Original Article

First study of *Salmonella* in meat in Romania

Liora Mihaiu[1], Alexandra Lapusan[2], Rodica Tanasuica[3], Rodica Sobolu[2], Romolica Mihaiu[4], Ovidiu Oniga[1], Marian Mihaiu[2]

[1] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania*
[2] *Department of Animal Production and Food Safety, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania*
[3] *Veterinary Public Health Institute, Bucharest, Romania*
[4] *Department of Management, Faculty of Economics and Business Administration, Babeş-Bolyai University, Cluj-Napoca, Romania*

Abstract

Introduction: The increasing antimicrobial resistance of *Salmonella* isolates is of major public health concern, but information regarding these aspects is still lacking in Romania. This study focused on a detailed and accurate investigation concerning prevalence, serotypes, and antimicrobial resistance patterns of *Salmonella* strains, isolated from pork and chicken meat, collected from all regions of Romania in 2011.

Methodology: The research was conducted on 650 samples of chicken and pork meat collected from production units and retail markets located in various regions of Romania. A total of 149 *Salmonella* isolates were recovered (22.92%), serotyped, confirmed by PCR, and further tested for antimicrobial susceptibility.

Results: Thirteen *Salmonella* serovars were identified; predominant serovars included Infantis, Typhimurium, Derby and Colindale. Multiple resistance was found in 83.22% (n = 124) of the isolates. The isolates were frequently resistant to tetracycline (80.53%), streptomycin (81.21%), sulfamethoxazole (87.25%), nalidixic acid (65.10%), and ciprofloxacin (42.95%). Additionally, a markedly lower resistance rate was observed for ampicillin (20.81%), chloramphenicol (16.78%), and ceftazidime (11.41%). Among 137 resistant *Salmonella* isolates, 35 different resistance patterns were found.

Conclusion: A high prevalence of *Salmonella* spp. and a relatively high resistance rate to multiple antimicrobials was found. This data indicates that chicken and pork meat could constitute a source of human exposure to multidrug-resistant *Salmonella* and therefore could be considered a potential vehicle of resistant *Salmonella* foodborne diseases. Further actions are needed to succesfully implement a national surveillance program for better monitoring of these resistant pathogens.

Key words: *Salmonella*; public health; prevalence; antimicrobial; resistance; food safety

J Infect Dev Ctries 2014; 8(1):050-058. doi:10.3855/jidc.3715

(Received 22 April 2013 – Accepted 17 August 2013)

Introduction

Numerous epidemiological studies have implicated foods of animal origin as major vehicles associated with illnesses caused by foodborne pathogens, which lead to the development of antimicrobial-resistant pathogens [1]. *Salmonella* species are considered to be among the most important foodborne pathogens in the world and salmonellosis is still one of the most widespread foodborne bacterial illnesses in humans, with clinical manifestations ranging from asymptomatic state to severe disease [2]. The majority of infections are associated with the ingestion of contaminated foods such as poultry, beef, pork, eggs, milk, cheese, seafood, fruits, juices, and vegetables [3,4], although most infections caused by multidrug-resistant *Salmonella* are acquired through contaminated foods of animal origin.

Strains of *Salmonella* that are resistant to antimicrobial agents have become a worldwide public health concern, since resistance in *Salmonella* limits the therapeutic options available to physicians in the treatment of salmonellosis in humans.

Surveillance of antimicrobial resistance in zoonotic bacteria such as *Salmonella* is essential for providing information on the magnitude and trends of resistance in foodborne pathogens in each country, because the resistance prevalence varies widely between and within countries and over time [5]. The use of antimicrobials in one country affects the spread of resistance in others.

Information about the prevalence of multidrug-resistant (MDR) *Salmonella* isolated from chicken and pork meat and about *Salmonella* resistance trends is lacking in Romania. Moreover, little is known about the potential role of meat in the dissemination of MDR *Salmonella*, because very limited research work concerning these factors had been done in our country.

The present study was undertaken to provide baseline data on antimicrobial resistance in *Salmonella* strains isolated from chicken and pork meat in Romania. We reported prevalence, serotypes, and antibiotic resistance patterns of *Salmonella* strains isolated from pork and chicken meat, collected from all regions of the country, in 2011.

Methodology

The study was conducted on 650 food samples, including pork (n = 208) and chicken (n = 442), randomly collected from production units and retail markets in Romania, during the period of January to December 2011.

Salmonella spp. isolation

The isolation protocol followed the steps recommended by the International Organization for Standardization (ISO) 6579 and previously described by Molla and Mesfin (2003) [6]. Briefly, the meat samples (25 g) were previously homogenized in buffered peptone water (225 mL) with a laboratory blender (Stomacher 400, Seward Ltd., Worthing, England) for approximately two minutes. After incubation for 18 to 24 hours at 37°C, 0.1 mL was inoculated in 10 mL Rappaport-Vassiliadis (RV) green broth (LabM Limited, Heywood, England) and incubated for 18 to 24 hours at 42°C. Another 1 mL from the culture obtained was inoculated into 10 mL of selenite cysteine (SC) broth (LabM Limited, Heywood, England) and incubated at 37°C for 18 to 24 hours. From both enrichment broths obtained, 1 mL was streaked onto brilliant green-phenol red-lactose-sucrose (BPLS) agar (Merck, Darmstadt, Germany) and xylose lysine deoxycholate (XLD) agar (Oxoid, Basingstoke, England). Following the incubation at 37°C for 24 hours, presumptive *Salmonella* colonies were characterized by their biochemical properties through slide agglutination using standard protocols. The positive colonies where then identified as *Salmonella* using the Sensititre Automated Microbiology System Aris 2X (Thermo Scientific, Waltham, USA) following the protocol stated by the producer.

Serotyping

The serovar was established with the antisera commercially available, *Salmonella* antisera test group (Denka Seiken Co., Tokyo, Japan), which provides specific agglutinins for each *Salmonella* antigen. The steps were followed in accordance with the protocol mentioned in the test performed.

PCR confirmation

All strains of *Salmonella* spp. were confirmed by PCR, targeting the common sequence *omp*C, using the set of primers previously described by Modaressi and Thong (2010) (F: 5′-ATCGCTGACTTATGCAATCG-3′, R: 5′ CGGGTTGCGTTATAGGTCTG-3′) [7]. The bacterial DNA extraction followed the basic steps previously described by Yang *et al*. (2008) with a few particularities [8]. Briefly, 150 µL of CHELEX (10%) reactive (BioRad, Berkeley, USA) was added in Eppendorff tubes (1.5 mL) (Ratiolab, Dreieich, Germany). The tubes were subjected to UV sterilization in a microbiological laminal flow, class II to remove any possible contaminants from the manipulation performed earlier. One to two colonies were harvested with a sterile microbiological loop and immersed in the CHELEX reactive. The following extraction temperatures were used: 57ºC - 30'; 94ºC - 5'. The last step included a high-speed centrifugation (14.000 rotations per minute) for one minute.

The amplification for *omp*C was carried out in a final volume of 25 µL containing 25 pmol of each primer, 12.5 µL of MasterMix (Bioline, London, UK), 4 µL of DNA template, and 6.5 µL PCR water grade (Sigma, Saint Louis, USA). For each experiment, a negative control containing the same reactive except for the DNA template, was used. For the positive control, *Salmonella* Typhimurium ATCC 14028 was used.

The PCR conditions were adjusted for the primers and *Taq*-polymerase used, consisting of an initial denaturation at 95°C for 4 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and 1 minute at 72°C; the final elongation was performed at 72°C for 5 minutes.

Given the fact that *Salmonella* serovars Typhimurium and Enteritidis are the most common cause of human salmonellosis worldwide, the accurate confirmation of these strains was made by PCR, amplifying specific sequences for *S.* Enteritidis (ENT) and *S.* Thyphimurium (STM). This multiplex PCR was selected to insure the accuracy of the results.

For the differentiation of *Salmonella* Typhimurium and *Salmonella* Enteritidis, a multiplex PCR was done, incorporating specific primers for *S.* Enteritidis (ENTF: 5′- TGTGTTTTATCTGATGCAAGAGG-3′, ENTR: 5′-TGAACTACGTTCGTTCTTCTGG-3′ (304 bp), *Salmonella* Typhimurium (STMF: 5′-TTGTTCACTTTTTACCCCTGAA-3′, STMR: 5′CCCTGACAGCCGTTAGATATT-3′ (401 bp) previously described by Modaressi and Thong (2010) [7]. PCRs were performed in a final volume of 25 µL that contained an optimized PCR mixture: 1 µL of each primer, 12.5 µL of MasterMix (Bioline, London, England), 4 µL of DNA template, and 2.5 µL PCR water grade. The PCR protocol consisted of the following steps: an initial denaturation step of 4 minutes at 94°C; 30 cycles of 30 seconds at 94°C; 30 seconds at 58°C; and 1 minute at 72°C. The final elongation step was performed at 72°C for 4 minutes. For the positive control, *Salmonella* Typhimurium ATCC 14028 and *Salmonella* Enteritidis ATCC 13076 strains were used.

Susceptibility testing

Eleven antimicrobials, some of them generally used in animal and human therapy, were tested on the isolated strains of *Salmonella* using the classical disk diffusion method, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [9]. The isolates were tested against ampicillin (AMP, 10 µg), cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), chloramphenicol (CHL, 30 µg), ciprofloxacin (CIP, 5 µg), gentamicin (GEN, 10 µg), nalidixic acid (NA, 30 µg), streptomycin (S, 10 µg), sulfamethoxazole (SMX, 300 µg) trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 µg), and tetracycline (TET, 30 µg). The CLSI breakpoints were applied for the interpretation of the results.

The statistical analysis

The statistical package SPSS Statistics version 19 was used. The differences were considered significant at a p value less than 0.05.

Results

Prevalence of Salmonella in chicken and pork meat

A total of 149 *Salmonella* isolates were recovered, representing 22.92% of the samples tested. Out of the 149 *Salmonella* isolates, 48 were from pork samples (32.21%) while 101 (67.78%) were from chicken samples.

Serotyping

All *Salmonella* isolates belonged to the subspecies *enterica* and were serotyped into 13 serovars of *Salmonella enterica* subsp. *enterica*: *S.* Infantis (n = 73), *S.* Typhimurium (n = 19), *S.* Derby (n = 15), *S.* Colindale (n = 14), *S.* Rissen (n = 6), *S.* Ruzizi (n = 5), *S.* Virkow (n = 5), *S.* Brandenburg (n = 4), *S.* Bredeney (n = 4), *S.* Calabar (n = 1), *S.* Enteritidis (n = 1), *S.* Muenchen (n=1), and *S.* Kortrijk (n = 1). Table 1 shows the serotyping results.

Antimicrobial susceptibility

The results of the antimicrobial resistance tests of 149 *Salmonella* isolates are shown in Table 2.

Isolates showing resistance to three or more antimicrobials were classsified as multidrug resistant (MDR).

Out of the 149 *Salmonella* isolates, 137 (91.94%) were resistant to at least one antimicrobial agent (single type of resistance), while 124 (83.22%) were considered to be MDR. The categories of antimicrobial agents tested included □-lactams, phenicols, tetracyclines, aminoglycosides, sulfonamides, and quinolones.

Resistance to tetracycline (80.53%), streptomycin (81.21%), sulfamethoxazole (87.25%), nalidixic acid (65.10%), and ciprofloxacin (42.95%) were most often observed. Markedly lower resistance rates were observed for ampicillin (20.81%), chloramphenicol (16.78%), and ceftazidime (11.41%). A small percentage of the isolates demonstrated resistance to gentamicine (0.67%), and no cefotaxime resistance was detected in any of the isolates.

The resistance to multiple antimicrobial agents was predominantely seen in serovars Infantis, Colindale, Derby and Typhymurium. Among the 19 *S.* Typhimurium isolates, 11 were MDR, all of them showing resistance to more than five antimicrobials (Table 3).

MDR was seen in 64.58% of the pork isolates and in 92.07% of the chicken isolates. Among 137 resistant *Salmonella* isolates, 35 different resistance patterns were found, and most of them were represented by five strains. More than 37% of the isolates showed resistance to five antimicrobials, while 22.8% were resistant to six antimicrobials. Two isolates (one isolate of *S.* Colindale from pork and one chicken isolate of *S.* Typhimurium) were resistant to eight antimicrobials, the highest number of resistant traits shown by the isolates. Antimicrobial resistance patterns exhibited by *Salmonella* isolates are presented in Table 3.

Table 1. Serotype of *Salmonella* isolates

Serotype	No.	%
Infantis	73	70.87
Typhimurium	19	18.45
Derby	15	14.56
Colindale	14	13.59
Rissen	6	5.83
Ruzizi	5	4.85
Virkow	5	4.85
Brandenburg	4	3.88
Bredeney	4	3.88
Muenchen	1	0.97
Kortrijk	1	0.97
Enteritidis	1	0.97
Calabar	1	0.97
Total	**149**	**100**

Table 2. Antimicrobial resistance of *Salmonella* isolates from chicken and pork meat

Antibiotic	Number of resistant (R) and intermediate (I) susceptibility isolates (%) from:					
	Pork (n = 48)		Chicken (n = 101)		All sources (n = 149)	
	R	I	R	I	R	I
Ampicillin	18 (37.5)	0	13 (12.87)	0	31 (20.80)	0
Cefotaxime	0	0	0	0	0	0
Ceftazidime	7 (14.58)	0	10 (9.90)	0	17 (11.40)	0
Gentamicin	0	0	1 (0.99)	0	1 (0.67)	0
Streptomycin	34 (70.83)	0	87 (86.13)	0	121 (81.20)	0
Sulfamethoxazole	37 (77.08)	0	93 (92.07)	0	130 (87.24)	0
Sulfamethoxazole / trimethoprim	8 (16.66)	0	40 (39.60)	0	48 (32.21)	0
Nalidixic acid	12 (25)	0	85 (84.15)	0	97 (65.10)	0
Ciprofloxacin	4 (8.33)	11 (22.91)	60 (59.40)	20 (19.8)	64 (42.95)	31 (20.8)
Tetracycline	16 (33.33)	0	88 (87.12)	0	120 (80.53)	0
Chloramphenicol	12 (25)	7 (14.58)	13 (12.87)	13 (12.87)	25 (16.77)	20 (13.42)

Table 3. Antimicrobial resistance patterns exhibited by *Salmonella* isolates

	***Salmonella* serovar**	**Resistance pattern**	**No. of isolates (%)**
One type of antimicrobial	Typhimurium, Derby, Ruzizi	SMX	7 (5.10)
	Typhimurium	TET	2 (1.45)
	Typhimurium, Brandenburg	S	2 (1.45)
Two types of antimicrobials	Rissen	SMX, S	1 (0.72)
	Brandenburg	AMP, S	1 (0.72)
Three types of antimicrobials	Infantis, Enteritidis, Colindale	SMX, NA, S	3 (2.18)
	Typhimurium	SXT, AMP, TET	1 (0.72)
	Derby, Muenchen	SMX, S, TET	5 (3.64)
	Derby	S, AMP, TET	1 (0.72)
	Calabar	S, SMX, CAZ	1 (0.72)
	Bredeney	SXT, AMP, SMX	2 (1.45)
Four types of antimicrobials	Infantis	SMX, NA, CIP, TET	1 (0.72)
	Infantis	SMX, NA, S, TET	7 (5.10)
	Infantis, Typhimurium	SMX, AMP, S, TET	3 (2.18)
Five types of antimicrobials	Infantis, Colindale	SMX, NA, CIP, S, TET	31 (22.62)
	Infantis	SMX, NA, CIP, TET, SXT	2 (1.45)
	Derby, Typhimurium	SMX, CHL, S, AMP, TET	5 (3.64)
	Typhimurium, Bredeney	SMX, S, AMP, TET, SXT	4 (2.91)
	Kortrijk, Infantis	SMX, NA, S, CHL, TET	2 (1.45)
	Derby	SMX, NA, S, TET, SXT	2 (1.45)
	Virkow	SMX, NA, S, CHL, TET	1 (0.72)
	Virkow, Ruzizi, Infantis	SMX, NA, S, CAZ, TET	8 (5.83)
	Typhimurium	SMX, S, CIP, AMP, TET	1 (0.72)
Six types of antimicrobials	Infantis, Colindale	SMX, NA, S, CIP, TET, SXT	20 (14.59)
	Typhimurium	SMX, S, CHL, AMP, TET, SXT	2 (1.45)
	Brandenburg	SMX, NA, S, AMP, TET, SXT	1 (0.72)
	Ruzizi, Infantis	SMX, NA, S, CAZ, CHL, TET	5 (3.64)
	Typhimurium	SMX, NA, S, AMP, CHL, TET	1 (0.72)
	Infantis	SMX, NA, S, CAZ, TET, SXT	3 (2.18)
	Infantis	SMX, NA, S, CHL, TET, SXT	1 (0.72)
	Bredeney	SMX, NA, CIP, GEN, CHL, TET	1 (0.72)
Seven types of antimicrobials	Colindale	SMX, NA, S, CIP, AMP, TET, SXT	3 (2.18)
	Infantis	SMX, NA, S, CIP, CHL, TET, SXT	1 (0.72)
	Typhimurium, Derby	SMX, S, CIP, CHL, AMP, TET, SXT	2 (1.45)
Eight types of antimicrobials	Typhimurium	SMX, NA, S, CIP, CHL, AMP, TET, SXT	2 (1.45)
			137 (100)

SMX, sulfamethoxazole; NA, nalidixic acid; CIP, ciprofloxacin; S, streptomycin; TET, tetracycline; AMP, ampicillin; SXT, sulfamethoxazole-trimethoprim; CHL, chloramphenicol; CAZ, ceftazidime; GEN, gentamicin; CTX, cefotaxime

The most prominent pattern was sulfamethoxazole, streptomycin, ciprofloxacin, nalidixic acid, and tetracycline (20.8% of the isolates).

Discussion

The increasing antimicrobial resistance in the foodborne zoonotic bacteria *Salmonella* is a major public health concern. Although in 2010, the number of reported human *Salmonella* cases in 14 countries – all members of the European Union – showed a significant decreasing level, Malta and Romania presented an increasing trend [10].

The present study, the first report about substantial MDR in *Salmonella* serovars isolated from Romania, revealed a high antimicrobial resistance in *Salmonella enterica* subsp. *enterica* isolated from chicken and pork meat There are few other reports about resistance to antimicrobial agents, but these reports focused on particular products in Romania [11].

Our study demonstrated that pork and chicken meat were contaminated by *Salmonella*; a total of 149 *Salmonella* isolates were recovered, representing 22.92% of the samples tested. Different prevalence rates of *Salmonella* spp. in food, especially poultry and poultry products, have been reported by different authors [12-17]. *Salmonella* contamination rates observed in our country are relatively high (22.92%) and confirm the widespread contamination of pork and chicken meat with *Salmonella* spp. The data reported by Romania in 2010 in the European Surveillence Program showed that samples tested at retail were less contaminated than samples tested earlier in the food chain [10], which shows that even higher levels are expected at farms or slaughterhouses. Moreover, the high contamination rates obtained in our study suggest that both poultry and pork meat could be a potential vehicle of transmission of *Salmonella* spp. from animals to humans. Given our results, a microbiological risk assessment (MRA) must be taken into consideration in this country, in order to improve food control systems and to produce safer food, reduce the number of foodborne illnesses, and facilitate the European trade.

All isolated strains belonged to *S. enterica* subsp. *enterica,* serotyped into 13 serovars: Infantis, Typhimurium, Derby, Colindale, Rissen, Ruzizi, Virchow, Brandenburg, Bredeney, Calabar, Enteritidis, Muenchen, and Kortrijk. Among these, 19 (18.45%) were identified as *S.* Typhimurium*,* which, according to the World Health Organization (WHO), is one of the most important serovars of *Salmonella* found in animals and their products responsible for the majority of human infections [5]. In 2000, the European Comission stated that the most frequently reported *Salmonella* serovars in humans, based upon reports from nine countries (not including Romania), were *S.* Enteritidis (59.1%), *S.* Typhimurium (13.0%), *S.* Hadar (1.8%), *S.* Virchow (1.7%), *S.* Infantis (0.9%), *S.* Agona (0.8%), *S.* Brandenburg (0.7%), and *S.* Newport (0.5%) [18]. Also, in sub-Saharan African countries, an increasing number of cases of multidrug-resistant (MDR) non-typhoidal *Salmonella* (NTS) infections have been reported [19].

Among the 149 Salmonella isolates, 83.22% were MDR. Multidrug resistance was seen in 64.58% of the pork isolates and in 92.07% of the chicken isolates.

The level was much higher than earlier figures reported by Thong and Modaressi (2011) in Malaysia (67%) [12], Yan *et al*. (2010) in China (20.9%) [15], Bouchrif *et al*. (2009) in Morocco (44%) [20], Hao Van *et al*. (2007) in Vietnam (34%) [21], and lower than figures reported by Yildirim *et al.* (2011) (97% of isolates from raw chicken carcasses exhibited multidrug resistance) [13]. When comparing the two types of meat sampled (pork and chicken), a significant difference ($p = 0.001$) was found in the multidrug resistance pattern; it was much higher in chicken (92.07%) than in pork (64.58%). The high rates of resistance revealed by this study can be explained by the extensive use of antimicrobial agents given in large units of animal growth, especially chickens, as prophylaxis, growth promoters, or treatment. The use of antimicrobials is under strict surveillance in Romania and growth promoters have been banned, but as it is well observed, farmers still use antimicrobials to achieve their production goals with minimal losses, regardless of the health costs [22].

The high resistance to sulfamethoxazole was not surprising, since sulfamethoxazole (in combination with trimethoprim) is widely used in Romania, both in human and veterinary medicine.

The widespread occurence of antimicrobial resistance to sulfonamides and tetracycline was also demonstrated in *Salmonella* strains isolated from fresh pork sausages by Murmann *et al.* in 2009 [15].

Resistance to tetracycline (80.53%) and streptomycin (81.20%) tended to occur at relatively high frequencies, higher than reported by Thong and Modaressi in 2011 (73.8% and 57.9%) [11], Yildirim *et al.* in 2011 (67.6% and 61.7%) [13], and Zhao *et al.* in 2009, who observed resistance of *S.* Typhimurium to streptomycin (63%) and tetracycline (61%) [23]. A higher resistance rate to tetracycline was reported by

Mahmud *et al.* in 2011 (93%) [14]. *S.* Typhimurium and *S.* Infantis isolates had higher resistance rates to these antibiotics. One of the serovars commonly involved in foodborne diseases, *S.* Typhimurium from chicken, was resistant to eight antimicrobials, the greatest number of resistant phenotypes. Lower rates (62%-65%) were mentioned in a number of studies focused on these specific strains [24,25] while higher rates of 84%-97% were mentioned less frequently [26]. Other studies concluded that this resistance prevalence for tetracycline and streptomycin is due to their frequent administration in veterinary medicine [27].

Nalidixic acid resistance was especially prevalent in chicken isolates (84.15%). This finding is in accordance with studies from other countries [12,15]. The prevalence of *Salmonella* isolates resistant to nalidixic acid was lower than the one reported by Shrestha *et al.* (2010) in Nepal [28]. The emergence of quinolone-resistant *Salmonella* isolates from food animals in Europe has increased substantially following the licensing of fluoroquinolones such as enrofloxacin for veterinary use [29]. Since enrofloxacin is the second most used antibiotic in veterinary practice in Romania after tetracycline, an even higher resistance was expected. Resistance to nalidixic acid is a matter of concern since nalidixic acid resistance has been associated with a decreased susceptibility to fluoroquinolones, which are used to treat salmonellosis in humans [30]. Treatment of serious human enteric infections with an effective fluoroquinolone can reduce the duration of illness, and most likely prevent complications and adverse outcomes, including hospitalization [31]. Therefore, resistance to fluoroquinolones can be considered an important public health concern, since these antibiotics are being widely used in veterinary medicine as well as in poultry production, which causes the resistence genes to be transmitted to humans through the food chain [32].

Resistance to ciprofloxacin (42.95%) was found to be high. Ciprofloxacin is widely used in Romania, especially in the treatment of human urinary tract infections, respiratory tract infections, and infections of the gastrointestinal tract. Simple observations of current medical practice reveal that many fluoroquinolones are used even in minor infections where no proper diagnosis has been made; the criteria set for a prudent use of antimicrobials, therefore, is not being observed.

In our study, few strains of *Salmonella* (11.40%) were resistant to third-generation cephalosporins (ceftazidime). However, the resistance rate is higher than the one reported by Yan *et al.* (2010) [15], Chao *et al.* (2007) [33], and Thong and Modarressi (2011) [12]. Concerning food-borne *Salmonella*, the resistances of most concern are those against quinolones and cephalosporins, both of which are mentioned in the WHO list of critically important antibiotics for human medicine [5].

In this study, the resistance to ampicillin was found to be only 20.80%, notably lower than the resistance reported in other countries by Mahmud *et al.* (2011) in Bangladesh (100%) [14] and Thai *et al.* (2012) in Vietnam [34]. This finding is not surprising, since ampicillin is not frequently used in animal therapy, although it is still prefered in the classical therapeutical protocol of salmonellosis in humans.

Lower resistance rates to chloramphenicol were observed (16.78%), similar to the rates reported previously by Murungkar *et al. (*2005) [35].

Among 137 resistant Salmonella isolates, 35 different resistance patterns were found; most of them were represented by five strains of antimicrobials. One of the most prominent patterns was sulfamethoxazole, streptomycin, ciprofloxacin, nalidixic acid, and tetracycline (SMX, NA, CIP, S, TET).

Conclusion

Overall, the frequencies and resistance patterns tend to vary remarkably from one country to another. Given the fact that, in Romania, this is the first report concerning the prevalence of MDR *Salmonella* isolates, we could not establish a correlation with the year, area, and environmental factors, but the general trends have been established regarding *Salmonella* spp. sensitivity to individual antimicrobial agents. The relatively high MDR *Salmonella* spp. isolates from chicken and pork meat observed in our study could be considered as one of the potential sources of human salmonellosis in Romania.

In conclusion, chicken and pork meat obtained in Romania pose a risk in the dissemination of MDR *Salmonella* in the European market. The results obtained provide baseline data for further antimicrobial resistance studies and also for the epidemiological inquiries that lack information about our country and that could be important, not only for epidemiologists monitoring the spread of MDR *Salmonella* in Romania, but also beyond our borders. From this study, it is evident that MDR *Salmonella* is more prevalent in poultry than in pork meat, which demonstrates the necessity for stricter surveillance of antimicrobial use in poultry production in Romania.

This single investigation might not be enough for a complete risk assessment concerning the possible *Salmonella* threat in food products, but it can draw a warning signal for further actions. This study recommends a closer cooperation between the parties involved in the prevention and control of diseases transmitted from food to humans.

References

1. Swartz MN (2002) Human diseases caused by foodborne pathogens of animal origin. Clin Infect Dis 34: S111-S122.
2. Galanis E, Lo Fo Wong DM, Patrick ME, Binsztein N, Cieslik A, Chalermchikit T, Aidara-Kane A, Ellis A, Angulo FJ, Wegener HC (2006) Web-based surveillance and global Salmonella distribution, 2000–2002. Emerg Infect Dis 12: 381-388.
3. Brands DA, Inman AE, Gerba CP, Maré CJ, Billington SJ, Saif LA, Levine JF, Joens LA (2005) Prevalence of Salmonella spp. in oysters in the United States. Appl Environ Microbiol 71: 893-897.
4. Zhao S, White DG, Friedman SL, Glenn A, Blickenstaff K, Ayers SL, Abbott JW, Hall-Robinson E, McDermott PF (2008) Antimicrobial resistance in Salmonella enterica serovar Heidelberg isolates from retail meats, including poultry, from 2002 to 2006. Appl Environ Microbiol 74: 6656-6662.
5. WHO 2001 (WHO Global Strategy for Containment of Antimicrobial Resistance) WHO, Geneva. WHO/CDC/CSR/DRS/2001.2.
6. Molla B, Mesfin A (2003) A survey of Salmonella contamination in chicken carcass and giblets in central Ethiopia. Revue Méd Vét 154: 267-270.
7. Modarressi SH, Thong KL (2010) Isolation and molecular subtyping of Salmonella enterica from chicken, beef and street foods in Malaysia. Sci Res Essays 5: 2713-2720.
8. Yang JL, Wang MS, Cheng AC, Pan KC, Li CF, Deng SX (2008) A simple and rapid method for extracting bacterial DNA from intestinal microflora for ERIC-PCR detection. World J Gastroenterol 14: 2872-2876.
9. Clinical and Laboratory Standards Institute (2006) Performance Standards for Antimicrobial Disk Susceptibility Tests. CLSI document M2-A9. CLSI: Wayne, PA.
10. EFSA (2012) The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010. EFSA J 10: 2597.
11. Ivana S, Bogdan A, Ipate J, Tudor L, Bărăităreanu S, Tănase A, Popescu AN, Caplan DM, Daneş M (2009) Food microbial contamination - the main danger in the catering type food industry in Romania. Rom Biotechnol Lett 14: 4260-4266.
12. Thong KL, Modarressi S (2011) Antimicrobial resistant genes associated with Salmonella from retail meats and street foods. Food Res Int 44: 2641-2646.
13. Yildirim Y, Gonulalan Z, Pamuk S, Ertas N (2011) Incidence and antibiotic resistance of Salmonella spp. on raw chicken carcasses. Food Res Int 44: 725-728.
14. Mahmud S, Bari L, Hossain MA (2011) Prevalence of Salmonella serovars and antimicrobial resistance profiles in poultry in Savar area, Bangladesh. Foodborne Pathog Dis 8: 1111-1118.
15. Yan H, Li L, Alam MJ, Shinoda S, Miyoshi S, Shi L (2010) Prevalence and antimicrobial resistance of Salmonella in retail foods in northern China. Int J Food Microbiol 143: 230-234.
16. Murmann L, Dossantos M, Cardoso M (2009) Prevalence, genetic characterization and antimicrobial resistance of Salmonella isolated from fresh pork sausages in Porto Alegre, Brazil. Food Control 20: 191-195.
17. Fakhr MK, Sherwood JS, Thorsness J, Logue CM (2006) Molecular characterization and antibiotic resistance profiling of Salmonella isolated from retail turkey meat products. Foodborne Pathog Dis 3: 366-374.
18. EC (European Commission) (2002) Trends and Sources of Zoonotic Agents in Animals, Feedstuffs, Food and Man in the European Union and Norway to the European Commission in accordance with Article 5 of the Directive 92/117/EEC, prepared by the Community Reference Laboratory on the Epidemiology of Zoonoses, BgVV, Berlin, Germany. Working document SANCO/927/2002; 1: 145-122.
19. Wain J, Keddy KH, Hendriksen RS, Rubino S (2013) Using next generation sequencing to tackle non-typhoidal Salmonella infections. J Infect Dev Ctries 7: 1-5.
20. Bouchrif B, Paglietti B, Murgia M, Piana A, Cohen N, Ennaj MM, Rubino S, Timinoun M (2009) Prevalence and antibiotic-resistance of Salmonella isolated from food in Morocco. J Infect Dev Ctries 3: 35-40.
21. Hao Van TT, Moutafis G, Istivan T, Tran LT, Coloe PJ (2007) Detection of Salmonella spp. in Retail Raw Food Samples from Vietnam and Characterization of Their Antibiotic Resistance. Appl Environ Microbiol 73: 6885-6890.
22. Chitescu CL, Nicolau A (2010) Contamination of food with residues of antibiotics in the sulphonamide class, risk can be avoided. Veterinary Drug 4: 1-11.
23. Zhao S, Blickenstaff K, Glenn A, Ayers SL, Friedman SL, Abbott JW, McDermott PF (2009) Beta-Lactam resistance in Salmonella strains isolated from retail meats in the United States by the National Antimicrobial Resistance Monitoring System between 2002 and 2006. Appl Environ Microbiol 75: 7624-7630.
24. Dogru AK, Ayaz ND, Gencay YE (2009) Serotype identification and antimicrobial resistance profiles of Salmonella spp. isolated from chicken carcasses. Trop Anim Health Prod 42: 893-897.
25. Iseri O, Erol I (2010) Incidence and antibiotic resistance of Salmonella spp. in ground turkey meat. Br Poult Sci 51: 60-66.
26. Asai T, Namimatsu T, Osumi T, Kojima A, Harada K, Aoki H, Toshiya S, Takahashi T (2010) Molecular typing and antimicrobial resistance of Salmonella enterica subspecies enterica serovar Choleraesuis isolates from diseased pigs in Japan. Comp Immunol Microbiol Infect Dis 33:109-119.
27. De Oliveira FA, Pasqualotto AP, da Silva WP, Tondo EC Characterization of Salmonella enteritidis isolated from human samples. Food Res Int. In press.
28. Shrestha A, Regmi P, Dutta RK, Khanal DR, Aryal SR, Thakur RP, Karki D, Singh UM (2010) First report of antimicrobial resistance of Salmonella isolated from poultry in Nepal. Vet Microbiol 144: 522-524.
29. Malorny B, Schroeter A, Helmuth R (1998) Incidence of quinolone resistance over the period 1986 to 1998 in veterinary Salmonella isolates from Germany. Antimicrob Agents Chemother 43: 2278-2282.
30. Gorman R, Adley CC (2004) Characterization of Salmonella enterica serotype Typhimurium isolates from human, food,

and animal sources in the Republic of Ireland. J Clin Microbiol 42: 2314-2316.
31. Food and Drug Administration (2000) Enrofloxacin for poultry; Opportunity for hearing. Federal Register 65 (211).
32. Gonzalez F, Pallecchi L, Rossolini GM, Araque M (2012) Plasmid-mediated quinolone resistance determinant qnrB19 in nontyphoidal Salmonella enterica strains isolated in Venezuela. J Infect Dev Ctries 6: 462-464.
33. Chao GX, Zhou XH, Jiao XN, Qian XQ, Xu L (2007) Prevalence and antimicrobial resistance of foodborne pathogens isolated from food products in China. Foodborne Pathog Dis 4: 277-284.
34. Thai TH, Lan NT, Hirai T, Yamaguchi R (2012) Antimicrobial resistance in Salmonella serovars isolated from meat shops at the markets in north Vietnam. Foodborne Pathog Dis 9: 986-991.
35. Murungkar HV, Rahman H, Kumar A, Bhattacharya D (2005) Isolation, phage typing and antibiogram of Salmonella from man and animals in northeastern India. Indian J Med Res 122: 237-242.

Corresponding author
Lapusan Alexandra, PhD
Faculty of Veterinary Medicine
University of Agricultural Sciences and Veterinary Medicine
3-5 Manastur Street, Cluj-Napoca, Romania
Tel: +40740140463
Email: lapusan_alexandra@yahoo.

Conflict of interests: No conflict of interests is declared.