



## Review Article



# Investigation of Salmonellosis during and after the COVID-19 Pandemic (2020-2023)

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### ABSTRACT

With the Coronavirus disease pandemic in 2020-2023, *Salmonella* distribution has gained great concerted attention since COVID-19 treatment and salmonellosis was conditioned by antibiotics. The present study aimed to investigate the incidence and clinical characteristics of Salmonellosis (salmonellosis gastroenteritis infection) in Armenia during the COVID-19 pandemic, between 2020 and 2023, without age restrictions.

The genus *Salmonella* was identified using biochemical tests and serological methods at Laboratories of Armenia. Near 100 stool samples were cultured on SS agar and selenite aquatic agar. The study identified 57 infected cases with *Salmonella* species and the majority of infection was in Yerevan people (n = 37 patients). The oldest patient was 71 years old from the Stepanavan region. Common symptoms included high temperature, diarrhea, restraint, weakness, pus residue in stool, unformed stool, headache, and dizziness. Two children from the same family were infected with *Salmonella* during the study. The patients were treated with different antibiotics for 3-10 days, with rifampicin, cephalosporins (2nd and 3rd generation), and carbapenems being the most commonly administered drugs. The antibiotic susceptibility index was determined using the EUCAST documents. After the antibiotic therapy, the patients' health was monitored for a month. Additionally, the study found the Extended Spectrum Beta-Lactamase (ESBL) enzymes in newborn, 10-year-old, and 33-year-old patients.

## 1. Introduction

According to the National Center for Disease Control and Prevention of Armenia, more than 350 annual cases of salmonellosis were estimated, associated with a large number of hospitalizations<sup>1</sup>. Moreover, as in recent years there is a tendency of increasing the consumption of chicken meat in Armenians' diet, new problems might arise regarding.

Salmonellosis is the second most commonly reported gastrointestinal infection, and an important cause of food-borne outbreaks in the EU/EEA<sup>2</sup>. The EU/EEA notification rate was 14.2 cases per 100 000 population. *Salmonella gastroenteritis* is a common cause of Salmonellosis, which typically manifests as diarrhea and abdominal cramps<sup>2</sup>. Pathogenic *Salmonella* ingested in food survives the gastrointestinal passage through the gastric acid barrier and invades the mucosa of the small and large intestines, producing toxins. As a result, this bacteria can disseminate from the intestines to cause systemic disease<sup>3</sup>. Therefore,

Salmonellosis should be considered a possible diagnosis in any acute diarrheal or febrile illness without an obvious cause<sup>3</sup>. Diagnosis is confirmed by isolating the organism from clinical specimens obtained from the stool<sup>4</sup>. While the normal intestinal microflora protects against *Salmonella*, the laboratory identification of the genus *Salmonella* is made by biochemical tests (Figure 1) and serological testing (Figure 2), with the stool typically plated on *Salmonella Shigella* (SS) agar or selenite aquatic agar.

Specific therapy for Salmonellosis consists of antibiotic administration<sup>5</sup>. The discovery of potent, relatively nontoxic antimicrobial therapeutic agents was perhaps the foremost medical advance of the twentieth century<sup>2</sup>. Antibiotics can be natural products, semisynthetic variants of natural products, or synthetic chemicals designed to block key biochemical pathways or interfere with cellular structures<sup>5</sup>. Cell walls are not found in mammalian cells

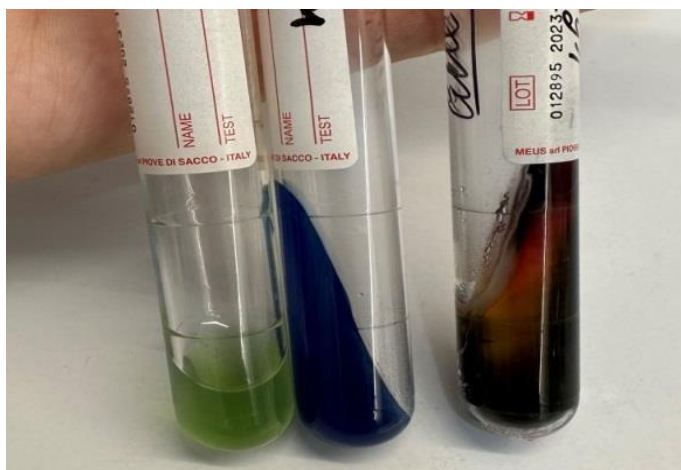


Figure 1. The biochemical tests of *Salmonella* spp.

and differ in composition among various bacterial species. Therefore, cell wall synthesis provides some potential therapeutic targets for anti-infective drugs, such as antibiotics that target anabolic cellular processes like cell wall synthesis, DNA replication, RNA transcription, and messenger RNA translation<sup>5,6</sup>. However, different studies have reported antimicrobial-resistant *Salmonella* isolates from food samples<sup>5</sup>. In the therapy of *Salmonella* infection, the natural and semisynthetic forms of beta-lactams and synthetic antibiotics are commonly used<sup>6</sup>.

According to the search results, the COVID-19 pandemic had a significant impact on the incidence of salmonellosis. In 2020, salmonellosis notification rates in the last five years, preceding the COVID-19 pandemic, have been stable<sup>7</sup>. The number of cases in 2020 were significantly lower than previously reported numbers, primarily as a consequence of the pandemic<sup>8,9</sup>.

Research conducted in the Netherlands found that salmonellosis incidence decreased significantly after March 2020<sup>8</sup>. However, there were also reports of salmonellosis continuing to be a problem during the pandemic, especially in endemic areas such as Pakistan<sup>9</sup>. Additionally, there have been recent outbreaks of *Salmonella* in sprouts sold in the US<sup>10</sup>. Overall, the impact of the COVID-19 pandemic on salmonellosis incidence appears to



Figure 2. The serological test of *Salmonella* spp. by SMES 3

be complex and may vary depending on the region and other factors.

The present study aimed to investigate the incidence and clinical characteristics of Salmonellosis in Armenia during the COVID-19 pandemic, between 2020 and 2023.

## 2. Materials and Methods

The data in the current study were collected from Armenia during January 2022 to March 2023. Bacterial cultures were obtained from near 100 stool specimen. The patients aged 2 years and above who tested positive for *Salmonella* were identified from the records of the clinical microbiology laboratory at Davidyants Laboratories. Information on age, sex, specimen source, clinical manifestations, and antimicrobial susceptibility were retrospectively reviewed and analyzed, and the amount of *Salmonella* species were calculated.

To identify *Salmonella*, fresh fecal samples were inoculated on SS agar plates at 35°C for 18-48 hours and Triple Sugar Iron (TSI) agar for biochemical identification. Isolates identified as the genus *Salmonella* were further tested using slide agglutination with antisera. Additional testing was conducted for TSI, lysine, citrate utilization (Simmons), disodium malonate, and H<sub>2</sub>S production. The antibiotic susceptibility index was determined using EUCAST documents.

### 2.1. Pathogenesis and clinical manifestations

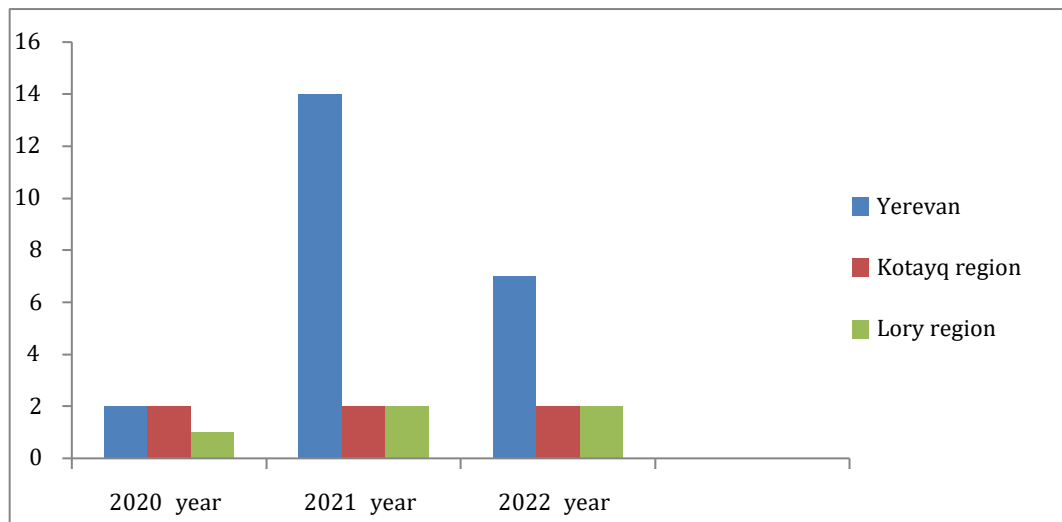
The severity of *Salmonella* infections in humans varies depend on the serotype and the health status of the host<sup>11</sup>. In human infections, the four different clinical manifestations are the enteric carrier state<sup>12</sup>.

Enteric fever is characterized by an incubation period of one week or more<sup>13</sup>. *Salmonella* infections are characterized by gastroenteritis or stomach flu, an inflammatory condition of the gastrointestinal tract that is accompanied by symptoms such as non-bloody diarrhea, vomiting, nausea, headache, followed by the onset of fever 7 and abdominal cramps<sup>14,15</sup>. Diarrhea is more commonly observed in children<sup>14</sup>. During the illness, enteric fever displays a specific fever pattern with an initial low-grade fever (37.5°C to 38.5°C), which slowly develops into a high-grade fever (38.5°C to 41.5°C) in the second week<sup>16</sup>.

The chronic carrier status is defined as the shedding of bacteria in the stool for more than a year after the acute stage of *Salmonella* infection<sup>17</sup>. Approximately 4% of patients may become chronic carriers.

## 3. Results and Discussion

Salmonellosis, a zoonosis, remains the most common disease affecting humans in Armenia<sup>18</sup>. However, the number of cases reported in 2020 was significantly lower than in previous years, likely due to the impact of the COVID-19 pandemic<sup>19</sup>. The higher proportion of symptomatic infections among young children may explain



**Diagram 1.** The epidemiology of *Salmonella* in Armenia (2020-2023)

why the incidence rate of salmonellosis in this population is 10 times higher than in adults<sup>19</sup>. Food has been identified as the main source of human salmonellosis outbreaks in Europe according to epidemiological surveys<sup>20</sup>. Although some people infected with *Salmonella* have mild symptoms and recover without treatment, others may require hospitalization due to the severity of their symptoms.

Salmonellosis is one of the more common causes of gastroenteritis with more than 2300 cases occurring in Georgia each year<sup>21</sup>. Between February 2021 and May 15, 2022 were most common salmonellosis that cause gastroenteritis in humans in Baghdad, Iraq<sup>21</sup>. However, it can be severe in young and elderly people, as well as individuals with impaired immunity<sup>21</sup>.

The bacterial cultures were obtained from stool specimens, and *Salmonella* (a Gram-negative bacillus) was isolated and cultured on SS agar plates. The incubation period for *Salmonella* spp. is generally 8 to 24 hours. *Salmonella* possesses several virulence factors that aid in overcoming host immune defenses during infection, which could increase the risk to human health. Further testing was conducted, including the TSI Triple Sugar Iron test, lysine test, citrate utilization - Simmons test, disodium malonate test, and H<sub>2</sub>S production test.

The disodium malonate test resulted in a positive outcome, as evidenced by the increase in alkalinity from utilization of the ammonium sulfate, causing the indicator to change from green to blue (Figure 2). The citrate test was used to determine if the organism could use sodium citrate as a sole carbon source. The alkaline pH resulting from the use of ammonium salts changed the pH indicator (bromthymol blue) in the medium from green to blue.

Lysine was decarboxylated by the enzyme lysine decarboxylase to cadaverine, a diamine, and CO<sub>2</sub>. A positive result in the lysine test was indicated by a purple color in the medium. If the organism does not possess the specific decarboxylase, the medium remains yellow,

indicating a negative result. Results were recorded within 24 hours.

The H<sub>2</sub>S production test was a two-step process. In the first step, H<sub>2</sub>S was formed from sodium thiosulfate. As H<sub>2</sub>S was a colorless gas, an indicator such as ferrous sulfate was necessary to visually detect its production (Figure 1). Finally, serological testing was conducted using SMES 3 (Figure 2).

The infection of *Salmonella* was discovered in the stool of 57 patients, the majority of whom were from Yerevan (n= 37 patients) and aged from 5 month to 63 years. The oldest patient was 71 years old from the Stepanavan region of Armenia (Diagram 1).

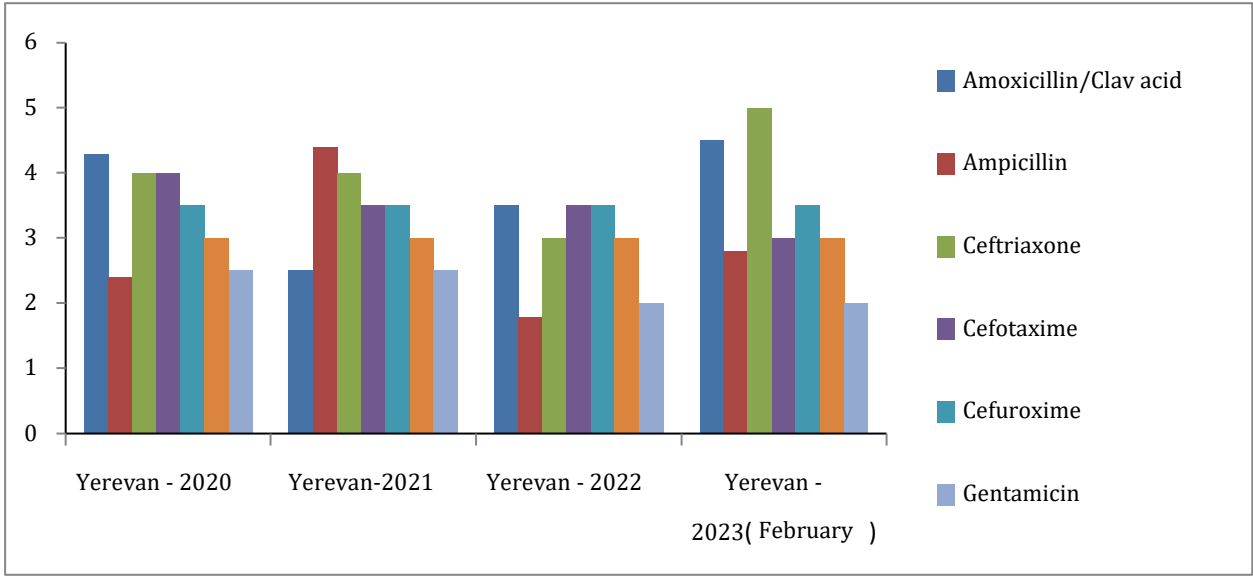
### 3.1. Antibiotic therapy of salmonellosis and antimicrobial-resistant bacteria of *Salmonella*

Moreover, the human colon serves as a reservoir for antimicrobial resistance, which could result in significant health risks. The infection often occurs after ingestion of contaminated food or water, emphasizing the importance of hand hygiene through regular hand washing with soap and warm water or using hand sanitizer.

Regional differences in resistance were observed during the examination of the antimicrobial susceptibility of *Salmonella* isolates. In Yerevan, high prescription rates for Ciprofloxacin and other fluoroquinolones were detected (Diagrams 1 and 2).

Nifuroxazide, an intestinal disinfecting drug, is a component of Enterofuryl. It is used to treat acute diarrhea in individuals over 6 years old that is caused by intestinal infection and aids in rehydration.

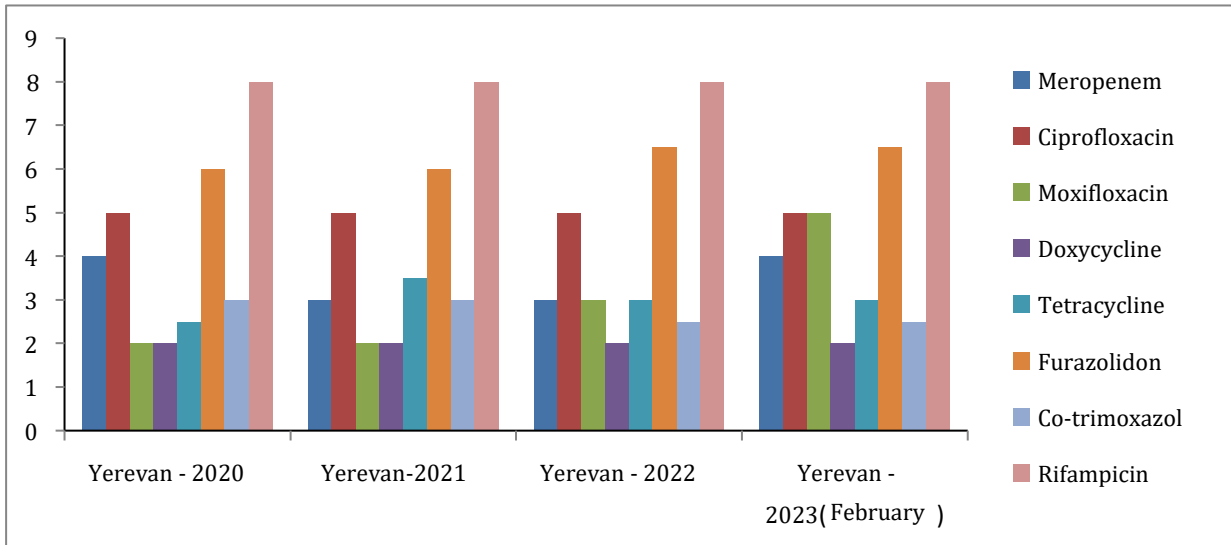
Antibiotics have revolutionized medicine in many ways and have saved millions of lives. Unfortunately, antimicrobial resistance is a natural selection outcome. The most common resistance mechanisms used by bacteria include enzymatic degradation or mutation in the antimicrobial target site, decreased cell wall permeability to antimicrobials, and active efflux of the antimicrobial.



**Diagram 2.** In 2020-2023, therapy of antibiotic during salmonellosis in Yerevan city

Resistance to antibiotics can cause treatment failure, increase the burden of illness, and lead to higher mortality, making it a significant public health concern. In this regard, Extended Spectrum Beta-Lactamase (ESBL) *Salmonella* strains were discovered in the stool

of newborn, 10, and 33-year-old patients. Most ESBL infections are transmitted through direct contact with an infected person; therefore, Meropenem, Furazolidone, and Rifampicin A/B were used for treatment (Diagram 3).



**Diagram 3.** In 2020-2023, therapy of antibiotic during salmonellosis in Yerevan city

#### 4. Conclusion

This study investigated the incidence and clinical characteristics of Salmonellosis in Armenia during the COVID-19 pandemic, between 2020 and 2023.

#### Declarations

##### Competing interests

There was no conflict of interest between the authors of

this study.

##### Authors' contribution

All authors were involved in data collection, design of the article, interpretation of results, review, and manuscript preparation.

##### Funding

Not applicable.

### Availability of data and materials

All data generated or analyzed during this study are included in this published article.

### Ethical considerations

The authors checked for plagiarism and consented to the publishing of the article. The authors have also checked the article for data fabrication, double publication, and redundancy.

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