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Prevalence and antibiotic resistance of *Salmonella* spp. in meat products, meat preparations and minced meat

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Abstract. This study aimed to determine *Salmonella* spp. prevalence in meat products, meat preparations and minced meat. Over a period of three years, a total of 300 samples were taken (100 RTE meat products, 100 meat preparations and 100 minced meat) and examined for the presence of *Salmonella* spp. Sampling was carried out at the warehouses of the food manufacturers. *Salmonella* spp. were not detected in RTE meat products, while 7% of semi-finished meat products (fresh sausages, grill meat formed and unformed) contained *Salmonella*, as did 18% of minced meats (minced pork II category, minced beef II category, mixed minced meat). The 25 *Salmonella* isolates obtained were examined for antibiotic resistance by the disk diffusion test, according to the NCCLS and CLSI guidelines. Isolates showed resistance to ampicillin and nalidixic acid (80%), tetracycline (72%), cefotaxime/clavulanic acid (48%), but not to gentamicin (8%) or trimethoprim/sulfamethoxazole (0%).

1. Introduction

Salmonella is an important cause of foodborne disease in humans throughout the world and is a significant cause of morbidity, mortality and economic losses. *Salmonella* problems can occur in all segments of the food chain [1]. According to the European Food Safety Authority (EFSA) report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015, a total of 94,625 confirmed human salmonellosis cases were reported by 28 European Union (EU) member states, resulting in an EU notification rate of 21.2 cases per 100,000 population [2]. This was a 1.9% increase in the EU notification rate compared with 2014. There was a statistically significant decreasing trend of salmonellosis in the 8-year period between 2008 and 2015 [2]. Meat during production, preparation and in retail comes into direct contact with microorganisms (different types and strains) which affect its shelf life and safety [3]. Foodborne pathogens are major causes of human illnesses in developing countries, causing high medical and hospitalization expenses [4]. A large number of foods, particularly

meat and broiler meat products, are the most important sources of human *Salmonella* contamination [5].

In recent years, an additional health problem is the emergence of multi-resistant strains of pathogenic bacteria, including *Salmonella* and especially in foods [6]. Extensive and intensive use of antibiotics for preventive and therapeutic purposes in veterinary medicine, as well as growth promoters in animal feedstuffs, contributed to the emergence of resistant bacteria, including zoonotic pathogens, in animals, and that can be transmitted in the food chain to humans [7]. *Salmonella* spp. possess highly efficient mobile genome parts (plasmids, genomic islands, transposons) with ability to exchange and keep different genes responsible for antimicrobial resistance [8,9]. Almost every day, new mutations in genes responsible for antimicrobial resistance are happening, induced by antibiotics used to combat *Salmonella* spp. [10]. EFSA and the European Centre for Disease Prevention and Control recommended antibiotics of interest for determining *Salmonella* spp. resistance: ampicillin, cefotaxime, chloramphenicol, gentamicin, nalidixic acid, sulphonamides and tetracycline [11].

The aim of this study was to determine the prevalence and antibiotic resistance of *Salmonella* spp. in retail-ready RTE meat products, and in meat preparations and minced meat (these latter two categories were not RTE).

2. Materials and Methods

In order to determine the presence of Salmonella spp. in RTE meat products, and in non-RTE meat preparations and minced meat, during a three-year period, 300 samples were taken in producers' warehouses. The meats were originally-packed products, taken from the batches intended for transport to retail. The meats were transported in a cold chain and delivered to the laboratory within a day. Determination of the Salmonella prevalence in the meats was performed in accordance with ISO standard [25].

Samples were suspended with 250 ml of buffered peptone water, homogenized for 30 seconds, and after that, were incubated at 37°C for 24 h. After pre-enrichment and incubation, 0.1 ml of the slurry was transferred into 10 ml Rappaport-Vassiliadis medium (bioMérieux, France), which was then incubated at 42°C, for a period of 24-48 h. After incubation, enrichment cultures were seeded onto differential Rambach and XLD agars, which were incubated overnight at 37°C. Colonies with typical growth and clearly differentiated were transferred into cryogenic vials for further testing.

Antibiotic resistance testing was performed by disk diffusion according to NCCLS recommendations using commercial discs and Mueller-Hinton agar (Bio-Rad, USA). The investigated isolates were first subcultured on trypticase soy agar (TSA) and incubated for 24 h at 37°C. Typical *Salmonella* colonies were suspended in physiological saline to 0.5 McFarland standard density. *Salmonella* suspensions were transferred by sterile swabs onto Mueller-Hinton agar, followed by antibiotic disk application (automatic applicator, Oxoid, UK). The following antibiotic disks (Oxoid Ltd., Basingstoke, UK) were used: nalidixic acid (quinolones) 30 μg, ampicillin (penicillin) 10 μg, cefotaxime/clavulanic acid (cephalosporins) 30 μg, gentamicin (aminoglycoside antibiotics) 10 μg, tetracycline (tetracycline) 30 μg, trimethoprim/sulfamethoxazole (inhibitors of folic acid) 30 μg. After 18 h of incubation, inhibition of *Salmonella* growth was measured, and the results were interpreted according to NCCLS (National Committee for Clinical Laboratory Standards) or CLSI (Clinical Laboratory Standard Institute) 2006 recommendations as sensitive, intermediate sensitive and resistant (Table 1).

Table 1. Limits of *Salmonella* growth inhibition for determining antimicrobial resistance in accordance with CLSI recommendations

Antibiotics	Inhibition growth zone (mm)		
	Resistant	Intermediate sensitivity	Sensitive
Ampicillin 10 μg	≤ 13	14 – 16	≥ 17

Nalidixic acid 30 µg	≤ 13	14 – 18	≥ 19
Cefotaxime 30 µg	≤ 22	23 – 25	≥ 26
Gentamicin 10 µg	≤ 12	13 – 14	≥ 15
Tetracycline 30 µg	≤ 11	12 – 14	≥ 15
Trimethoprim/Sulfamethoxazole 30 µg	≤ 10	11 – 15	≥ 16

3. Results

Results of *Salmonella* spp. presence in the meats examined are presented in Table 2.

Table 2. *Salmonella* spp. presence in RTE meat products, meat preparations and minced meat

Meat type	No. of samples	*Salmonella* spp. positive samples	
		Number	%
RTE meat products	100	0	0
Meat preparations	100	7	7
Minced meat	100	18	18
Total	*300*	*25*	*8.33*

Antibiotic resistance results of *Salmonella* spp. isolates from the meat examined are presented in Table 3.

Table 3. Antibiotic resistance of *Salmonella* isolates from meat preparations and minced meat

Antibiotic	No. of Isolates	Sensitive		Intermediate		Resistant	
		Number	%	Number	%	Number	%
Nalidixic acid	25	0	0	5	20	20	80
Ampicillin	25	2	8	3	12	20	80
Tetracycline	25	2	8	5	20	18	72
Cefotaxime	25	7	28	6	24	12	48
Gentamicin	25	18	72	5	20	2	8
Trimethoprim/Sulfamethoxazole	25	20	80	5	20	0	0

4. Discussion

RTE foods pose a direct risk to consumers, and according to an EFSA annual report[2], in 2015, 1.1% and 0.7% positive samples were found for RTE food from broilers and pig meat, respectively, whereas one positive sample and no positive samples were found for RTE food from turkey and cattle meat, respectively. In our study, there were no positive samples for Salmonella spp. in RTE meat products (boiled sausages, cooked sausages, pâtés) which were thermally processed and originally packed. The results obtained indicate that any Salmonella spp. presence in RTE meats could be more related to inappropriate conditions or use of RTE food in retail (secondary contamination, disruption of cold chain during storage and contamination after package is opened). Similar results to those in our study were found in Latvia [12]. There, a total of 3,152 samples of raw and RTE meats were collected during the official control and in-house control procedures in 2015. The prevalence of Salmonella was

0.8% (25/3152). The highest prevalence (1.5%) of Salmonella was found in minced meat and meat preparations (7/481), while the lowest (0%) was in frozen meat, meat preparations (0/349) and RTE meats (0/364) [12]. In a ten year study in the United States (US), the Food Safety and Inspection Service conducted microbiological testing programs for RTE meat and poultry products produced at approximately 1,800 federally inspected establishments [28]. The cumulative 10-year Salmonella prevalences were as follows: jerky, 0.31%; cooked, uncured poultry products, 0.10%; large-diameter cooked sausages, 0.07%; small-diameter cooked sausages, 0.20%; cooked beef, roast beef, and cooked corned beef, 0.22%; salads, spreads, and pâtés, 0.05%; and sliced ham and luncheon meat, 0.22%. The cumulative 3-year Salmonella prevalence for dry and semidry fermented sausages was 1.43%. [28]. The prevalence data have certain limitations that restrict statistical interpretations, because these RTE product-testing programs are strictly regulatory in nature and not statistically designed.

In the EU, the highest occurrence of samples not-compliant with *Salmonella* criteria was found in foods of meat origin which are intended to be cooked before consumption [2]. Among these foods, 'minced meat and meat preparations from poultry' had a notable level of noncompliance (6.8% of single samples and 5.1% of batches) [2]. A study in the US in the period 2005-2007 determined an overall *Salmonella* prevalence of 4.2% in minced beef meat [14]. Enumeration showed that 94.2% of these contained *Salmonella* levels below 2 CFU/g. Regional monthly prevalences of *Salmonella* varied from 1.8% to 6.5% but were not statistically different [14]. The results obtained in our study show a similar *Salmonella* prevalence in meat preparations (7% in semi-finished meat preparations), but in minced meat, our *Salmonella* prevalence was significantly higher (18%) compared to other studies [12,14,26]. In a similar study in Belgium, *Salmonella* prevalences in minced meat at retail level ranged from 0.3% to 4.3% [26]. In an investigation in Poland [29], significantly lower prevalences of *Salmonella* spp. in meat preparations were determined (0.4-0.7% in porcine meat preparations and fresh sausages) than in our study.

The presence of *Salmonella* spp. in meat preparations and minced meat poses a risk to human health. The in-laboratory testing for *Salmonella* using reference method EN ISO 6579:2008 [25], lasts 4-5 days, so there is not enough time to prevent exposure of consumers to contaminated meat preparations or minced meat, if these are sold soon after production. Although meat preparations and minced meat are intended to use after thermal processing, and *Salmonella* is thermally sensitive, the presence of *Salmonella* spp. is considered a food safety problem, as is stated in Serbian legislation [27].

The presence of *Salmonella* spp. in minced meat, meat preparations and meat products is related to the origin of meat used in production (epizootic situation, primary production, slaughterline, cutting, cold storage, hygiene practice of employees). *Salmonella* contamination in the food chain was examined in Brazil using a meta-analysis model, and Monte Carlo simulation estimated the *Salmonella* prevalence in beef cuts from processing plants was ~6.1% (95% probability) [13]. This was in reasonable agreement with a pool (n = 105) of survey data for *Salmonella* prevalence in Brazilian beef cuts (~4.9%; 95% probability) carried out in commercial establishments. The results not only underscored the significant increase in *Salmonella* prevalence that can occur during evisceration/splitting and boning but also reinforced that, when hygienic slaughter procedures are properly implemented, the load of *Salmonella* can be reduced at dehiding, rinsing and chilling [13]. A slightly better situation was determined in Belgium [15]. A constant and significant decrease in *Salmonella* prevalence was observed for pork carcasses, trimmings, and minced meat and for beef minced meat. Less than 3% of beef carcasses and trimming samples were positive for *Salmonella* spp. From 1997 to 1999, the prevalence of *Salmonella* spp. was assessed at different stages through the pork, poultry, and beef meat production chains, and initial prevalences were 20 to 26% [15]. Based on this introductory study, a new sampling plan was used from 2000 to 2003. This new plan was suitable for monitoring zoonoses, because it was representative of nationwide production processes, covered all periods of the year, was executed by trained samplers and the analyses were carried out by recognized laboratories using an identical analytical method [15].

In our study, *Salmonella* isolates were sensitive (at intermediate or sensitive levels) to trimethoprim/sulfamethoxazole (100%) or gentamicin (92%), were sensitive to a lesser degree to cefotaxime (52% of isolates were sensitive) but very low percentages of our isolates were sensitive to tetracycline (28%), ampicillin (20%) or nalidixic acid (20%). Similar results were gained by Wang et. al [16] when they determined their *Salmonella* isolates had low ampicillin sensitivity (21.7%). In contrast, Nogrady et. al [17] determined *Salmonella* isolates originating from primary production in Hungary were largely resistant to sulphonamides [17]. In Italy during 2005-2006, *Salmonella* isolates from primary production and retail showed multiresistance to ampicillin, chloramphenicol, streptomycin, sulphonamides, tetracycline, kanamycin and trimethoprim/sulfamethoxazole [18]. In Poland during 2008-2012, 106 *Salmonella* isolates were sensitive to nalidixic acid (47%), tetracycline (68%), ampicillin (72%), sulphonamides (74%) and cefotaxime (100%) [19]. Finally, in Thailand *Salmonella* isolates were sensitive to tetracycline (27%), nalidixic acid (46%), gentamicin (64%), ampicillin (73%) and trimethoprim/sulfamethoxazole (73%) [20].

Salmonella presence in primary production is a significant public health problem, particularly in countries without adequate control measures or in the areas where climate favours *Salmonella* survival and growth [21]. Biosecurity measures in primary production are a line of well-designed obstacles in order to prevent *Salmonella* contamination and spread [22,23]. Continual education of employees is the basis for implementing biosecurity measures, as is stated in primary production biosecurity protocols [24].

5. Conclusion

This study shows that the presence of Salmonella spp. in meat preparations and in minced meat, together with the high prevalence of antibiotic-resistant strains, is a significant public health issue in Serbia (18% of minced meat and 7% of meat preparations contained the organism). Salmonella spp. were not detected in RTE meat products.

The presence of *Salmonella* spp. in minced meat and meat preparations is a safety issue, although these types of foods are intended to be used after thermal processing (as stated on the food declarations).

"*Salmonella* free" status, under current production conditions for Serbian fresh meat, meat preparations and minced meat, is most likely unachievable. Therefore, *Salmonella* prevalence in meat production at different production stages in the food chain must be determined and monitored under the *Salmonella* National Control Program.

The testing time using the reference method for *Salmonella* determination [25] lasts longer (4-5 days) than the shelf life of minced meat or meat preparations (usually 48-72 h), so there is not enough time to take any corrective measures, in order to prevent exposure of consumers to potentially harmful food. Therefore, other analytical methods to determine *Salmonella*, which provide results in a shorter time and with similar levels of reliability as the reference method, must be applied. This would enable appropriate corrective measures to be taken, and should result in less risk to consumers' health.

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