DIVERSITY AND DYNAMICS OF THE SPREAD OF MICROORGANISMS FROM THE ENTEROBACTERIACEAE FAMILY WITH AN ETILOGICAL ROLE IN ACUTE DIARRHEA DISEASES

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Abstract. The Enterobacteriaceae family is very numerous and represents the largest taxonomic unit, which includes 44 genera, 25 of which are involved in human pathology. Acute diarrheal disease presents a major public health issue, and identifying the pathogens helps both in detecting the sources of infection spread and in establishing the correct treatment. In this context, the aim of this research was to identify the microorganisms from the Enterobacteriaceae family most frequently involved in acute diarrheal diseases and the dynamics of their spread at the Public Health Medical Institution AMT Buiucani, Chișinău. The research was conducted between 2011 and 2023. A total of 2,393 Enterobacteriaceae strains (17.9%) were isolated and identified from 13,359 clinical samples received for the diagnosis of acute diarrheal disease outbreaks. Ten microorganisms from the Enterobacteriaceae family were identified – 8 conditional pathogens (*Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *Escherichia coli* cu proprietăți hemolizante, *Morganella* spp., *Serratia* spp. and *Providencia* spp.) with a share of 94.15%, and 2 pathogens (*Shigella* spp., *Salmonella* spp.) with a share of 5.85%. The etiological spectrum of conditional pathogens was dominated by *Klebsiella* spp. (43.71%), while for pathogens, Salmonella spp. (5.35%) predominated. Microorganisms from the Enterobacteriaceae family were isolated annually, with the exception of the genera *Morganella* and *Providencia* (conditional pathogens) and *Shigella* (pathogens), which were not identified in certain years. The highest circulation of Enterobacteriaceae was observed between 2011 and 2014 (10.2%-11.58%), with the lowest in 2020 (3.97%). The dynamics of the circulation of microorganisms from the Enterobacteriaceae family showed a slow decrease, with *Klebsiella* spp. dominating the etiological spectrum. The results obtained highlight the importance of monitoring the incidence of Enterobacteriaceae with etiological roles in acute diarrheal diseases to prevent acute bacterial diarrhea.

Keywords: microorganisms, Enterobacteriaceae, diversity, dynamics, acute diarrheal diseases.

Rezumat. Diversitatea și dinamica răspândirii microorganismelor din familia Enterobacteriaceae cu rol etiologic în boli diareice acute. Familia Enterobacteriaceae este foarte numeroasă și reprezintă cea mai vastă unitate taxonomică, care include 44 genuri, dintre care 25 sunt implicate în patologia umană. Boala diareică acută prezintă o problemă majoră de sănătate publică, iar identificarea agenților patogeni servește atât la depistarea surselor de răspândire a infecției, cât și la stabilirea tratamentului corect. În acest context scopul acestei cercetări a constat în identificarea microorganismelor din familia Enterobacteriacea cel mai frecvent implicate în bolile diareice acute și dinamica răspândirii în Instituția Medicală Sănătate Publică AMT Buiucani, municipiul Chișinău. Cercetările au fost efectuate în anii 2011-2023. Au fost izolate și identificate 2393 de tulpini (17,9%) de enterobacterii din cele 13359 de probe clinice recepționate pentru diagnosticul epizoadelor de boală diareică acută. Au fost identificate 10 microorganisme din familia Enterobacteriaceae – 8 agenții condiționat patogeni (*Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *Escherichia coli* cu proprietăți hemolizante, *Morganella* spp., *Serratia* spp. și *Providencia* spp.), cu o pondere de 94,15% și 2 agenți patogeni (*Shigella* spp., *Salmonella* spp.), cu o pondere de 5,85%. Spectrul etiologic al agenților condiționat patogeni a fost dominat de *Klebsiella* spp. (43,71%), al celor patogeni – de *Salmonella* spp. (5,35%). Microorganismele din familia Enterobacteriaceae au fost izolate anual, cu excepția genurilor *Morganella* și *Providencia* (condiționat patogene) și *Shigella* (patogene), care în unii ani n-au fost identificate. Cea mai mare circulație a enterobacteriilor a fost evidențiată în perioada 2011-2014 (10,2%-11,58%), cea mai mica – în 2020 (3,97%). Dinamica circulației microorganismelor din familia Enterobacteriaceae a înregistrat o scădere lentă, spectrul etiologic a fost dominat de *Klebsiella* spp. Rezultatele obținute relevă importanța monitorizării incidenței enterobacteriilor cu rol etiologic în BDA pentru a preveni diareea bacteriană acută.

Cuvinte cheie: microorganisme, Enterobacteriaceae, diversitate, dinamică, boli diareice acute.

INTRODUCTION

The Enterobacteriaceae family is very numerous, with various features, and represents the largest taxonomic unit, which includes 44 genera, of which 25 are involved in human pathology. They inhabit the intestines of humans and animals, being spread with feces everywhere in the environment and can cause food poisoning and acute diarrheal disease.

Among the conditional pathogens, the microorganisms from the *Klebsiella* genus are the most frequent cause of acute diarrheal diseases (ADD). They are ubiquitous and commonly found in the etiology of acute diarrheal diseases (GALEŢCHI et al., 1997; EVTODIENCO et al., 2010; GUZMAN-OTAZO et al., 2019; JELAMSCHI et al., 2023). *Klebsiella* bacteria are normally found in the intestinal tract. The species *K. pneumoniae* and *K. oxytoca* have a significant impact on human pathology (RUSU et al., 2009). Species from the *Enterobacter* genus are mainly associated with healthcare-associated infections. Out of the 22 species in the *Enterobacter* genus, the clinically most significant are *E. cloacae* and *E. aerogenes*. These conditional pathogens cause gastrointestinal diseases, which are transmitted through water or food (REBEDEA, 2000). The ability of the *Enterobacter* genus to survive in a wide range of environmental conditions leads to the outbreak of various infections in clinical settings (CHAVDA et al., 2016).

Among the pathogenic enterobacteria, the genera *Salmonella* and *Shigella* are responsible for millions of cases of foodborne illness. In developing countries, diseases caused by these pathogens are not only the most widespread but are also associated with a higher mortality rate (DEKKER & FRANK, 2015). The worsening socio-economic conditions in these countries play an important role in increasing the risk of contracting shigellosis (COVRIC et al., 2013).

Globally, about 10% of the population is affected by acute diarrheal disease annually, leading to over 420,000 deaths. According to the World Health Organization (WHO) and UNICEF, approximately 2 billion cases of diarrheal disease are recorded worldwide each year, and 1.9 million children under 5 years of age die from acute bacterial diarrhea, particularly in developing countries (GRIMONT & GRIMONT, 2006).

In the Republic of Moldova, the incidence of overall morbidity due to acute bacterial diarrhea (ABD) in 2022 was 450.6 per 100,000 population, which is 2.7 times higher compared to 2021 – 170.1 cases per 100,000 (2020 – 113.5, 2019 – 509.4 per 100,000 population). The overall morbidity indices for ADD are also on the rise, reaching 490.5 in 2022 (a 2.4-fold increase) compared to the previous year (2021 – 203.6%, 2020 – 144.2% per 100,000 population). During 2022, 21 ADD outbreaks were reported, affecting 310 individuals, including 235 children. This is in contrast to 2021, which had 8 outbreaks with 117 patients, 2020 with 7 outbreaks and 140 patients, and 2019 with 34 outbreaks and 590 patients. In 2022, 446 multiple outbreaks of ABD with 2-4 cases were recorded, showing an increase compared to previous years (2021 – 220, 2020 – 132), indicating a potentially higher risk of outbreaks (JELAMSCHI et al., 2023).

The purpose of this study was to identify the microorganisms from the Enterobacteriaceae family most frequently involved in acute diarrheal diseases and to analyze the dynamics of their spread at the MTA Buiucani Public Health Medical Institution, Chișinău municipality.

MATERIAL AND METHODS

The research was conducted between 2011 and 2023. A total of 2,393 strains (17.9%) of enterobacteria were isolated and identified from the 13,359 clinical samples received at the Microbiological Laboratory of the Public Health Medical Institution AMT Buiucani, Chișinău, for the diagnosis of acute diarrheal disease outbreaks. The basis for the bacteriological analysis was the isolation of a pure culture of the pathogen and its subsequent study. The etiological confirmation of the disease was made based on coproculture, following the methodological instructions "Microbiological Diagnosis of Intestinal Infections" recommended by the Ministry of Health of the Republic of Moldova (EVTODIENCO et al., 2013). Before inoculation, each stool sample was homogenized by mixing 1 g with 2 ml of sterile saline solution (NaCl 0.9%). The inoculating loop was loaded with the biomaterial and then streaked on the surface of the agar plate in perpendicular lines.

Primary inoculation of the biomaterial was carried out on plates with the following culture media: Columbia agar + 5% blood, Endo agar, Chromatic Detection, Bismuth sulfite agar, SS agar, and then incubated at 36±1°C for 24 hours. After the incubation period, the plates were examined macroscopically.

Preliminary identification of the microorganisms was done based on cultural characteristics (S, M, or R colony types). The replication of lactose-negative colonies was carried out on Kligler Iron agar, a disaccharide medium used to differentiate microorganisms from the Enterobacteriaceae family based on their ability to ferment glucose, lactose, and produce H_2S.

Final identification was performed based on biochemical tests using the API EnteroPluri-Test, which includes tests for glucose (fermentation to acid or acid/gas), lysine and ornithine decarboxylation, hydrogen sulfide or indole production, fermentation of adonitol, lactose, arabinose, sorbitol, Voges-Proskauer (acetoin production), dulcitol/PA (phenylalanine deamination), urease hydrolysis, and sodium citrate utilization. For the pathogenic agents *Salmonella* spp. and *Shigella* spp., serological tests were performed using the agglutination method (a drop of polyvalent serum and a drop of sterile saline solution were placed on a microscope slide) (REBEDEA, 2000). Confirmation of pathogenic microorganisms (*Shigella* spp., *Salmonella* spp.) was done using specific polyvalent and monovalent immune sera on the microscope slide.

RESULTS AND DISCUSSION

During the study period, from the total number of samples taken (13359) from patients with acute diarrheal diseases, 2393 (17.9%) strains of microorganisms from the Enterobacteriaceae family were isolated and eight conditionally pathogenic agents were identified – *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *Escherichia coli* with hemolytic properties, *Morganella* spp., *Serratia* spp. and *Providencia* spp., with a share of 94.15% and 2 pathogens – *Shigella* spp. and *Salmonella* spp., with a share of 5.85%.

The etiological spectrum of conditionally pathogenic agents was dominated by *Klebsiella* spp. (43.71%), followed, with a smaller percentage, by *Proteus* spp. (14.33%), *Citrobacter* spp. (13.12%), *Enterobacter* spp. (10.99%) and Escherichia coli with hemolytic properties (8.73%). The lowest share belonged to *Serratia* spp. (1.42%), *Providencia* spp. (1.09%) and *Morganella* spp. (0.75%) (Fig. 1). The etiological spectrum of pathogens was dominated by *Salmonella* spp. (5.35%), while *Shigella* spp. had a share of 0.51%.

The quantitative indices of strains from the *Enterobacteriaceae* family show that acute diarrheal disease was most frequently caused by *Klebsiella* spp., with the highest value recorded in 2017 (54.93%) and the lowest in 2023 (31.9%) (Fig. 2). The proportion of conditional pathogen indices during the study period showed a slow decrease in values, except for *Citrobacter* spp. (20.22%) and *E. coli* with hemolytic properties (15.85%), which recorded their highest values in 2022. The *Proteus* genus reached its peak in 2012 (27.57%), *Enterobacter* spp. in 2018 (28.16%), and *Serratia* spp. in 2019 (4.21%). The *Morganella* genus was recorded during the study period in 2012, 2014, 2017, and between 2019-2022, with values ranging from 0.41% to 3.68%. In other years, it was not identified.

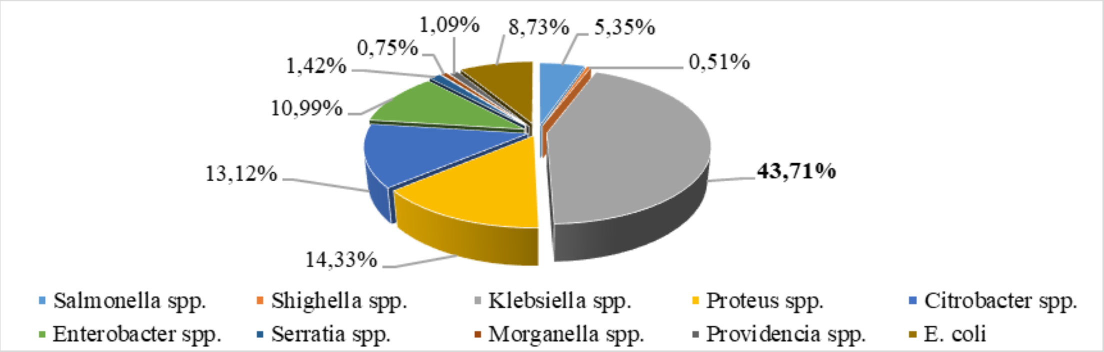

Figure 1. Etiological spectrum of strains from the Enterobacteriaceae family isolated during the study period.

The proportion of quantitative indices for pathogenic agents also showed a slow decrease during the study period. *Salmonella* spp. recorded its highest value in 2015 (15.79%). The *Shigella* genus was only recorded in 2011, 2015, and between 2017-2020, with the highest value in 2017 (1.88%). It was not identified in other years.

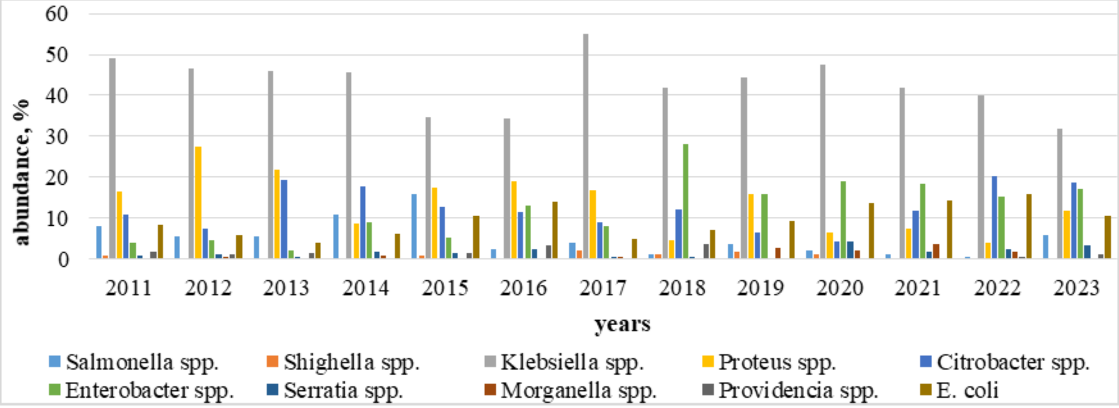

Figure 2. Share of quantitative indices of representatives of the Enterobacteriaceae family identified during the study period.

The highest circulation of microorganisms from the Enterobacteriaceae family was registered in the period 2011-2014 (10.2%-11.58%), the lowest – in 2020 (3.97%) (Fig. 3). Starting with 2021, a slow increase in positive results is observed. Microorganisms from the Enterobacteriaceae family were isolated annually, with the exception of the genera *Morganella* and *Providencia* (conditionally pathogenic) and *Shigella* (pathogenic), which in some years were not identified.

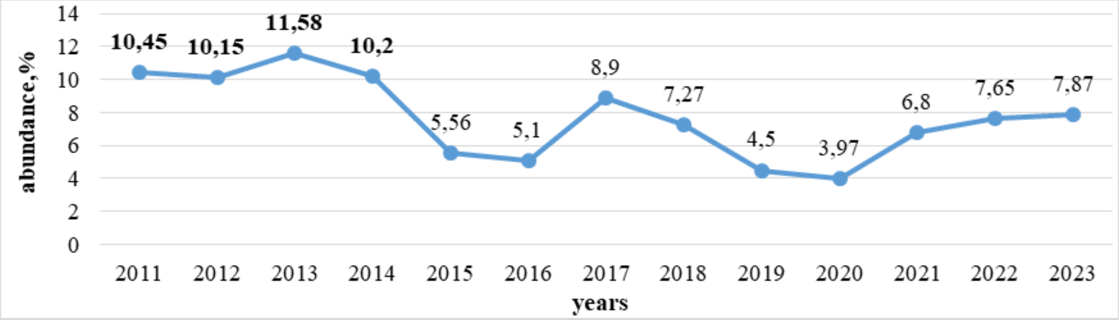

Figure 3. Dynamics of the circulation of microorganisms from the Enterobacteriaceae family during the study period.

In the Republic of Moldova, the incidence of overall morbidity due to ADD in the period 2002 – 2023 was increasing, except for the years 2020, 2021 when it was the lowest. In 2022 it amounted to 450.6 per 100 thousand population, increasing by 2.7 times compared to 2021 - 170.1 cases per 100 thousand, (2020 - 113.5, 2019 - 509.4 per 100 thousand population) (https://statbank.statistica.md/PxWeb/pxweb/ro/30%20Statistica%20sociala/30%20Statistica%20sociala__08%20SAN__SAN020/SAN020400rcl.px/table/tableViewLayout1/). This can be explained by the fact that during the period 2020-2021, sanitary and epidemiological rules in institutions and public transport were tightened, various disinfectant solutions were used. Compliance with personal hygiene rules is a sure condition for preventing illness.

CONCLUSIONS

In total, during the study period, 2,393 strains of microorganisms from the Enterobacteriaceae family were isolated, which include eight conditional pathogens – *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *Escherichia coli* with hemolytic properties, *Morganella* spp., *Serratia* spp., *Providencia* spp., and two pathogens – *Shigella* spp. and *Salmonella* spp.

During the study period, a fluctuation in the quantitative indices of *Enterobacteriaceae* microorganisms was observed, ranging from 0.51% to 43.71%. The etiological spectrum was dominated by the conditional pathogen *Klebsiella* spp. (43.71%).

The highest circulation of Enterobacteriaceae microorganisms was recorded between 2011 and 2014 (10.2%-11.58%), and they were isolated annually, with the exception of the genera *Morganella* and *Providencia* (conditional pathogens) and *Shigella* (pathogens), which were not identified in some years.

The results obtained during the study period highlight the importance of monitoring the incidence of Enterobacteriaceae with etiological roles in (ADD) to prevent acute bacterial diarrhea.

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