Prevalence and antimicrobial resistance of Salmonella in meat and meat products in Latvia

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Abstract

Introduction and objective. Salmonella is a foodborne pathogen which causes gastrointestinal illness in consumers, and exhibits resistance to antimicrobials of veterinary and clinical significance. The aim of this study is to detect the prevalence and antimicrobial resistance of *Salmonella* isolates from meat in Latvia.

Materials and method. A total of 3,152 samples of raw and ready-to-eat (RTE) meats were collected during the official control and in-house control procedures in 2015. Samples were tested in accordance with ISO 6579:2002. All *S*. Typhimurium, *S*. Enteritidis and other isolates recovered from the official control samples (*S*. Derby, *S*. Give) were tested for antimicrobial resistance. The minimum inhibitory concentration (MIC) values were investigated in line with the requirements of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Results. 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%) in frozen meat and meat preparations (0/349) and RTE meats (0/364). The most common serovars were *S*. Typhimurium (36%, 9/25) and *S*. Derby (32%, 8/25). In total, 62% (13/21) of *Salmonella* isolates were resistant to at least one antimicrobial agent. Altogether, 40% (8/20) of isolates were resistant to sulfamethoxazole, 25% (5/20) to nalidixic acid, ciprofloxacin, ampicillin and 20% (4/20) to tetracycline. All isolates were susceptible to ceftazidime, cefotaxime, meropenem, azithromycin and tigecycline. *S*. Typhimurium exhibited antimicrobial resistance more often (87.5%) than other serovars.

Conclusion. The study shows that the presence of *Salmonella* in meat, together with the high prevalence of resistant strains, is a significant public health related issue in Latvia.

Key words

Salmonella, pork, poultry, minced meat preparations, antimicrobial resistance, Latvia

INTRODUCTION

Human salmonellosis is one of the most important foodborne infections worldwide, and the second most frequently reported in the European Union [1, 2]. The disease is caused by *Salmonella enterica* subsp *enterica*, which is divided into more than 2,500 serovars with non-typhoidal *Salmonella* serovars S. Typhimurium, S. Enteritidis and S. Infantis were the most frequently found in clinical cases and foodstuffs in the EU [2, 3].

Although salmonellosis is characterized by gastrointestinal disorders, severe infections, such as bacteraemia and focal manifestations in the form of meningitis, septic arthritis, osteomyelitis, cholangitis and pneumonia, have also been reported [4]. The reported incidence of salmonellosis in the EU was 20.4 cases per 100,000 inhabitants during 2009–2013 [2]. Salmonellosis still remains the most relevant foodborne infection in Latvia with the incidence of 19.0 – 48.0 cases per 100,000 inhabitants during 2009–2013 [2].

Salmonella may be present in animals and spread from the animal host to food originating from the animal, resulting in the contamination of foodstuffs. Therefore, foods of animal

origin remain the main source of *Salmonella* and the disease is mostly attributed to the consumption of such contaminated products as meat, milk and eggs [2]. In previous studies, *Salmonella* was isolated from retail raw eggs, raw and processed milk, and meat and meat products [5, 6, 7, 8]. Since salmonellosis may require systemic treatment, including antimicrobial drug therapy, the increasing resistance of *Salmonella* to clinically significant antimicrobial drugs is an issue for concern [4]. The antimicrobial resistance could make antibiotic therapy ineffective for patient treatment and, consequently, the resistance of *Salmonella* isolates should be monitored [9].

Studies on the epidemiology of *Salmonella* through the food chain are important for drawing out the specific patterns of distribution and antimicrobial resistance of the pathogen. Meat and its products have been reported to be important sources of *Salmonella* [7, 8]; therefore, the aim of this study is to study the prevalence and antimicrobial resistance of *Salmonella* in meats in Latvia.

MATERIALS AND METHOD

Sampling. In 2015, a total of 3,152 samples of meat and meat products were collected from slaughterhouses, meat processing plants and retail outlets. Samples were obtained during official controls and food safety monitoring of meat

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industry enterprises, including in-house control procedures. Samples included raw meat and raw meat preparations (n=1,010), minced meat and minced meat preparations (n=481), frozen meat and frozen meat preparations (n=349), slaughterhouse carcass samples (n=948) and ready-to-eat (RTE) meats (n=364).

Isolation and identification of Salmonella. Samples were investigated according to the ISO 6579:2002 [10]. An amount of 25 g of sample was pre-enriched in 225 ml of Buffered Peptone Water (Biolife, Italy) for 18±2 h at 37 °C. After incubation, an aliquot of 0.1 ml of suspension was transferred into 10 ml of Rappaport-Vassiliadis soya (Biolife) and 10 ml of Mueller-Kauffmann broths, which were incubated for $24 \text{ h} \pm 3 \text{ at } 41.5 \text{ °C}$ and 37 °C, respectively. Then, a 10 µl loop of the enriched suspension was plated out on Xylose Lysine Deoxycholate (XLD, Biolife) and Brilliant-Green Phenol-Red Lactose (BPLS, Biolife) agars, with subsequent incubation for $24 \text{ h} \pm 3 \text{ at } 37 \text{ °C}$. After incubation, the plates were examined for the presence of Salmonella typical colonies, identified by a pink or red colour surrounded with red zone, or red colour with a black centre, on BPLS and XLD, respectively. Selected presumptive colonies were confirmed biochemically according to ISO 6579:2002 requirements [10].

Salmonella subtyping. After the confirmation of *Salmonella* spp., all isolates were stored at -80 °C in a mix of Brain-Heart Infusion (Biolife) and 20% glycerol. *Salmonella* colonies were serotyped according to the White-Kauffmann-Le Minor scheme by slide agglutination with specific O- and H- antigen sera (Staten Serum Institute, Denmark). Phage typing was performed in accordance with the Colindale systems.

Detection of antimicrobial resistance. All *S*. Typhimurium, *S*. Enteritidis isolated from in-house and official control samples, as well as other *Salmonella* serovars recovered from the official control samples, were tested for the detection of antimicrobial resistance with the minimum inhibitory concentration (MIC) method [11]. Before the testing, *Salmonella* isolates were plated out on Nutrient agar (Biolife) and incubated for 18–24 h at 37 °C. The colonies were then mixed with saline until the bacterial suspension in saline with the density of 0.5 McFarland was obtained (Nephelometer Sensititre, UK). An amount of 50 µl of bacterial suspension in saline was transferred into a tube containing 11 ml of cation-adjusted Mueller-Hinton broth (Sensititre), which was used for further testing.

For the detection of MIC, the EUVSEC panels were applied (TREK Diagnostic Systems Ltd., UK) in accordance with Commission Decision (EC) No 652/2013 [12]. The antimicrobial resistance testing panel contained ampicillin (1-64 mg/l, AMP), azithromycin (2-64 mg/l, AZT), cefotaxime (0.25-4 mg/l, CTX), ceftazidime (0.5-8 mg/l, CAZ), chloramphenicol (8-128 mg/l, C), meropenem (0.03-16 mg/l, MER), nalidixic acid (4-128 mg/l, NA), ciprofloxacin (0.015-8 mg/l, CIP), tetracycline (2-64 mg/l, TE), tigecycline (0.25–8 mg/l, TI), colistin (1–16 mg/l, COL), gentamicin (0.5-32 mg/l, CN), trimethoprim (0.25-32 mg/l, W) and sulfamethoxazole (8-1024 mg/l, SMX). Each well of the panel was inoculated with 50 μ l of bacterial suspension in Mueller-Hinton broth and the panel incubated for 18 -20 h at 37 °C. MIC was detected according to the EUCAST ECOFF after incubation [12].

Statistical analyses. Chi-square test was used for calculation of significance of differences between the prevalence of *Salmonella* in different categories of meats.

RESULTS

Prevalence of *Salmonella* in meats. Overall, the prevalence of *Salmonella* in raw meat and meat products was 0.8 % (25/3152). The highest prevalence of 1.5 % was found in minced meat and meat preparations (7/481), while the lowest of 0% in frozen meat and meat preparations 0/349) and RTE meats (0/364). There were no differences (P>0.05) in the prevalence (0.9%) of *Salmonella* between the raw meat and meat preparations (9/1,010) and carcass samples (9/948) (Tab. 1). Poultry (7.8%) and lamb (3.4%) slaughterhouse carcass samples, as well as minced pork and minced pork preparations (2.8%), were the most prevalent, while the cattle

Table 1. Prevalence of Salmonella in raw meat and meat preparations in Latvia in 2015

Meat category	Meat type or product	No. of samples	No. of positive samples (%)		
	pork	317	5 (1.6)		
Raw meat	beef	134	2 (1.5)		
and meat	poultry ^a	392	2 (0.5)		
preparations	lamb	14	0 (0)		
	mixed meat	No. of samples samples (% 317 5 (1.6) 134 2 (1.5) 392 2 (0.5) 14 0 (0) 153 0 (0) 1010 9 (0.9) ^h 17 0 (0) 211 6 (2.8) 90 0 (0) 163 1 (0.6) 481 7 (1.5) 3 0 (0) 7 0 (0) 41 0 (0) 298 0 (0) 349 0 (0) 297 1 (3.4) 488 2 (0.4) 367 1 (0.3) 64 5 (7.8) 948 9 (0.9) 21 0 (0)	0 (0)		
Subtotal		1010	9 (0.9) ^h		
Minced	beef	17	0 (0)		
meat and	pork	pork 317 beef 134 oultry ^a 392 lamb 14 sed meat 153 1010 beef beef 17 pork 211 oultry 90 ed meat ^b 163 000000000000000000000000000000000000	6 (2.8)		
minced meat	poultry	90	0 (0)		
preparations	mixed meat ^b	163	1 (0.6)		
Subtotal		481	7 (1.5)		
	pork	3	0 (0)		
Frozen meat and meat	beef	7	0 (0)		
preparations	poultry	41	0 (0)		
	mixed meat ^c	298	0 (0)		
Subtotal		349	0 (0)		
	lamb	29	1 (3.4)		
Carcassd	pig	488	2 (0.4)		
Carcassu	cattle	367	1 (0.3)		
	poultry	64	5 (7.8)		
Subtotal		948	9 (0.9)		
	cooked and grilled poultry ^e	21	0 (0)		
	smoked sausages ^f	92	0 (0)		
RTE meat	cooked sausages, frankfurters	948 9 (0.9) illed poultrye 21 0 (0) ausages ^f 92 0 (0) es, frankfurters 101 0 (0)			
	smoked meat ^g	130	0 (0)		
	pâté	20	0 (0)		
Subtotal		364	0 (0)		
Total		3152	25 (0.8)		

^a – chicken, duck and turkey

^b – kebab, raw sausages, cutlet, meatballs ^c – including meat dumplings (n=243)

^c – including meat dumplings (n=243)
^d - slaughterhouse samples taken by the competent authority

^e – chicken

f - cold-smoked, hot-smoked, dried and half-smoked sausages

⁹ – chicken and pork

^h – diferences in the prevalence of *Salmonella* between the slaughtehouse carcass and raw meat and raw meat preparations were not significant (P>0.05)

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carcass samples (0.3%) the less contaminated with Salmonella (Tab. 1). The majority of Salmonella isolates originated from pork, poultry (chicken and duck) and beef, comprising 52% (13/25), 28 % (7/25) and 12 % (3/25), respectively (Tab. 2). Altogether, 7 Salmonella serovars were recovered from meat and meat products (Tab. 2). The most common serovars were S. Typhimurium (36%), S. Derby (32%) and S. Enteritidis(12%). Other serovars were S. Braenderup, S. Give, S. Stanley and S. Virchow, which comprised 4% each (1/25). The highest variety of serovars was found in pork, where the serovars S. Derby, S. Typhimurium, including monophasic variant 4, 12; i:-;,S. Enteritidis, S. Braenderup, S. Stanley and S. Virchow were confirmed. S. Derby was more often isolated from pork, while S. Typhimurium and S. Enteritidis from poultry (Tab. 2).

Table 2. Distribution of Salmonella serovars in different meat types in Latvia

Calmanalla	Food category No. of isolates									
Salmonella serovar	Beef	Pork	Poultry	Lamb	Mixed meats	Total (%)				
Typhimurium	1	3	4	-	1	9 (36)				
Derby	2	6	-	-	-	8 (32)				
Enteriditis	-	1	2	-	-	3 (12)				
Braederup	-	1	-	-	-	1 (4)				
Give	-	-	1	-	-	1 (4)				
Stanley	-	1	-	-	-	1 (4)				
Virchow	-	1	-	-	-	1 (4)				
Non-specified O:9,12	-	-	-	1	-	1 (4)				
Total (%)	3 (12)	13 (52)	7 (28)	1 (4)	1 (4)	25 (100)				

Antimicrobial resistance of Salmonella isolated from meat.

Among 21 Salmonella isolates used for antimicrobial testing, 13 (62%) exhibited resistance to at least one antimicrobial agent. All isolates were susceptible to ceftazidime, cefotaxime, meropenem, azithromycin and tigecycline. Mostly, the Salmonella isolates were resistant to sulfamethoxazole (38%, 8/21), nalidixic acid, ciprofloxacin and ampicillin (24%, 5/21) and tetracycline (19%, 4/21) (Tab. 3). Altogether, 5 of 13 (38%) Salmonella isolates were resistant to one antimicrobial agent (colistin), or to one group of antimicrobial drugs (fluoroquinolones). Another 8 isolates (62%) showed resistance to 2-4 groups of antimicrobial drugs, including 5 Salmonella multi-resistantisolates (resistant to 3 or more classes of antimicrobial drugs).

S. Typhimurium exhibited antimicrobial resistance (89%, 8/9) more often than S. Enteritidis (66.6%, 2/3). S. Derby displayed antimicrobial resistance less frequently, and 25% (2/8) of isolates were found to be resistant to tetracycline and sulfamethoxazole (Tab. 3). S. Enteritidis and S. Typhimurium showed resistance to the widest range of antimicrobials, and resistance to 4 groups of antimicrobial drugs was identified with resistance phenotypes AMP-SMX-W-COL and AMP-CN-C-SMX, respectively (Tab. 4).

Resistant Salmonella isolates were identified in poultry (55%; 7/13) more frequently than pork (31%, 4/13), beef and minced meat (7%; 1/13 each). However, only 2 of 7 poultry isolates exhibited multi-resistance, while all the pork, beef and mixed meat isolates were multi-resistant. The most frequent resistance phenotype was TE-SMX identified in S. Derby in pig carcass, and AMP-TE-SMX in S. Typhimurium found in retail pork isolates (Tab. 4).

Table 4. Antimicrobial resistance phenotype of Salmonella in Salmonellapositive raw meat samples

Serovar	Source	MR (%)	No. of isolates	Resistance phenotype
Derby	Pig carcass	0 (0)	2	TE-SMX
Enteritidis	Chicken meat FT 12 ^a	0 (0)	1	COL
	Poultry carcass FT 12	1 (100)	1	AMP-SMX-W-COL-
Give	Duck ^a	1 (100)	1	AMP-W-SMX
Typhimurium	Beef carcass DT 12	1 (100)	1	AMP-CN-C-SMX
	Poultry carcass DT 141	0 (0)	4	NA-CIP
	Pork ^b	2 (100)	2	AMP-TE-SMX
	Mixed meat DT 104 ^c	0 (0)	1	NA-CIP-SMZ
-				

MR - resistant to three or to more than two groups of antimicrobial drugs according to EUCAST ECOFF

- products were imported from Lithuania

Products were imported input language
 phagotypes were not detected, one of samples was imported from Poland and contained monophasic S. Typhimurium

- monophasic S. Typhimurium

DISCUSSION

The prevalence of 0.8% Salmonella in meat in the presented study was comparable with the prevalence of Salmonella in raw meats (0.89%) and RTE products (0%) in Estonia [8]. Minced meats and minced meat preparations (1.5%) were mostly found to be contaminated with Salmonella; however, the reported prevalence was less than in ground meats in Greece (3.4%), Poland (2%), and in raw pork sausages

Table 3. Antimicrobial resistance of Salmonella servyars isolated from raw meats and meat preparations

Salmonella serovar	No. of isolates	NO. OF TESISIAIL ISOIALES													
		AMP	CAZ	CTX	MER	AZT	COL	С	CN	NA	CIP	TE	TI	W	SMX
Derby	8	0	0	0	0	0	0	0	0	0	0	2	0	0	2
Enteritidis	3	1	0	0	0	0	2	0	0	0	0	0	0	1	1
Give	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1
Typhimurium	9	3	0	0	0	0	0	1	1	5	5	2	0	0	4
Total	21	5	0	0	0	0	2	1	1	5	5	4	0	2	8

Key to antimicrobial drugs: AMP – Ampicillin, CAZ - Ceftazidime, CTX - Cefotaxime, MER – Meropenem, AZT – Azithromycin, COL – Colistin, C – Chloramphenicol, CN – Gentamicin, NA- Nalidixic adic, CIP- Ciprofloxacin, TE- Tetracycline, TI – Tigecycline, W- Trimethoprim, SMX – Sulfamethoxazole

(3.5%) in Italy [13, 14, 15]. Despite the overall prevalence of *Salmonella* in meats in the EU, there is a trend in its decrease which is attributable to the effective *Salmonella* control programme in the food chain [16, 17]. However, the presence of *Salmonella*-positive raw meats, raw minced meats and preparations on the retail market still represents a concern for public health.

Poultry, lamb and pork were found to be the meats most contaminated with *Salmonella*. The presented findings are in good agreement with previous reports on the high prevalence of *Salmonella* in poultry meat and poultry carcasses [8, 14, 18]. Pork has also been identified as an important source of *Salmonella* [8, 16]. The prevalence of *Salmonella* in meat is attributed to the transmission of the pathogen from the animal during slaughter, indicating the significance of good hygienic practice in both pork and poultry meat production in Latvia.

The most common *Salmonella* serovars were *S*. Typhimurium (36%), *S*. Derby (32%) and *S*. Enteritidis (12%). *S*. Enteritidis, *S*. Typhimurium, *S*. Braenderup, *S*. Stanley and *S*. Virchow were isolated from pork and poultry meat in previous studies, which is in agreement with the results of the current stuidy [8, 13,14, 18]. The identification of monophasic *S*. Typhimurium supports previous reports that pork is the main food category mostly contaminated with monophasic *S*. Typhimurium 4, [5], 12; i:-;. [8,19,20, 21]. *S*. Give is an unusual serovar for Latvia, and rarely reported in foods [8, 22]. *S*. Give and monophasic *S*. Typhimurium-positive products were imported from Lithuania and Poland, thus, the appearance of new serovars should be considered in Latvia.

Clinical salmonellosis in Latvia is mostly caused by *S*. Typhimurium and *S*. Enteritidis; however, the infections with *S*. Derby, *S*. Virchow and *S*. Stanley have also been registered [23, 24, 25]. In general, the majority of *Salmonella* clinical cases in Latvia have been attributed to serovars identified in meat in Latvia [25]. Additionally, the isolated serovars were among the most frequently implicated in human cases in Europe, highlighting the emergence of certain serovars in the EU, including Latvia [2]. This covers the increases in the prevalence of *S*. Derby and *S*. Stanley in food and clinical cases, and confirmation of the monophasic *S*. Typhimurium variant 4, [5], 12; i:-;, which became one of the most predominant in several European countries [2, 19, 26].

A total of 62% of *Salmonella* isolates exhibited resistance to at least one antimicrobial drug. This was less than 68.9% and 84% reported in Poland and the USA, respectively, but higher than 57.7% observed in Austria [13, 27, 28]. In comparison, the maximum number of resistances of *Salmonella* isolates in another study (4) was less than the 11 and 12 reported previously [7, 13].

Salmonella resistance to ceftazidime, cefotaxime, carbapenems was not identified in the presented study, which is in agreement with Mąka et al. [13]. In contrast, White et al. [27] reported the isolation of ceftiofur and ceftriaxone-resistant strains from ground turkey and chicken meat in the USA. The resistance to carbapenems – imipenem was reported previously in *Salmonella* in retail chicken meat in Germany [7]. In the present study, the resistance to sulfamethoxazole (38%), ampicillin (24%) nalidixic acid (24%), ciprofloxacin (24%) and tetracycline (19%) was identified the most frequently. These antimicrobial drugs

were among those to which *Salmonella* exhibited the most antimicrobial resistance, and the presented results are in line with the previously reported [5, 13, 27, 28, 29].

In Latvia, all 4 S. Typhimurium poultry stains originated from the same farm, sharing the same phagotype DT 141 and resistance phenotype nalidixic acid-ciprofloxacin. The pattern of antimicrobial resistance of *Salmonella* isolates in meat may reflect the specific antibiotic usage pattern in animal husbandry. Fluoroquinolones belong to critically important antimicrobials that are applied in human medicine and treatment of severe *Salmonella* infection [9]. Assessment of their usage in productive poultry husbandry should be evaluated in Latvia. Altogether, 3 of 13 (23%) *Salmonella* were isolated from imported meat from Lithuania and Poland, with 2 of 3 isolates were multi-resistant; therefore, the introduction of multi-resistant *Salmonella* serovars with imported meat should be considered.

CONCLUSION

This is the first report on the prevalence of *Salmonella* in meat in Latvia and shows that the presence of *Salmonella* in the food chain is still a problem: minced meats and minced meat preparations (1.5%) were mostly found to be contaminated. *S.* Enteritidis, *S.* Derby and *S.* Typhimurium, including the monophasic variant, were the predominant serovars isolated from meat. *Salmonella* exhibited antimicrobial resistance to at least one antimicrobial agent in 62% of isolates; however, the number of multi-resistant strains was less than reported previously. This could be attributable to the low application of antimicrobial drugs in Latvia, compared to the average application in the EU. The antimicrobial resistance pattern was in agreement with that previously reported, and could reflect the specific usage of antimicrobial agents in animal husbandry in Latvia.

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