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To cite this article: J Kureljuši *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **854** 012049

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## ***Salmonella* - foodborne pathogen and antimicrobial resistance**

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**Abstract.** Foodborne diseases encompass a wide spectrum of illnesses and are a growing public health problem worldwide. They are caused by consumption of food or water contaminated by pathogenic (disease-causing) microorganisms such as bacteria, viruses and parasites. The contamination of food can occur at any stage in the process from food production to consumption (“farm to fork”) and can result from environmental contamination (water, soil or air). They enter the body through the gastrointestinal tract where the first symptoms often occur like nausea, vomiting, stomach cramps, and diarrhoea. However, symptoms differ among the different types of foodborne diseases and the patient’s immune status. Symptoms can sometimes be severe and some foodborne illnesses can even be fatal. Commonly recognized foodborne infections are: campylobacteriosis, *Escherichia coli* O157:H7 infection and haemolytic uremic syndrome (HUS), salmonellosis, cryptosporidiosis, listeriosis, giardiasis, norovirus infection, scombroid fish poisoning, shigellosis, toxoplasmosis, *Vibrio* infection and yersiniosis. One of the top three germs that cause illnesses from food eaten in EU is *Salmonella*.

### **1. Introduction**

Nearly one in three foodborne outbreaks in the EU in 2018 was caused by *Salmonella*. Salmonellosis was the second most commonly reported gastrointestinal infection in humans in the EU (91,857 cases reported), after campylobacteriosis (246,571). *Salmonella* infections are an important public health problem worldwide and antibiotic resistance is one of the biggest public health challenges of our time [1].

#### *1.1. Salmonella species and typing*

Salmonellosis is an infectious disease of domestic and wild animals caused by gram-negative bacteria of the genus *Salmonella*. To date, over 2,400 different serotypes of this genus have been isolated from different vertebrate species, of which more than 200 have also been isolated in humans. A small number of serotypes are highly adapted to certain host species, causing severe septicemic forms of the disease. *Salmonellosis* is important from the aspect of food safety of animal origin due to its zoonotic character. In addition, there is also an impact of this infection on pig health and production economics. Within the EU countries, there is legislation that obliges member states to monitor *salmonellosis* in breeding stock



as well as fattening pig farms. *Salmonella* spp. are a group of bacteria which reside in the intestinal tract of humans and warm blooded animals and are capable of causing a disease. *Salmonella* are members of the *Enterobacteriaceae* family, and the genus *Salmonella* contains two species:

1. *Salmonella enterica*
2. *Salmonella bongori*

*Salmonella enterica* is one of the three most important agent of foodborne illness. This species is sub-classified into six subspecies: *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae* and *S. enterica* subsp. *indica* of which *S. enterica* subsp. *enterica* is the most important for human health. The genus *Salmonella* can be subdivided into more than 2,400 serotypes. Different salmonella serotypes have different hosts. Some serotypes are pathogenic exclusively to humans such as *S. Typhi* and *S. Paratyphi*, while other serotypes (*S. Typhimurium* and *S. Newport*) infect different species. Non-typhoid salmonellosis in humans is usually caused by several dozen serotypes. Meanwhile, more and more serotypes are being isolated, especially from immunocompromised patients. A key factor that defines a particular salmonella serotype as a successful pathogen is its ability to enter a non-phagocytic host cell through certain molecular mechanisms, and to adapt to a wide range of hosts.

### 1.2. Transmission of *Salmonella*

*Salmonella* can be transmitted to humans in different ways. First, through a direct contact with infected persons or animals occurs, second, by eating food infected with the bacteria i.e. ingestion of contaminated food and third by eating raw or undercooked meat which is the most common way *Salmonella* is spread. Normally, *Salmonella* bacteria cause infections via fecal-oral transmission. *Salmonella* infection can be prevented by good hand hygiene and food handling procedures.

A significant moment of the bacterium of the genus *Salmonella* is its ability to survive outside the host. Research shows that in stored samples of feed, grass or dust, spiked with  $10^6$  to  $10^8$  *S. Typhimurium* per gram, survival times of one year are not uncommon and the survival up to four years is also observed [7], while in liquid manure, *S. Typhimurium* was re-isolated after 140 days at +10°C [8]. In field experiments, the survival times have not been quite that long, but are still at least weeks to months, depending on temperature and humidity. For example, *Salmonella* can survive in salt solution for several months, and over 80 days in dust. *Salmonella* can be present in rivers, wastewater, sewage and other waters and fertilizers [9].

### 1.3. Antimicrobial resistance

Antimicrobial drugs, including antibiotics, antifungals, antiparasitics and antivirals are compounds used to prevent and treat infections in humans, animals and plants. Microorganisms that develop antimicrobial resistance are sometimes referred to as superbugs [10]. Antimicrobial resistance occurs when bacteria, viruses, fungi and parasites change over time and no longer respond to antimicrobials, making infections harder to treat and increasing the risk of disease spread, severe illness and death. The largest number of isolated strains of *Salmonella* Enteritidis originates from humans and animals sensitive to most antibiotics, so the occurrence of resistance is very small. The mentioned causative agent is most often isolated from infected people, and it originates from poultry. Because poultry do not cause significant clinical symptoms, the infection is usually not treated with antibiotics, so there is no selective pressure and no development of resistance.

## 2. Methods

One hundred samples swabs pig carcass were collected in a pig slaughterhouse. *Salmonella* was isolated from the swabs and identified according to the international standard method [11]. First, pre-enrichment was in buffered peptone water (BPW), then selective enrichment was performed in Rappaport Vassiliadis Single Component Enrichment Broth (RVS) and Mueller Kauffman Tetrathionate Broth

(MKTTn). The isolation media used were Brilliant Green Agar (BG) and Xylose Lysine Decarboxylase Agar (XLD) [11].

Antibiotic resistance was determined using the disc diffusion method for each *Salmonella* isolate on Mueller-Hinton agar (OXOID, England). We pour the agar into a sterile glass or plastic petri dish on a flat surface to a uniform depth of 4 mm and left at 37°C overnight to check for sterility. From a pure bacterial culture (not more than 48 hours old except for slow growing organisms), we took individual *Salmonella* isolates with a wire loop and transferred colonies to 5 ml of Trypticase soy broth or 0.9% saline and incubated at 35°C for 4 h, then each isolate was compared with 0.5 McFarland turbidity standards. The cultures were inoculated onto the agar by streaking with the swab containing the inoculum. *Salmonella* isolates were tested for susceptibility to the following nine antibiotics: ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (30 µg), gentamycin (10 µg), nalidixic acid (30 µg), nalidixic acid(30 µg), trimethoprim/sulfamethoxazole, ceftazidime (30 µg), meropenem (10 µg) and tetracycline (30 µg), using the disk diffusion method according to guidelines set by the Clinical Laboratory Standards Institute (CLSI) [12]. Antibiotic impregnated discs were dispensed on the surface of cultures of Muller-Hinton agar and incubated at 35°C for 20 h.

### 3. Results

All *Salmonella* isolates tested were sensitive to five antimicrobial drugs (ceftazidime, ciprofloxacin, sulfamethoxazole-trimethoprim, meropenem and gentamicin) and were resistant to tetracycline. For all other tested antimicrobial drugs, high sensitivity of the tested *Salmonella* isolates was established. Resistance ranged from 7.41% for ampicillin and chloramphenicol, to 12.96% for nalidixic acid. (Table 1)

**Table 1.** Results of susceptibility testing of *Salmonella* to antimicrobial drugs obtained by the disk diffusion method

Antimicrobial drugs	Sensitive isolates %	Resistant isolates %
Ampicillin (10 µg)	92.60	7.41
Chloramphenicol (30 µg)	92.60	7.41
Ciprofloxacin (30 µg)	100	0
Gentamycin (10 µg)	100	0
Nalidixic acid (30 µg)	87.04	12.96
Trimethoprim/Sulfamethoxazole	100	0
Ceftazidime (30 µg)	100	0
Meropenem (10 µg)	100	0
Tetracycline (30 µg)	0	100

### 4. Conclusion

To date, various antibiotics have been useful in both human and veterinary medicine. Examples of such antibiotics are gentamicin, ampicillin and amoxicillin. Some antimicrobial drugs, such as enrofloxacin and flumequine, have only been developed for veterinary use. The use of antibiotics as growth promoters in intensive pig and poultry farming has influenced the development of resistance in some bacteria. For these reasons, since January 2006, EC Regulation No. 1831/2003 has banned the use of all antimicrobial drugs as feed additives.

Pathogens that are antibiotic resistant and that are in food could spread through the food chain to humans, in whom they can cause infections. The determined resistance rates of *Salmonella* to the antibiotics studies are in line with the findings of other authors [13]. Of the antimicrobial drugs tested, tetracyclines and ampicillin are particularly commonly used in veterinary clinical practice, while ciprofloxacin is very commonly used in human medicine, and another fluoroquinolone drug not studied, enrofloxacin, has

been developed specifically for veterinary use. This is especially important because it is known that resistance to one antibiotic from a group can sometimes impart resistance to an entire group of antibiotics.

In order to reduce the number of resistant pathogenic microorganisms, farmers, veterinarians, meat processors, doctors and government agencies, all the way to consumers who should be educated, should work together to prevent improper and excessive use of antimicrobial drugs. In primary production, the principles of precondition programs should be applied, which imply the application of GHP and GMP, (good hygiene and good manufacturing practices). This will prevent or reduce the risk of disease in animals, and thus the need for antimicrobial therapy. If therapy is still necessary, it must be applied only in the correct way and under the supervision of a veterinarian. In slaughterhouses and meat processing facilities, the possibility of contamination of meat with the contents of the digestive tract or resistant zoonotic pathogens can be significantly reduced by applying precondition programs and the HACCP concept.

### Acknowledgment

This paper was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (No 451-03-9/2021-14/ 200030)

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