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Molecular characterization and antimicrobial susceptibility of *Salmonella* Enteritidis and *Salmonella* Typhimurium originating from the food chain

Molekularna karakterizacija i antimikrobna osetljivost *Salmonella* Enteritidis i *Salmonella* Typhimurium poreklom iz lanca hrane

Marko DMITRIĆ[1], Vladimir KURĆUBIĆ[2] (✉), Dejan VIDANOVIĆ[1], Saša ŽIVKOVIĆ[1], Matija MUNJIĆ[2], Ivan VIĆIĆ[3], Nedjeljko KARABASIL[3]

[1] Veterinary Specialized Institute Kraljevo, Žička 34, 36000 Kraljevo, Republic of Serbia

[2] University of Kragujevac, Faculty of Agronomy, Department of Food Technology, Cara Dušana 34, 32000 Čačak, Republic of Serbia

[3] University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, 11000 Belgrade, Republic of Serbia

✉ Corresponding author: vkurcubic@kg.ac.rs

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ABSTRACT

Salmonella Enteritidis and *Salmonella* Typhimurium (*S. Enteritidis* and *S. Typhimurium*) represent epidemiologically important serotypes; therefore, from the aspect of public health, their characterization is extremely important. During this research, molecular characterization and antimicrobial susceptibility testing of 60 isolates of *S. Enteritidis* and 60 isolates of *S. Typhimurium* originating from food, animal feed and poultry feces was performed. Antimicrobial susceptibility was tested using the disc diffusion method (EUCAST protocol), while the E test was used to determine the minimum inhibitory concentration (MIC). Molecular characterization was performed using the pulsed-field gel electrophoresis (PFGE) method. Confirmation was previously performed using the real-time PCR method, detecting the *safA* gene of *S. Enteritidis* and the *fliA-IS200* gene of *S. Typhimurium*. Using the enzyme *XbaI*, 20 different PFGE profiles of *S. Enteritidis* and 21 different PFGE profiles of *S. Typhimurium* were determined. The results of testing 60 *S. Typhimurium* isolates using the disc diffusion method showed that 50% of the isolates were resistant to Ampicillin; 46.67% to Tetracycline; 31.67% to Chloramphenicol; 11.67% on Trimetoprim and 3.33% on Pefloxacin. The results of testing 60 isolates of *S. Enteritidis* using the disk diffusion method showed that 10% of the isolates were resistant to Pefloxacin; 5% to Ampicillin and 1.67% to Tetracycline. All resistant isolates, after the disc diffusion method, were also tested using E test strips. The results of this study justify the continued importance of *Salmonella* monitoring and control for the food chain to maintain food safety and protect public health.

Keywords: *Salmonella*, pulsed-field gel electrophoresis, disc diffusion method, antimicrobial susceptibility, E test

APSTRAKT

Salmonella Enteritidis i *Salmonella Typhimurium* predstavljaju epidemiološki važne serotipove, stoga je sa aspekta javnog zdravlja njihova karakterizacija od izuzetnog značaja. Tokom ovog istraživanja izvršena je molekularna karakterizacija i ispitivanje antimikrobne osetljivosti 60 izolata *S. Enteritidis* i 60 izolata *S. Typhimurium* poreklom iz hrane, hrane za životinje i fecesa živine. Ispitivanje antimikrobne osetljivosti izvršeno je disk difuzionom metodom (EUCAST protokol), dok je za određivanje minimalne inhibitorne koncentracije primenjen E test. Molekularna karakterizacija izvršena je primenom PFGE metode. Prethodno je izvršeno potvrđivanje primenom real-time PCR metode, detekcijom *safA* gena *S. Enteritidis* i *fliA-IS200* gena *S. Typhimurium*. Upotrebom enzima *XbaI*, utvrđeno je 20 različitih PFGE profila

S. Enteritidis i 21 različit PFGE profil. *S. Typhimurium*. Rezultati ispitivanja 60 izolata *S. Typhimurium* disk difuzionom metodom pokazali su da je 50% izolata rezistentno na ampicilin; 46,67% na tetraciklin; 31,67% na hloramfenikol; 11,67% na trimetoprim i 3,33% na pefloksacin. Rezultati ispitivanja 60 izolata *S. Enteritidis* disk difuzionom metodom pokazali su da je 10% izolata rezistentno na pefloksacin; 5% na ampicilin i 1,67% na tetraciklin. Svi rezistentni izolati, nakon disk difuzione metode ispitani su i primenom E test traka. Rezultati ove studije opravdavaju kontinuirani značaj praćenja i kontrole *Salmonella* u lancu hrane, kako bi se održala bezbednost hrane i zaštitilo javno zdravlje.

Ključne reči: *Salmonella*, PFGE, real-time PCR, disk difuziona metoda, antimikrobna osetljivost, E test

INTRODUCTION

Salmonella is a rod-shaped, Gram-negative, facultatively anaerobic, non-spore-forming bacterium belonging to the *Enterobacteriaceae* family (Waldman et al., 2020). The genus *Salmonella* represents one of the leading foodborne pathogenic microorganisms (Karabasil et al., 2025). They have significant diversity (more than 2600 serotypes) and can cause gastrointestinal infections in humans and animals, which can be lethal, especially in the young and elderly population (Hagras et al., 2024). According to EFSA (2021), 52,702 human salmonellosis cases were reported in the EU in 2020. Among the 42,203 cases with confirmed serotype, *Salmonella Enteritidis* (48.7%), *Salmonella Typhimurium* (12.4%), and the monophasic *S. Typhimurium* variant (11.1%) accounted for 72.2% of cases, consistent with previous years. As the ultimate goal within the EU is to achieve a reduction in the prevalence of *Salmonella* in poultry, a strict microbiological criterion has been established, which, in accordance with Regulation (EU) No. 1086/2011, implies the absence of serovars *S. enterica* subsp. *enterica* Enteritidis and Typhimurium, including its monophasic variant with seroformula 4,[5],12:i:– in 25 g of samples of poultry neck skin and fresh meat, as *Salmonella enterica* is one of the most common zoonotic pathogens of humans and animals globally (Maurischat et al., 2015). *S. Typhimurium* and *S. Enteritidis* are the most common *Salmonella* serotypes that cause gastroenteritis (GE) in humans, as well as acute GE, typhoid, paratyphoid, and systemic infections (Kübra Dindar Demiray and Sayar, 2024; Hashemi et al, 2025). Dar et al. (2017) reported that the diseases caused by *S. Typhimurium* are of public health importance, as human salmonellosis is mainly caused by *S. Typhimurium* (through food poisoning). The foodborne pathogenesis of *S. Typhimurium* highlights the importance of understanding how it affects eggs and what strategies are needed for more effective infection control. Recent reviews emphasize that *S. Typhimurium* remains a significant public health threat, driven by its widespread global distribution and exceptional adaptability (Perez-Sepulveda and Hinton, 2025). A growing threat to human health is posed by new lineages of invasive non-typhoidal *Salmonella* (iNTS), causative agents of bloodstream infections that cause approximately 77,000 deaths worldwide annually, predominantly in sub-Saharan Africa. An additional complication is the finding that they are often multidrug-resistant (MDR) and target immunocompromised individuals, especially children under 5 years of age. *S. Typhimurium* ST313 strains are considered responsible for the high level of bloodstream infections in patients with weakened or paralyzed immune responses (HIV epidemics in Africa during the 1980s) (Crump et al., 2023).

To reduce the possibility of *Salmonella* infections, it is necessary to permanently implement good practices (effective food hygiene, adequate selection of an efficient and effective disinfectant in the entire food chain, water, sanitation and limiting the use of antibiotics in animals used for food) (Billah and Rahman, 2024; Bun and Nugroho, 2024). To efficiently and effectively prevent the spread of antimicrobial resistance (AMR) infections and the pressure of iNTS infections, it is imperative to permanently conduct genomic surveillance, develop and improve diagnostic tools for new *S. enterica* infections, and implement integrated interventions in the public health sector (Shaji et al., 2023; Muturi et al., 2025).

During epidemiological studies, it is generally not enough to type *Salmonella* isolates to the species and serotype level, but the application of typing methods that can differentiate between genetically related isolates is also necessary. Classical serotyping has been largely superseded by molecular methods (which are generally very successful as alternatives). A wide range of methods have been developed for subtyping *Salmonella*, and development is still ongoing, as each method has its own advantages and disadvantages (cost, speed, robustness and sensitivity). Whether typing is assessed by classical or modern methods, the classification into serovars has proven useful, and there is a consensus that this classification should be maintained (Wattiau et al., 2011). Despite the large number of methods available for typing *Salmonella* spp. (Steve et al., 2004). There is currently no single method that is optimal for all types of research (Foxman et al., 2005; Francisco et al., 2024). Therefore, the best results are often obtained by combining two or more methods. Such an approach was also applied during this study, the main objectives of which were:

1. To form a collection of *S. Enteritidis* and *S. Typhimurium* serotypes.
2. To examine the susceptibility of *S. Enteritidis* and *S. Typhimurium* serotypes to antimicrobial (AM) drugs.
3. To perform molecular characterization of *S. Enteritidis* and *S. Typhimurium* serotypes using Pulsed Field Gel Electrophoresis (PFGE).

MATERIAL AND METHODS

Formation of a collection of S. Enteritidis and S. Typhimurium isolates.

During this study, a collection of 60 isolates of *S. Enteritidis* and 60 isolates of *S. Typhimurium* was formed. Of the 60 isolates of *S. Enteritidis* and the same number of isolates of *S. Typhimurium*, 24 were isolated from food and animal feed, 24 from poultry feces and 12 from clinical material of sick people. The number and origin of the isolates are shown in Tables 1 and 2.

Isolation of Salmonella spp.

Detection of *Salmonella* spp. in food, feed and poultry feces was performed in accordance with the method "Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp. (SRPS EN ISO 6579-1:2017)". Selective isolation was performed using Xylose Lysine Deoxycholate (XLD) (Oxoid, UK) and *Salmonella Shigella* (SS) agar (Oxoid, UK). For final confirmation, presumptive *Salmonella* colonies were cultured onto nutrient agar (HiMedia, India) and their identity was confirmed using classic biochemical and serological tests. *Salmonella* isolates were stored at −80 °C with glycerol as a cryoprotectant to maintain long-term viability and genetic stability.

Serotyping

Serotyping of *Salmonella* spp. was performed in accordance with the standard *"Horizontal method for the detection, enumeration and typing of Salmonella – Part 3: Guidelines for the typing of Salmonella spp."* (SRPS CEN ISO/TR 6579-3:2014). Polyvalent antiserum (Biorad, USA) and monovalent antisera (Statens Serum Institute, Denmark) were used.

Real-time PCR detection of S. Enteritidis and S. Typhimurium

The detection of *S. Enteritidis* and *S. Typhimurium* genomes using the real-time PCR method was performed using primers and probes for the detection of *safA* (*S. Enteritidis*) and *fliA-IS200* genes (*S. Typhimurium*) (Maurischat et al., 2015; Dmitric et al., 2025). The *S. Enteritidis*-specific probe was labeled at the 5′ end with the reporter dye 6-carboxyfluorescein (FAM) and at the 3′ end with a dark quencher (BHQ-1). The *S. Typhimurium*-specific probe was labeled at the 5′ end with the reporter Hexachloro-Fluorescein (HEX) and at the 3′ end with a dark quencher (BHQ-1). The polymerase chain reaction (PCR) was carried out in a final volume of 25 µL, containing 2× Brilliant III Ultra-Fast QPCR Master Mix (Agilent Technologies, USA), 400 nM of each primer, and 250 nM of each probe.

Table 1. Origin of *S. Enteritidis* isolates

Isolate	Origin	Isolation done by
1 - 12	Sick people	Institute for Public Health of Serbia (IPHS) "Dr M. J. Batut"
13	Chicken skins	Veterinary Specialized Institute "Kraljevo"
14	Chicken liver	
15	Cattle carcass swab	
16	Chicken liver	
17	Chicken mechanically separated meat	
18	Chicken mechanically separated meat	
19 - 21	Chicken skins	
22 - 24	Complete fodder mixtures for feeding animals	
25, 26	Laying hen carcass	Veterinary Specialized Institute "Jagodina"
27	Chicken meat	
28	Fresh sausage	
29 - 31	Chicken mechanically separated meat	
32 - 33	Poultry liver and spleen	Institute of Food Technology in Novi Sad (FINS)
34	Chicken meat	
35	Minced chicken meat	
36	Chicken mechanically separated meat	
37 - 60	Poultry feces	Veterinary Specialized Institute "Kraljevo"

Table 2. Origin of *S. Typhimurium* isolates

Isolate	Origin	Isolation done by
61 - 72	Sick people	Institute for Public Health of Serbia (IPHS) "Dr M. J. Batut"
73	Pljeskavica (Burger)	Veterinary Specialized Institute "Jagodina"
74	Ćevapičići (Kebab)	
75	Minced unshaped meat	
76 - 79	Complete fodder mixtures for feeding animals	Veterinary Specialized Institute "Kraljevo"
80 - 83	Minced pork	Institute of Food Technology in Novi Sad (FINS)
84 - 88	Slaughter line	
89 - 93	Minced mixed meat	
94	Minced pork	
95 - 96	Minced pork	Veterinary Specialized Institute "Kraljevo"
97 - 120	Poultry feces	Veterinary Specialized Institute "Kraljevo"

Two µL of extracted DNA was used as template. Amplification conditions included a 3-minute "hot start" at 95 °C, followed by 45 cycles of 15 s at 95 °C and 30 s at 61 °C. Instead of an internal amplification control (IAC, pUC18/19), the DNA Extraction Control Mix 610 (Bioline, UK) was used as IAC. The internal amplification control, as well as primers and probe for IAC, were added to the reaction according to the manufacturer's instructions. Fluorescence levels were analyzed using the real-time PCR instrument software (Agilent AriaMx Software v1.1, Agilent Technologies, USA). Primers and TaqMan probes were ordered from Metabion (Germany) (Dmitric et al., 2025).

Antimicrobial susceptibility testing (AST)

Disc diffusion method

AST using the disc diffusion method was performed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) protocol (Matuschek et al., 2014). Briefly, several *Salmonella* colonies grown on nutrient agar (Oxoid, UK) for 16–24 h at 37 °C were transferred using a sterile swab into physiological saline (0.9% NaCl). The bacterial suspension was adjusted to a turbidity equivalent to 0.5 McFarland standard and used within 15 minutes. The inoculum was applied to the surface of Mueller-Hinton agar (MH agar, Oxoid, UK) using a sterile swab by streaking in three directions, rotating the plate by 60° each time to ensure even distribution. Prior to inoculation, excess liquid was removed from the swab by rotating it against the inner upper wall of the tube to prevent over-inoculation of the MH agar surface. Antibiotic disks were applied within 15 minutes after inoculation. A total of six antibiotic disks were placed on each 90 mm Petri dish. Disks were applied using sterile forceps under aseptic conditions. The prepared plates were incubated in an upright position at 35 ± 1 °C for 16–20 h. After incubation, inhibition zones clearly visible to the naked eye from a distance of 30 cm were measured using a vernier calliper. To perform the disc diffusion method, discs from the manufacturer "Oxoid" (Great Britain) were used: ampicillin (AMP10), azithromycin (AZM15), cefotaxime (CTX5), chloramphenicol (C30), gentamicin (CN10), pefloxacin (PEF5), sulfamethoxazole (RL100), tetracycline (TE30), tigecycline (TGC15), trimethoprim (W5), meropenem (MEM10) and ceftazidime (CAZ10) produced by "Bioanalyse" (Turkey).

E-test

The minimum inhibitory concentration (MIC) was determined using the E-test. Inhibition readings were interpreted according to EUCAST recommendations (EUCAST, 2018) and according to the manufacturer's instructions for the E-test strips (Liofilchem, 2017). This method is based on the application of strips impregnated with an antibiotic in a gradient of concentrations expressed in µg/mL (micrograms per milliliter). On the same side of the strip where the antibiotic concentration scale is printed, the code identifying the antimicrobial agent is also indicated. Mueller-Hinton agar (MH agar, Oxoid, UK) was used as the reference medium. Prior to use, the test strips were equilibrated to room temperature.

The inoculum was prepared in the same manner as for the disk diffusion method. Briefly, 4–5 well-isolated *Salmonella* colonies grown on nutrient agar (Oxoid, UK) for 16–24 h at 37 °C were transferred with a sterile swab into 5 mL of physiological saline (0.9% NaCl). The bacterial suspension was adjusted to a turbidity equivalent to 0.5 McFarland standard and used within 15 minutes. The inoculum was applied to the surface of Mueller-Hinton agar (MH agar, Oxoid, UK) using a sterile swab by streaking in three directions. Excess liquid was removed from the swab beforehand by rotating it against the inner upper wall of the tube to prevent over-inoculation of the agar surface.

Antibiotic strips were applied within 15 minutes after plate inoculation using sterile forceps under aseptic conditions, with the antibiotic concentration scale facing upward and the code identifying the antibiotic oriented toward the edge of the Petri dish. The strips were placed so that they were in full contact with the agar surface along their entire length, without the presence of air bubbles, and were not moved after placement. The pre-

pared plates were incubated in an upright position at 35 ± 1 °C for 16–20 h.

After incubation, an elliptical zone of inhibition formed around the strip. The point at which the edge of the ellipse intersected the strip was read as the MIC value. Quality control was performed in accordance with the manufacturer's instructions for MIC Test Strip (Liofilchem, Italy).

The following test strips (MIC Test Strip, Liofilchem ,Italy) were used to perform the E-test: ampicillin (AMP 0.016-256 µg/mL), chloramphenicol (C 0.016-256 µg/mL), ciprofloxacin (CIP 0.002-32 µg/mL), tetracycline (TE 0.016-256 µg/mL), trimethoprim (TM 0.002-32 µg/mL).

Pulsed-Field Gel Electrophoresis (PFGE)

Pulsed-field gel electrophoresis (PFGE) was performed according to the *PulseNet USA Standardized PFGE Protocol* (CDC, 2017; Ribot et al., 2006). Briefly, *Salmonella* isolates were streaked onto tryptone soy agar (TSA, Oxoid, UK) and incubated at 37 °C for 18–24 h. After incubation, grown *Salmonella* colonies were transferred with a sterile swab into Cell Suspension Buffer (CSB; 100 mM Tris:100 mM EDTA, pH 8.0). The concentration of bacterial cells in the suspension was adjusted by dilution with CSB or by adding bacterial cells. The absorbance of the prepared suspensions was measured using a spectrophotometer (UV-1800, SHIMADZU, Japan) at a wavelength of 610 nm and ranged from 1.3 to 1.4.

From the prepared suspension, 400 µL was transferred into plastic tubes (Eppendorf, Germany), and 20 µL of proteinase K (20 mg/mL, Thermo Scientific, USA) was added to each tube. Previously prepared 1% SeaKem Gold agarose (Lonza, USA) was maintained in a water bath at 55–60 °C. Subsequently, 400 µL of 1% SeaKem Gold agarose was added to the Eppendorf tube containing 400 µL of the bacterial suspension, and the components were mixed by pipetting up and down several times. Immediately thereafter, the mixture was transferred into plastic molds (Disposable Plug Mold, Bio-Rad Laboratories, USA), where the agarose was allowed to solidify at ambient temperature for 15 minutes.

The prepared plugs were then pushed out of the molds using a metal spatula and transferred into tubes containing cell lysis buffer (CLB; 50 mM Tris:50 mM EDTA, pH 8.0 + 1% Sarcosyl). Lysis was performed in a thermo-shaker (BioSan, Latvia) at 55 °C for 2 hours with constant agitation. This step aimed to disrupt the bacterial cell wall under the action of proteinase K and the lysis buffer without breaking the DNA strands, thereby allowing the intact DNA to be exposed to restriction enzymes.

After lysis, the plugs were washed twice in pre-warmed (54–55 °C) ultrapure water (HPLC grade) and four times in TE buffer (10 mM Tris:1 mM EDTA, pH 8.0), also pre-warmed to 54–55 °C. The purpose of this step was to obtain a highly purified *Salmonella* genome by removing unwanted lysis products and residual proteinase.

Prior to restriction enzyme digestion, an incubation step was performed using restriction buffer (10× Tango buffer with BSA, Thermo Scientific, USA) diluted 1:10 with ultrapure water (HPLC grade). A volume of 200 µL of the diluted buffer was added to 1.5 mL Eppendorf tubes. The plugs were transferred to sterile Petri dishes using a spatula and then cut with a scalpel into slices 2.5 mm in width. The plug slices were subsequently transferred into tubes containing the diluted restriction buffer. Plugs of *Salmonella* ser. Braenderup, used as the standard, were prepared in the same manner. Samples and control plugs were incubated at ambient temperature for 10–15 minutes, after which the buffer was removed with a pipette, taking care not to damage or aspirate the plug slices.

The restriction enzyme master mix was prepared by combining ultrapure (HPLC-grade) water, 10× restriction buffer supplemented with bovine serum albumin ((Thermo Scientific, USA), and the restriction enzyme *XbaI* (10 U/µL, Thermo Scientific, USA). The components were mixed gently to ensure homogeneity. A total volume of 200 µL of the prepared restriction enzyme mix was added to each tube, completely covering the plug slices. Enzymatic digestion was then carried out by incubating the samples at 37 °C for 2 hours.

After enzymatic digestion, the plugs were subjected to electrophoresis in 1% SeaKem Gold agarose using the CHEF DR-III system (Bio-Rad, USA). Electrophoresis was performed for 18 hours under the following conditions: initial switch time 2.2 seconds, final switch time 63.8 seconds, voltage 6 V, and buffer temperature 14 °C. TBE buffer (0.5× Tris-Boric acid-EDTA; Tris 0.04 M, Boric acid 0.04 M, EDTA disodium 0.001 M) was used for gel preparation and as the running buffer.

After electrophoresis, the gel was stained with ethidium bromide (Bio-Rad, USA). Gel imaging was performed using the GelDoc XR system (Bio-Rad, USA). Primary gel analysis was conducted using GelDoc software, while the dendrogram showing the degree of genetic similarity among isolates was generated using FPQuest software (Bio-Rad, USA).

Profiles are assigned labels consisting of the first letter of the bacterial species, three letters of the serotype, two letters of the restriction enzyme used and a four-digit number starting from 0001 (For example: "STYPXB0001" - genotype 1 of *S. Typhimurium* obtained using the restriction enzyme *XbaI*).

RESULTS

Genotyping of S. Enteritidis isolated from the food chain (PFGE)

Analysis of 60 *S. Enteritidis* isolates using enzyme *XbaI* revealed 20 distinct PFGE genotypes (Figure 1). Isolates within the same profile showed 100% similarity, while inter-genotype similarity ranged from 78–97%. The most common genotype, **SENTXB0001**, included 22 isolates (7 human, 13 food/feed, 2 poultry feces). Subtyping by antimicrobial profiles showed minor variations: isolate 17 (AMP, TE), isolate 31 (AMP) and isolate 4 (PEF). **SENTXB0002** comprised 8 isolates (1 human, 1 feed, 6 poultry feces); only isolates 53 (PEF) and 55 (AMP, PEF) showed resistance. **SENTXB0003** (4 isolates; 2 food, 2 feces): isolates 32 and 54 resistant to PEF. **SENTXB0004** - **SENTXB0006** (3 isolates each) and **SENTXB0007** - **SENTXB0008** (2 isolates each) showed no antimicrobial resistance. Single-isolate genotypes **SENTXB0009** - **SENTXB0020** (12 in total) originated from various sources (human, food, feces). Only isolate 27 (**SENTXB0019**) was resistant (PEF); all others were susceptible to the tested antibiotics.

Genotyping of Salmonella Typhimurium isolated from the food chain (PFGE)

Analysis of 60 *S. Typhimurium* isolates with enzyme *XbaI* identified 21 **PFGE genotypes** (Figure 2). Isolates within the same profile showed 100% similarity, while similarity between profiles ranged 77-98%. The most frequent genotype, **STYPXB0001**, included 21 isolates (17 from food, 4 from poultry feces). Based on antimicrobial profiles, three subgroups were observed: AMP, C, TE (majority of isolates); AMP, TE (isolates 104, 107); sensitive (isolates 94, 100, 117). **STYPXB0002** contained 9 isolates (5 human, 1 feed, 3 feces) showing variable resistance: AMP, TE, W (main group); AMP, TE (isolate 76); AMP (isolate 120); others sensitive. **STYPXB0003** - **STYPXB0008** (total 17 isolates) included food, feed, and fecal origins, all susceptible to tested antibiotics. **STYPXB0009** (2 food isolates) showed TE resistance. Single-isolate genotypes **STYPXB0010** - **STYPXB0021** (12 total) originated from various sources; resistance detected in: **STYPXB0010** (AMP, C, PEF, TE), **STYPXB0013** (AMP, C, TE), **STYPXB0016** (AMP), **STYPXB0018** (AMP, TE), **STYPXB0019** (AMP, C, PEF, TE, W). All other isolates were sensitive to the tested antibiotics.

Susceptibility testing of S. Enteritidis using the disk diffusion method

All *S. Enteritidis* isolates from human clinical specimens were susceptible to all tested antimicrobials, with the exception of pefloxacin, to which resistance was detected in one isolate. Isolates from food and animal feed were fully susceptible to azithromycin, cefotaxime, ceftazidime, chloramphenicol, gentamicin, meropenem, tigecycline, and trimethoprim, while resistance was observed to pefloxacin (two isolates), amoxicillin (two isolates), and tetracycline (one isolate).

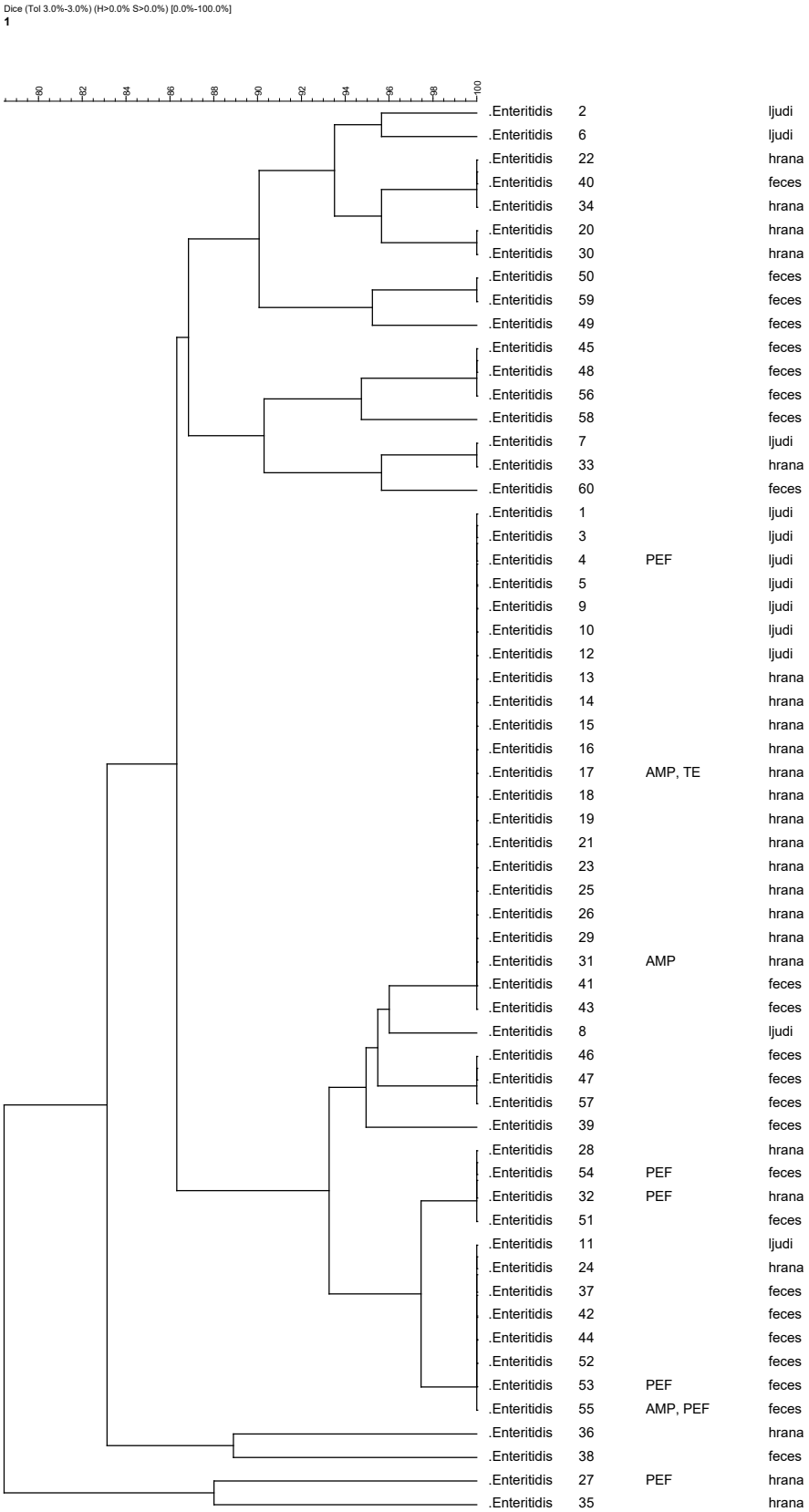

Figure 1. Dendogram of the electrophoretic scheme obtained with the enzyme *Xbal* - gene of 60 isolates of *S. Enteritidis* from the FPQuest program, which shows the similarity coefficient between the tested isolates, with a display of isolates resistant to AM drugs from the applied palette - ampicillin (AMP), pefloxacin (PEF), tetracycline (TE). Abbreviations: hrana – *Salmonella* isolates from food or animal feed; ljudi – clinical *Salmonella* isolates from infected patients; feces – *Salmonella* isolates from poultry feces.

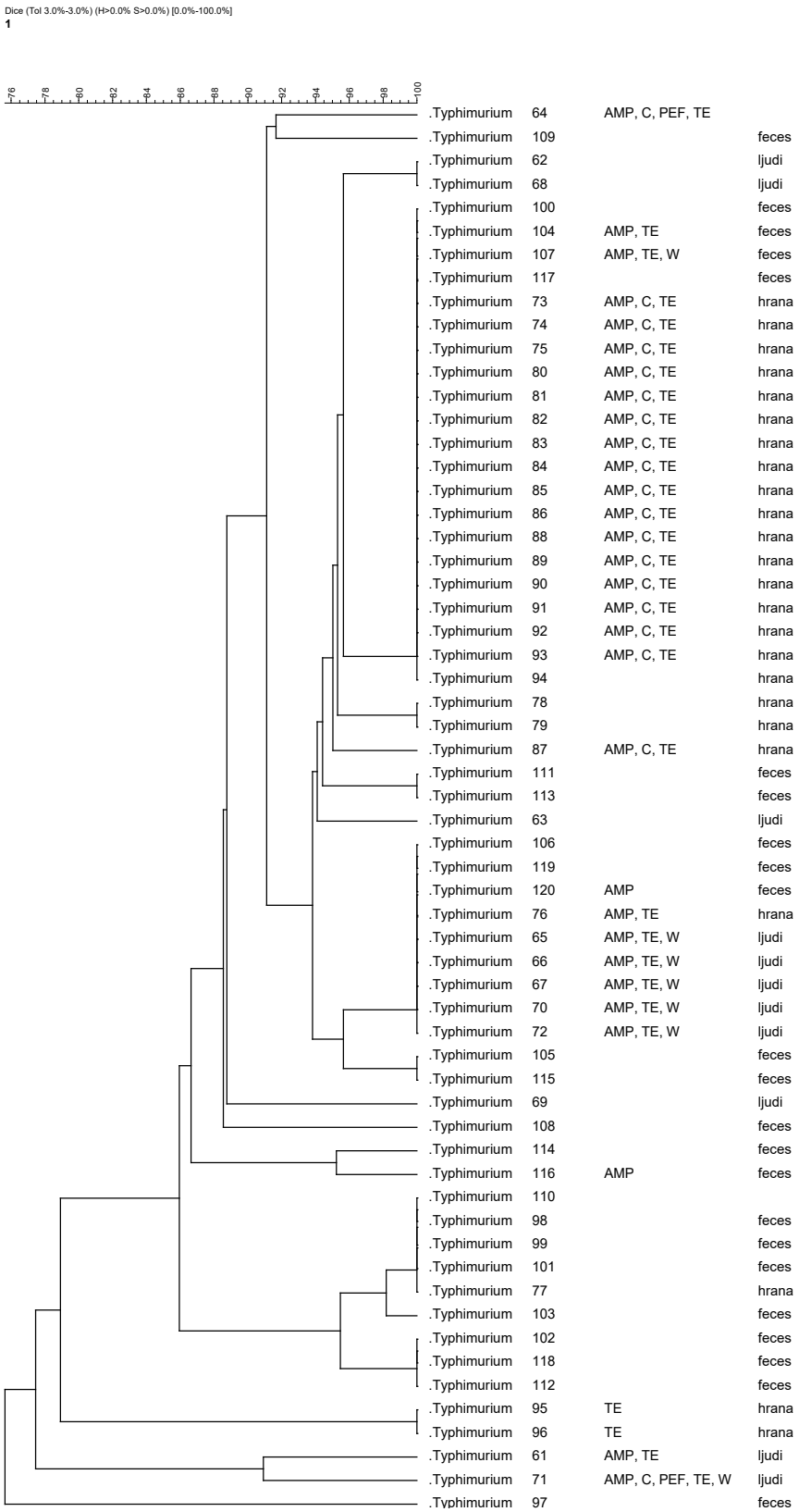

Figure 2. Dendogram of the electrophoretic scheme obtained with the enzyme *Xbal* - gene of 60 isolates of *S. Typhimurium* from the FPQuest program, which shows the similarity coefficient between the investigated isolates, with a display of isolates resistant to AM drugs from the applied palette - ampicillin (AMP), chloramphenicol (C), pefloxacin (PEF), tetracycline (TE), trimethoprim (W). Abbreviations: hrana – *Salmonella* isolates from food or animal feed; ljudi – clinical *Salmonella* isolates from infected patients; feces – *Salmonella* isolates from poultry feces.

All isolates from poultry feces were susceptible to azithromycin, cefotaxime, ceftazidime, chloramphenicol, gentamicin, meropenem, tetracycline, tigecycline, and trimethoprim; resistance was detected to pefloxacin in three isolates and to amoxicillin in one isolate. The sensitivity of 60 *S. Enteritidis* isolates to 12 AM drugs is shown in Table 3.

Susceptibility testing of S. Typhimurium using the disk diffusion method

After applying the disk diffusion method, all *S. Typhimurium* isolates were sensitive to azithromycin, cefotaxime, ceftazidime, gentamicin, meropenem, and tigecycline. Resistance was observed to pefloxacin, trimethoprim, chloramphenicol, tetracycline, and amoxicillin at varying levels. Food-derived isolates exhibited a high degree of resistance to chloramphenicol, ampicillin, and tetracycline, while human isolates showed notable resistance to ampicillin, tetracycline, and trimethoprim. The susceptibility of 60 *S. Typhimurium* isolates to 12 antimicrobial agents is presented in Table 4.

Examination of the sensitivity of S. Enteritidis to AM drugs by the E test

S. Enteritidis isolates that were resistant according to disk diffusion testing were further examined using the E-test. The E-test was applied to isolates that showed resistance to ciprofloxacin (six isolates), ampicillin (three isolates), and tetracycline (one isolate). The results of antimicrobial susceptibility testing of *S. Enteritidis* obtained by the E-test showed that 3/3 (100%) isolates were resistant to ampicillin (MIC>8 µg/mL), 1/1 (100%) was resistant to tetracycline (MIC≥16 µg/mL) and 3/6 (50%) were resistant to ciprofloxacin (MIC>0.06 µg/mL). The results are presented in Table 5.

Table 3. Summary of *S. Enteritidis* susceptibility test results (disc diffusion method)

Antimicrobial (AM) drugs	Origin of isolate / number			
	Humans / n = 12	Food / n = 24	Feces / n = 24	Total
	R (%)	R (%)	R (%)	R (%)
Ampicillin (AMP10)	0	2 (8,33)	1 (4,17)	3 (5)
Azithromycin (AZM15)	0	0	0	0
Cefotaxime (CTX5)	0	0	0	0
Ceftazidime (CAZ10)	0	0	0	0
Chloramphenicol (C30)	0	0	0	0
Gentamicin (CN10)	0	0	0	0
Meropenem (MEM10)	0	0	0	0
Pefloxacin (PEF5)	1(4,17)	2 (8,33)	3 (12,5)	6 (10)
Sulfamethoxazole (RL100)	ND	ND	ND	ND
Tetraciklin (TE30)	0	1 (4,17)	0	1 (1,67)
Tigecycline (TGC15)	0	0	0	0
Trimetoprim (W5)	0	0	0	0

Abbreviations: R – resistant, ND – no defined criterion for AMR assessment

Table 4. Summary of *S. Typhimurium* susceptibility test results (disc diffusion method)

Antimicrobial (AM) drugs	Origin of isolate / number			
	Humans / n = 12	Food / n = 24	Feces / n = 24	Total
	R (%)	R (%)	R (%)	R (%)
Ampicillin (AMP10)	8 (66,67)	18 (75)	4 (16,67)	30 (50)
Azithromycin (AZM15)	0	0	0	0
Cefotaxime (CTX5)	0	0	0	0
Ceftazidime (CAZ10)	0	0	0	0
Chloramphenicol (C30)	2 (16,67)	17 (70,83)	0	19 (31,67)
Gentamicin (CN10)	0	0	0	0
Meropenem (MEM10)	0	0	0	0
Pefloxacin (PEF5)	2 (16,67)	0	0	2 (3,33)
Sulfamethoxazole (RL100)	ND	ND	ND	ND
Tetraciklin (TE30)	8 (66,67)	18 (75)	2 (8,33)	28 (46,67)
Tigecycline (TGC15)	0	0	0	0
Trimetoprim (W5)	6 (50)	0	1(4,17)	7 (11,67)

Abbreviations: R – resistant, ND – no defined criterion for AMR assessment

Table 5. Results of susceptibility testing of *S. Enteritidis* obtained by the E-test

Antimicrobial (AM) drugs	Resistant isolates after the disk diffusion method	Resistant isolates after the E test	
		Designation	(%)
Ampicillin (AMP 0.016-256 µg/mL)	**3** (17, 31, 55)	**3** (17, 31, 55)	100
Ciprofloxacin (CIP 0.002-32 µg/mL)	**6** (4, 27, 32, 53, 54, 99)	**3** (27, 32, 55)	50
Tetracycline (TE 0.016-256 µg/mL)	**1** (17)	**1** (17)	100

Examination of the sensitivity of S. Typhimurium to AM drugs using the E test

S. Typhimurium isolates that were resistant according to disk diffusion testing were further examined using the E-test. The E-test was applied to isolates showing resistance to ampicillin (30 isolates), tetracycline (30 isolates), chloramphenicol (19 isolates), trimethoprim (7 isolates), and ciprofloxacin (2 isolates). The results of antimicrobial susceptibility testing obtained by the E-test showed that 2/2 (100%) isolates were resistant to ciprofloxacin (MIC>0.06 µg/mL), 19/19 (100%) to chloramphenicol (MIC>8 µg/mL), and 7/7 (100%) to trimethoprim (MIC≥4 µg/mL), while 29/30 (96.7%) were resistant to ampicillin (MIC>8 µg/mL) and tetracycline (MIC≥16 µg/mL). The results are presented in Table 6.

Table 6. Results of susceptibility testing of *S. Enteritidis* obtained by the E-test

Antimicrobial (AM) drugs	Resistant isolates after the disk diffusion method	Resistant isolates after the MIC Test Strip	
		Designation	(%)
Ampicillin (AMP 0.016-256 µg/mL)	**30** (61, 64, 65, 66, 67, 70, 71, 72, 73, 74, 75, 76, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 104, 107, 116, 120)	**29** (61, 64, 65, 66, 67, 70, 71, 72, 73, 74, 75, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 104, 107, 116, 120)	96,7
Ciprofloxacin (CIP 0.002-32 µg/mL)	**2** (64, 71)	**2** (64, 71)	100
Chloramphenicol (C 0.016-256 µg/mL)	**19** (64, 71, 73, 74, 75, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93)	**19** (64, 71, 73, 74, 75, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93)	100
Tetracycline (TE 0.016-256 µg/mL	**30** (61, 64, 65, 66, 67, 70, 71, 72, 73, 74, 75, 76, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 95, 96 104, 107)	**29** (61, 64, 65, 66, 67, 70, 71, 72, 73, 74, 75, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 95, 96 104, 107)	96,7
Trimetoprim (TM 0.002-32 µg/mL)	**7** (65, 66, 67, 70, 71, 72, 107)	**7** (65, 66, 67, 70, 71, 72, 107)	100

DISCUSSION

Characterization of S. Enteritidis and S. Typhimurium

During this study, the characterization of *S. Enteritidis* and *S. Typhimurium* isolates included molecular characterization (Pulsed Field Gel Electrophoresis - PFGE), testing of sensitivity to AM drugs (Disc - diffusion method) and determination of the minimum inhibitory concentration (E test). Typing of 60 isolates of *S. Enteritidis* and 60 isolates of *S. Typhimurium* was previously performed using the duplex real-time PCR protocol for the detection of the *safA* gene of *S. Enteritidis* and the *fliA-IS200* gene of *S. Typhimurium* (Dmitric et al., 2025).

Detection of *Salmonella* spp. in samples of animal and human origin, as well as in samples from the environment in the area of food production and handling, represents only the initial step in identifying the source and defining the transmission routes of this pathogenic microorganism. During epidemiological studies, it is generally not enough to type *Salmonella* isolates to the level of species and serotype, but it is also necessary to apply typing methods that can distinguish between epidemiologically different but genetically related isolates (Deng et al., 2015). Tracking the sources of microbiological contaminants has always been important in the food production chain, however, advances in the development of molecular typing methods have provided tools that enable faster and extremely reliable determination of the source of food contamination (Moorman et al., 2010). The imperative is more than obvious to improve *Salmonella* detection techniques because older techniques are mostly labor-intensive and time-consuming. Nucleic acid detection and gene editing technology, immunological techniques, Raman spectroscopy, biosensors and rapid detection devices are recognized as innovative *Salmonella* detection techniques today (Patel et al., 2024; Yang et al., 2025). Maurischat et al. (2015) developed and internally validated a method that detects and differentiates between SE and STM serovars of *Salmonella* based on a 5-plex real-time PCR assay (24 hours after sampling).

A recent study by Ibrahim (2024) reported a 12% prevalence of *Salmonella* infection in frozen poultry imported into Iraq (13/110 samples). Among the positive samples, 38% were identified as *S. Enteritidis* (5/13) and 30% as *S. Typhimurium* (4/13), indicating that frozen poultry represents a significant source of zoonotic *Sal-*

monella infections. In contrast, Tarabees et al. (2017) investigated 100 raw chicken meat samples and detected *Salmonella* spp. using biochemical, serological, and MALDI-TOF methods. *S. Enteritidis* and *S. Typhimurium* were identified in 2% and 3% of samples, respectively. Multiplex PCR revealed the presence of several virulence genes in both serovars, including *sitC*, *sopB*, *sifA*, *lpfC*, *spaN*, *sipB*, and *invA*. Although the prevalence was relatively low, the authors emphasized the importance of strict food safety and public health measures to reduce the risk of salmonellosis.

Another study aimed to determine the presence of *Salmonella* spp., *S. Enteritidis* and *S. Typhimurium* in raw chicken meat in Selangor (Malaysia) and their antibiotic susceptibility profile, and a combination of most probable number (MPN) and multiplex polymerase chain reaction (mPCR) methods was used for their quantification. *Salmonella* spp., *S. Enteritidis* and *S. Typhimurium* were detected in 20.80%, 6.70% and 2.50%, respectively, in 120 chicken meat samples, and the estimated amount ranged from <3 to 15 MPN/g. Antibiograms revealed different resistance of *S. Enteritidis* and *S. Typhimurium* isolates to multiple AM drugs. All isolates were resistant to Erythromycin, Penicillin and Vancomycin, while susceptibility was recorded to Trimethoprim, Amoxicillin/Clavulanic acid, Gentamicin and Tetracycline. The results of the study reveal that retail chicken meat may be aantimicrobial-resistant *Salmonella*, a public health concern (Thung et al., 2016).

A study to investigate food contamination in hospitals in different parts of Iran included the identification of *Salmonella Enteritidis*, *Salmonella Typhimurium*, *Bacillus cereus*, *Bacillus subtilis*, and *Clostridium perfringens* as the main foodborne pathogens. Out of 360 food samples, *Salmonella* was confirmed in 21 (5.82%) food samples, and the ratio of *S. Typhimurium*: *S. Enteritidis*: other *Salmonella* species was 12 (3.33%): 4 (1.11%): 5 (1.38%), respectively. The most contaminated hospital foods were salad, kebab, and rice samples (36%, 16%, and 12%), respectively. In this study, two strains of *S. Typhimurium* and *S. Enteritidis* were the primary causes of food contamination among the pathogens tested, indicating the need to apply stricter control measures, especially for unprocessed foods (salads) (Hashemi et al., 2025).

Genotyping of S. Enteritidis and S. Typhimurium using the PFGE method

Pulsed-field gel electrophoresis was first described in 1983 and quickly became a popular method for typing pathogenic foodborne microorganisms (Schwartz et al., 1983). When typing *Salmonella*, the primary restriction endonuclease is *XbaI*. If, after restriction with the primary enzyme, PFGE profiles are obtained that cannot be distinguished, secondary (*BlnI/AvrII*) or tertiary enzymes (*SpeI*) are used. Standardization of the PFGE method by "PulseNet International" has resulted in PFGE becoming the most commonly used method for identifying the source of epidemics, monitoring and testing the most important foodborne pathogenic microorganisms, including *Salmonella* spp. (Ribot et al., 2006). The discriminatory power of PFGE depends on the number and distribution of restriction sites across the genome, which determine the number and size of DNA bands. It can be enhanced by using multiple restriction enzymes, and results are highly reproducible between laboratories (Swaminathan et al., 2001). However, PFGE is labor-intensive, time-consuming (about three days), and requires costly equipment, high-quality reagents, and skilled personnel (Steve et al., 2004; Herschleb et al., 2007).

By examining the genetic similarity of 60 *S. Enteritidis* isolates, 12 of human origin, 24 of food origin and 24 of poultry faeces origin, using the *XbaI* enzyme, 20 different PFGE profiles (genotypes) were determined. PFGE results revealed that *S. Enteritidis* in this research were genetically heterogeneous and diverse, as well as in a similar study by Abd Hazis et al. (2022), in which 27 unique patterns known as pulsotypes were generated, using PFGE analysis. Within isolates belonging to the same profile, the genetic similarity was 100%, while the similarity between different genotypes ranged from 78% to 97%. The most numerous genotype, with a 100% identical PFGE profile (SENTXB0001), includes a total of

22 isolates (36.67%), of which 7 isolates originated from humans (58.33%), 13 isolates originated from food and animal feed (54.17%), and two isolates originated from poultry feces (8.33%). The second largest genotype (SENTXB0002) included 8 isolates (13.33%), one originating from humans (8.33%), one originating from animal feed (4.17%) and six originating from poultry feces (25%). Genotype SENTXB0003 included 4 isolates (6.67%), two originating from food (8.33%) and two originating from poultry feces (8.33%). Genotype SENTXB0004 included 3 isolates (5%), 2 originating from food and animal feed (8.33%) and one originating from poultry feces (4.17%). Three isolates (5%) were assigned to the genotype SENTXB0005 and SENTXB0006, all 3 (12.5%) originating from poultry feces. Three genotypes (SENTXB0007, SENTXB0008, SENTXB0009) included 2 (3.33%) isolates each. Eleven genotypes (from SENTXB00010 to SENTXB0020) included 1 isolate each (1.67%). It is noted that the first two genotypes included 50% of the total number of isolates, 33% of isolates from feces, 58.34% of isolates from food and animal feed and even 66.7% of isolates originating from sick people.

By examining the genetic similarity of 60 *S. Typhimurium* isolates, 12 of human origin, 24 of food origin and 24 of poultry feces origin, using the *XbaI* enzyme, 21 different PFGE profiles (genotypes) were determined. Within isolates belonging to the same profile, the genetic similarity was 100%, while the similarity between different genotypes was 77% to 98%. The most numerous genotype, with a 100% identical PFGE profile, is the group designated as STYPXB0001, with a total of 21 isolates (35%), of which 17 (70.83%) isolates were of food origin, and 4 (16.67%) from poultry feces. The second largest genotype, STYPXB0002, included 9 isolates (15%), five from humans (41.67%), one from animal feed (4.17) and three from poultry feces (12.5%). 5 isolates (8.33%), one originating from animal feed (4.17%) and 4 originating from poultry feces (16.67) were assigned to the STYPXB0003 genotype. Three isolates were assigned to the genotype STYPXB0004 (5%), all originating from poultry feces (12.5%). Five genotypes (from STYPXB0005 to STYPXB0009) included 2 isolates each (3.33%), and 12 genotypes (from STYPXB0011 to STYPXB0021) included 1 isolate each (1.67%). It is noted that the first two genotypes included 50% of the total number of isolates, 29.17% of isolates from feces, 41.67% of isolates originating from sick people and even 75% of isolates from food and animal feed (with 70.83% belonging to the first genotype). Similar to our findings, Wiesner et al. (2006) discovered 34 PFGE patterns (the dominant pattern was *S.* Type0001). The heterogeneity of this serotype has also been demonstrated in studies by Tamada et al. (2001).

Antimicrobial susceptibility testing of S. Enteritidis and S. Typhimurium

According to the data published by EFSA in the countries of the EU during 2016, isolates of *Salmonella* spp. coming from infected people, they were resistant in a high percentage to sulfonamides (34.6%), ampicillin (29.5%) and tetracyclines (29.2%). A high percentage of *S. Typhimurium* isolates showed resistance to ampicillin (60.6%), sulfonamides (50.0%) and tetracyclines (51.3%), while *S. Enteritidis* isolates were resistant to nalidixic acid (18.4%), colistin (17.5%) and ciprofloxacin/pefloxacin (12.3%). On the other hand, isolates of *Salmonella* spp. originating from poultry showed a high percentage of resistance to ciprofloxacin (64.7%), nalidixic acid (61.5%), sulfamethoxazole (55.6%) and tetracycline (46.1%), while resistance to ampicillin amounted to 19.7% and to trimethoprim 14.8% (EFSA and ECDC, 2018).

Laboratories responsible for typing *Salmonella* in humans need to harmonise the methods used for susceptibility testing in order to ensure the continued effectiveness of surveillance of resistance to AM drugs. The international surveillance network Enter-net (funded by the EU, with 18 national reference laboratories for *Salmonella*) conducted a study of resistance or susceptibility to a panel of 11 AM drugs (ampicillin, cefotaxime, chloramphenicol, gentamicin, kanamycin, streptomycin, sulfonamides, tetracyclines, trimethoprim, nalidixic acid and ciprofloxacin), examining forty-eight strains of *Salmonella enterica*. Over 8,500 tests were evaluated by disk diffusion (DD), agar breakpoint (BP) or full minimum

inhibitory concentration (MIC) methods. There was a high degree of agreement for the detection of resistance to most AM drugs, except for reduced susceptibility to ciprofloxacin, where significant disagreement was noted. All isolates with reduced susceptibility to ciprofloxacin were resistant to nalidixic acid, and it is proposed to determine MICs for ciprofloxacin for isolates resistant to nalidixic acid. Disagreement was primarily observed in the testing of susceptibility to streptomycin. The common position of the Enter-net network members is that there is a real possibility to create databases on AM susceptibility. Harmonized antibiogram data for *Salmonella* can be exchanged between national reference laboratories for *Salmonella* within the EU, except for reduced susceptibility to ciprofloxacin (Threlfall et al. 1999).

During the experimental part of this study, 60 isolates of *S. Enteritidis* were examined using the disc-diffusion method, whereby 10% of the isolates showed resistance to pefloxacin, 5% to ampicillin and 1.67% to tetracycline. Isolates of *S. Enteritidis*, resistant after testing with the disk-diffusion method, were tested with the E-test. The E test was applied to isolates that showed resistance to ciprofloxacin (6 isolates), ampicillin (3 isolates) and tetracyclines (1 isolate). The results of testing the sensitivity of *S. Enteritidis* to AM drugs obtained by the E-test showed that 3/3 (100%) were resistant to ampicillin, 1/1 (100%) were resistant to tetracycline, and 3/6 (50%) were resistant to ciprofloxacin. Fluoroquinolones are widely used in the treatment of complicated cases of salmonellosis in adults, while cephalosporins of the third generation are mainly used in children (EFSA, 2017). However, immediately after the beginning of the use of fluoroquinolones in therapy, *Salmonella* strains resistant to them were also detected (Mølbak et al., 1999; Chen et al., 2007). Occurrence of serotypes of *Salmonella* spp. with reduced sensitivity to fluoroquinolones, such as ciprofloxacin, often results from a chromosomal mutation of DNA gyrase, most commonly in the *gyrA* gene region (Griggs, 1996), and very rarely in the *gyrB* gene region (Piddock, 2002). Other resistance mechanisms can also lead to reduced susceptibility of *Salmonella* spp. to fluoroquinolones, including changes in cell membrane permeability and activation of efflux pumps (Ruiz, 2003).

From the aspect of public health, a particular problem is represented by non-typhoidal *Salmonella* resistant to extended-spectrum cephalosporins, such as ceftriaxone, which is mainly used to treat children (Miriagou et al., 2004, Su et al., 2005). Alternatively, azithromycin or imipenem can be used (Stoycheva and Murdjeva, 2006). Some serotypes of *Salmonella* spp. have begun to develop resistance to broad-spectrum cephalosporins as a result of mutated genes encoding *β*-lactamases, enzymes that hydrolyze antibiotics with *β*-lactam rings such as cephalosporins (Carattoli, 2003). Many types of *β*-lactamases (CTX-M, TEM, OXA, SHV) have been identified in *Salmonella* isolates (Fernandez Vazquez et al., 2006; González-Sanz et al., 2009). Also, research has shown that some *Salmonella* isolates on plasmids possess the *blaCMY-2* gene that produces *AmpC β*-lactamase that leads to resistance to cephalosporins and amoxicillin/clavulonic acid (Keelara and Thakur, 2014). By examining 60 isolates of *S. Enteritidis* and the same number of isolates of *S. Typhimurium*, no resistance to the applied cephalosporins (cefotaxime and ceftazidime) was found during the performance of the disk-diffusion method in the experimental part of this research.

After applying the disk diffusion method, all 60 isolates of *S. Typhimurium* showed sensitivity to azithromycin, cefotaxime, ceftazidime, gentamicin, meropenem and tigecycline. Resistance ranged from 3.33% to pefloxacin, 11.67% to trimethoprim, 31.67% to chloramphenicol, 46.67% to tetracycline to 50% to amoxicillin. Isolates from food showed a high degree of resistance to chloramphenicol (70.83%), ampicillin (75%) and tetracycline (75%). Also, human isolates in high estimation showed resistance to ampicillin (66.67%), tetracycline (66.67%) and trimethoprim (50%). Isolates of *S. Typhimurium*, resistant after testing with the disk-diffusion method, were tested with the E-test. The E test was applied to isolates that showed resistance to ampicillin (30 isolates), tetracyclines (30 isolates), chloramphenicol (19 isolates), trimethoprim (7 isolates) and ciprofloxacin (2 isolates). The results of testing the sensitivity of *S. Typhimurium* to AM drugs obtained by the E-test showed that 2/2 (100%) were resistant to ciprofloxacin, 19/19 (100%) to chloram-

phenicol and 7/7 (100%) to trimethoprim, while 29/30 (96.7)% were resistant to ampicillin and tetracycline.

In the study of Dishan et al. (2024), 112 (38.22%) of the 293 chicken samples contained *S. enterica*, with five (4.46%) and one (0.89%) of the isolates identified as *S. Enteritidis* and *S. Typhimurium*, respectively. All isolates examined of antibiotic resistance showed that they were sensitive to Meropenem and Aztreonam. Doxycycline (96.42%) and Trimethoprim-sulfamethoxazole (71.42%) were most resistant antibiotics. In the study by Yousefi Amin et al. (2024), antimicrobial resistance patterns of the *S. Enteritidis* and *S. Typhimurium* strains were evaluated and the most resistant were colistin (100%) and nalidixic acid (75%), out of a total of 18 antibiotics tested.

In Iraq, Al-Shafee and Abdulwahid (2024) analyzed 300 samples (150 chicken products and 150 human feces) following ISO 6579 and Global Network protocols. *Salmonella enterica* was isolated in 8.66% of chicken samples and 4.6% of human samples. *S. Typhimurium* predominated in liver and minced meat, while *S. Typhi* was found in humans. Antimicrobial testing (disk diffusion and VITEK® 2) showed multidrug resistance in human isolates and variable resistance patterns in chicken isolates, mainly to *β*-lactams, aminoglycosides, and fluoroquinolones.

Putturu et al. (2013) examined *S. Enteritidis* isolates obtained from different samples of chicken, mutton, turkey meat, faecal and cloacal samples of poultry and turkey, eggs, water and feed on sensitivity and resistance to selected antibiotics. *S. Enteritidis* isolates were less sensitive to sulfonamides and more sensitive to ciprofloxacin, chloramphenicol, amikacin, gentamycin, streptomycin, amoxicillin and tetracyclines. Higher resistance was observed with sulfonamide, ampicillin, and nalidixic acid, respectively (Putturu et al., 2013).

The increase in the prevalence of *Salmonella* spp. resistant to multiple antibiotics (Multi Drug Resistant - MDR), but also resistance to clinically important AM agents, such as fluoroquinolones and third-generation cephalosporins, represents a serious problem worldwide. AM drugs were widely used in intensive livestock production as growth promoters, as well as in disease prevention. The link between the use of AM drugs in animals and the emergence of resistant bacteria that can lead to human disease has been confirmed (Araque, 2009; Gousia et al., 2011; El-Tayeb et al., 2017; Velhner et al., 2018; Jajere, 2019). Therefore, in the countries of the European Union, EC No 1831/2003 regulation prohibits the use of all antimicrobial drugs as feed additives (OJEU, 2003).

The study by Zhang et al. (2024) identified *Salmonella enterica* ST11 as the cause of the food poisoning outbreak, highlighting the importance of next-generation sequencing (NGS) tools and phenotypic characterization in epidemiological studies, and highlighting the potential risk of a new multi-antibiotic ST11 clone for *S. Enteritidis*. The study by Zheng et al. (2025) was created since the emergence of multidrug-resistant (MDR) *S. Enteritidis* highlighted the importance of regular monitoring of the emergence of antibiotic-resistant strains, and included combined phenotypic analysis and whole genome sequencing (WGS) of 95 *S. Enteritidis* isolates from retail and environmental meat samples in China (2014–2019), to clarify the association between genetic characteristics and antibiotic resistance phenotypes (ARP).

The distribution of *Salmonella* serotypes varies from region to country, and in southern China, *S. Typhimurium* was found to be the main and dominant serotype detected in patients (Hung et al., 2017; Liang et al., 2019; Chen et al., 2023), with a proportion of over 50%. In Southeast Asia, in Vietnam, a relevant study found that *S. Typhimurium* was the most dominant serotype (41.8%) in NTS isolates (Duong et al., 2020). In Denmark, the predominant non-typhoidal *Salmonella* serotypes were *S. Enteritidis*, monophasic *S. Typhimurium* and *S. Typhimurium* (Aarø et al., 2024). In the Netherlands, the leading serotypes of invasive NTS were *S. Enteritidis* and *S. Typhimurium* (Mughini-Gras et al., 2020). A large-scale outbreak of MDR monophasic *S. Typhimurium* infection during 2022 was monitored in several countries, and in 10 of them it was investigated and linked to contaminated chocolate products (Larkin et al., 2022).

A major public health concern was the emergence of S. Tyhimurium DT 104, which was first identified in Great Britain in 1984 (Threlfall et al., 1996) . This phagotype exhibits resistance to five AM agents: ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline (Gebreyes et al., 2004; Rayamajhi et al., 2008). Studies show that serotypes of *Salmonella* spp., which are MDR, have the ability to produce different types of hybrid plasmids. Most of the genetic cassettes located within these plasmids consist of resistance genes that lead to reduced susceptibility to AM drugs such as chloramphenicol, tetracycline, ampicillin and streptomycin (Guerra et al., 2002). A very significant contribution to the deepening and systematization of knowledge about genes and mechanisms of resistance and the mode of action of *Salmonella* was made by a recent review by Punchihewage-Don et al. (2024).

Due to the constant and growing concern about (uncontrolled, inappropriate or unauthorized) use of antibiotics and antimicrobials, alternative methods of combating microorganisms or the application of natural agents are being investigated (Abou Elez et al., 2021; El-Saadony et al., 2022), so the most recent findings are of importance suggesting that the phage *vB_Sal_TmvP009* has high potential as an AM agent against *S. Typhimurium* monophasic variant, i.e. for the control of *Salmonella* in the food industry (Liu et al., 2025).

CONCLUSIONS

This study showed notable genetic diversity among *S. Enteritidis* and *S. Typhimurium* isolates, with a few dominant PFGE genotypes. *S. Enteritidis* isolates were mostly susceptible to tested antibiotics, while *S. Typhimurium* showed higher resistance, particularly to ampicillin, tetracycline and chloramphenicol. The findings emphasize the need for continuous surveillance of antimicrobial resistance and molecular typing of *Salmonella* within the food chain.

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