Incidence of *Salmonella* Infantis in poultry meat and products and the resistance of isolates to antimicrobials

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Abstract. Globalisation, climate change, changes in eating habits and the food industry, modern animal husbandry and market demands often have a negative impact on quality assurance, food safety and animal health. After the eradication of some zoonotic diseases that previously often jeopardized the human population, today in developed countries, the focus is mainly on the control of zoonoses transmitted by food. *Salmonella* is one of the most common pathogens that can be transmitted from animals to humans, and its reservoirs are poultry, cattle and pigs, so one transmission route to humans is from contaminated food of animal origin. Multidrug-resistant isolates of *Salmonella*, which can transfer their resistance genes to other microorganisms, are considered a serious threat to public health. Control of *Salmonella* primarily depends on a good monitoring system and knowledge of the presence of serovars and strains in an epizootiological area. During the first nine months of 2016, 1321 samples of poultry meat and products were examined, among which 108 harboured *Salmonella*. Altogether, 29 of the 108 isolates (26.85%) were *Salmonella* Infantis. For all 29 *S*. Infantis isolates, antimicrobial resistance was tested by the disc diffusion method. The isolates showed 100% resistance to amoxicillin, and nalidixic acid.

1. Introduction

Bacteria of the genus *Salmonella* belong to the family *Enterobacteriaceae*. The *Salmonella* genus includes over 2,570 different serotypes. All species of this genus are pathogenic to humans and cause different types of illnesses, from intestinal infections such as diarrhoea to generalized infections which are life-threatening such as typhoid fever. Infectious diseases in humans are mainly caused by *Salmonella enterica* subspecies *enterica* (*S*.), serotype Typhi or Paratyphi. Other serotypes mostly cause intestinal infections, but can also cause septicaemia, and often the existence of long-term carrier status.

Apart from in humans, *Salmonella* can cause disease in many animal species, and human disease is commonly associated with the consumption of food of animal origin. Humans can become infected through the faecal-oral route, by consuming different kinds of contaminated food and water or through contact with animals. *Salmonella enterica* subsp. *enterica* serotype Enteritidis (*S*. Enteritidis), *S*. Typhimurium, *S*. Infantis and *S*. Hadar are ubiquitous serotypes with a wide range of hosts. They rarely cause systemic disease in healthy adults, but they are able to colonize gastrointestinal tract of many animal species. Thanks to frequent colonization and a high level of excretion in faeces of
animals reared for human consumption, ubiquitous serotypes can enter the food chain and cause salmonellosis cases in humans [1]. The ubiquity of Salmonella and its prevalence in the natural environment, on farms and in the food chain, as well as its adaptability to many animal reservoirs and routes of transmission, makes epidemiological studies of these bacteria very complex. For most epidemics among humans, the source of infection can be detected relatively easily, because they are usually foodborne, caused by one serotype and mainly of limited duration, but hospital epidemics are exceptions. When animals are bred for human consumption on farms, sources, routes and methods of transmission are usually more complex, especially as multiple strains and serotypes of Salmonella can persist concurrently on farms for a long time. Hence, the eradication of Salmonella on farms is a challenge. The first step in preventing the spread of infection between animals and humans is monitoring the movement of individual strains of Salmonella in both populations. For this purpose, it is not enough to identify isolates just to the level of Salmonella serovars, because not all isolates within each serotype are identical. The divisions at different levels within the serotypes have significance in epidemiological studies and at the local level, in the context of supervision of these pathogens.

The role of poultry in the epidemiology of human Salmonella infections is recognized to be due to the development of intensive poultry production worldwide. Infected poultry is one of the most important reservoirs of Salmonella that are transmitted to humans through the food chain. In the past, the main motive for the control of salmonellosis in poultry was to reduce production losses. Today, the interest in protecting public health, plus political and consumer pressures, have resulted in the prevention of foodborne salmonellosis being a top priority for poultry producers [2]. The presence of Salmonella in poultry (compared to other domestic food-source animals), means poultry products have far greater significance as potential sources of infection for humans. Therefore, monitoring the presence and movement of Salmonella in poultry flocks is the first step in the control of this zoonosis, which later in the poultry chain is continued by microbiological control of poultry meat, eggs and their products [3].

Paratyphoid infections in poultry are caused by motile Salmonella serotypes, of which the most common causes are S. Enteritidis and S. Typhimurium. These organisms can infect a variety of hosts, including humans. In poultry, paratyphoid infections are generally asymptomatic, while in humans they cause symptoms of food poisoning [4].

In Serbia, foodborne salmonellosis outbreaks were linked commonly with cakes (22.2%), eggs (8.9%) or fried chicken (4.4%). However, according to information on the presence of Salmonella in some types of foods in Belgrade in the last twenty years, Salmonella was more likely to be isolated from chicken (49.2%), but was detected significantly less often in cakes and pies (8.8%) [5].

S. Enteritidis is, together with S. Typhimurium, a leading cause of salmonellosis in humans in the world. However, according to available data, in recent years, the number of isolates of S. Enteritidis and S. Typhimurium has decreased by half, but a constant increase in the number of isolates of S. Infantis has been recorded with its main sources being pigs, poultry and animal food [6]. The S. Infantis presence in human and animal food, as well as in animals, has led to this being one of the ten most frequently identified Salmonella serotypes in the European Union (EU) [6]. The European Food Standards Agency (EFSA) [6] stated that, according to results from the European Centre for Disease Control (ECDC), for the period from 2012 to 2014, S. Infantis was the fourth most common Salmonella serovar in the EU. Furthermore, it was revealed that S. Infantis contains isolates of several different clones which are present not only in food, but also in humans [7].

Human infection caused by S. Infantis via food is increasingly being monitored worldwide. S. Infantis is also increasing among human isolates in Europe (Germany, Hungary, Netherlands, Finland), but the rise of this serovar is also noticed in countries around the world (Australia, New Zealand, Russia, Argentina, Brazil, Canada, Japan [6,8,9,10]. In Hungary, in recent years, there has been an increased occurrence of S. Infantis not only in poultry production, but also in humans [11,12]. Likewise in Germany, S. Infantis is the third most common Salmonella serovar in recent years in humans, and poultry and pork meat are major sources of infection [7,13].
According to available literature, the Republic of Srpska and neighbouring countries have not conducted studies that include isolates from poultry, people and food of poultry origin, in an attempt to investigate their possible clonal association as evidence of circulating strains in the poultry food chain. However, available data from Croatia, where S. Infantis has been monitored for several years in broiler flocks, showed isolations of S. Infantis are increasing in absolute terms, but also in relation to other pathogenic strains of Salmonella that are being monitored [14].

Many scientific studies in various countries have recognized poultry meat as a potential source of S. Infantis, and the presence of this organism as the predominant Salmonella serovar in poultry in Israel was associated with an increase in the number of people with diabetes during 2007-2009 [9,15]. Also, over a period of five years (2004-2009), 76 S. Infantis isolates were obtained from broilers from nine countries in Central and Eastern Europe [10,16]. A significant increase (up 162%) of human infection by S. Infantis was reported in the United States in 2014, compared to the previous reporting period (2011-2013) [17].

EFSA and ECDC [18], reporting on antimicrobial resistance among pathogens and indicator bacteria in humans, animals and food, stated that S. Infantis showed resistance to more than 90% of the tested antimicrobials. The greatest resistance (including to ciprofloxacin, streptomycin, sulphonamides and tetracyclines) was shown by isolates from broiler meat and hens. In countries where vaccination against S. Enteritidis and S. Typhimurium is carried out with good results, it can be assumed that this could allow other Salmonella to enter the poultry at farm level; S. Infantis is a potential candidate for this [7].

The Republic of Srpska’s Policy on Microbiological Criteria for Foods [19] requires serotyping of S. Enteritidis and S. Typhimurium only when isolates are from fresh broiler chicken meat, or from broilers, hens, or breeding or fattening flocks of turkeys, while all other Salmonella isolations are recorded as contamination with Salmonella spp., and do not require further subtyping.

Good farming and good hygienic practices (GFP and GHP) are a set of measures that are applied to control Salmonella spp. [20]. The basis of food production with minimal contamination with Salmonella is good manufacturing practice as well as the implementation of hazard analysis and critical control points (HACCP) from preparation to delivery [21].

The aim of this study was to determine the overall incidence of S. Infantis in poultry meat and poultry meat products, frozen and fresh in the Republic of Srpska, as well as to determine the resistance of the S. Infantis isolates to antimicrobial drugs.

2. Materials and methods
Microbiological testing of fresh and frozen poultry meat was carried out from January to October 2016 as part of the normal business activities in the Laboratories for Microbiology of Food, Animal Feed and Water, Veterinary Institute of the Republic of Srpska “Dr Vaso Butozan”. In total, 1321 samples were tested including: 39 grilled chickens, 244 chicken fillets, 54 chicken wings, 24 chicken meat samples, 136 fresh neck skins, 234 frozen neck skins, 40 livers, 265 samples of mechanically deboned meat, 56 thighs together with drumsticks, 14 marinated meats, 6 chicken breasts with skin and 215 eggs. All samples were tested for Salmonella according to the method ISO 6579/Cor 2:2010 [22].

Further biochemical identification and serological typing was conducted on suspected colonies grown on solid media. Biochemical reactions (triple sugar iron, urea, lysine decarboxylase test, ONPG, indole production and Voges-Proskauer) were used for the biochemical identification which confirmed the physiological characteristics of the genus Salmonella. Serological typing of Salmonella isolates was by the Kauffman-White-Le Minor scheme [23], with polyvalent and monovalent antisera. Subtyping of isolates was conducted using the antisera O6, O7, H1:r, and H2;5 for S. Infantis, and O9, H1:g, and H1:m for S. Enteritidis.

Disk diffusion according to Kirby-Bauer [25] was used for testing the susceptibility of the S. Infantis isolates to antimicrobial drugs. The tested isolates were first streaked on trypticase soy agar (TSA) and incubated for 24 h at 37°C. Salmonella suspensions were prepared in saline from the grown colonies corresponding to the density of 0.5 McFarland standard. Salmonella suspensions were
swabbed onto Mueller Hinton agar with sterile swabs (HiMedia, India), and then commercial antibiotic discs (Liofilchem, Italy) were placed on the plates. The S. Infantis isolates were examined for resistance to: streptomycin, at the level of 10 μg, cefotaxime 30 μg, cephaclor 30 mg, cephalxin 30 mg, ceftazidime 30 mg, ceftriaxone 30 mg, kanamycin 30 mg, pipemidic acid 20 mg, amoxicillin 30μg and nalidixic acid 30 μg. After 24 h of incubation at 37°C, inhibition zones were measured. Isolates that produced intermediate inhibition zones were considered resistant.

3. Results and discussion
In the period from 1 January to 1 September 2016, 1321 samples of poultry meat and products were tested for the presence of Salmonella. A total of 108 (8.18%) of the samples contained Salmonella. Biochemical characterization confirmed their belonging to the genus Salmonella. Of the 108 Salmonella isolates recovered, 6 (5.55%) were S. Enteritidis, and 29 (26.85%) were S. Infantis, according to the antisera used. Table 1 shows the number of isolates of S. Infantis, S. Enteritidis and total Salmonella according to the number and type of samples.

Table 1. The incidence of S. Infantis, S. Enteritidis and Salmonella spp. according to poultry meat and product type.

| Sample                          | Sample size | S. Infantis | S. Enteritidis | Salmonella spp. |
|---------------------------------|-------------|-------------|---------------|-----------------|
|                                 | N   | +  | %  | +  | %  | +  | %  | +  | %  |
| Grilled chicken                 | 39  | 1  | 2.56 | 1  | 100.00 | 0  | 0.00 | 0  | 0.00 |
| Chicken fillet                  | 244 | 24 | 9.83 | 5  | 20.83 | 0  | 0.00 | 19 | 79.16 |
| Chicken wings                   | 54  | 9  | 16.66 | 5  | 55.55 | 1  | 11.11 | 3  | 33.33 |
| Chicken meat                    | 24  | 5  | 20.83 | 1  | 20.00 | 0  | 0.00 | 4  | 16.66 |
| Fresh neck skins                | 130 | 16 | 12.30 | 3  | 14.28 | 0  | 0.00 | 13 | 12.30 |
| Frozen neck skins               | 234 | 21 | 8.97  | 3  | 14.28 | 5  | 23.80 | 13 | 12.30 |
| Liver                           | 40  | 2  | 5.00  | 1  | 50.00 | 0  | 0.00 | 1  | 25.00 |
| Mechanically deboned meat       | 265 | 15 | 5.66  | 4  | 26.66 | 0  | 0.00 | 11 | 41.81 |
| Drumsticks together with thighs | 56  | 9  | 16.07 | 6  | 66.66 | 0  | 0.00 | 3  | 33.33 |
| Marinated meat                  | 14  | 0  | 0.00  | 0  | 0.00 | 0  | 0.00 | 0  | 0.00 |
| Chicken breasts with skin       | 6   | 4  | 66.66 | 0  | 0.00 | 0  | 0.00 | 4  | 66.66 |
| Eggs                            | 215 | 2  | 0.93  | 0  | 0.00 | 0  | 0.00 | 2  | 100.00 |
| **Total**                       | **1321** | **108** | **8.18** | **29** | **26.85** | **6** | **5.55** | **73** | **67.59** |

The most frequently Salmonella-positive types of poultry were chicken breasts with skin (66.66%), chicken meat (20.83%), chicken wings (16.66%), drumsticks together with thighs (16.07%) and fresh neck skin (12.30%).

S. Infantis, compared to the total number of isolated Salmonella, was predominant in drumsticks together with thighs (66.66%) and chicken wings (55.55%).

Table 2 shows the results of testing the resistance the S. Infantis isolates to antimicrobials by the disc diffusion method.
Table 2. Antimicrobial resistance of *S. Infantis* isolates from poultry meat and products.

| Antimicrobial      | Number of isolates tested | Number of resistant isolates | % | Number of sensitive isolates | % |
|--------------------|----------------------------|-------------------------------|---|-----------------------------|---|
| Streptomycin       | 26                        | 9                             | 34.61 | 17                        | 65.38 |
| Cefotaxime         | 29                        | 16                            | 55.17 | 13                        | 44.82 |
| Cefaclor           | 29                        | 12                            | 41.37 | 17                        | 58.62 |
| Cephalexin         | 29                        | 9                             | 31.03 | 20                        | 68.96 |
| Ceftazidime        | 29                        | 20                            | 68.96 | 9                         | 31.03 |
| Ceftriaxone        | 29                        | 0                             | 0.00  | 29                        | 100.00 |
| Kanamycin          | 29                        | 0                             | 0.00  | 29                        | 100.00 |
| Pipemidic acid     | 29                        | 15                            | 51.72 | 14                        | 48.27 |
| Amoxicillin        | 26                        | 26                            | 100.00 | 0                          | 0.00 |
| Nalidixic acid     | 29                        | 29                            | 100.00 | 0                          | 0.00 |

The *S. Infantis* isolates showed resistance to: amoxicillin and nalidixic acid (100%), ceftazidime (68.96%), cefotaxime (55.17%), pipemidic acid (51.72%), chloramphenicol (41.37%), streptomycin (33.33%) and cephalexin (31.03%), but not to ceftriaxone or kanamycin. These results coincide with the results of other researchers who have studied this issue [26].

For 20 (68.97%) of the *S. Infantis* isolates, multiple resistance was found to four or more antimicrobial drugs. The results are shown in table 3.

Table 3. Multidrug-resistant *S. Infantis* (number of isolates) according to poultry product sources.

| Antimicrobials      | Grilled chicken (1) | Chicken wings (2) | Chicken fillet (5) | Fresh skins (3) | Drumsticks together with thighs (6) | Frozen neck skin (3) | Mechanically deboned meat (4) | Liver (1) | Chicken meat (1) |
|---------------------|---------------------|-------------------|-------------------|-----------------|-------------------------------------|----------------------|--------------------------------|---------|-------------------|
| Streptomycin        | NT a                | R b               | S c               | R               | R                                   | S                    | S                              | NT      | NT b              |
| Amoxicillin         | NT b                | R b               | S c               | S               | S                                   | NT b                 | R                              | NT      | R c               |
| Cefotaxime          | R b                 | S                 | S                 | R               | R                                   | S                    | S                              | S       | R b              |
| Kanamycin           | S                   | S                 | S                 | S               | S                                   | S                    | S                              | S       | S                 |
| Cefahlor            | R                   | S                 | S                 | R               | S                                   | S                    | R                              | S       | S                 |
| Cephalexin          | S                   | R                 | S                 | S               | S                                   | R                    | S                              | S       | S                 |
| Ceftazidime         | R                   | R                 | R                 | R               | R                                   | S                    | R                              | R       | R                 |
| Pipemidic acid      | R                   | R                 | R                 | R               | R                                   | S                    | R                              | R       | R                 |
| Nalidixic acid      | R                   | R                 | R                 | R               | R                                   | S                    | R                              | R       | R                 |
| Ceftriaxone         | S                   | S                 | S                 | S               | S                                   | S                    | S                              | S       | S                 |
| **Total**           | 4                   | 7                 | 3                 | 6               | 5                                   | 0                    | 6                              | 2       | 4                 |

a NT – not tested
b R – resistant
c S – sensitive

Research into resistance to antimicrobial drugs among foodborne microorganisms that cause disease is very topical. The level of antimicrobial resistance in some *Salmonella* is 100%, e.g. resistance to tetracycline, chloramphenicol, streptomycin, and sulphonamides in *S. Typhimurium* DT104 isolates. The occurrence of so-called related resistance, such as that in some *S. Typhimurium* DT104, is of particular importance since the use of one antimicrobial drug selectively acts on all resistance genes present, and thus, the isolates have a selective advantage in the case of administration of ampicillin, chloramphenicol, streptomycin, sulphonamides or tetracyclines [25]. The selection of resistant isolates among bacterial pathogens whose are primary reservoirs are animals is achieved by
selective pressure based on unjustified and excessive use of antimicrobials (or antimicrobial growth promoters) in the treatment or prevention of animal diseases.

Many different *Salmonella* isolates exhibit multiple resistances to streptomycin, kanamycin, sulphonamides, tetracyclines and some β-lactams (penicillins and cephalosporins). It has been shown that the plasmids that carry resistance to several antimicrobials are common among *Salmonella* and that these genes are often grouped in integrons. Integrons in *Salmonella* can carry genes for resistance to chloramphenicol, sulphonamides, tetracyclines and streptomycin. The usual resistance genes, class one integrons, which are common in many bacterial species, were also found in *Salmonella* [27]. Altogether, 11% of human *Salmonella* isolates in the US carry antimicrobial resistance genes, and some isolates have acquired resistance to gentamicin and the third generation of cephalosporins [8].

Since there are no results of previous surveillance of poultry meat or products in the markets in the Republic of Srpska, we clearly cannot comment on changes in the presence of *Salmonella* in poultry meat and products, but the finding of 8.18% *Salmonella*-positive samples is still a warning. Among the poultry isolates, 26.83% were *S*. *Infantis*, which is worrying, since it confirms the significant exposure of the human population in the Republic of Srpska to this pathogen. The occurrence of antimicrobial resistant, and in particular multiple-resistant, isolates of *S*. *Infantis* is also alarming.

The presence of *S*. *Infantis* in poultry meat could be a result of poor manufacturing and hygiene practices in slaughterhouses and manufacturing plants, but it could also be a consequence of the implementation of vaccination against *S*. *Enteritidis* and *S*. *Typhimurium*, which some researchers have already confirmed [7,28]. Some experts define the generally increasing incidence of *S*. *Infantis* as the emergence of a new pathogenic microorganism causing infectious disease in humans, and which has occurred in a new host; alternatively, the incidence of the pathogen could be significantly increasing as a result of long-term changes in the epidemiology of *S*. *Infantis* [29].

4. Conclusions

Based on the results, the following conclusions can be made:

1. In the first nine months of 2016, the incidence *Salmonella* in samples of poultry meat and meat products was 8.18%, of which 5.55% were *S*. *Enteritidis* and 26.85% were *S*. *Infantis*.
2. *S*. *Infantis* isolates showed 100% resistance to amoxicillin and nalidixic acid. Multiple resistances to four or more antimicrobials were found in 68.97% of the *S*. *Infantis* poultry isolates.
3. Having in mind that the sources, routes and methods of transmission of *Salmonella* in the food chain are inextricably linked to the risks of infection and illness in humans, epidemiological studies should include monitoring and typing of these pathogens in humans, and in animals intended for human consumption, in all phases of food production process and distribution.
4. Poultry meat contaminated with *S*. *Infantis* poses a risk to human health, particularly children, the elderly and people with immunodeficiency.
5. Raising consumer awareness regarding methods of preparing food, especially poultry meat and meat products (heat treatment destroys *S*. *Infantis*) is required, but also the importance of good hygiene practice for the food business operators, in order to avoid cross-contamination, must constantly be stressed.

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