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Study regarding *Escherichia coli* serotypes isolated in meat products from Alba County, Romania

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Abstract

50 samples of minced meat and prepared raw meat commercialized on Alba County were analyzed to determinate the microbial growth of *Escherichia coli* positive to β-glucuronidase. Results indicate a high percentage of positive samples (36%) due to inadequate conditions of hygiene during handling. Positive samples had undergone further investigations by isolation of *E. coli* strains and their serological analysis. Isolated strains showed positive reactions with certain types of O antisera agglutinative, which could be assigned to different virulent groups.

Keywords: meat products, contamination, *Escherichia coli*, serotypes

1. Introduction

In recent decades, certain food pathogenic bacteria have been highlighted as new threats to human health. Some of these bacteria can be part of natural microflora. They can live in the host body for a while, and then multiplying by new means of transmission or to new ecological niches.

Escherichia coli is a ubiquitous bacteria, present naturally in large numbers in the human digestive tract. However, there are pathogenic strains capable of producing illness. *E. coli* are important for food sanitation; its presence is considered a hygiene indicator of health and fecal contamination. All food can be contaminated with *E. coli* and its presence is an important indicator of hygiene in handling, processing and storage of food products. Also, it is recognized the bacilli coli resistance to environmental factors [1]. *E. coli* contamination sources are animal foods (meat, milk) or feed contaminated with faeces during slaughter or milking, respectively during handling and processing [2].

In present more serological variants of *E. coli* are know. They contain a somatic O, flagellar H and capsular K antigens. *E. coli* are classified into several virulent groups as they produce pathological reaction. In each group, bacteria are part of certain serotypes O, depending on their reactions with specific antibodies. Although identification of *E. coli* requires checking their pathogenicity, *E. coli* presents often specific serotypes.

Enteroaggregative E. coli (EAEC) produce enterotoxin and citotoxin causing diarrhea especially at children.

Enterohaemorrhagic E. coli (EHEC) affect mainly the colon and produce large amounts of toxin (Shiga-like toxins and verotoxin). The prototype of this group is *Escherichia coli* O157: H7. It cause bloody diarrhea (hemorrhagic colitis), severe abdominal cramps, vomiting and in some cases can affect kidney function. At some people can also cause haemolytic uraemic syndrome (HUS).

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The reservoir of this pathogen appears to be mainly cattle and other ruminants.

Enteroinvasive E. coli (EIEC) infection causes a syndrome that is identical to *Shigellosis*, with diarrhea and high fever. EIEC are highly invasive, and they use adhesin proteins to bind and to enter into intestinal cells. They produce no toxins, but severely damage the intestinal wall through mechanical cell destruction. Main serotypes are O124 and O164, and could be linked with certain foods (Brie and Camembert cheeses made from raw milk or hamburger meat). Other important serotypes are: O28, O112, O136, O143, O144, O152. Any food contaminated with human feces from a sick person, either directly or through contaminated water could cause disease. One major poisoning caused by EIEC was held in 1973 in the U.S. due to a batch of cheese imported from France [3].

Enteropathogenic E. coli (EPEC) are not generated of toxins, but cause diarrhea and intestinal mucosal lesions at infants. Most commonly serotypes are: O26, O44, O55, O86, O111, O114, O119, O125, O126, O127, O142 and O158. EPEC outbreaks involve foods like beef and chicken, but any food exposed to fecal contamination may be suspected [4].

Enterotoxigenic E. coli (ETEC) produce enterotoxins causing diarrhea (traveler's diarrhea – TD) in the country under developing. ETEC was rarely isolated in developed countries, although occasional outbreaks have occurred in Europe and USA [5]. The main source is represented by different types of food and contaminated water. Enterotoxins produced by ETEC include heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST) or both. O-groups from ETEC are: O8, O6, O15, O25, O27, O78, O115, O148, O159, etc. These organisms were rarely isolated from dairy products such as soft cheese [6].

Frequency of *E. coli* is higher at children, and the infections caused by EPEC are found only at children up to two years. EIEC cause diseases or sporadic form of epidemic outbreaks and usually spreads by food. ETEC is frequently encountered in the summer months. In a study conducted in a region of northwestern Spain, ETEC colonies were isolated in 13 of 19 positive samples from children with diarrhea. The most common ETEC serotypes were O153, O27 and O6 [7].

Other studies show that ETEC strains O153: H45 are the source of sporadic and mass outbreaks of diarrhea. Other strains isolated were O27 and O6 [8].

The purpose of the study was to analyze microbial growth of *Escherichia coli* in some meat products from Alba County. The serotypes of *E. coli* were also established.

2. Materials and Method

50 samples of minced meat and prepared raw meat, manufactured in different production units (butcher's shops) from Alba County were taken for analysis. Refrigerated samples were collected after meat processing (five samples each of about 300g). Raw material is represented by pigs and cattle meat. Samples were subjected to microbiological examination of *Escherichia coli* positive β-glucuronidase [9].

10 g of samples is homogenized with 90 ml Peptone Saline Water in order to obtain the initial dilution (10^{-1}). Decimal dilutions are made till 10^{-3}. 1 ml of resulting sample is introduced into two Petri plates for each dilution. 15 ml tryptone bile x-glucuronide (TBX) medium (melted and cooled at 44°C) are added. The Petri plates are allowed to solidify on a cold horizontal surface. A blank sample with 15 ml of medium is made in order to check the sterility. The Petri plates are placed with the lid down, in an incubator at 44°C for 18h to 24h.

After the incubation, typical CFU (colony-forming unit) of *Escherichia coli* positive to β-glucuronidase are counted in each plate containing less than 150 CFU (blue) and less than a total of 300 CFU (figure 1). Strains of *E. coli* that are not growing at 44°C (negative to β-glucuronidase), especially *E. coli* O157 will not be detected by this method.

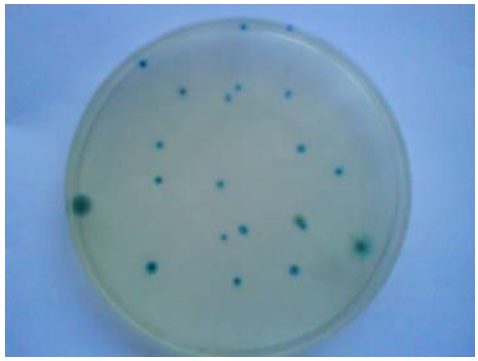

Figure 1. *E. coli* colonies positive to β-glucuronidase on the TBX medium

The number of CFU (N) is calculated according to relation 1:

$$N = \frac{\sum a}{V \times (n_1 + 0{,}1 n_2) d} \quad (1)$$

were:

Σa - CFU counted on all Petri plates held in two successive dilutions; V - volume of inoculums added on each Petri plate [ml]; n_1 - number of Petri plates used for the first dilution; n_2 - number of Petri plates used for the second dilution; d - dilution factor of the first dilution retained. According to EC Regulation 2073/2005 on microbiological criteria for foods [10], amended by EC Regulation 1441/2007 [11], the microbiological criteria for *E. coli* are presented in table 1:

Table 1. Microbiological criteria for *E. coli*

Food Category	Sampling plan		Limits	
	n	c	min, cfu/g	Max, cfu/g
Minced meat	5	2	50	500
Prepared raw meat	5	2	500	5000

n = number of units contained in the sample; c = number of sample units that have values between min and Max.

Results interpretation:
- satisfactory, if all observed values are smaller than min.;
- acceptable, if the maximum value of c/n is between min and Max, while the remaining values are smaller than min;
- unsatisfactory, if one or more observed values are higher than Max or more c/n are between min and Max.

Unsatisfactory samples, each of 5 colonies/sample were selected for serological identification with *E. coli*. antiserum (Denka Seiken Co. manufacturer., LTD) (table 2). These are liquid products containing specific somatic (O) antibodies (polyvalent sera: pig; monovalent sera: rabbit) and 0.08 w/v% sodium azide as preservative.

Table 2. Specific somatic (O) antibodies

Polyvalent Sera	Monovalent sera						
Polyvalent 1	O1	O26	O86	O111	O119	O127	O128
Polyvalent 2	O44	O55	O125	O126	O146	O166	
Polyvalent 3	O18	O114	O142	O151	O157	O158	
Polyvalent 4	O6	O27	O78	O148	O159	O168	
Polyvalent 5	O20	O25	O63	O153	O167		
Polyvalent 6	O8	O15	O115	O169			
Polyvalent 7	O28	O112	O124	O136	O144		
Polyvalent 8	O29	O143	O152	O164			

When the reagent is made in contact with a strain of *E. coli* witch presents the right antigen, the antigen-antibody reaction and agglutination occurs. This reaction is observed macroscopically.

Each selected colony is transfer into a Petri plate with nutritive agar (non-selective medium that does not generate the autoagglutination or insufficient antigen production) and incubated at 44°C. The results are a pure culture witch will be tested.

A small quantity of bacterial culture (3-5 times the amount of a match head) is suspended in 3 ml physiological saline and heated at 100°C for one hour (to remove the possibility of false-positive or negative reactions).

The heated suspension is spin at 900 rpm for 20 minutes. The supernatant is removed and the precipitate is added in 0.5 ml physiological saline. This solution is used like antigen suspension. One drop of each polyvalent serum is placed on glass slides and over each of these are drop 5-10 µl of antigen suspension. The glass slides for one minute and agglutination is observed. If positive reaction with a polyvalent serum appears, then the agglutination with each monovalent serum constituent of respectively polyvalent serum is followed.

3. Results and Discussion

Analysis results of minced meat and raw meat samples are presented in table 3.

From Table 3 we can notice that, 32 samples showed good results according to EC Regulation 1441/2007 and 18 samples, were inadequate.

As regards the samples we can be observed that in both cases the results are similar: 41.2% inadequate samples for minced meat and 33.3% inadequate samples for raw meat.

There is a difference in the proportion of samples with no detectable *E. coli* (<10/g), which is higher in minced meat (23.5%) than raw meat (12.1%) (figure 2).

Since the samples that showed adequate results can not be considered a threat to public health (even if *E. coli* is present in some samples), only the 18 inadequate samples were subjected to further investigations regarding serological identification of *E. coli* isolated strains. The results are presented in Table 4.

Table 3. Analysis results of minced meat and raw meat samples

Sample	Food sample	Results				
		Test sample A	Test sample B	Test sample C	Test sample D	Test sample E
1	Minced meat	<10	<10	<10	<10	<10
2	Prepared raw meat	$3.3x10^2$	$2.5x10^2$	$3.2x10^2$	$2.7x10^2$	$2.8x10^2$
3	**Minced meat**	**80**	**$1.0x10^2$**	**$1.2x10^2$**	**40**	**90**
4	Prepared raw meat	$4.7x10^2$	$4.9x10^2$	$5.2x10^2$	$4.9x10^2$	$4.8x10^2$
5	**Prepared raw meat**	**$1.4x10^3$**	**$1.1x10^3$**	**$1.0x10^3$**	**$1.9x10^3$**	**$2.0x10^3$**
6	**Prepared raw meat**	**$7.0x10^2$**	**$7.9x10^2$**	**$9.3x10^2$**	**$1.1x10^3$**	**$7.5x10^2$**
7	Minced meat	<10	20	<10	<10	10
8	Prepared raw meat	<10	<10	<10	<10	<10
9	Prepared raw meat	$1.7x10^2$	$1.0x10^2$	$1.3x10^2$	$1.9x10^2$	$1.8x10^2$
10	**Minced meat**	**$2.1x10^2$**	**$2.5x10^2$**	**$3.2x10^2$**	**$2.9x10^2$**	**$2.8x10^2$**
11	Prepared raw meat	90	90	80	70	50
12	Minced meat	<10	<10	30	<10	<10
13	Prepared raw meat	$3.2x10^2$	$3.1x10^2$	$3.8x10^2$	$3.5x10^2$	$3.7x10^2$
14	Prepared raw meat	<10	<10	<10	<10	<10
15	Prepared raw meat	<10	<10	<10	<10	<10
16	**Prepared raw meat**	**$3.4x10^3$**	**$3.7x10^3$**	**$4.0x10^3$**	**$2.9x10^3$**	**$3.5x10^3$**
17	Minced meat	20	<10	<10	20	<10
18	Prepared raw meat	$1.1x10^2$	$1.0x10^2$	$1.9x10^2$	$1.9x10^2$	$1.6x10^2$
19	Prepared raw meat	90	90	60	50	50
20	**Prepared raw meat**	**$6.8x10^3$**	**$7.5x10^3$**	**$6.2x10^3$**	**$6.7x10^3$**	**$7.1x10^3$**
21	Minced meat	10	90	$1.2x10^2$	30	40
22	**Prepared raw meat**	**$7.8x10^2$**	**$8.1x10^2$**	**$9.0x10^2$**	**$7.9x10^2$**	**$8.0x10^2$**
23	**Prepared raw meat**	**$2.4x10^3$**	**$2.1x10^3$**	**$3.0x10^3$**	**$2.9x10^3$**	**$2.7x10^3$**
24	Prepared raw meat	$5.5x10^2$	$5.3x10^2$	$4.8x10^2$	$4.5x10^2$	$4.7x10^2$
25	**Minced meat**	**60**	**90**	**40**	**30**	**90**
26	**Prepared raw meat**	**$3.8x10^3$**	**$3.7x10^3$**	**$4.2x10^3$**	**$3.9x10^3$**	**$3.6x10^3$**
27	Minced meat	<10	<10	<10	<10	<10
28	Prepared raw meat	$2.5x10^2$	$2.6x10^2$	$3.0x10^2$	$3.1x10^2$	$2.4x10^2$
29	**Minced meat**	**$6.6x10^2$**	**$6.2x10^2$**	**$7.0x10^2$**	**$6.9x10^2$**	**$6.5x10^2$**
30	Prepared raw meat	$1.9x10^2$	$1.2x10^2$	$1.1x10^2$	$1.0x10^2$	$1.4x10^2$
31	**Minced meat**	**60**	**80**	**90**	**90**	**60**
32	Prepared raw meat	50	50	50	40	10
33	**Prepared raw meat**	**$2.8x10^3$**	**$2.7x10^3$**	**$2.2x10^3$**	**$2.5x10^3$**	**$2.9x10^3$**
34	**Prepared raw meat**	**$1.1x10^4$**	**$1.3x10^4$**	**$1.3x10^4$**	**$1.2x10^4$**	**$1.2x10^4$**
35	Prepared raw meat	<10	<10	<10	<10	<10
36	**Minced meat**	**$1.8x10^2$**	**$1.2x10^2$**	**$2.0x10^2$**	**$1.7x10^2$**	**$1.8x10^2$**
37	Minced meat	<10	<10	<10	<10	<10
38	Prepared raw meat	$1.3x10^2$	$1.6x10^2$	$2.0x10^2$	$2.1x10^2$	$1.7x10^2$
39	Prepared raw meat	10	10	50	30	40
40	**Prepared raw meat**	**$3.5x10^3$**	**$4.1x10^3$**	**$4.0x10^3$**	**$3.5x10^3$**	**$3.9x10^3$**
41	Minced meat	10	20	20	30	60
42	**Prepared raw meat**	**$1.0x10^4$**	**$1.1x10^4$**	**$1.0x10^4$**	**$1.3x10^4$**	**$1.8x10^4$**
43	Minced meat	<10	<10	<10	<10	<10
44	Prepared raw meat	$2.2x10^2$	$1.6x10^2$	$2.1x10^2$	$1.5x10^2$	$1.2x10^2$
45	Prepared raw meat	90	$1.0x10^2$	70	$1.1x10^2$	60
46	Minced meat	30	40	50	40	10
47	Prepared raw meat	$4.4x10^2$	$4.2x10^2$	$3.7x10^2$	$3.5x10^2$	$4.2x10^2$
48	Prepared raw meat	<10	<10	<10	<10	<10
49	**Minced meat**	**$2.5x10^2$**	**$2.8x10^2$**	**$2.7x10^2$**	**$3.1x10^2$**	**$2.5x10^2$**
50	Prepared raw meat	<10	20	<10	<10	<10

Table 4. Serological results obtained by isolated strains analysis

No.	Sample number	Food sample	Agglutination	
			Polyvalent Sera	Monovalent Sera
1	Sample 3	Minced meat	Polyvalent 4	O159
2	Sample 5	Prepared raw meat	Polyvalent 2	O125
3	Sample 6	Prepared raw meat	Polyvalent 6	O8
4	Sample 10	Minced meat	Polyvalent 4	O159
5	Sample 16	Prepared raw meat	Polyvalent 1	O1
6	Sample 20	Prepared raw meat	Polyvalent 4	O78
7	Sample 22	Prepared raw meat	Polyvalent 6	O8
8	Sample 23	Prepared raw meat	Polyvalent 8	O29
9	Sample 25	Minced meat	Polyvalent 4	O6
10	Sample 26	Prepared raw meat	Polyvalent 4	O27
11	Sample 29	Minced meat	Polyvalent 4	O6
12	Sample 31	Minced meat	Polyvalent 4	O6
13	Sample 33	Prepared raw meat	Polyvalent 7	O112
14	Sample 34	Prepared raw meat	Polyvalent 7	O112
15	Sample 36	Minced meat	Polyvalent 1	O1
16	Sample 40	Prepared raw meat	Polyvalent 6	O8
17	Sample 42	Prepared raw meat	Polyvalent 6	O8
18	Sample 49	Minced meat	Polyvalent 6	O8

The most common serological reaction was the one with polyvalent serum 4 (7 samples). Five samples of 18 reacted positively with the polyvalent serum 6. Two samples showed a positive reaction with polyvalent serum 1 and 7, and one sample with polyvalent serum 2 and 8. No sample reacted positively with polyvalent serum 3 and 5.

Three samples from the ones witch reacted with polyvalent serum 4, were positive to monovalent serum O6, two were positive to O159 and one to O78 and O27. Five samples reacted positively with polyvalent serum 6. All were agglutinated with the monovalent O8. All these strains agglutinated with polyvalent serum of two groups belong to virulent ETEC.

Two of the strains showed positive reaction with antibodies of polyvalent serum 1, O1 -group.Also, one strain agglutinated with polyvalent 2, O125-group. They are part of virulent EPEC group.

Two other strains were agglutinated with polyvalent serum antibodies 7, O112-group and one strain reacted positively with polyvalent serum 8, O29-group. These strains are virulent EIEC group.

Quantitatively, in only two samples (prepared raw meat) results showed a *E. coli* growth of 10^4.

The other ones, even in the inadequate-ones, the microbial growth was up to 10^3. According to some authors, food poisoning can not occur only for a microbial growth of 10^6-10^{10}/g. But, according to E.U. regulation, the 18 samples showed unsatisfactory results. The results are worrying because these meat products are designed to be eaten cooked, but in many cases, heat treatment is deficient and micro-organisms can not be destroyed.

High percentage of inadequate samples is mainly due to poor conditions of hygiene in production but may be due to the raw materials too. Faces contamination can occur during slaughter of animals. Thus, to avoid the microbial growth the hygiene rules should be followed at all stages of production.

Regarding virulent groups it can be notice that most isolates stains belong to ETEC group (66.6%), and the rest to EPEC and EIEC groups (16.6% each). The highest frequency was for the serotype O8, followed by O6, both belonging to ETEC group.

These results suggest that strains of ETEC group are present in high proportion in our country. Moreover, in recent decades, ETEC is the most studied serotypes being present at populations with a low economic level [12].

All these results suggest that there are public health risks associated with consumption of these foods.

Thermal processing is required, since these products are part of the food intended to be cooked before consummation.

4. Conclusion

This study allowed a microbiological analysis of minced meat and prepared raw meat samples sold on the market of Alba County.

64% of total analyzed samples were suitable to EC Regulation 2073/2005 regarding microbiological criteria for foodstuffs, amended by EC Regulation 1441/2007.

The results showed the importance of hygiene rules in all stages of production starting from the animal slaughter up to the finished product.

Most *E. coli* strains isolated from inadequate samples (submitted to serological investigations) showed positive reaction with polyvalent serum 4, then 6 and the rest with 1, 7, 2 and 8.

Monovalent sera which showed the more agglutination reaction with isolated strains were O8 and O6.

Most isolates strains belong to virulent ETEC group (66.6%), then EPEC and EIEC (16.6% each).

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