DYNAMICS OF THE ETIOLOGICAL STRUCTURE AND SENSITIVITY TO ANTI-BACTERIAL AGENTS OF SALMONELLOSIS IN BULGARIA FOR THE PERIOD 2016 – 2019

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ABSTRACT

Background. In recent years, some features of salmonellosis have been noted: an increase in morbidity all around the world, which is accompanied by an increase in the number of isolated serotypes from humans, animals and food; an increase in sporadic diseases and multi-countries Salmonella outbreaks.

Material and methods. For the period 2016–2019 in the National Reference Laboratory for Enteric Infections, Pathogenic Cocci and Diphtheria 2 696 Salmonella isolates from all over the country have been confirmed. All of the strains were epidemiologically and microbiologically examined, and tested for antimicrobial resistance.

Results. Salmonellosis is caused by several dominant for Bulgaria serotypes - is for S. Enteritidis and S. Typhimurium take the leading place, followed by Salmonella 1,2,5,12: i: -, Infantis, Paratyphi B Java, Kottbus, Corvallis, Derby, Dublin.

Conclusions. Given the widespread transfer of Salmonella through the exchange of food and animals, and international tourism, the microbial landscape of salmonellosis is becoming more diverse, though the leadership of S. Enteritidis and S. Typhimurium has been maintained in the recent years, both worldwide and Bulgaria.

Keywords: salmonellosis, serotype, salmonella, antibiotic susceptibility.

INTRODUCTION

Salmonella infections are a major cause of gastroenteritis worldwide. Approximately 2.8 billion cases of diarrhoea are reported each year. The transmission of Salmonella is often associated with the consumption of contaminated water and food of animal origin (eggs, meat, dairy products, etc.) and this is facilitated by poor hygiene conditions (1). S. Enteritidis is the most commonly isolated Salmonella serotype, followed by S. Typhimurium according to WHO, CDC, ECDC and FoodNet. Besides, they are the leading cause of food epidemics, followed by S. Agona, S. Infantis, and last but not least S. Typhi. The latter is still taking thousands of children’s lives in endemic areas of Asia and Africa (2, 3, 4). The number of antibiotic-resistant Salmonella strains is increasing every year. Resistance to ampicillin, amoxicillin and trimethoprim/sulfamethoxazole is more common in developing countries. In all cases, a strict assessment of the actual needs for antibiotic therapy, consideration of the current aetiology and the antibiotic susceptibility of the dominant Salmonella serotypes are required (5, 6). The leading place of salmonellosis among intestinal bacterial infections, the growing incidence of salmonellosis all over the world, the high susceptibility of children, as well as the severity of the clinical course, determine the importance of the problem and the need for its thorough investigation.

Our study presents the etiological dynamics of salmonellosis in Bulgaria for the period 2016 - 2019 and the sensitivity of the isolated strains to the most commonly used antibacterial agents.

MATERIALS AND METHODS

For the period 2016 - 2019 in the National Reference Laboratory for Enteric Infections, Pathogenic Cocci and Diphtheria 2 696 Salmonella isolates from all over the country were confirmed, of which: 718 in 2016, 796 in 2017, 586 in 2018 and 596 in 2019. To trace the etiological dynamics of salmonellosis in Bulgaria, we conditionally divided the period into two smaller...
ones - first period January 2016 - December 2017, the second period January 2018 - December 2019. The identification of Salmonella serotypes was performed phenotypically - agglutination of a slide with antisalmonella sera (Sifin - Germany) and genetically - PCR analysis for identification of S. Enteritidis and S. Typhimurium, as well as for differentiation of S. Paratyphi B var. Java, as described in our previous studies (7, 8). Demonstration of epidemiological relatedness of Salmonella isolates and confirmation of epidemic outbreaks was performed by PFGE analysis (pulse gel-electrophoresis) according to the protocol of PulsNet, CDC, Atlanta (9).

Antimicrobial susceptibility
Due to the widespread prevalence of resistant Salmonella strains worldwide, treatment must be guided by the antimicrobial susceptibility of each specific isolate of Salmonella sp. The isolates of Salmonella sp. (from faeces, urine, blood and wounds) were tested for antimicrobial susceptibility to cefotaxime- CTX (30µg), cefoxitin- FOX (30µg), ceftazidime- CAZ (30µg), ampicillin- AMP (10µg), amoxicillin/clavulanic acid- AUG (20/10µg), amikacin- AMK (30µg), gentamicin- GEN (10µg), tetracycline- TE (30µg), chloramphenicol- CL (30µg), ciprofloxacin- CIP (5µg) and trimethoprim/sulfamethoxazole- SXT (1,25/ 23,75 µg) by the disk diffusion method according to EUCAST.

RESULTS AND DISCUSSION
During the first subperiod (January 2016 - December 2017) the number of salmonellosis cases in the country was 1,514, 468 of which in children aged 0-12 years; the incidence was 10.63%, and the mortality rate was 0.14% with two deaths of salmonellosis according to the data from the Epidemiology Department of NCIPD and NCPHA. During this period, the relative spread of S. Typhimurium increased (35.17%) at the expense of S. Enteritidis (41.01%). Salmonella 1,4,5,12: i:- comprised 13,02% of the cases, due to several registered food epidemics among children. Salmonella Infantis (5.98%) maintained its dominant position with a slight increase in isolated cases. During the same period 2018 - 2019 an epidemic outbreak of a rare for Bulgaria serotype - London was registered (3.89%), which dramatically changing dramatically of serotype dynamics for the country. The remaining 13.95% of salmonellosis cases are detailed in Figure 1b. A total of 11 epidemic outbreaks were reported during the second period, (six fewer than the first period ), as follows: 5 epidemics caused by S. Enteritidis, 3 epidemic outbreaks - by S. Typhimurium, 2 - by Salmonella 1,4,5,12: i:- and one with a total of 198 patients - by S. London,. The etiological dynamics of the reported salmonellosis outbreaks in the country for the two compared periods 2016 - 2017 and 2018 – 2019 is shown in Figure 2.
Salmonellosis cases are reported throughout the year, with the highest number of affected - 916 cases (60.45%) in June - October for the first period and 734 cases (62.06%) in May - September for the second period . This is due to the seasonal occurrence of food epidemics during the warmer months. In both periods the group of children (0-12 years) was the most affected, and the incidence in men was higher than in women, as published by other authors (10, 11).
In Bulgaria, cases of Typhoid fever are mostly sporadic. The last reported epidemic of S. Typhi was in 2014, affecting a risk group of elderly people in a closed group. For the two compared periods three sporadic, laboratory-proven cases of S. Typhi were reported in Bulgaria, each of them imported from
Figure 1a: Etiological structure of Salmonellosis in Bulgaria for the period 2016 - 2017.

Figure 1b: Etiological structure of Salmonellosis in Bulgaria for the period 2018 - 2019.
an endemic region - India, Morocco, Uganda. All the three isolates of S.Typhi demonstrated sensitivity to the tested antibiotics, which facilitated the treatment of this dangerous systemic infection.

It is important to note that a large part of the Bulgarian population in neglects the diarrhoea syndrome, and does not look for medical help, thus leaving an important part of diarrhoeas etiologically indeterminate, reflecting on the number and dynamics of salmonellosis cases in the country.

Despite the frequent reports of a worldwide increase in *Salmonella* resistance to fluoroquinolones (12, 13), the antimicrobial susceptibility demonstrated by *Salmonella* isolates studied in this investigation, was encouraging - 90% of isolates were sensitive to ciprofloxacin. According to the data of the NRL for Enteric Infections for the two compared periods, the studied representatives of *S. Enteritidis* showed a good sensitivity to the most often used antibacterial agents - CTX, FOX, CAZ, AMP, AUG, AMK, GEN, TE, CL, CIP, SXT (90%). Still, some Enteritidis strains were resistant to these antibiotics, most often to GEN and AMP. *S. Typhimurium* is often resistant to AUG and SXT, and more rarely – to CIP, which could be associated with the animal origin of the serotype and the frequent use of antibiotics in agriculture. It is impressive The 100% antibiotic sensitivity of certain epidemiologically related *Salmonella* isolates from the various epidemic outbreaks in the country should be mentioned (*S. Infantis*, and *S. Enteritidis* from the first period and *S. London* and *Salmonella 1,4,5,12:i:-* from the second period). The antibiotic susceptibility of isolates from an epidemic is one of the phenotypic criteria for proving the relatedness of these isolates (14). In our study, the phenotypic method was only complementary to the established gold standard for bacterial outbreaks examination - PFGE analysis, which has been successfully applied in NRL for Enteric Infections for years. The antibiotic susceptibility of the other serotypes did not change significantly.

Particular attention is paid to isolates belonging to *S. Paratyphi B* var. Java due to the possible manifestation of more severe clinical symptoms resembling Typhoid fever. The most common Java strains resistance is to

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**Figure 2:** Dynamics of the etiological causes of the registered epidemics of salmonellosis in Bulgaria for the two compared periods 2016 - 2017 and 2018 - 2019.
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AMK and GEN (30% of isolates).

It is of The need for antibiotic therapy requires careful assessment in order to avoid the development of dysbacteriosis and the appearance of resistance among the circulating Salmonella serotypes. During the studied period 2016 - 2019, the largest relative share in Bulgaria had S. Enteriditis among the isolated strains in Bulgaria, which is also the most prevalent salmonella serotype in Europe and the world according to ECDC, CDC and WHO. Salmonella Enteritidis is a leading etiological cause of food and water-borne epidemics, both in Bulgaria and in other EU member states (15, 16, 17). There is an obvious tendency of increase the number of laboratory-confirmed single-phase isolates of Salmonella Typhimurium (1,4,5,12:i:-) but at the moment the two-phase Typhimurium (1,4,5,12:i:1,2) is still leading in Bulgaria. The sensitivity of both varieties S. Typhimurium to AUG, CIP and SXT are lower, which hardly justifies their inclusion in the therapeutic process.

In general, the susceptibility of Salmonella to antibacterial drugs remains high, despite the growing number of resistant strains in the world. That could be due to a more moderate consumption of antibiotics in Bulgaria compared to other European countries as France, Italy, Belgium and others. (18, 19, 20).

CONCLUSION

The etiological structure of salmonellosis in Bulgaria does not differ significantly from that in other European countries. The relatively high susceptibility of Salmonella strains to the most commonly used antibiotics in the country should not be reassuring. A constant control and monitoring of salmonellosis is indispensable for the good medical practice.

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