Resistance of *Salmonella* spp. isolated from poultry meat to antimicrobial drugs

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Abstract. Uncontrolled use of antimicrobials in the prophylaxis and treatment of human and animal diseases leads to the appearance of residues in the environment and food. The use of antimicrobials as growth promoters in breeding affirms the importance of their residual finding in foods of animal origin. Bacteria of the genus *Salmonella* are one of the most common causes of food poisoning. All species of this genus are pathogenic to humans and cause various diseases known as salmonellosis. Humans can become infected through the faecal-oral route by consuming contaminated food and water or by direct contact with an animal. *Salmonella* that are resistant to antimicrobial drugs can transfer resistance genes to other microorganisms. In this work, the resistance of 10 *Salmonella* isolates from poultry meat to 8 different antimicrobial substances was examined by the disk diffusion method. All *Salmonella* isolates were sensitive to trimethoprim-sulfamethoxazole and chloramphenicol. All isolates were resistant to amoxicillin and significant percentages were resistant to other antimicrobial drugs. Also, multi-drug resistance of *Salmonella* isolates was found. The best prevention of salmonellosis in humans is constant and comprehensive control of this hazard in food products during production, processing, storage, and sale.

1. Introduction

Salmonellosis is a major health and economic problem. The disease occurs in all animals and intensifies with more intensive farming. In young animals, it usually occurs in the form of septicaemia, while in older animals, it is characterized by acute and chronic enteritis. Despite progress made in *Salmonella* control, this pathogen is still present in food and is the most common cause of foodborne diseases [1],[2].

According to a 2018 report by the European Food Safety Authority (EFSA) and the European Center for Disease Prevention and Control (ECDC), *Salmonella* was the cause of every third food related epidemic reported in European Union countries in year 2018. Salmonellosis is the second most commonly reported gastrointestinal infection in human in the EU (91857 reported cases). In 2018, EU countries reported 5146 food-related epidemics involving 48365 cases. Slovakia, Spain and Poland reported 67% of their total 1581 food-related epidemics were caused by *Salmonella*, and the source of the epidemics was mainly associated with eggs [3]. When it comes to food production all over the world and in Serbia, food safety comes first. Safe food is free of residues, contaminants and pathogenic microorganisms. In food production, primary production itself has a great impact on safety.
For *Salmonella* infections, morbidity in humans is almost 100%, and mortality occurs in environments where general hygiene conditions are poor and where there is no health care [4]. It is estimated that 93.8 million people worldwide are diagnosed with *Salmonella*-induced gastroenteritis each year, with 155000 deaths. *Salmonella* is the most common bacterial cause of foodborne illness in the United States, causing about 44% of confirmed bacterial foodborne infections, and in Europe *Salmonella* infection is immediately behind *Campylobacter* infection [5]. *S. Enteritidis* and *S. Typhimurium* are the serotypes that most commonly cause salmonellosis in humans. Frequent use of antimicrobial drugs results in the occurrence of resistance to various antimicrobial drugs in bacteria, including *Salmonella*. In the 2018 EFSA/EDCA report, antimicrobial resistance was confirmed as one of the main threats to public health [3].

Bacteria can be resistant to one or more different antimicrobial drugs. It is also important to note that antimicrobial drug resistance can be transmitted from resistant to non-resistant bacteria, but also to those that already carry resistance to the same or different antimicrobial drugs, thus creating multiple resistance. As a consequence of *Salmonella* antimicrobial resistance, there is an increase in unsuccessful antimicrobial treatments, an increase in the number of hospitalized cases and increase in mortality [6]. Excessive use of antibiotics in the medical and veterinary industries is one of the main causes for the emergence of multi-resistant foodborne pathogens that are often difficult to treat. In recent years, epidemics caused by multidrug-resistant *Salmonella* have been documented [7].

In cases of invasive salmonellosis, antimicrobial drugs (amoxicillin, chloramphenicol, ceftriaxone, ciprofloxacin, trimethoprim-sulfamethoxazole) are used in therapy [8]. Salmonellosis is known to be a major public health issue, and the aim of this study was to examine the occurrence and prevalence of *Salmonella* in food and their resistance to antimicrobial drugs.

### 2. Materials and methods

Ten isolates of *Salmonella* from poultry meat were used in this study. The submitted samples were sown on selective-differential media [9]. Suspect colonies grown on solid substrates were sown first on nutrient agar and then on triple sugar iron agar. Biochemical tests (triple sugar, urea agar, lysine decarboxylase broth, indole formation and VP reactions) were used for biochemical identification, thus confirming the physiological characteristics of the genus *Salmonella*. The disk diffusion method according to [10] was used to test the susceptibility of isolates to antimicrobial drugs. The test isolates were first sown on trypticase soy agar and incubated for 24 h at 37°C. *Salmonella* suspensions in saline were prepared from the grown colonies, which corresponded to a density of 0.5 McFarland standard. *Salmonella* suspensions were applied with sterile swabs to Mueller Hinton agar (HiMedia, India), and then commercial antibiotic discs (Liofilchem, Italy) with specific amounts of active substance (amoxicillin 30µg, cefuroxime 30 µg, trimethoprim sulfamethoxazole 25 µg, chloramphenicol 30 µg, tetracycline 30 µg, ciprofloxacin 5 µg, gentamicin 10 µg, nalidixic acid 30 µg) were introduced. After 24 h incubation at 37°C, the results were read by determining the diameter of the inhibition zone and the mean value was calculated. Isolates that were read as intermediate were considered resistant.

### 3. Results and discussion

In the Laboratory for Microbiology and Food, Animal Feed and Water of the Dr Vaso Butozan Public Veterinary Institute of RS, a total of 1240 food samples were examined for the presence of *Salmonella*. Of the examined food samples, 54 (4.34%) did not meet the prescribed microbiological criteria for *Salmonella* [11]. The table (Table 1) shows the results of *Salmonella* testing for the examined food samples.

| Table 1. Results of testing foods for the presence of *Salmonella* |
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Of the 54 isolates of *Salmonella*, up to 10 isolates were tested for resistance/susceptibility to eight antimicrobial drugs, and the results obtained are shown in Table 2.

**Table 2. Antimicrobial resistance of isolated *Salmonella* to antimicrobial drugs**

| Antimicrobial drug               | Number of *Salmonella* examined | Susceptible | %   | Resistant | %   |
|----------------------------------|---------------------------------|-------------|-----|-----------|-----|
| Amoxicillin                      | 10                              | 0           | 0.00| 10        | 100 |
| Cefuroxime                       | 7                               | 5           | 71.42| 2         | 28.57|
| Trimethoprim-sulfamethoxazole    | 10                              | 10          | 100.00| 0         | 0.00 |
| Chloramphenicol                  | 9                               | 9           | 100.00| 0         | 0.00 |
| Tetracycline                     | 10                              | 6           | 60.00| 4         | 40.00|
| Ciprofloxacin                    | 10                              | 5           | 50.00| 5         | 50.00|
| Gentamicin                       | 10                              | 9           | 90.00| 1         | 10.00|
| Nalidixic acid                   | 10                              | 5           | 50.00| 5         | 50.00|

All 10 (100%) *Salmonella* isolates included in our study were resistant to amoxicillin, 50% of isolates were resistant to ciprofloxacin and nalidixic acid, 40% to tetracycline, 28.57% to cefuroxime and 10% to gentamicin. Results of this study are in agreement with the results of other researchers when it comes to *Salmonella* resistance to nalidixic acid, tetracycline and ciprofloxacin to which different *Salmonella* serotypes show resistance worldwide [12, [13], [14], [15]. According to [16], in EU Member States, *Salmonella* isolated from broiler production was resistant to nalidixic acid, ciprofloxacin, tetracycline and sulphonamides. This current study did not show resistance to trimethoprim-sulfamethoxazole (10 isolates tested) or chloramphenicol (9 isolates tested). Some studies of antimicrobial resistance in non-typhoid *Salmonella* have shown a high incidence of resistance in *S. Typhimurium* (77%) to tetracycline, medium resistance (20-30%) to chloramphenicol, sulfamethoxazole-trimethoprim, ampicillin, and nalidixic acid and low resistance (less of 5%) to ciprofloxacin and third generation of cephalosporins [17]. The resistance of 84 *Salmonella* isolates to eight antimicrobial drugs was examined using the disk diffusion method, and it was found that all isolates were resistant to one or more tested antimicrobial drugs. All isolates were sensitive to chloramphenicol. Resistance ranged from 11.9% to sulfamethoxazole-trimethoprim (SXT) to 100.0% resistance to erythromycin [18]. Resistance to amoxicillin, doxycycline, kanamycin, gentamicin and tetracycline was found in 81.81% of *Salmonella* isolates, while 54.54% of isolated *S. enterica* serovars were highly sensitive to ciprofloxacin [19]. In the period between 2007-2011 [20], antibiotic resistance was assessed for 12,582 strains of *Salmonella*, and the results of the study showed increased resistance to ampicillin (12.4 to 18.9%), tetracycline (≈ 15.2 to ≈ 18.9%) and gentamicin (7.0 to ≈ 9.6%). In a study [20] of antimicrobial susceptibility profiles of 1234 strains, resistance to eight antimicrobial drugs was found in 54.5% of isolates. Multidrug resistance (three or more groups) was observed in 16.4% of strains, with 190 different patterns [20]. Resistance to at least two groups of antibiotics was found in most isolates of *Salmonella enterica*. 

![Image]

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**Total number of examined samples**

| Satisfactory (no *Salmonella*) | Non satisfactory (contain *Salmonella*) |
|---------------------------------|-----------------------------------------|
| n | % | n | % |
| 1240 | 1186 | 95.64 | 54 | 4.35 |
Four multidrug-resistant isolates were isolated from animal feed and poultry products [21]. Figure 1 shows results of antimicrobial resistance testing of the *Salmonella* in the current study.

![Figure 1. Results of antimicrobial resistance of *Salmonella*](image)

Of the 10 isolates tested, 7 (70%) showed multiple resistance, i.e. resistance to 2 or more examined antimicrobial drugs. Three of the 10 *Salmonella* isolates were resistant to just one antimicrobial drug. Figure 2 shows the number of multidrug-resistant isolates to five, four, three and two antimicrobial drugs.

![Figure 2. Number of multiresistant *Salmonella* isolates among 10 isolates examined](image)

The results of this study are consistent with other studies on multiple resistance [3], [4], [12]. A particular problem is the occurrence of resistance to key antimicrobial drugs, because it was proven that such plasmids can transfer resistance and create resistance to multiple types of antimicrobial drugs [13]. Available data on antimicrobial drug resistance differ, and the reason could be the distribution and prevalence of certain serotypes in different countries and in different animals. Certain *Salmonella* strains within serotypes could have a specific and characteristic pattern of antimicrobial resistance to a particular antimicrobial drug, which means the drug is ineffective. These results indicate the importance of food chain surveillance and detection of modified patterns in foodborne zoonotic bacteria important to public health.

Inadequate and excessive use of antimicrobial drugs, whether by inappropriate doses, inadequate length of treatment or unnecessary use, results in the occurrence of bacterial resistance to antimicrobial drugs. The problem of resistance itself is mostly associated with the occurrence and spread of the mechanism of resistance in humans, because the appearance of resistance in zoonotic bacteria has begun to affect human therapy. Uncontrolled use of antimicrobial drugs and non-compliance with the
withdrawal time for elimination of drugs in animals used in human nutrition has led to the transmission of resistant bacteria through the food chain to humans and the occurrence of diseases that are difficult to treat. Most drugs used in animal therapy (ampicillin, tetracycline, gentamicin) belong to the same group of antimicrobial drugs used in human medicine, so resistance to one drug from the group could induce resistance to the whole group. The occurrence of resistant strains of *Salmonella* in animals is very significant since poultry, pigs and cattle are known to be the primary reservoirs of *Salmonella*. According to the European Centre for Disease Prevention and Control (ECDC), European Food Safety Authority (EFSA) and European Medicines Agency, resistance to nalidixic acid, ciprofloxacin, quinolones and cephalosporins have been identified as the most significant public health danger when it comes to *Salmonella* [4]. The results of this study are worrying because they showed that, in our area, there are multi-resistant strains of *Salmonella* that were imported through food or infected poultry.

4. Conclusion
The results of this study clearly show that all (100%) tested isolates of *Salmonella* were resistant to amoxicillin, as one of the most commonly used antimicrobial drugs, and that 70% of isolates were resistant to two or more antimicrobial drugs. Uncontrolled, improper and unprofessional use of antimicrobial drugs results in the occurrence of resistant microorganisms to one or more antimicrobial drugs. In addition to application of preventive and hygienic-sanitary measures at all levels from the field to the table, routine testing of isolated *Salmonella* for antimicrobial drug resistance should be introduced. It is only on the basis of continuous monitoring of bacterial resistance/sensitivity to antimicrobial drugs that adequate therapy for bacterial diseases can be maintained. A national strategy to control consumption and rational use of antimicrobials in animals is needed. The best prevention of salmonellosis in humans is constant and comprehensive microbiological control of food products during production, processing, storage and sale.

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