PUBLIC HEALTH

# ANTIMICROBIAL SUSCEPTIBILITY OF SALMONELLA ENTERITIDIS ISOLATED IN THE PERIOD FROM 2020 TO 2022 IN THE SHTIP REGION

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**Key words:** infection, food, antimicrobial susceptibility, *Salmonella enteritidis*

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## Abstract

Bacteria of the genus *Salmonella* are widely distributed in nature. They can colonize different hosts and are the most common causes of intestinal infections in humans and animals. The main route of *Salmonella* transmission to humans is by contaminated food, or undercooked eggs and meat. The aim of this study was to analyze the antimicrobial susceptibility of *Salmonella enteritidis*, which was the most frequently isolated Salmonella in the Microbiological Laboratory of the Center of Public Health in Shtip. Materials and methods: A total of 1839 samples (12 rectal swabs and 1827 feces), from persons suspect for salmonellosis in the region of Shtip were investigated for the period 2020 to 2022 year. SS (Salmonella Shigella) agar, Rambach agar, and Selenit F media were used for detection of Salmonella spp., and antimicrobial susceptibility testing of the isolated bacteria is performed with disk diffusion method according EUCAST. Results: *Salmonella enteritidis* is detected in 62 samples (3.37%), isolated strains of *Salmonella enteritidis* demonstrate high resistance to ampicillin 2 μg (37.09%), and multi-resistant strains (6.45%) were also detected. Conclusion: The appearance of ampicillin and multi-resistant strains of *Salmonella enteritidis* in the samples analyzed in our study is a sign of concern and an alarm to initiate a change in the algorithm for the treatment of Salmonella enteritidis infections, especially with the use of fluoroquinolones and cephalosporins (of the third generation). It is recommended to introduce a system for the rational use of antimicrobials in human and veterinary health.

ЈАВНО ЗДРАВЈЕ

# АНТИМИКРОБНА ОСЕТЛИВОСТ НА SALMONELLA ENTERITIDIS ИЗОЛИРАНА ВО ПЕРИОДОТ ОД 2020 ДО 2022 ГОДИНА ВО ШТИПСКИОТ РЕГИОН

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## Извадок

Бактериите од родот *Salmonella* се широко распространети во природата. Можат да бидат колонизатори на различни домаќини и се најчести предизвикувачи на цревни инфекции кај луѓето и кај животните. Главниот пат на пренесување на бактериите од родот *Salmonella* кај луѓето е преку контаминирана храна или недоволно термички обработени јајца и месо. Цел-та на трудот беше да се анализира антимикробната осетливост на детектираните соеви на *Salmonella enteritidis*, која беше најчест детектиран вид во микробиолошката лабораторија на Центарот за јавно здравје во Штип. Материјал и методи: Вкупниот број на испитани примероци во микробиолошката лабораторија на Центарот за јавно здравје во Штип во периодот од 2020 до 2022 година е 1839, од кои 12 ректални брисеви и 1827 копрокултури од лица за кои постои сомневање дека имаат салмонелоза во Штипскиот регион. За детекција на Salmonella spp. се користени: SS (Salmonella Shigella) агар, Rambach агар, Selenit F бујон, а детекцијата на антимикробната осетливост на изолираната бактерија се врши со диск дифузиона мето-да согласно ЕУКАСТ методологијата. Резултати: *Salmonella enteritidis* е детектирана кај 62 примероци (3.37%) во регионот на Штип. Изолираните соеви *Salmonella enteritidis* покажуваат висока резистенција кон ампицилин 2 μg (37.09%), а детектирани се и мултирезистентни со-еви (6.45%). Заклучок: Појавата на висока резистенција кон ампицилин и мултирезистентни соеви на Salmonella enteritidis во анализираните примероци претставува знак за загриженост и аларм за иницирање на промена на алгоритамот за лекување на инфекциите со *Salmonella enteritidis*, особено со примената на флуорокинолоните и цефалоспорините (од трета гене-рација). Се препорачува воведување на систем за рационална употреба на антимикробните средства во хуманото и ветеринарно здравје

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## Introduction

Bacteria of the genus *Salmonella* are widely distributed in nature. They can colonize different hosts and are the most common causes of intestinal infections in humans and animals. The main route of Salmonella transmission to humans is by contaminated food, or undercooked eggs and meat.[1,2] Salmonellosis caused by non-typhoidal serotypes of *Salmonella* usually presents as self-limiting mild diarrhea, with cramps and fever, however it can cause severe invasive infections. *Salmonella* infection is the second most reported zoonotic disease in humans, with 60,050 reported cases in the EU in 2021, and it is a bacterium which is the cause of the largest number of food-borne outbreaks.[3] According to the report by the Institute of Public Health of North Macedonia, in the group of intestinal acute infectious diseases, salmonellosis is the third most common infectious disease with incidence of 9.1.[4]

Samples from people suspected of having salmonellosis from the Shtip region, which includes the settlements around the towns of Shtip, Probishtip and Radovish, are sent to the Microbiological Laboratory of the Center of Public Health in Shtip for detection of the bacteria and their serotype and for determination of the antimicrobial sensitivity which is relevant for appropriate patients' therapy.

The aim of this paper was to show the antimicrobial susceptibility of *Salmonella enteritidis* isolated from people with symptoms suspicious of having salmonellosis in the Shtip region.

## Materials and methods

The study was carried out in the Microbiological Laboratory of the Center of Public Health in Shtip in the period from 2020 to 2022. Data from the laboratory examination of human samples (coproculture and rectal smear) from persons with salmonellosis symptoms were analyzed. During the period of examination, the total number of examined samples was 1839, of which 12 were rectal swabs and 1827 coprocultures. *Salmonella spp*. were detected by using nutrient media: SS (Salmonella Shigella) agar, Rambach agar, Selenite F broth. After incubation at 37°C for 18 to 24 hours in aerobic conditions, a plate latex agglutination was performed to identify bacterial growth. Antimicrobial susceptibility of the isolated bacteria was detected by disk diffusion method using Mueller Hinton agar by EUCAST methodology[5].

The following antibiotics were used: Ceftriaxone (30), Cefixime (5), Cefalexin (30), Cefuroxime (30), Ampicillin (2), Ampicillin (10), Amoxicillin / Clavulanic Acid (20-10), Piperacillin/Tazobactam (30-6), Gentamycin (10), Amikacin (30), Ciprofloxacin (5), Levofloxacin (5), Moxifloxacin (5), Norfloxacin (10), Trimethoprim/Sulfamethoxazole (1.25-23.75), Imipenem (10), Ertapenem (10), Meropenem (10). After incubation for 18-24 hours at 37°C, the zones of inhibition/diameter were measured with a ruler which interpreted *Salmonella enteritidis* susceptibility to the antibiotic.

## Results

In the analyzed period, there were 1839 examined samples suspected of salmonellosis. *Salmonella enteritidis* was isolated in 62 samples (3.37%) or in 3 rectal swabs and 59 coprocultures.

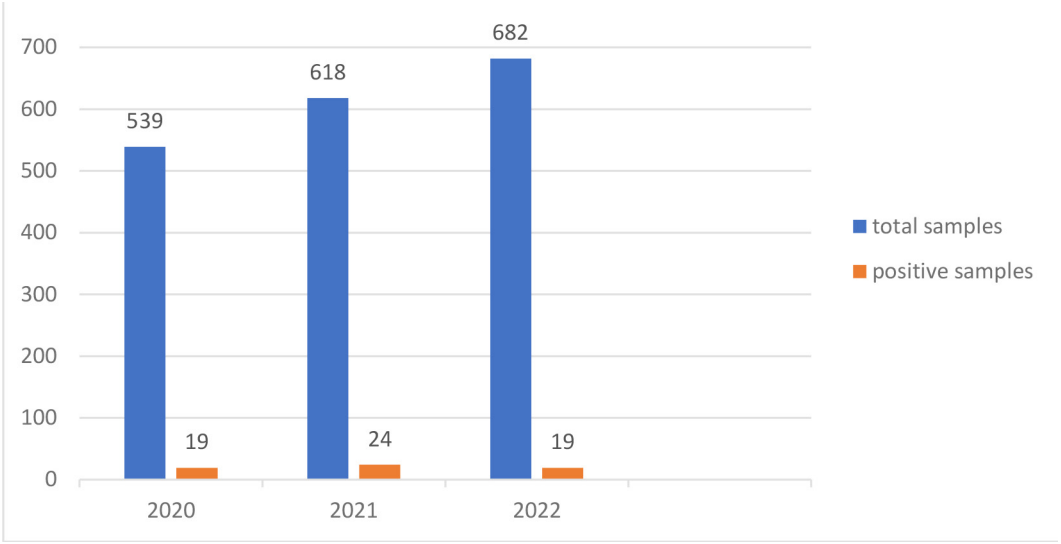


**Figure 1.** Total number of examined and positive samples of *Salmonella enteritidis*

As shown in Figure 1, in 2020, *Salmonella enteritidis* was isolated in 19 (3.53%) samples of a total of 539 samples received. In 2021, 24 (3.88%) samples of 618 tested samples were positive. In 2022, there were 19 (2.79%) positive samples of 682 tested samples.

**Table 1** Antimicrobial susceptibility of isolated cases of *Salmonella enteritidis*

| Antibiotic (µg) | Number of samples with isolated *Salmonella enteritidis* | Number of bacteria sensitive to the antibiotic | Number of bacteria resistant to the antibiotic |
|---|---|---|---|
| Ceftriaxone (30) | 62 | 62 | / |
| Cefixime (5) | 62 | 62 | / |
| Cefalexin (30) | 62 | 62 | / |
| Cefuroxime (30) | 62 | 62 | / |
| Ampicillin (2) | 62 | 39 | 23 |
| Ampicillin (10) | 62 | 57 | 5 |
| Amoxicillin/Clavulanic Acid (20-10) | 62 | 62 | / |
| Piperacillin/Tazobactam (30-6) | 62 | 62 | / |
| Gentamycin (10) | 62 | 62 | / |
| Amikacin (30) | 62 | 62 | / |
| Ciprofloxacin (5) | 62 | 58 | 4 |
| Levofloxacin (5) | 62 | 58 | 4 |
| Moxifloxacin (5) | 62 | 58 | 4 |

| | | | |
|---|---|---|---|
| Norfloxacin (10) | 62 | 58 | 4 |
| Nalidixic Acid | / | / | / |
| Trimethoprim/ | 62 | 62 | / |
| Sulfamethoxazole (1.25-23.75) | 62 | 62 | / |
| Imipenem (10) | 62 | 62 | / |
| Ertapenem (10) | 62 | 62 | / |
| Meropenem (10) | 62 | 62 | / |

According to the results shown in Table 1, it can be seen that:

- 23 (37.09%) *Salmonella* isolates were resistant to Ampicillin 2.
- 5 (8.06%) *Salmonella* isolates were resistant to Ampicillin 10 μg.
- 4 (6.45%) *Salmonella* isolates were resistant to fluroquinolones (Ciprofloxacin, Levofloxacin, Moxifloxacin, Norfloxacin).

The Laboratory in the Center of Public Health in Shtip detected *Salmonella enteritidis* in 36 men (58.06%) and 26 women (41.94%). The largest number of positive results were found among patients in the age group of 0-9 years, a total of 18 people (29.03%). In terms of residence area, a larger number of patients were urban residents compared to rural residents, or 48 (77.42%) versus 11 persons (17.74%). Regarding hospitalization, 17 persons (27.42%) were treated in hospital and 45 persons (72.58%) on an outpatient basis. Although the bacteria was detected throughout the year, most of the positive isolates were found during summer, i.e. in July and August (Table 2).

**Table 2** Number of patients with isolated *Salmonella enteritidis* according to months in the year

| Year/ month | 2020 | 2021 | 2022 | Total |
|---|---|---|---|---|
| January | 3 | / | 1 | 4 |
| February | / | / | 1 | 1 |
| March | / | / | 3 | 3 |
| April | / | / | 4 | 4 |
| May | / | 1 | 3 | 5 |
| June | / | 4 | 3 | 7 |
| July | / | 10 | / | 10 |
| August | 4 | 4 | 2 | 10 |
| September | 1 | / | / | 1 |
| October | 7 | / | 1 | 8 |
| November | 3 | 3 | / | 6 |
| December | 1 | 2 | 1 | 4 |

## Discussion

*Salmonella* infections are challenge and public health problem for each country regardless of its development and availability of health care services. Sometimes, several countries are affected at the same time, as confirmed by the latest European Center for Disease Control report, from October 2023.[6,7] Well-developed microbiological capacities for rapid and early detection of *Salmonella spp.*, as well as determination of their antimicrobial sensitivity are of significant importance for prevention of disease spread and for patients treatment in a timely manner. *Salmonella enteritidis* was detected in the Shtip region in each of the analyzed years found in examined samples. The examination of the local susceptibility/resistance of *Salmonella enteritidis* by the Center of Public Health in the Shtip region (the towns of Shtip, Probishtip and Radovish) is important for patients' adequate empiric therapy and for development of local guidelines for therapy. Forty-five percents of the isolated strains in Shtip showed resistance to Ampicillin, which is in agreement with the equally high resistance (from 20 – 50%) in a number of European countries.[8,9] The high resistance (from 20 – 50%) to Ciprofloxacin[8,10] and Erythromycin[8,11] was reported by 5 countries in Europe, and only one country (Poland) reported an extremely high resistance (more than 70%)[8.] The appearance of multiresistant strains of *Salmonella enteritidis* (6.45%) in the analyzed samples in our study raises concern and an alarm to initiate modifications in the treatment algorithm for *Salmonella enteritidis* infections, especially in the usage of fluoroquinolones and cephalosporins (of third generation)8,10. According to the European Center for Disease Prevention and Control (ECDC), the resistance to these groups of drugs is hazardous to public health. Resistance to these Salmonella enteritidis types is related to the increased antibiotics usage in both animal and human populations[6,8,13]. Therefore, a better system for rational use of antimicrobial agents is recommended to be introduced in human and veterinary medicine[12,13].

## Conclusion

The most frequently isolated bacteria from the genus *Salmonella* in the Shtip region is *Salmonella enteritidis*. The strains isolated in the region by the Center of Public Health in Shtip showed a high resistance to Ampicillin. Multiresistant strains of *Salmonella enteritidis* were detected. This should be the reason for strengthened and organized public health response and taking measures for prudent use of antibiotics in veterinary and human medicine.

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