implicați în bolile diareice acute prin integrarea analizei structurii etiologice, diversității taxonomice, particularităților morfo-culturale și profilurilor de sensibilitate la antimicrobiene. Au fost evidențiate particularitățile circulației agenților patogeni în funcție de perioada de studiu, sezonalitate și structura pe grupe de vârstă a pacienților, fiind identificate tendințe ale modificării structurii etiologice și ale răspândirii antibioticorezistenței. Studiul completează datele existente privind spectrul agenților bacterieni implicați în bolile diareice acute și oferă o bază actualizată pentru interpretarea particularităților microbiologice și epidemiologice ale acestora în condițiile municipiului Chișinău.

Problema științifică soluționată. A fost soluționată problema științifică privind caracterizarea structurii etiologice și a rezistenței antimicrobiene a agenților implicați în bolile diareice acute în cadrul AMT Buiucani, prin identificarea spectrului etiologic, a particularităților epidemiologice și a tendințelor de circulație și rezistență, oferind suport științific pentru optimizarea supravegherii, diagnosticului și tratamentului acestor infecții.

Semnificația teoretică. Lucrarea contribuie la extinderea cunoștințelor privind diversitatea taxonomică, particularitățile morfo-culturale și profilurile de sensibilitate la antimicrobiene ale agenților patogeni implicați în bolile diareice acute și completează datele privind dinamica circulației acestora în condițiile municipiului Chișinău.

Valoarea aplicativă a lucrării. Rezultatele obținute contribuie la optimizarea identificării microbiologice a agenților patogeni implicați în bolile diareice acute, monitorizarea sensibilității și rezistenței la antimicrobiene și susținerea activităților de supraveghere și control al acestor infecții.

Implementarea rezultatelor științifice. Rezultatele cercetării sunt implementate în activitățile practice ale laboratorului microbiologic al Invitro Diagnostics SRL și sunt utilizate în procesul educațional și cercetarea științifică în domeniile biologiei, ecologiei și științelor aplicate.

АННОТАЦИЯ

Гуцу Надежда. «Возбудители острых диарейных заболеваний – морфокультуральные особенности, методы идентификации, антибиотикорезистентность и динамика распространения в Кишиневе», кандидатская диссертация по биологическим наукам, Кишинев, 2026.

Диссертация состоит из введения, 4 глав, общих выводов и рекомендаций, библиографии из 226 наименований, 4 приложения, 132 страницы основного текста, 44 рисунок, 32 таблиц. Полученные результаты опубликованы в 14 научных статьях.

Ключевые слова: патогены, острые диарейные заболевания, этиологическая структура, морфокультуральные особенности, динамика распространения, чувствительность к антимикробным препаратам

Область исследования:163.04.Микробиология

***Цель диссертации*:** Изучение таксономического разнообразия и морфокультуральных особенностей возбудителей острых диарейных заболеваний, внедрение методов микробиологической идентификации, определение профилей чувствительности и устойчивости к антибиотикам и анализ динамики их распространения в городе Кишиневе.

***Задачи*:** Определение этиологической структуры и таксономического разнообразия патогенов, вызывающих острые диарейные заболевания в пределах медицинского центра Буюканы, муниципалитета Кишинева; характеристика морфокультуральных особенностей выявленных патогенов и выделение их характеристик в зависимости от таксономической принадлежности; применение методов микробиологической идентификации и оценка их эффективности при идентификации патогенов и определении антимикробной чувствительности; анализ распределения и динамики циркуляции патогенов в зависимости от периода исследования, сезонности и возрастной структуры лиц с острыми диарейными заболеваниями; определение профилей чувствительности и резистентности к антибиотикам выделенных штаммов и выделение современных тенденций в антибиотикорезистентности.

Научная новизна и оригинальность. Впервые в Кишиневе на основе микробиологического мониторинга, проведенного в ТМА Буюканы в 2011–2024 гг., дана комплексная характеристика возбудителей острых диарейных заболеваний с учетом их этиологической структуры, таксономического разнообразия, морфокультуральных особенностей и антибиотикочувствительности. Выявлены особенности циркуляции патогенов по периодам исследования, сезонам и возрастным группам пациентов, а также тенденции изменения этиологической структуры и антибиотикорезистентности. Полученные данные расширяют представления о бактериальных возбудителях ОДЗ и служат актуальной основой для оценки их микробиологических и эпидемиологических особенностей в условиях Кишинева.

Разрешённая научная проблема. Научная проблема, касающаяся характеристики этиологической структуры и антимикробной резистентности возбудителей острых диарейных заболеваний в пределах ТМА Буюканы, была решена путем выявления этиологического спектра, эпидемиологических особенностей, а также тенденций циркуляции и резистентности, что обеспечило научную основу для оптимизации эпидемиологического надзора, диагностики и лечения этих инфекций.

Теоретическая значимость. Данная работа способствует расширению знаний о таксономическом разнообразии, морфокультуральных особенностях и профилях антимикробной чувствительности патогенов, вызывающих острые диарейные заболевания, а также дополняет данные о динамике их циркуляции в условиях Кишинева.

Практическая значимость исследования. Полученные результаты способствуют оптимизации микробиологической идентификации патогенов, вызывающих острые диарейные заболевания, мониторингу чувствительности и резистентности к антимикробным препаратам, а также поддержке мероприятий по эпидемиологическому надзору и контролю этих инфекций.

Внедрение научных результатов. Результаты исследования внедрены в практическую деятельность микробиологической лаборатории Invitro Diagnostics SRL и используются в учебном процессе и научных исследованиях в области биологии, экологии и прикладных наук.

ANNOTATION

Gutu Nadejda. „Pathogenic agents of acute diarrheal diseases – morpho-cultural particularities, identification methods, antibiotic resistance and spread dynamics in the municipality of Chisinau", PhD thesis in biological sciences, Chisinau, 2026.

The thesis consists of introduction, 4 chapters, general conclusions and recommendations, bibliography of 226 titles, 4 annexes, 132 basic text pages, 44 figures, 32 tables. The results obtained are published in 14 scientific papers.

Key words: pathogens, acute diarrheal diseases, etiological structure, morpho-cultural features, spread dynamics, antimicrobial sensitivity.

Field of study: 163.04. Microbiology.

***The purpose of the paper*:** Elucidating the taxonomic diversity and morpho-cultural peculiarities of pathogens of acute diarrheal diseases, implementing microbiological identification methods, determining antibiotic sensitivity and resistance profiles and analyzing the dynamics of their spread in the city of Chisinau.

***Objectives*:** Determination of the etiological structure and taxonomic diversity of pathogens involved in acute diarrheal diseases within TMS Buiucani, Chisinau municipality; Characterization of the morpho-cultural particularities of the identified pathogens and highlighting their characteristics in relation to taxonomic affiliation; Application of microbiological identification methods and evaluation of their efficiency in identifying pathogens and determining antimicrobial susceptibility; Analysis of the distribution and dynamics of pathogen circulation in relation to the study period, seasonality and age group structure of subjects with acute diarrheal diseases; Determination of antibiotic sensitivity and resistance profiles of isolated strains and highlighting current trends in antibiotic resistance.

Scientific novelty and originality. For the first time in Chisinau, based on microbiological monitoring carried out within the Buiucani TMS over an extended period (2011–2024), a complex characterization of pathogens involved in acute diarrheal diseases was carried out by integrating the analysis of the etiological structure, taxonomic diversity, morpho-cultural features and antimicrobial sensitivity profiles. The peculiarities of the circulation of pathogens were highlighted depending on the study period, seasonality and age group structure of patients, and trends in the change of the etiological structure and the spread of antibiotic resistance were identified. The study complements the existing data on the spectrum of bacterial agents involved in acute diarrheal diseases and provides an updated basis for interpreting their microbiological and epidemiological features in the conditions of Chisinau.

Scientific problem. The scientific problem regarding the characterization of the etiological structure and antimicrobial resistance of agents involved in acute diarrheal diseases within the Buiucani TMS was solved, by identifying the etiological spectrum, epidemiological particularities and circulation and resistance trends, providing scientific support for optimizing the surveillance, diagnosis and treatment of these infections.

Theoretical significance. The work contributes to expanding knowledge regarding the taxonomic diversity, morpho-cultural peculiarities and antimicrobial sensitivity profiles of pathogens involved in acute diarrheal diseases and completes data regarding the dynamics of their circulation in the conditions of Chisinau municipality.

The applicative value of the work. The results obtained contribute to optimizing the microbiological identification of pathogens involved in acute diarrheal diseases, monitoring antimicrobial sensitivity and resistance, and supporting surveillance and control activities of these infections.

Implementation of scientific results. The research results are implemented in the practical activities of the microbiological laboratory of Invitro Diagnostics SRL and are used in the educational process and scientific research in the fields of biology, ecology and applied sciences.

GUȚU NADEJDA

PATHOGENIC AGENTS OF ACUTE DIARRHEAL DISEASES - MORPHO-CULTURAL PECULIARITIES, IDENTIFICATION METHODS, ANTIBIOTIC RESISTANCE AND THE SPREAD DYNAMICS IN CHISINAU

163.04. Microbiology

Abstract of PhD Thesis in Biological Sciences

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