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Enterobacteria from the genera *Salmonella* and *Shigella* with an etiological role in acute diarrheal diseases

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Abstract. The research was carried out during the years 2011-2022 in PHMI TMA Buiucani, 12496 samples were processed and 129 strains (1.03%) were isolated. The major causative agents of acute diarrheal disease were pathogenic enterobacteria *Salmonella* spp. (90.7%) and *Shigella* spp. (9.3%). The dominant serovars were *Salmonella* Enteritidis (53.49%) and S. Typhimurium (37.21%), and *Shigella* serogroups constituted 4.65% each. The isolation rate of *Salmonella* and *Shigella* strains decreased ranging from 1.67% (year 2011) to 0.12% (year 2022), except for 2014 (2.01%) and 2015 (1.78%). To mitigate acute bacterial diarrhea, it is necessary to apply infection prevention strategies.

Keywords: pathogens, Enterobacteriaceae, *Salmonella*, *Shigella*, acute diarrheal diseases.

Enterobacteriile din genurile *Salmonella* și *Shigella* cu rol etiologic în bolile diareice acute

Rezumat. Cercetările au fost efectuate în perioada anilor 2011-2022 în IMSP AMT Buiucani, au fost prelucrate 12496 de probe și izolate 129 tulpini (1,03%). Agenții cauzali majoritari ai bolii diareice acute au fost enterobacteriile patogene *Salmonella* spp. (90,7%) și *Shigella* spp. (9,3%). Dominante au fost serovarurile *Salmonella* Enteritidis (53,49%) și S. Typhimurium (37,21%), iar serogrupurile de *Shigella* au constituit 4,65% fiecare. Rata izolării tulpinilor de *Salmonella* și *Shigella* a scăzut variind de la 1,67% (anul 2011) până la 0,12% (anul 2022), cu excepția anilor 2014 (2,01%) și 2015 (1,78%). Pentru atenuarea diareii bacteriene acute este necesar de a aplica strategii de prevenire a infecțiilor.

Cuvinte cheie: agenți patogeni, Enterobacteriaceae, *Salmonella*, *Shigella*, boli diareice acute .

1. Introduction

Among the enteric pathogens of bacterial origin, which endanger human life worldwide, there are included *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Vibrio cholerae*, *Escherichia coli, Yersinia enterocolitica*. Among these, the genera *Salmonella* and *Shigella* continue to be the major cause of acute diarrheal disease (ADD) in many countries, thus presenting a serious challenge to health authorities. The aforementioned pathogens cause a well-characterized spectrum of disease in humans, ranging from asymptomatic states to hemorrhagic colitis and fatal typhoid fever [2]. Bacteria of the genus *Salmonella* are actively fermentative and cause the disease called salmonellosis in humans. Salmonellosis remains a serious socio-economic problem for the most countries of the world. Actually, even in all economically developed countries of the world, salmonellosis is widespread. Currently, more than 2500 serotypes of *Salmonella* are described, some of them having host specificity, found only in humans or only in certain species of animals and birds [10]. In the United States, 4 serotypes represent nearly half of all reported human isolates, namely *Salmonella* Typhimurium (19%), *Salmonella* Enteritidis (14%), *Salmonella* Newport (9%), and *Salmonella* Javiana (5%) [1].

All representatives of the genus *Shigella* are capable of causing a widespread disease - dysentery. Dysentery has been reported since ancient times, today recognized as "dysenteric syndrome" (muco-sanguineous stools, tenesmus and abdominal cramps, frequent stools) characteristic for several diarrheal diseases. Since the last century, it has also traditionally been called "bacillary dysentery", to differentiate it from amoebic dysentery. *Shigella* are gram-negative, aerobic, non-sporulating, immobile microorganisms. They differ in antigenic structure, biochemical activity, pathogenicity and virulence. A major virulence is possessed by *Shigella dysenteriae*, especially the S. *shigae* serotype, which secretes exotoxin with hemolytic properties. Fairly high virulence possesses S. *flexneri* and less pronounced – S. *sonnei*. Shigelloses are typical anthroponoses. Both the reservoir and the sources of the pathogen are hosted by the sick person, with an acute or chronic form of infection. A major risk represent the patients with mild or cleared forms of infection, especially people who are part of the socially vulnerable contingents [8].

In the Republic of Moldova, in 2021 the dysentery morbidity increased insignificantly to 0.06 cases per 100 thousand, compared to 2020 (0.03). In the Eastern districts, morbidity remains at the same level, constituting 1.71 cases per 100 thousand. During the years 2021 and 2020, no outbreak of dysentery was reported, the last outbreak being recorded in 2017. After the worsening of the epidemiological situation due to salmonellosis in 2014 (52.9 per 100 thousand), when the maximum indicators were reached after zero in 1992, in 2021 the morbidity increased insignificantly to 7.31 (by 12.4% compared to 2020), including by increasing the number of epidemic outbreaks – 8 (2020 – 7, 2019 – 34). As in previous years, in 2021 there were no significant changes in the etiological structure of salmonella in the population and in the environment, predominant are S. Enteritidis – 84.5% (2020 – 84.6%, 2019 – 76.9%) and S. Typhimurium – 11.6% (2020 – 14.1%, 2019 – 17.2%). According to National Agency for Food Safety (ANSA) data, 36 strains of salmonella were detected in animal products (raw material and finished products) in 2021, including: S. Infantis – 25.0%, S. Kentucky – 13.9%, S. Fillmore – 5.6%, S. Enteridis – 5.6%, S. Montevideo – 11.7%, *Salmonella* spp. – 33.4%, etc. [6].

Morbidity through salmonellosis is mainly recorded among the urban population. The multi-year dynamics of morbidity due to salmonellosis in the country has a cyclical character. In the last 70 years two periods are highlighted: the first one – 1961-1989, with a tendency of increasing morbidity, period associated with the intensification of animal breeding in zootechnical complexes, and the second – period marked by a tendency of decreasing morbidity, associated with the lowering of animal number maintained in complexes and their increasing in the private sector. The periodicity of the epidemic process is largely conditioned by the changes in the etiological structure. The morbidity increase in the 70s of the last century was caused by S. Typhimurium. The share of this *Salmonella* serovariant in the etiological structure in the given period was 60.8%. The increase in morbidity in the 80s was mostly ($\approx$70%) caused by S. Enteritidis. In the years 1999-2005, S. Typhimurium serovar prevailed in the etiological structure (60%), and in the last period (2006-2012) dominated S. Enteritidis (59.13%). Adequate sanitary-hygienic rules must be strictly followed when transporting, storing, preparing and marketing food products. An important measure concerns the deratisation and disinsection of storage and food processing objects [8].

Morbidity through shigellosis is also more frequently recorded in the urban population in comparison with the rural population. Most illnesses occur in the warm season of the year. In the summer-autumn months, 70-80% of the annual morbidity is registered. During the warm time of the year, the need for drinking water essentially increases and favorable conditions are created for the multiplication of shigella in food products, especially in dairy products, but also in water, conditions that can lead to more frequent occurrence of both sporadic cases and of outbreaks or epidemics [8].

The purpose of this work is to identify the pathogens from genera *Salmonella* and *Shigella* from the Enterobacteriaceae family, their share in the etiology of acute diarrheal disease and the dynamics of the spread in the Public Health Medical Institution TMA Buiucani, Chisinau.

2. Materials and Methods

The research was carried out during the years 2011-2022. In total, 12496 clinical samples were investigated in the microbiological laboratory of the Public Health Medical Institution (PHMI) TMA Buiucani. The etiological confirmation of the disease was carried out on the basis of coproculture, according to the methodological indication

”Microbiological diagnosis of intestinal infections” recommended by MH RM [4]. Before being inoculated, each fecal matter sample was homogenized with sterile saline solution NaCl 0.85% - 2 ml. The loop was loaded with fecal matter and then discharged on the surface of the agar plate in the form of perpendicular streaks. The primary seeding of the biomaterial was done in Petri dishes on culture media (Endo agar, UTI chromogenic agar, Bismuth sulfite agar, Columbia agar + 5% blood) and thermostated at 37°C for 24 hours. After the incubation period, the Petri dishes and the tubes containing the sample are removed from the thermostat and the lactose-negative colonies are examined. For the identification of microorganisms from the Enterobacteriaceae family, the Kligler Iron Agar medium was used, based on the double fermentation of sugar and the production of H2S, and biochemical tests on differential media Urea agar, Citrate agar, Simmons agar, Acetate agar. The confirmation of pathogenic bacteria (*Shigella* spp., *Salmonella* spp.) was performed by the serological method of slide agglutination with specific polyvalent and monovalent immune sera [4, 5].

3. Results and Discussion

From the total number of investigated clinical samples (12496) there were identified 129 strains (1.03%) in which the etiological agent was represented by pathogenic microorganisms from 2 genera of the Enterobacteriaceae family: *Salmonella* (S. Enteritidis, S. Typhymurium) and *Shigella* (*S. sonnei, S. flexneri*). Among the representatives of these two genera the *Salmonella* genus had the highest share with 90.7% (117 strains), and the *Shigella* genus – 9.3% (12 strains). The analysis of the share of pathogenic strains identified during the study period showed that in most cases there were isolated the serotypes S. Enteritidis - 53.49% (69 strains) and S. Typhimurium - 37.21% (48 strains) (Fig.1) . The most frequently isolated *Shigella* serogroups were *S. flexneri* and *S. sonnei*, which recorded a share of 4.65% each (6 strains each).

Analyzing the etiological structure of the pathogenic strains depending on the frequency of isolation, during the study period the dominant serovars of the *Salmonella* enterica species were S. Enteritidis (83.33%) and S. Typhimurium (75%), and the species of the *Shigella* genus varied between 16.67% (*S. flexneri*) and 33.33% (*S. sonnei*) (Fig. 2).

One of the sources of salmonellosis infection are the products of sick poultry (meat, eggs) or clinically healthy poultry - carriers of different *Salmonella* serotypes pathogenic for humans [3]. Identifying the food source that caused the illness is sometimes difficult, but it is the most important measure to prevent the spread of salmonellosis infection. The European Center for Disease Prevention and Control (ECDC) reports that ”compared to 2015 there was a 23.6% increase in the number of outbreaks caused by S. Enteritidis

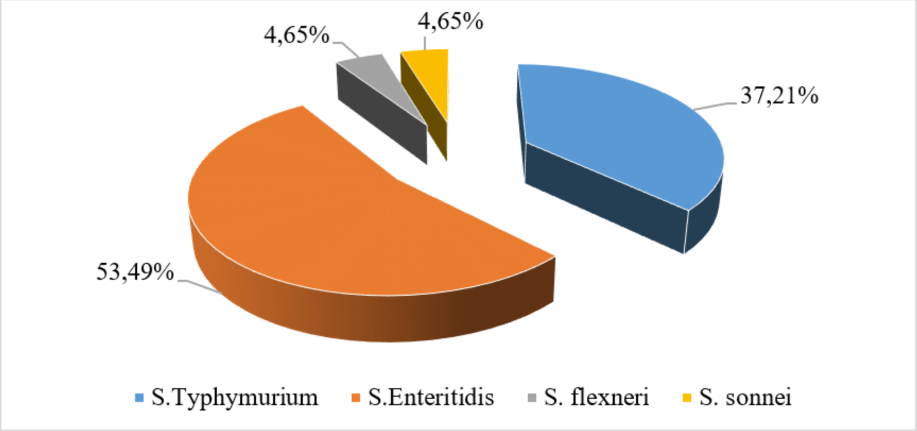

Figure 1. Etiological structure of pathogenic strains from the Enterobacteriaceae family isolated during the study period.

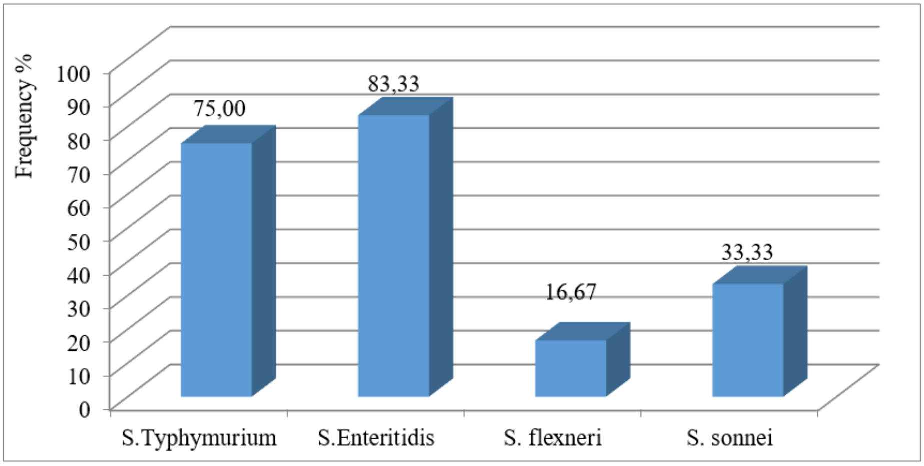

Figure 2. The etiological structure of pathogenic strains of the Enterobacteriaceae family depending on the frequency of isolation during the study period.

reported at EU level in 2016, with 13 Member States". The most important *Salmonella* serovars involved in the outbreaks were S. Enteritidis followed by S. Typhimurium [7]. *Shigella* spp. species are mainly transmitted by the fecal-oral route. The house fly (*Musca domestica*) acts as a vector of transmission of the pathogen. They are easily transmitted through personal contact with the infected person or by consuming contaminated food or water. Even a minimal bacterial load of 10-100 cells, can lead to the development of the infection. The pathogen transmission is influenced by the variations of environmental factors such as temperature and rainfall. The increase of the infection rate was observed during the summer months. They are highly contagious and occur especially in areas with unsatisfactory sanitary conditions [9].

Enterobacteriaceae pathogens *Shigella* spp. and *Salmonella* spp. were annually isolated. The highest circulation of pathogens *Shigella* spp. and *Salmonella* spp. was highlighted in the years 2014 (2.01%), 2015 (1.78%) and 2011 (1.67%) (fig. 3). Between 2013 and 2017, the share of positive results was practically similar, constituted about 1%, and in the years 2012, 2016, 2018-2022 – it varied from 0.89% to 0.12%.

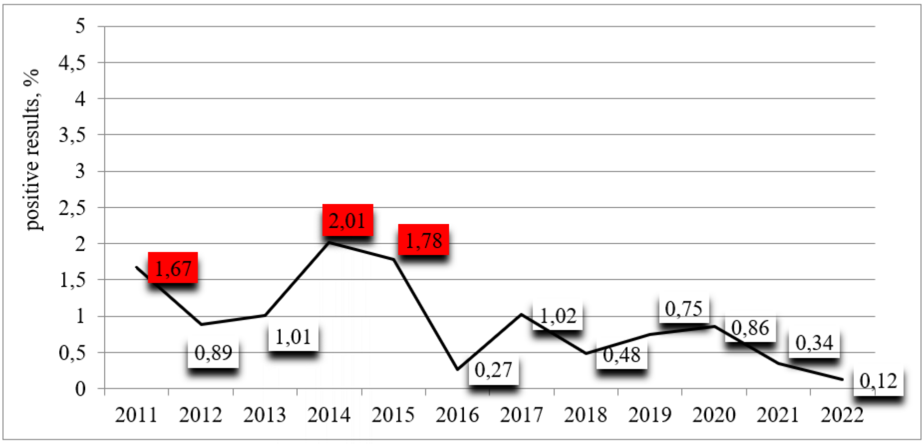

Figure 3. Dynamics of the pathogenic strains circulation of the Enterobacteriaceae family during the study period.

Analyzing the etiological structure of the pathogenic strains for each year, it was found that ADD were most frequently caused by 2 strains - 58.3%, less often 3 strains - 25% and very rarely 1 strain - 16.7% (fig. 4).

Throughout the study period ADDs were caused by both salmonella and shigella, but the analysis of the etiological structure demonstrated that salmonellosis was predominant, namely the strains *S. enterica* with the serovars S. Enteritidis and S. Typhymurium. In the last 2 years of study, all ADD cases investigated in the microbiological laboratory of PHMI TMA Buiucani were identified as salmonellosis - S. Enteritidis (2021) and S. Typhimurium (2022) with 100% each.

ADD caused by *Shigella* spp. strains were reported as sporadic cases in 2011 (*S. flexneri* 9.09%) and 2015 (*S. sonnei* 4.55%). The incidence of ADD caused by *Shigella* spp. serogroups has significantly increased in the following years. Thus, in 2017, 33.3% of disease cases were caused by *S. flexneri*, and during 2018-2020, the *S. sonnei* strain was identified as the causative agent of ADD in 33.3% - 50% of cases.

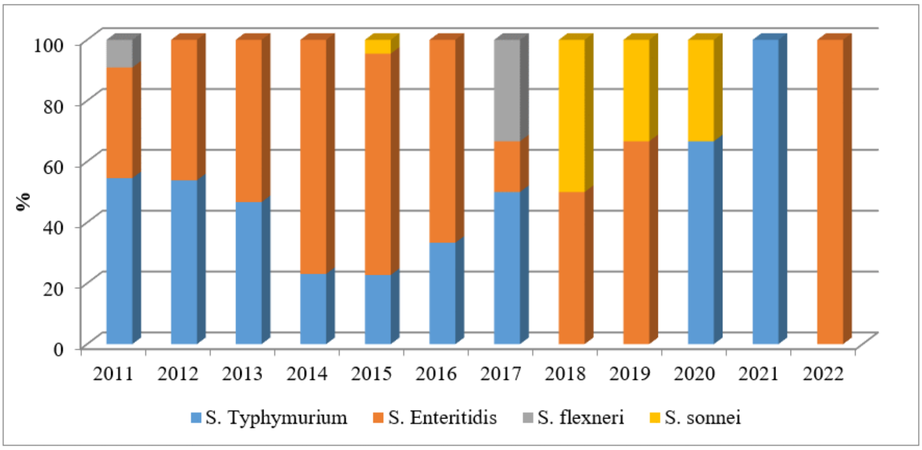

Figure 4. Etiological structure of pathogenic strains of the Enterobacteriaceae family determined during the study period.

The isolation rate of *Salmonella* and *Shigella* strains from ADD patients has decreased since 2019. During this period, the sanitary-epidemiological rules in institutions and public transport were strengthened, there were used various disinfectant solutions and compliance with personal hygiene rules is the safe condition of disease avoidance.

4. CONCLUSIONS

During the research period (2011-2022) it was established that in most cases the acute diarrheal disease has a bacterial etiology, the pathogens belonging to the Enterobacteriaceae family, the *Salmonella* and *Shigella* genera. The morbidity through salmonellosis constituted 90.7%, and through shigellosis 9.3%.

The isolation of pathogens in salmonellosis revealed the presence of the *Salmonella enterica* species, S. Enteritidis (53.49%) and S. Typhimurium (37.21%) serovars, and in shigellosis there were determined the *S. flexneri* and *S. sonnei* serogroups with a share of 4.65% each.

The bacteriological confirmations of ADD caused by enterobacteria had the highest incidence in 2011 (1.67%), 2014 (2.01%) and 2015 (1.78%), after which the dynamics of the circulation of these pathogens was decreasing continuously. In the etiological structure of ADD, 2 strains predominated more frequently - 58.3%, less often 3 strains - 25% and very rarely 1 strain - 16.7%.

The study was carried out as part of the doctoral project with the theme "Pathogenic agents of acute diarrheal diseases - morpho-cultural peculiarities, identification methods, antibiotic resistance and the spread dynamics in Chisinau".

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