Romanian Biotechnological Letters *Vol. 21, No. 5, 2016*

ORIGINAL PAPER

Prevalence of some foodborne pathogens in meat products in Romania

Received for publication, March 3rd 2015
Accepted, June 4th, 2015

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Abstract

An updated data analysis in the changing foodborne pathogens evolution in Romania and improvements in food chain traceability is provided for comparison with trends in the context of global food trade. This report represents a monitoring plan from 2013-2014, that was used to assess the prevalence of some foodborne pathogens from different meat (bovine, sheep, goat, pork, chicken, turkey carcasses, raw and ground meats) belonging to the intensive animal breeding farms, slaughterhouses and retail markets. A number of 41190 meat samples were collected from four different areas of Romania and examined. The foodborne pathogens Salmonella sp., Escherichia coli Biotype I, Escherichia coli serotype O157:H7, Listeria monocytogenes, coagulase-positive Staphylococcus sp. were studied. Salmonella sp. isolates proved to be resistant to 7 out of the tested 17 antibiotics. The prevalence of E. coli Biotype I was the highest in the investigated samples (1.14%), while for L. monocytogenes was 0.38%, and none of the tested products was positive for Escherichia coli VTEC O157:H7 and coagulase-positive Staphylococcus sp.

Keywords: antibiotics, *Escherichia coli*, meat, *Salmonella sp.*

1. Introduction

Foodborne diseases represent a global problem that involves a wide spectrum of illnesses caused by contaminated food and water (NEWELL & al. [1]). Continuous increasing of the global food trade led to the consumer concern related to the food safety, foodborne pathogens, methods for monitoring the microbiological pathogens. Evaluation of epidemiological data of foodborne diseases in different countries is of general interest, especially for global manufacturers and exporters of many agricultural foods. Foodborne pathogens are real threats to the safety of the food supply worldwide, requiring a better understanding of prevalence through national surveys at appropriate intervals. *Salmonella sp., Listeria monocytogenes, Escherichia coli O157:H7* and coagulase-positive foodborne pathogens with highest incidence worldwide that cause human illness by ingestion of contaminated food causing around of 40% of the approximately 50 million annual deaths mostly in the developing countries (GALLHOFF [2]; VAZQUEZ-BOLAND & al. [3]). There have been progresses in alternative electrochemical biosensors for detection of pathogenic bacteria in the food industry (INVITSKI & al. [4]). The screening made in 2008 by the European Union, reported that 131,468 confirmed human cases suggesting that salmonellosis was again the second most

often reported zoonotic disease (EFSA [5]). The summary report on zoonoses and zoonotic agents stated a decrease in human salmonellosis by 8.8% in 2010 compared with 2009. The same report revealed fresh broiler and turkey meat as the most contaminated food with *Salmonella sp.* (EFSA [5]). In chicken carcasses, originated from developing countries, the realities about the prevalence of food pathogens reveal that they are at higher levels of distribution (WILFRED & al. [6]). The prevalence of *Salmonella sp.* in chicken carcasses in Romania (1.8%) is in high contrast with the results obtained in India and Northern Thailand. Salmonellosis is a very important foodborne bacterial illness, with more than 1.3 billion cases reported annually worldwide. *S. typhimurium* and *S. enteritidis* are known to be the serotypes with the highest prevalence in human illness (TAN and SHELEF [7]). By comparison with other EU countries, EFSA report shows for Romania there is an increasing trend in salmonellosis in humans. The number of human salmonellosis, worldwide, has doubled in 2010 compared with 2006 (EFSA [5]). Also, the prevalence of *Salmonella sp.* in poultry meat and meat products increased from 0.6% in 2006 to 5.2% in 2010, in pork meat and meat products increased from less than 0.1% to 2.1%. In 2010 no *Salmonella sp.* was declared in bovine meat and meat products (EFSA [5]). Due to this fact a large number of samples was analyzed in this study in order to detect the prevalence of *Salmonella sp.* among other foodborne pathogens, the serotypes were detected and their susceptibility to antibiotics was tested. For Romania, the incidence of *Salmonella sp.* occurrence in humans between 2007 and 2009 was 11 per 100,000 people (PIRES and HALD [8]). *Salmonella sp.* serotypes *S. typhimurium* and *S. enteritidis* are the most important agents in foodborne salmonellosis in humans (PIRES and HALD [8]). In the last decade several *Salmonella sp.* outbreaks are recorded in Romania and their frequency do not exceed outbreaks studied in worldwide, including European countries like Denmark, Sweden and the Netherlands (HALD & al. [9]; PIRES and HALD [8]; VALKENBURGH & al. [10]; WHALSTRÖM & al. [11]).

Also, in the last decade, infections with *Escherichia coli* VTEC have become a major health problem in the developed countries (EFSA [5]). Most outbreaks and sporadic cases of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) have been attributed to *E. coli* VTEC strains (MAHMOUD [12]). *E. coli* O157:H7 is traditionally associated with outbreaks of diarrhea, hemorrhagic colitis and hemolytic-uremic in humans. Most clinical signs of disease arise as a consequence of production of Shiga-like toxin 1 and 2 (*Stx1*, *Stx2*) or combinations of these toxins (GEHUA & al. [13]). For a long period of time, *L. monocytogenes*proved to be a significant foodborne pathogen of humans (DONGYOU [14]). Various foods and environmental samples have been involved in the spread of *L. monocytogenes* via meat and meat products, vegetables and fish (VAZQUEZ-BOLAND & al. [3]). The safety of the food supplies is very important due to their role in altering the risk of human illness and the occurrence of foodborne disease outbreaks. Although there have been progresses in reducing pathogens in meat products, additional efforts are ongoing to continually improve the safety of all meat products manufactured in Romania. This study presents data on contamination status of the raw and ground meats obtained through the national surveillance system on the territory of Romania during 2013-2014 and to provide a better understanding of the Romanian food safety issues. In addition these results will supply valuable data for future EFSA reports.

2. Materials and Methods

These studies have been done according to the National Program of Surveillance and Control in The Field of Animal Food Safety on the Romanian territory. The samples were collected from four different areas of Romania, in which important laboratories are present

(South-East Area from Braila county, North-East Area from Iasi county, North-West Area from Cluj county and South-West Area from Arad county). A large number of samples were analyzed in this study in order to detect the prevalence of *Salmonella sp.* among other foodborne pathogens, the serotypes were detected and their susceptibility to antibiotics was tested. The analysis was performed according to standardized protocols. This report represents a monitoring plan from 2013-2014, that was used to assess the prevalence of some foodborne pathogens from types of meat from different animal species (bovine, sheep, goat, pork, chicken, turkey carcasses, raw and ground meats) belonging to the intensive animal breeding farms, slaughterhouses and retail markets. All these samples were obtained from several Romanian production plants. These samples were chosen either because studies had shown that these foods contained *Salmonella sp.*, *Listeria monocytogenes*, *Escherichia coli* Biotype I, *Escherichia coli* VTEC O157:H7 and coagulase-positive *Staphylococcus sp.*, or as a means of assessing the prevalence of foodborne in most types of meat produced in Romania (Table 1).

Table 1. Prevalence of food pathogens in different types of meats

Type of meats	Total	Contaminated	*Salmonella sp.*	*Listeria monocytogenes*	*Escherichia coli*Biotype I	*Escherichia coli* VTEC O157	Coagulase-positive *Staphylococcus sp.*
Raw meats							
Carcasses of bovine, sheep, goat	2538	2	2/2499 (0.08)	-	0/26 (0)[a]	0/11 (0)	0/2
Carcasses of chicken (broilers and turkey)	1905	34	34/1888 (1.8)	-	0/17 (0)	-	-
Pork muscle parts	7280	54	51/6442 (0.8)	2/292 (0.7)	1/458 (0.21)	0/35 (0)	0/53
Ground meats							
Mechanically processed meats from poultry intended to be eaten cooked	1216	22	16/833 (1.92)	0/29 (0)	6/353(1.69)	-	0/1
Mechanically processed meats from other species than poultry intended to be eaten cooked	2212 4	160	37/12797 (0.28)	4/374 (1.06)	119/8504 (1.39)	0/403 (0)	0/42
Meat products for immediate consumption (ham, sausages, etc) intended to be eaten without cooking	6127	5	4/3172 (0.1)	1/1146 (0.08)	0/1690 (0)	0/5 (0)	0/114
Total	**4119 0**	**277 (0.67)**	**144/27631 (0.52)**	**7/1841 (0.38)**	**126/11025 (1.14)**	**0/454 (0)**	**0/212 (0)**

For bovine, sheep, goat and pork, there were approximately 110 slaughterhouses and more than 300 production plants for meat products and ground meat. The total number of poultry establishments consisted of approximately 40 abattoirs (of which 30 were high capacity slaughterhouses) and 100 cutting plants. This sampling plan was implemented to allow representative sampling of the entire Romanian meat production process, including the retail level (supermarkets). The total number of samples (41190) taken at the production level was proportional to the capacity of the establishment (number of carcasses slaughtered or quantity of meat produced during 6 months) and took into account the representative distribution of production in all important Romanian counties (Cluj county, Arad county, Constanta county, Iasi county). Samples were placed in a Coleman flask on ice, transported to the laboratory for analysis within 2-4 h after collection. Detection of *Salmonella sp.* was made

following the working procedure described in EN ISO 6579, namely the enrichment of 25g homogenized sample in 225ml buffered peptonate water (BPW) using a stomacher, incubation at 37°C ± 1°C for 18h ± 2h, followed by enrichment of the culture obtained in liquid selective media (Rappaport-Vassiliadis broth with soy – RVS broth, Thermo Fisher Scientific Inc, US) incubated at 41.5°C ± 1°C for 24h ± 3h and tetrathionate Muller-Kauffmann broth with added novobiocin (MKTTnbroth, Thermo Fisher Scientific Inc, US), incubated at 37°C ± 1°C for 24h ± 3h. For isolation, the cultures that were obtained, were dispersed on xylose-lysine-deoxycholate agar (XLD agar, Becton Dickinson GmbH, Germany) and Rambach agar (chromogenic medium, Merck KgaA, Germany) that were incubated at 37°C for 18-24h. Typical colonies were subject of confirmation using biochemical tests (study of biochemical equipment on Triple Sugar Iron agar, Christensen agar, medium LIA byThermo Fisher Scientific Inc, US). Serotyping of the isolates was accomplished by agglutination reactions using polyvalent and monovalent O and H antisera (Denka Seiken Co., Ltd. Tokyo, Japan). The obtained results are presented in Table 2. Susceptibility of *Salmonella sp.* to antimicrobial substances was made according to EUCAST – European Committee on Antimicrobial Susceptibility Testing, 2010. The study of *Salmonella sp.*isolates behavior in the presence of antimicrobial substances was made using the diffusion method with discs impregnated with antimicrobial substances in different concentrations (Oxoid LTD., Basingstoke, Hampshire, England). They were applied on the surface of Mueller Hinton agar previously inoculated with the bacterial suspension brought to a turbidity of 0.5 McFarland and previously prepared from fresh cultures (24 hours) of the target strain. The antibiotic disseminates in the inoculated medium with the tested bacterial strain, resulting in the appearance of a circular area of inhibition with a diameter depending on the tested bacterial strain sensitivity. The applications of this technique, reading and interpretation of the results were made according to NCCLS's recommendations (National Committee for Clinical Laboratory Standards, USA-2003). The results are reported in Table 3. Detection of *Listeria monocytogenes*, was made according to EN ISO 11290:1 following the primary enrichment steps in a selective medium (demi-Fraser broth, Thermo Fisher Scientific Inc, US) by mixing 25g sample in 225 ml medium incubated at 30°C for 24h. The secondary enrichment was made on Fraser broth followed by incubation at 37°C for 48h. For isolation and identification, the obtained cultures were dispersed using OttavianiAgosti agar and Palcam agar and incubated for 24-48h at 37°C. The confirmation of genus affiliation was made by cell and colony appearance morphology observation, mobility test and catalase reaction; the confirmation of the species affiliation was accomplished by the ability to ferment xylose and rhamnose, hemolytic activity and the CAMP reaction. The detection of β-glucuronidase-positive *E. coli* was made according to ISO 16649-2:2007, consisting in duplicate inoculation of agar with tryptone-bile-glucuronide (TBX, Thermo Fisher Scientific Inc, US) plates with 1 ml initial dilution and / or decimal dilutions and the aerobic incubation at 44 ± 1°C for 18-24h. After incubation, the typical CFU of β-glucuronidase-positive *Escherichia coli* (blue colonies) are counted in each dish from two consecutive decimal dilutions. The detection of *Escherichia coli* serotype O157:H7, all isolates were examined for verotoxin virulence genes (S*tx1* and S*tx2*) using PCR and the confirmation of the results was accomplished using classical microbiological techniques. Due to the relation of this pathotype with foodborne outbreaks, the analysis has an utmost importance for Romanian society. The samples were processed in accordance with EN ISO 16654:2002, namely enrichment of 25g homogenized sample in 225 ml medium with modified tryptone soy extract, containing novobiocin (mTSB + N) using a stomacher and incubation at 41.5 ± 1°C. Culture cells were used as samples for PCR reactions. All suspected single colonies were subcultured on Tryptic Soy Agar (TSA) (BD)

and analyzed by PCR. Total DNA was isolated from culture cells using PureLink genomic DNA mini kit (Invitrogen, USA). DNA samples (2µl nucleic acids) were amplified in a 25µl reaction mix containing the following components: GeneAmp 10x PCR Buffer II with $MgCl_2$, 1.25 U AmpliTaq Gold DNA Polymerase (Invitrogen, USA), 2.5mM dNTP and 2 pM of each STX-specific oligonucleotide primer, *Stx1* f CTT CGG TAT CCT ATT CCC GG, *Stx1* r GGA TGC ATC TCT GGT CAT TG, *Stx2* f GCG GTT TTA TTT GCA TTA GC, *Stx2* r TCC CGT CAA CCT TCA CTG TA – as published by GEHUA [13]. Oligonucleotide primers were provided by TibMolBiol, Germany. The final volume was adjusted with nuclease free water. PCR was performed using a iCycler-BIO-RAD thermocycler, with the following program: initial denaturation step at 95°C for 8 min followed by 30 cycles of amplification with 30 s at 94°C for denaturation, 30 s at 56°C for annealing of primers, 30 s at 72°C for primer extension, ending with a final extension at 72°C for 7 min for *Stx1* and an initial denaturation step at 95°C for 8 min, followed by 30 cycles of amplification with denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s, ending with a final extension at 72°C for 7 min, for *Stx2*. The PCR products were visualized on 2% agarose gels stained with ethidium bromide, with a negative control represented by water and a positive control provided by National Food Safety Authority, represented by *E. coli* O157:H7, strain positive for *Stx1* and *Stx2*. Microbiological techniques were used for confirmation. Separation and concentration of the microorganisms using immunomagnetic particles coated with anti-*E. coli* O157:H7 antibodies were performed after 6 hours and again after 18 hours. The isolation was performed by immunomagnetic particles grown on MacConkey agar with cefixime-tellurite-sorbitol (CT-SMAC,Thermo Fisher Scientific Inc, US) and MacConkey agar with sorbitol and BCIG (SMAC with BCIG, Thermo Fisher Scientific Inc, US) media that have been incubated at 37°C for 18-24h. Confirmation of the absence of typical colonies was made by biochemical (indole production) and serological tests (agglutination with *E. coli* O157 antiserum). Detection and counting of coagulase positive *Staphylococcus* sp. (*S. aureus* and other species) were made according to EN ISO 6888-2:2002. The plates of agar containing fibrinogen and rabbit plasma/Baird Parker agar (Thermo Fisher Scientific Inc, US) were inoculated with 1ml initial dilution and/or decimal dilutions of the samples and incubated at 37°C for 24-48h. For the agar plates containing fibrinogen and rabbit plasma, the colonies can be observed after incubation for 24 ± 2 hours and possibly an additional 24 hours at 37 ° C. Coagulase positive *Staphylococcussp.* form black or gray (or white) colonies, surrounded by a halo of precipitate, indicating clotting activity. The test is considered positive when the presence of at least one colony that indicates clotting activity is observed. The use of agar with fibrinogen and rabbit plasma is not required to confirm the colonies by coagulase test, because the composition and characteristics include the coagulase activity. For the Baird Parker agar, typical colonies are black and gray, bright and convex (between 1 mm and 1.5 mm in diameter, after incubation for 24 hours, and between 1.5 mm and 2.5 mm in diameter after incubation for 48 hours at 37° C) surrounded by a clear area that can be partially opaque. After incubation for at least 24 hours an opalescent ring may occur in immediate contact with the colonies in the clear area. It is necessary to confirm these colonies by coagulase test using rabbit plasma citrate.

3. Results and Conclusions

This report presents a monitoring plan from 2013-2014, for measuring the prevalence of some foodborne pathogens from different meats of animal species (Table 1). The presence of foodborne microorganisms has been evaluated in many studies all over the world (ALALI & al.[15]; EL-MALEK & al. [16]; HARA-KUDO & al. [17]; HASSAN & al. [18]). The reports

have a wide range of results depending on the region where the studies were applied, the types of facilities investigated, seasonality or processing methods.

Table 2. *Salmonella* serotypes found in different types of meats

Serotype / Isolation source	*S.* Agona	*S.* Blockley	*S.* Bredeney	*S.* Choleraesuis	*S.* Colorado	*S.* Concord	*S.* Derby	*S.* Enteritidis	*S.* Hadar	*S.* Heidelberg	*S.* Ifantis	*S.* Manchester	*S.* Newport	*S.* Norwich	*S.* Parkroyal	*S.* Reading	*S.* Rissen	*S.*Saintpaul	*S.* Tennessee	*S.* Typhimurium	Total
Carcasses of chicken (broilers and turkey)	1	1	3		2	1	2	4	1	1	1			1		1	1	9	1	4	34
Mechanically processed meats from poultry intended to be eaten cooked	3		3			1		3		1			1					4			16
Pork muscle parts			12	1		5	3	6	3								4	15		2	51
Mechanically processed meats from other species than poultry intended to be eaten cooked	7		2			2		2	1				1					13		9	37
Carcasses of bovine, sheep, goat												2									2
Meat products for immediate consumption (ham, sausages, etc) intended to be eaten without cooking									2						2						4
Total	**11**	**1**	**20**	**1**	**2**	**9**	**5**	**15**	**7**	**2**	**1**	**2**	**2**	**1**	**2**	**1**	**5**	**41**	**1**	**15**	**144**

In our study *S. typhimurium* and *S. enteritidis*were found with a high frequency in the analyzed samples, especially in pork muscle parts (*S. enteritidis*) and mechanically processed meats from other species than poultry (*S. typhimurium*). Interestingly, *S.* Saintpaul was the most frequent serotype found in the analyzed samples in Romania, as shown in Table 2. The situation reported internationally positioned Romania as a country with low contamination with *Salmonella sp.* in meat products. A study in India reported a 69% frequency of isolation of *Salmonella sp.* from chicken carcasses, also in Northern Thailand *Salmonella sp.* prevalence was 57% (BAJAJ & al. [19], PADUNGTOD and KANEENE [20]). Contrary to these findings, in the United States, *Salmonella sp.* was isolated only from 3% of the analyzed meats (ZHAO & al. [21]), with a range from 1.9% in beef meat to 4.2% in chicken meat. In a study elaborated in the United Kingdom between 2003-2005 (LITTLE & al. [22]) an average of 2.4% of the analyzed meats were contaminated with *Salmonella sp.*, pork having the highest contamination (3.9%) and beef having the lowest contamination (1.3%). Our study is similar with the findings from the United States and United Kingdom, evidencing low percentages of contamination with *Salmonella sp.* Although this serotype is not the most important agent of foodborne Salmonellosis in humans, it produced a serious outbreak that occurred in the United States in 2008 (BARTON & al. [23]). *S.* Bredeney, the second most important serotype found in our study has the highest prevalence in pork. Our results are in concordance with the results of a previous study that analyzed *Salmonella sp.* prevalence in meats, water, fruit and vegetables in Ireland from 2005 to 2009 and found the highest frequency of this serotype in raw meat and raw products of porcine origin (DUGGAN & al. [24]). *Salmonella sp.* isolates were tested for 17 antibiotics (Table 3), seven of them did not inhibit growth of the investigated samples. Some strains of *Salmonella sp.* were highly susceptible to gentamicin, ampicillin / sulbactam, ciprofloxacin and ofloxacin. The isolates were most likely to be resistant to flumequine (84.7 percent of isolates), nalidixic acid (78.5 percent), colistinsulfate (78.5 percent) and to a lesser extent, enrofloxacin (15.3 percent). In

addition, no strains were multi resistant (Table 3). *L. monocytogenes*, the overall prevalence of this food pathogen was 0.38, ranging from 0.08% to 1.06% in different groups of meats. The present study reveals similar results with those indicated before. *Listeria* infection, unlike infection with other common foodborne pathogens, is associated with a high case-fatality rate of approximately 20–30% and can resist to different meat processing technologies that involve low temperatures or the use of acids or salts (WATSON [25]).This is why its presence is reported by our study in meat subproducts (0.7%). An interesting result was obtained for the ground meats for immediate consumption (0.08%), as a result of well applied safety procedures in the intensive production plants and slaughtering units. In the United States the prevalence of *Listeria* in ready-to-eat meat is much lower, ranging from 0.52% to 5.16%. These results were similar to other results obtained in 2003 that indicates an overall *Listeria* prevalence of 1.82% (GOMBAS & al. [26]). International situation for *L. monocytogenes* was reported especially in poultry, red meat, and meat products (EL-MALEK & al. [16]; NYENJE & al. [27]) in a variety of countries worldwide (CORDANO and ROCOURT [28]; MENA & al. [29]; RØRVIK & al. [30]; UYTTENDAELE & al. [31]). Studies recorded in several countries mention a high occurrence of *Listeria* in different types of meats. In Brazil, contamination rate with *L. monocytogenes* in ground beef was found to be 25% and 66.7% with *Listeria* ssp. (BARROS & al. [32]). In Egypt, the average contamination rate of meat was 41% (EL-MALEK & al. [16]) and even higher in Malaysia (73.9%) (HASSAN & al. [33]), and Turkey (83.3%) (YUCEL & al.[34]).

Table 3. Susceptibility of *Salmonella* isolates from food products to antimicrobial agents

Antibiotic	Total number of tested isolates	Susceptible		Moderately susceptible		Resistant	
		No.	%	No.	%	No.	%
Ampicillin / Sulbactam	88	80	90.9	0	0.0	8	9.1
Ceftazidime	86	67	77.9	8	9.3	11	12.8
Cefuroxime	23	5	21.7	11	47.8	7	30.5
Ciprofloxacin	144	99	68.7	8	5.6	37	25.7
Colistinsulfate	144	31	21.5	0	0.0	113	78.5
Enrofloxacin	131	110	76.4	12	8.3	22	15.3
Flumequine	144	19	13.2	3	2.1	122	84.7
Gentamicin	144	131	91.0	3	2.1	10	6.9
Nalidixic acid	144	25	17.3	6	4.2	113	78.5
Neomycin	110	19	17.3	16	14.5	75	68.2
Nitrofurantoin	72	25	34.7	27	37.5	20	27.8
Norfloxacin	100	53	53	13	13	34	34
Ofloxacin	114	95	83.3	2	1.7	17	15
Spectinomycin	142	27	19	22	15.5	93	65.5
Streptomycin	144	16	11.1	42	29.2	86	59.7
Tetracycline	144	11	7.6	41	28.5	92	63.9
Ticarcillin	121	46	59.7	13	16.9	18	23.4

The prevalence of *E. coli* Biotype I is low comparing with the data reported in 2001 (ZHAO et al. [21]). In our study, *E. coli* Biotype I was isolated from 1.14% of the analyzed meats with a minimal detection in pork (0.21%) and the highest detection rate in mechanically processed meats from poultry (1.92%). In the United States the frequency of isolation of *E. coli* Biotype I from meats was 21.7% (38.7% from chicken, 19% from beef, 16.3% from

beef). A possible explanation for such a low prevalence is related on the traditional feeding practices in our country, on grain based diet. More advanced research can indicate that the diet may greatly influence the evolution of *E. coli* Biotype I. Although *E. coli* is a part of normal flora of humans and other animals, some strains can lead to diseases (DUNDAS & al. [35]). There are reports about severe illness outbreaks linked to verocytotoxin producing *E. coli* O157:H7 (CONEDERA & al. [36]; GAUTHIER & al. [37]; MEAD & al. [38]). In this investigation no *E. coli* O157:H7 isolate was detected in any of the 454 meat samples analyzed (selected from the four main county laboratories, from Romanian area, in accordance with the National Program of Surveillance and Control in The Field of Animal Food Safety). All the tested samples were negative by conventional PCR. Although there are many reports about coagulase-positive *Staphylococcussp.* as an important contaminant of animal origin food, being also responsible for food shock (CAPITA & al. [39]; GHOSH & al. [40]; KREYENSCHMIDT & al. [41]; KUMAR & al. [42]; NYENJE & al. [27]), no contamination was found in the meat samples collected in 2011-2012 from the designated Romanian slaughtering units and retail sales units. No detections of coagulase-positive *Staphylococcussp.* were made using the tested methods. Although the sensitivity of the test, using BPA, can be questionable there were present only negative colonies. Following 48 hours of incubation the sensitivity increased and no RFP+ colonies were observed. In conclusion, investigating the meat samples collected during 2013-2014 in Romania, the overall prevalence of foodborne pathogens was 0.67%. Two types of foodborne pathogens were detected: *Salmonella sp.* (0.52%), *Listeria monocytogenes* (0.38%) and *Escherichia coli* Biotype I had the highest frequency 1.14% (Table 1). Taking into account that the foodborne pathogens are important determinants of human illness, a close monitoring of food safety under a surveillance program at appropriate intervals is needed.

4. Acknowledgements

The research was supported by the Project QualiMeat – PN-II-PT-PCCA-2011-3.2-0509 and by the strategic grant POSDRU/159/1.5/S/133391, Project "Doctoral and Post-doctoral programs of excellence for highly qualified human resources training for research in the field of Life sciences, Environment and Earth Science" cofinanced by the European Social Found within the Sectorial Operational Program Human Resources Development 2007 – 2013.

References

1. D.G. NEWELL, M. KOOPMANS, L. VERHOEF, E. DUIZER, A.A. KANE, H. SPRONG, M. OPSTEEGH, M. LANGELAAR, J. THREFALL, F. SCHEUTZ, J. GIESSEN, H. KRUSE. Foodborne diseases-the challenges of 20 years ago still persist while new ones continue to emerge. *Inter. J. Food Microbiol*, 139: 3 (2010).
2. G. GALLHOFF. Community strategy against antimicrobial resistance. *Int. J. Med Microbiol*, 296: 7 (2006).
3. J.A. VAZQUEZ-BOLAND, M. KUHN, P. BERCHE, T. CHAKRABORTY, G. DOMÍNGUEZ BERNAL, W. GOEBEL, B. GONZÁLEZ-ZORN, J. WEHLAND, J. KREFT. *Listeria* pathogenesis and molecular virulence determinants. *Clin. Microbiol*, Rev. 14: 584 (2001).
4. D. INVITSKI, I. ABDEL-HAMID, P. ATANASOV, E. WILKINS. Biosensors for the detection of pathogenic bacteria. *Biosens Bioelectron*, 14: 624 (1999).
5. European Food Safety Authority and European Centre for Disease Prevention and Control. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Foodborne Outbreaks in the European Union in 2010. *EFSA J.*, 10: 2597 (2012).
6. R.S. WILFRED, P.K. NITHIN, K.G.S. NAVEEN. Prevalence of foodborne pathogens in market samples of chicken meat in Bangalore. *Inter. Food Res. J*, 194: 1763 (2012).

7. W. TAN, L. A. SHELEF. Automated detection of *Salmonella spp*. in foods. *J. Microbiol. Meth*, 37: 87 (1999).
8. S.M. PIRES, T. HALD.Assessing the differences in public-health impact of *Salmonella* subtypes using a Bayesian microbial subtyping approach for source attribution.*Food.Path.Dis*, 7: 143 (2010).
9. T. HALD, D. VOSE, H.C. WEGENER, T. KOUPEEV. A Bayesian approach to quantify the contribution of animal-food sources to human salmonellosis.*RiskAnal*, 24: 255 (2004).
10. S. VALKENBURGH, R. OOSTEROM, O. VAN STENVERS, M. AALTEN, M. BRAKS, B. SCHIMMER, A. GIESSEN, W. VAN PELT, M. LANGELAAR. Zoonoses and Zoonotic Agents in Humans, Food, Animals and Feed in The Netherlands 2003-2006. RIVM rapport number: 330152001. ISBN-13, 978-90-6960-184-7 (2007).
11. H. WHALSTRÖM, Y. ANDERSSON, L. PLYM-FORSHELL, S.M. PIRES. Source attribution of human *Salmonella* cases in Sweden. *Epidemi. Infect*, (2010).
12. A. MAHMOUD. Molecular characterization of diarrheagenic*Escherichia coli* isolated from meat products sold at Mansoura city, Egypt. *Food Contr*., 25: 159 (2012).
13. W. GEHUA, C.G. CLARK, F.G. RODGERS. Detection in *Escherichia coli* of the genes encoding the major virulence factors, the genes defining the O157:H7 serotype and components of the Type 2 shiga toxin family by Multiplex PCR. *J. Clin. Microbiol*, 40: 3613 (2002).
14. L. DONGYOU.Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen.*J. Med. Microbiol*, 55: 645 (2006).
15. W.Q ALALI, S. THAKUR, R.D. BERGHAUS, M. P. MARTIN, W. A. GEBREYES. Prevalence and distribution of *Salmonella* in organic and conventional broiler poultry farms. *Food. Path. Dis*, 7: 1363 (2010).
16. A.M.A. EL-MALEK, S.F. H. ALI, R. HASSANEIN, A. M. MOEMEN, K. I.ELSAYH. Occurrence of *Listeria* species in meat, chicken products and human stools in Assiut city, Egypt with PCR use for rapid identification of *Listeria monocytogenes*. *Vet. W*., 3: 353 (2010).
17. Y. HARA-KUDO, H. KONUMA, Y. KAMATA, M. MIYAHARA, K. TAKATORI, Y. ONOUE, Y. SUGITA-KONISHI, T. OHNISHI.Prevalence of the main foodborne pathogens in retail food under the national food surveillance system in Japan.*FoodAdditiv.Contam*., A. 745097: 1 (2012).
18. A.N. HASSAN, A. FAROOQUI, A. KHAN, A.Y. KHAN, S.U. KAZMI. Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan. *J. Infect. Develop*. Ctries. 6: 382 (2010).
19. B.K. BAJAJ, S. VIBHOR, K. SANJANA, R.L. THAKUR. Prevalence of *Salmonella* in poultry and meats and growth inhibition of *Salmonella enteritidis* by organic acids.*J. Food Sci. Technol*. 40: 556 (2003).
20. P. PADUNGTOD, J. B. KANEENE. *Salmonella* in food animals and humans in northern Thailand.*Inter. J. Food Microbiol*. 108: 346 (2006).
21. C. ZHAO, B. GE, J. DE VILLENA, R. SUDLER, E. YEH, S. ZHAO, D. G. WHITE, D. WAGNER, J. MENG. Prevalence of *Campylobacter* spp., *Escherichia coli* and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, D.C., area. *Appl. Environ. Microbiol*. 67: 5431 (2001).
22. C.L. LITTLE, J.F. RICHARDSON, R.J. OWEN, E. DE PINNA, E.J. THRELFALL. *Campylobacter* and *Salmonella* in raw red meats in the United Kingdom: prevalence, characterization and antimicrobial resistance pattern, 2003-2005. *Food Microbiol*.3: 538 (2008).
23. B. C. BARTON, R.K. MODY, J. JUNGK, L. GAUL, J.T. REDD, S. CHEN, S. COSGROVE, E. HEDICAN, D. SWEAT, L. CHÁVEZ-HAUSER. Outbreak of *Salmonella* Saintpaul infections associated with raw produce. *New Engl. J. Med*. 10: 918 (2008).
24. S. DUGGAN, E. JORDAN, M. GUTIERREZ, G. BARRETT, T. O'BRIEN, D. HAND, K. KENNY, J. FANNING, N. LEONARD, J. EGAN.*Salmonella*in meats, water, fruit and vegetables as disclosed from testing undertaken by Food Business Operators in Ireland from 2005 to 2009. *I. Vet. J.*, 65: 17 (2012).
25. R. WATSON. Deaths from listeriosis remains a cause for concern in Europe. *Brit. Med. J*. 338: 319 (2009).
26. D. E. GOMBAS, Y. CHEN, R. S. CLAVERO, V. N. SCOTT. Survey of *Listeria monocytogenes* in ready-to-eat foods. *J. Food Protect*. 66: 559 (2003).
27. M.E. NYENJE, C.E. ODJADJARE, N.F. TANIH, E. GREEN, R. N. NDIP. Foodborne pathogens recovered from ready-to-eat foods from roadside cafeterias and retail outlets in Alice, Eastern Cape Province, South Africa: public health implications. *Inter. J. Environ. Research Pub. Health*. 9: 2608 (2012).

28. A.M. CORDANO, J. ROCOURT.Occurrence of *Listeria monocytogenes* in food in Chile. *Inter. J. Food Microbiol.*70: 175 (2001).
29. C. MENA, G. ALMEIDA, L. CARNEIRO, P. TEIXEIRA, T. HOGG, P.A. GIBBS.Incidence of *Listeria monocytogenes* in different food products commercialized in Portugal. *Food Microbiol*.21: 213 (2004).
30. L. M. RØRVIK, B. AASE, T. ALVESTAD, D. A. CAUGANT. Molecular epidemiological survey of *Listeria monocytogenes* in broilers and poultry products.*J. Appl.Microbiol*. 94: 6333 (2003).
31. M. UYTTENDAELE, P. DE TROY, J. DEBEVERE.Incidence of *Listeria monocytogenes* in different types of meat products on the Belgian retail market.*Inter.J. Food Microbiol*. 53: 75 (1999).
32. M.A BARROS, L.A. NERO, L.C. SILVA, L. D'OVIDIO, F.A. MONTEIRO, R. TAMANINI, R. FAGNANI, E. HOFER, V. BELOTI.*Listeriamonocytogenes*: Occurrence in beef and identification of the main contamination points in processing plants. *Meat Sci*. 764: 591 (2007).
33. Z. HASSAN, P.E. ENDANG, R.S.R. ABDUL, G. RUSUL. Prevalence of *Listeria* species and *L. monocytogenes* in meat and fermented fish in Malaysia.*South.Asian J. Trop. Med. Pub. Health*.32: 99 (2001).
34. N. YUCEL, S. CITAK, M. ONDER.Prevalence and antibiotic resistance of *Listeria* species in meat products in Ankara, Turkey. *Food Microbiol*. 22: 241 (2005).
35. S. DUNDAS, W.T.A. TODD, A.I. STEWART, I.A. STEWART, P.S. MURDOCH, A.K.R. CHAUDHURI, S.J. HUTCHINSON. The Central Scotland *Escherichia coli* O157:H7 Outbreak: Risk Factors for the Hemolytic Uremic Syndrome and Death among Hospitalized Patients. *Clin. Infect. Dis*. 7: 923 (2001).
36. G. CONEDERA, E. MATTIAZZI, F. RUSSO, E. CHIESA, I. SCORZATO, S. GRANDESSO, A. BESSEGATO, A. FIORAVANTI, A. CAPRIOLI.A family outbreak of *Escherichia coli* O157 haemorrhagic colitis caused by pork meat salami.*Epidemiol. Infect*. 135: 311 (2007).
37. M. GAUTHIER, M. SIMARD, B.W. BLAIS. Prevalence of *Escherichia coli* O157: H7 and *Salmonella* in traditional meats derived from game animals in Nunavik. Rur. Remo. *Health*, 10: 1329 (2010).
38. P.S. MEAD, L. SLUTSKER, V. DIETZ, L.F. MCCAIG, J.S. BRESEE, C. SHAPIRO, P.M. GRIFFIN, R.V. TAUXE. Food-related illness and death in the United States. *Emerg. Infect. Dis*. 5: 607 (1999).
39. R. CAPITA, C. ALONSO-CALLEJA, M.D.C. GARCÍA-FERNÁNDEZ, B. MORENO. Microbiological quality of retail poultry carcasses in Spain. *J. Food Protect*. 64: 1961 (2001).
40. M. GHOSH, S. WAHI, M. KUMAR, A. GANGULI.Prevalence of enterotoxigenic *Staphylococcus aureus* and *Shigella* spp. in some raw street vended Indian foods. *Inter. J. Environ. Health Res.* 17: 151 (2007).
41. J. KREYENSCHMIDT, N. PETERS, B. PETERSEN, B. KUNZ.Characterisierung des Verderbs von Frischfleisch – Veränderung mikrobiologischer und biochemischer Parameter von Geflügelfleisch bei unterschidlichen Lagertempraturen. *Fleischwirtschaft International*. 82: 108 (2002).
42. M. KUMAR, D. AGARWAL, M. GHOSH, A. GANGULI. Microbiological safety of street vended fruit chats in Patiala city. *Ind. J. Med. Microbiol*. 24: 75 (2006).