ISSN: 1220-3173; **E-ISSN**: 2457-7618

RESEARCH ON THE ISOLATION AND IDENTIFICATION OF SOME BACTERIA FROM FOOD PRODUCTS OF ANIMAL ORIGIN

CERCETĂRI PRIVIND IZOLAREA ȘI IDENTIFICAREA UNOR BACTERII DIN PRODUSE ALIMENTARE DE ORIGINE ANIMALĂ

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ABSTRACT

Diseases due to the consumption of food of animal origin have an important role in the field of public health, since the food constitutes an essential way of contaminating humans with bacterial germs. In addition to possible contamination from an animal source, food is also subject to accidental contamination throughout processing until consumption. During five years, a total of 199 samples of poultry, pork, sheep meat, and dairy products were collected from butcheries, supermarkets, and grocery stores in Harghita County. Standard methods SR EN ISO 6579/2003 AC/2006, SR EN ISO 10272/2006, and EN/ISO 11290-1:2017 were used for serotyping the bacterial strains. The Vitek2 Compact system was used for the identification of the isolated strains, using specific cards for both Gram-positive and Gram-negative bacteria. The results showed the bacterial strains differed by year and type of product. Poultry and pig meat and meat products had the highest frequency of *Salmonella* spp. contamination, while milk samples and dairy products were predominantly contaminated with *Escherichia coli*. *Salmonella enterica* subsp. *enterica* serovar Coeln was identified for the first time in Romania.

Keywords: bacterial strains, dairy products, identification, meat products

REZUMAT

Îmbolnăvirile datorate consumului de alimente de origine animală dețin o pondere importantă în domeniul sănătății publice, întrucât alimentele constituie calea esențială de contaminare a omului cu germeni bacterieni. Pe lângă posibila contaminare dintr-o sursă animală, alimentele sunt supuse și unor contaminări accidentale, pe tot parcursul prelucrării până la introducerea în consum. Pe parcursul a cinci ani, au fost recoltate în total 199 de probe de carne de pasăre, porc, oaie, respectiv produse lactate de la măcelarii, supermarketuri și magazine alimentare din județul Harghita. Au fost utilizate metode standardizate SR EN ISO 6579/2003 AC/2006, SR EN ISO 10272/2006 și EN/ISO 11290-1: 2017. Sistemul Vitek2 Compact a fost utilizat pentru identificarea tulpinilor izolate, folosind carduri specifice atât pentru bacterii Grampozitive, cât și pentru Gram-negative. Rezultatele au arătat că tulpinile bacteriene diferă în funcție de an și tip de produs. Carnea de pasăre și porc, precum și produsele din carne au avut cea mai mare frecvență de contaminare cu *Salmonella* spp., în timp ce probele de lapte și produsele lactate au fost predominant contaminate cu *Escherichia coli*. *Salmonella enterica* subsp. enterica serovar Coeln a fost identificat pentru prima dată în România.

Cuvinte cheie: tulpini bacteriene, produse lactate, identificare, produse din carne

Among food pathogens, *Salmonella, Escherichia coli, Listeria*, and *Campylobacter* species are recognized as some of the most important food contaminants, which can cause severe infections in humans and large economic losses worldwide (1, 2, 8, 16, 18).

Currently, the World Health Organization (WHO) considers food poisoning caused by bacteria belonging to these genera as one of the most important diseases caused by the consumption of contaminated food. For example, globally, it is estimated that non-typhoidal *Salmonella* infections range from 200 million to 1.3 billion, with a mortality rate of approximately three million each year. Listeriosis affects vulnerable consumers (e.g., immunocompromised individuals, neonates, and the elderly) and pregnant women, producing invasive infections with a high case-fatality rate (20–30%) and miscarriages, whereas in healthy adults, the infection is self-limiting and is characterized only by a febrile gastroenteritis (6, 12).

The presence of microorganisms with an important role in food contamination (e.g., *Salmonella*, *E. coli*, *Listeria*, *Yersinia*, and *Campylobacter*), both in the food flow and at the point of sale, is also of particular importance for veterinary pathology.

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The positive evolution in identifying these food-borne pathogens, especially in recent years, is largely due to the modernisation of identification and diagnostic methods, as opposed to the increase in the degree of food contamination (5, 10, 13, 15, 19).

In addition, the importance of these germs, for animal and human pathology also results from the fact that the prevention of bacterial infections is very difficult, since, with few exceptions, they do not have host specificity and thus the possibility of their survival and multiplication, even in the abiotic environment (e.g., food, feed, and water). Moreover, man intervenes decisively through incorrect handling, transport, storage, or processing activities (8, 14, 17).

In this context, the aim of this study was to isolate the main potentially pathogenic bacterial species from animal products using classical and standardised bacteriological methods, followed by their identification using the Vitek2 Compact system.

MATERIALS AND METHODS

The research was carried out over five years (from 2017 to 2021) on a total of 199 samples of poultry, pork, sheep meat (fresh refrigerated meat, minced meat, mince paste, and sausages), and dairy products (traditionally kneaded cheese, curd, cream 12% fat, and urda) taken from sales units (butchery, supermarket, grocery store) in Harghita County. The refrigerated and minced meat samples came from three species, namely pork, poultry, and sheep, while dairy samples were of bovine and ovine origin.

National standardized methods SR EN ISO 6579/2003 AC/2006 (Romanian Standards Association, 2003), SR EN ISO 10272/2006 (Romanian Standards Association, 2006), and the EN/ISO 11290-1: 2017 two-step enrichment method were used for the isolation, identification, and serotyping of bacterial strains (20, 21).

Preliminary determination of *Salmonella* and *E. coli* was carried out following classical examination methods, respectively bacteriological examinations, including microscopic examination (Gram staining method) for morphological characters and examination of cultural characters following the culture on usual media (meat broth, nutrient agar, and blood agar). In the next step, Levine, Rambach, and Edel-Kampermacher selective media were used to identify the Gram-negative bacteria, while Oxford and Chapman media were used for the identification of Gram-positive bacteria. After inoculating, the media were incubated for 24 hours at 37°C. After incubation, the plates were read and the cultural characters of the main colonies developed, including pigmentogenesis, were assessed on isolated colonies.

From these isolated colonies, inoculations on blood agar were performed in order to obtain pure cultures and each culture was placed in the thermostat for 24 hours. Afterwards, from each culture, a bacterial suspension was made and it immersed in 3 ml of saline solution (0.9% NaCl) in a 12x75mm plastic tube. Each suspension was brought to an optimum density, between 0.5 - 0.63 McFarland (3).

Finally, the identification of bacterial strains was performed with the Vitek2 Compact system, using specific cards for both Gram-positive and Gram-negative bacteria.

RESULTS AND DISCUSSIONS

The primary identification allowed the classification of all isolated strains into Gram-negative species (159) and Gram-positive species (40), with bacillary and coccobacillary forms and sizes ranging from 2-4/0.5-0.7 µm, non-encapsulated, unsporulated, but ciliated. Subsequently, on Levine, Edel-Kampelmacher, and Rambach selective culture media, colony-forming strains with specific characteristics of *Salmo-*

Table 1

Results of bacterial identification in samples taken in 2017

Crt. no.	Sample category	Matrix	No. of isolated strains	Species	Serotyping result
1	Fresh meat	Chicken legs	3	*Salmonella* spp.	*Salmonella* Infantis
2	Fresh meat	Pork neck	3	*Salmonella* spp.	*Salmonella* Infantis
3	Fresh meat	Mince paste	2	*Salmonella* spp.	*Salmonella* Infantis
4	Fresh meat	Mince paste	2	*Salmonella* spp.	*Salmonella* Typhmurium
5	Fresh meat	Pork neck	3	*Salmonella* spp.	*Salmonella* Infantis
6	Fresh meat	Chicken legs	2	*Salmonella* spp.	*Salmonella* Derby
7	Fresh meat	Pork neck	2	*Salmonella* spp.	*Salmonella* Typhmurium
8	Fresh meat	Boneless pork leg	3	*Salmonella* spp.	*Salmonella* Infantis
9	Fresh meat	Boneless pork leg	2	*Salmonella* spp.	*Salmonella* Typhmurium
10	Fresh meat	Pork minced meat	3	*Salmonella* spp.	*Salmonella* Rissen
TOTAL STRAINS			25		

Table 2

Results of bacterial identification in samples taken in 2018

Crt. no.	Sample category	Matrix	No. of isolated strains	Species	Serotyping result
1	Fresh meat	Chicken legs	5	*Salmonella* spp.	*Salmonella* Goldcoast
2	Fresh meat	Chicken legs	5	*Salmonella* spp.	*Salmonella* Infantis
3	Dairy product	Cream 12% fat	5	*E. coli*	*E. coli O121*
4	Dairy product	Kneaded cheese	5	*E. coli*	*E. coli O121*
5	Fresh meat	Boneless pork leg	5	*Salmonella* spp.	*Salmonella* Brandemburg
6	Fresh meat	Boneless pork leg	5	*Salmonella* spp.	*Salmonella* Infantis
7	Fresh meat	Pork neck	2	*Salmonella* spp.	*Salmonella* Derby
8	Fresh meat	Boneless pork leg	5	*Salmonella* spp.	*Salmonella* Infantis
9	Fresh meat	Minced pork	5	*E. coli*	*E. coli* untypeable
10	Fresh meat	Pork neck	5	*Salmonella* spp.	*Salmonella* Typhmurium
TOTAL STRAINS			47		

nella spp. and *Escherichia coli* pathogens were successfully isolated. Colonies specific to *Listeria monocytogenes* were also obtained on Oxford agar, while on Chapman medium, the bacterial colonies were absent. Following bacterioscopic and cultural examination, the strains were included in three genera, namely *Salmonella*, *Escherichia*, and *Listeria*.

Examination with the Vitek2 Compact system was made in the shortest time after the samples reception, and the results obtained were centralised for each year and sample category. Thus, of the total samples taken in 2017, all 25 isolates obtained were included in the *Salmonella* genus, regardless of the matrix from which the samples were taken (chicken flesh, pork neck, mince, and boneless pork flesh), while serotyping revealed the presence of four serovars, namely *S.* Infantis (14 strains), *S.* Typhmurium (6 strains), *S.* Derby (2 strains), and *S.* Rissen (3 strains). The results showed that *S.* Rissen serotype was isolated only from minced pork meat, whereas the other serovars were present in both pork and poultry meat (Table 1).

In 2018, a total of 47 strains were isolated, out of which 15 (31.91%) were included in *E. coli* species, and respectively 32 (68.09%) in *Salmonella* species. Of the *E. coli* strains, ten were isolated from dairy products (cream, traditional kneaded cheese) and included in the O121 serotype, while the remaining five strains were isolated from minced pork (Table 2).

From the *Salmonella* strains, in 2018, compared to 2017, new serotypes such as *S.* Goldcoast and *S.* Brandemburg were identified, which together with *S.* Infantis, *S.* Derby, and *S.* Typhmurium, were identified in both pork and poultry. It is worth mentioning the fact that among the identified serovars, *Salmonella* Goldcoast was also identified by Bărbălan et al. in 2021, during the research carried out for the doctoral thesis, thus being identified for the second time in our country in products of animal origin (3).

In 2019, a number of 41 bacterial strains were isolated from fresh meat, minced meat, urda, and sheep curd. Among the isolates, *E. coli* (22 strains) showed a higher isolation frequency compared to previous years, followed by *Salmonella* (19 strains). Moreover, it was observed that, in addition to the *S.* Infantis and *S.* Ty-

Table 3

Results of bacterial identification in samples taken in 2019

Crt. no.	Sample category	Matrix	No. of isolated strains	Species	Serotyping result
1	Fresh meat	Minced pork	4	*Salmonella* spp.	*Salmonella* Typhmurium
2	Poultry meat	Chilled chicken legs	5	*Salmonella* spp.	*Salmonella* Kentucky
3	Poultry meat	Frozen chicken legs	5	*Salmonella* spp.	*Salmonella* Infantis
4	Poultry meat	Chicken breast	5	*Salmonella* spp.	*Salmonella* Infantis
5	Fresh meat	Minced pork	5	*E. coli*	*E. coli untypeable*
6	Fresh meat	Minced pork	5	*E. coli*	*E. coli untypeable*
7	Fresh meat	Minced pork	5	*E. coli*	*E. coli untypeable*
8	Dairy product	Urda	1	*E. coli*	*E. coli untypeable*
9	Fresh meat	Minced pork	5	*E. coli*	*E. coli untypeable*
10	Dairy product	Sheep curd	1	*E. coli*	*E. coli untypeable*
TOTAL STRAINS			41		

Table 4

Results of bacterial identification in samples taken in 2020

Crt. no.	Sample category	Matrix	No. of isolated strains	Species	Serotyping result
1	Meat product	Smoked pork sausages	5	*Listeria* spp.	*Listeria monocytogenes*
2	Fresh meat	Fresh pork sausages	5	*Listeria* spp.	*Listeria monocytogenes*
3	Meat product	Smoked pork sausages	5	*Listeria* spp.	*Listeria monocytogenes*
4	Fresh meat	Smoked pork sausages	5	*Listeria* spp.	*Listeria monocytogenes*
5	Dairy product	Cow curd	5	*Salmonella* spp.	*Salmonella* Typhmurium
6	Fresh meat	Minced pork	5	*Salmonella* spp.	*Salmonella* Enteritidis
7	Fresh meat	Dry mutton sausages	2	*Salmonella spp.*	*Salmonella* Coeln
8	Fresh meat	Chicken legs	2	*Salmonella* spp.	*Salmonella* Coeln
9	Meat product	Smoked pork sausages	5	*Listeria* spp.	*Listeria monocytogenes*
10	Meat product	Dry mutton sausages	5	*Listeria* spp.	*Listeria monocytogenes*
TOTAL STRAINS			44		

phimurium serotypes isolated from chicken legs and chicken breast, respectively minced meat, the *S.* Kentucky serotype was also isolated from five samples of chicken legs (Table 3). By analysing the results obtained in 2020, a number of 44 bacterial strains were identified, originating from samples of pork (minced meat, fresh and smoked pork sausages), chicken meat (chicken thighs), sheep meat (dried sheep sausages), and curd. The strains were classified into two genera, *Listeria* (30 strains) and *Salmonella* (14 strains).

In the case of *Listeria* genus, all 30 strains isolated from fresh pork sausages (5 strains), smoked pork sausages (20 strains), and smoked sheep sausages (5 strains) were included in the species *Listeria monocytogenes*. On the other hand, *Salmonella* serovars were identified in samples of chicken legs (4 strains), minced pork (5 strains), and cow curd (5 strains) (Table 4). Among the identified *Salmonella* serotypes, *S.* Enteritidis isolated from minced pork samples is commonly found in food products of animal origin. *Salmonella* Coeln*,* isolated and identified in four samples of chicken legs is mentioned for the first time in Romania. This serotype is very rarely identified in food products of animal origin (7), an aspect that once again emphasizes the sporadic spread of *Salmonella* serotypes in our country.

In 2021, a total of 42 bacterial strains were isolated and identified from samples of poultry (meat and skin), pork (respectively pork leg, with bone and boneless, minced meat, and fresh and smoked sausages), and cow curd. The isolated bacteria were included in *Salmonella* genus (32 strains), *S.* Enteritidis*, S.* Infantis*, S.* Risen*, S.* Typhimurium serovars*,* respectively in *Listeria monocytogenes* (10 strains) (Table 5).

Thus, analysing the results obtained in this research, it can be mentioned that two bacterial species were identified, namely *Listeria monocytogenes* (40 strains) and *Escherichia coli* O121 (ten strains) and non-typable *Escherichia coli* (27 strains), while the other strains, up to 199, were included as serovars of the *Salmonella* genus. Similar results to those obtained from the meat samples were also found in research carried out in Libia on 100 samples of raw meat products (minced meat and beef burger) collected from different

Table 5

Results of bacterial identification in samples taken in 2021

Crt. no.	Sample category	Matrix	No. of isolated strains	Species	Serotyping result
1	Fresh meat	Boneless pork leg	5	*Salmonella* spp.	*Salmonella* Rissen
2	Fresh meat	Pork leg	5	*Salmonella* spp.	*Salmonella* Rissen
3	Fresh meat	Pork sausages	5	*Salmonella* spp.	*Salmonella* Rissen
4	Fresh meat	Boneless pork leg	5	*Salmonella* spp.	*Salmonella* Rissen
5	Fresh meat	Cow curd	5	*Salmonella* spp.	*Salmonella* Typhmurium
6	Fresh meat	Minced pork	5	*Salmonella* spp.	*Salmonella* Enteritidis
7	Fresh meat	Poultry meat	1	*Salmonella* spp.	*Salmonella* Infantis
8	Fresh meat	Poultry skin	1	*Salmonella* spp.	*Salmonella* Infantis
9	Meat product	Smoked pork sausages	5	*Listeria spp.*	*Listeria monocytogenes*
10	Fresh meat	Fresh pork sausages	5	*Listeria spp.*	*Listeria monocytogenes*
TOTAL STRAINS			42		

markets and butcher shops. The researchers found that the incidence of *E. coli* in the samples was between 12 and 24%, and the isolated serotypes O91:H21, O121: H7, O78, O124, and O44:H18 had various rates. Also, the overall incidence of isolated strains of *Salmonella* spp. ranged from 4% to 6%, with the identification of *S.* Typhimurium, *S.* Enteritidis, and *S.* Inganda serovars. The authors concluded by highlighting the importance of personal hygiene of the sellers and the awareness about different sources of contamination in order to prevent cross contamination from the hide of slaughtered animals during skinning and evisceration to avoid contamination of such meat and offal by faecal matter (11). Also, in a study on the prevalence and antimicrobial resistance of *Listeria monocytogenes* isolated from beef meat, authors found that out of 140 samples collected from supermarkets, butcheries, and traditional markets, the prevalence of *L. monocytogenes* was 7.14%, with most of the strains detected in butcheries (6 strains) and supermarkets (4 strains). The authors stated that three strains were resistant to all nine tested antimicrobials, while all isolates were resistant to at least one antimicrobial, pointing out the importance of the severe application of quality systems at the slaughterhouse and retail site levels (4).

CONCLUSIONS

Based on cultural and morphological characteristics, 199 strains isolated from pork, poultry, sheep, and dairy samples were included in the *Salmonella* genus (122 strains), *Escherichia coli* (37 strains), and *Listeria monocytogenes* (40 strains). The bacterial strains identified as *Escherichia coli* species, were confirmed by the Vitek-2 Compact system, and after the serotyping, ten strains were included to O121 serotype, while the remaining 27 strains were untippable. Meat and meat products with the highest frequency of *Salmonella* spp. contamination were of poultry and pig origin, while milk samples and dairy products were predominantly contaminated with *Escherichia coli*. The incidence of *Salmonella* serovars was variable and there were nine types identified, among which can be mentioned the presence of some rare serovars, such as *Salmonella* Goldcoast or *Salmonella* Coeln, which was identified for the first time in Romania, an aspect that underlines the sporadic spread of serotypes in our country.

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