# Occurrence of *Escherichia coli* in meat preparations

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Professional paper

# **ABSTRACT**

Meat preparations are produced from one or more types of minced meat with added seasonings or additives, and are usually placed on the market under labels: ćevapčići, meat patty or hamburger/burger. The bacterial contamination of such products with Escherichia coli during production and distribution is invariably possible. In this paper, we have tested 50 samples of meat preparations for bacteria E. coli, whose presence points to the faecal contamination of food. Only 8 % of meat preparation samples had an E. coli count of less than 500 cfu/g, suggesting that all samples tested in this study yielded satisfactory results regarding the prescribed microbiological criteria for food.

Key words: Escherichia coli, meat preparations

# **UVOD**

Escherichia coli are a common food contaminant, especially in raw or insufficiently heat treated food. These bacteria usually reside in human and domestic animals' intestines, while their presence in water and food indicates faecal contamination. Some E. coli serovars are enteropathogens and responsible for a large number of food poisoning cases. The symptoms depend on the infectious dose and strain pathogenicity. Although many E. coli strains are considered harmless, some produce verotoxins (VTEC or verotoxin-producing Istrains) and cause serious illnesses. VTEC strains are classified as human pathogens and causes of alimentary infections, with the most commonly excreted serovar being O157:H7 (Marinculić et al., 2009). E. coli are fairly resistant

bacteria. Not only can they survive in water and on land for months, but they can very easily and quickly propagate in different foods. They are unaffected by low temperatures, can reproduce even at temperatures of 0 °C and below, up to – 5 °C, and rapidly become resistant to antibiotics. They are sensitive to temperatures of 60 °C/ 15-30 min and 90 °C / 4min. Moreover, *E. coli* can survive even the pasteurisation of milk. Like other bacteria from this family, they are sensitive to chlorine and chlorine compounds (Blount 2015).

Large retail chain customers, in addition to packaged fresh meat, often offer pre-prepared shaped minced meat. Meat preparations are produced from one or more types of minced meat with added seasonings or additives, and are placed on the

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market under labels: ćevapčići, meat patty, hamburger/burger, etc. The bacterial contamination of such products with E. coli during production and distribution is invariably possible. It is, therefore, of utmost importance to implement frequent controls and observe microbiological purity criteria in production facilities and butchers' shops prescribed by law. The testing of food for microbiological contaminants is the responsibility of all food business operators (FBOs). Related monitoring includes determining the total bacteria and Enterobacteriaceae count on carcass surfaces, as well as E. coli and Salmonella counts in minced meat batches (Paulsen et al., 2006). Varga et al. (2012) investigated the microbiological safety of minced meat products and established the E. coli count of less than 10 cfu/g in 38 samples of minced meat, 27 ćevapčići samples and 35 fresh grill sausage samples. Previous studies found these bacteria in 11.8 % (Kočila, 1995), that is, 16.7 % (Popović, 1998) of the tested samples of meat preparations collected from retail stores, grills and restaurants, in a count greater than the count allowed by then applicable criteria. Černelić (2002) investigated the bacteriological safety of shaped meat reduced to fragments (n = 40) originating from retail stores, grills and restaurants and found that three samples (7.5 %) tested positive for E. coli in a number > 103/g. The author emphasised

that implementing the hazard analysis and critical control point system (HACCP) into small production facilities may improve hygienic conditions.

# **MATERIALS AND METHODS**

We collected 50 samples of meat preparations for microbiological testing. Samples were sampled from food business operators' facilities of low production capacity. The number of elementary units taken into consideration and the amount of each collected sample had to correspond to those prescribed by the Ordinance on the frequency of sampling carcases, minced meat and meat preparations, and the conditions and manner of reducing the number of sample elementary units in facilities of low production capacity (Anon., 2012; Table 1).

The isolation of *E. coli* was performed in accordance with the procedure described in HRN ISO 16649-2:2001: Method for the enumeration of betaglucuronidase-positive *E. coli* – Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. The biochemical identification was carried out using the commercially available API 20E assay for identification and differentiation of strains from the *Enterobacteriaceae* family.

The results were, in accordance with the Regulation on Microbiological Criteria for Foods (Anon.,

**Table 1:** The frequency of sampling minced meat, meat preparations and mechanically separated meat (MSM) depending on production capacity (Anon., 2012)

| Monthly production                          |                                      | Sampling frequency   |  |  |
|---|--------------------------------------|--|--|--|
| capacity                                    | Type of meat                         | Initial sampling frequency   | Reduced sampling frequency (with satisfactory results) |  |
| from 5 to 10 tonnes of meat preparations    | all types of meat,<br>except poultry | E. coli: 1 sample every 2 weeks, until obtaining 6 consecutive satisfactory results                                    | <i>E. coli:</i><br>1 sample per month                  |  |
| from 1 to 5 tonnes of meat preparations     | all types of meat,<br>except poultry | E. coli: 1 sample per month, until obtaining 4 consecutive satisfactory results  | <i>E. coli:</i> 1 sample every 2 months                |  |
| from 501 kg to 1 tonne of meat preparations | all types of meat,<br>except poultry | E. coli: 1 sample every 2 months, until obtaining 3 consecutive satisfactory results                                   | <i>E. coli:</i> 1 sample every 4 months                |  |
| up to 500 kg of meat preparations           | all types of meat,<br>except poultry | <ul><li>E. coli:</li><li>1 sample every 4 months, until obtaining</li><li>3 consecutive satisfactory results</li></ul> | <i>E. coli:</i><br>1 sample every 6 months             |  |

**Tablica 2.** *Criteria for E. coli (Anon., 2005; Anon., 2011)* 

|  | Category of food | Microorganisms | Sample collection plan |   | Limit values         | Reference test method          | Stage in which the<br>criterion was applied | Measure in the case of<br>unsatisfactory results  |
|--|------------------|----------------|------------------------|---|----------------------|--------------------------------|---|---|
|  | Meat             | E. coli        | n                      | С | М                    | HRN EN ISO                     | End of production process                   | Improving the hygiene of                          |
|  | preparations     |                | 2                      | 5 | 500 cfu/g ili<br>cm2 | 16649-1 ili<br>HRN ISO 16649-2 |   | production, choice and/or origin of raw materials |

2005), interpreted as satisfactory (*E. coli* count of less than m - <500 cfu/g), acceptable (count between limit values m and M, i.e., 500-5000 cfu/g) and unsatisfactory (number of colonies greater than M, i.e., 5000 cfu/g), as shown in Table 2.

All tests were conducted in the laboratory for testing the safety of food and general use products Zin-lab in Zagreb.

# **RESULTS AND DISCUSSION**

No samples of meat preparations had an *E. coli* count greater than 5000 cfu/g, whereby four samples or 8 % (n = 50) had a count of less than 500 cfu/g. The results of bacteriological tests for samples 5, 14, 23 and 46, that were positive for *E. coli*, are presented in Table 3.

As Table 3 indicates, all samples positive for *E. coli* had a bacteria count of less than 500 cfu/g in all five examined elementary units and could, therefore, be considered compliant with health and safety standards in accordance with the Regulation 2073/2005. The count of bacteria in sample 5 amounted to 50-70 cfu/g, in sample number 14 to 60-100 cfu/g, in sample number 23 to 200-400 cfu/g and in sample number 46 to 120-140 cfu/g. As mentioned previously, no bacteria were detected in the remaining 46 samples.

Unlike Lindberg et al. (1998) and Varga et al. (2012), who detected either no bacteria or detected a count of less than 10 bacteria in a gram of a sample, our results demonstrated the occurrence of E. coli bacteria in meat preparations in a count lower than the count prescribed by limit criteria. In comparison, Černelić (2002) detected E.coli in fresh ćevapčići and burgers in a count greater than 1000/g. Moreover, Kočila (1995) and Popović (1998) in their research of microbiological safety of meat preparations established that 11.8 % and 16.7 %, respectively, of the examined samples did not comply with regulations due to the E. coli count that was greater than permitted. Although we, in our research, detected the bacteria in 8 % of the examined samples, the established count of E. coli was within the permitted limits and the samples, thus, considered safe according to the microbiological criteria.

# CONCLUSION

In accordance with the Regulation on Microbiological Criteria for Foods (Anon., 2005) and the Guidance on Microbiological Criteria for Foods (Anon., 2011), all tested samples of meat preparations complied with the food safety criteria. *E. coli* bacteria were detected in

**Tablica 3.** Results of bacteriological tests for E. coli

| Sample number | Method                   | Limit values | E. coli, cfu/g |     | Assessment |
|---------------|--------------------------|--------------|----------------|-----|------------|
|               |                          | 500 - 5.000  | EU* No. 1      | 70  | Accurate   |
|               |                          |              | EU* No. 2      | 50  |            |
| 5             | HRN ISO 16649-<br>2:2001 |              | EU* No. 3      | 60  |            |
|               |                          |              | EU* No. 4      | 50  |            |
|               |                          |              | EU* No. 5      | 60  |            |
|               | HRN ISO 16649-<br>2:2001 | 500 - 5.000  | EU* No. 1      | 100 | Accurate   |
|               |                          |              | EU* No. 2      | 80  |            |
| 14            |                          |              | EU* No. 3      | 60  |            |
|               |                          |              | EU* No. 4      | 70  |            |
|               |                          |              | EU* No. 5      | 100 |            |
|               | HRN ISO 16649-<br>2:2001 | 500 - 5.000  | EU* No. 1      | 200 | Accurate   |
|               |                          |              | EU* No. 2      | 300 |            |
| 23            |                          |              | EU* No. 3      | 400 |            |
|               |                          |              | EU* No. 4      | 200 |            |
|               |                          |              | EU* No. 5      | 300 |            |
|               | HRN ISO 16649-<br>2:2001 | 500 - 5.000  | EU* No. 1      | 120 | Accurate   |
|               |                          |              | EU* No. 2      | 140 |            |
| 46            |                          |              | EU* No. 3      | 100 |            |
|               |                          |              | EU* No. 4      | 110 |            |
|               |                          |              |                | 120 |            |

<sup>\*</sup> EJ- elementarna jedinica

four samples of meat preparations, in the count lower than permitted limit values (<500/g). Based on these results, in view of the good results of the analysis, it can be concluded that measures taken in order to prevent *E. coli* contamination during the production of meat preparations are considered satisfactory.

\* The paper is an extract from Ana Robina's graduate thesis (2018): Occurrence of Escherichia coli in meat preparations. Graduate thesis. Faculty of Agronomy, University of Zagreb. Graduate thesis. Manuscript, p. 36. Mentor: Prof. Lidija Kozačinski, PhD.

#### **REFERENCES**

**Anonimno (2005):** Uredba komisije (EZ) br. 2073/2005 o mikrobiološkim kriterijima za hranu (L 338/1).

**Anonimno (2011):** Vodič o mikrobiološkim kriterijima za hranu (3. izmijenjeno izdanje, ožujak, 2011)

Anonimno (2012): Pravilnik o učestalosti uzorkovanja trupova, mljevenog mesa i mesnih pripravaka te uvjetima i načinu smanjenja broja elementarnih jedinica uzorka u objektima manjeg kapaciteta proizvodnje (NN 30/10 i 38/12)

**Blount Z. D. (2015):** The unexhausted potential of *E. coli.* eLife 4: e05826 DOI: 10.7554/eLife.05826

**Černelić, S. (2002):** Kakvoća proizvoda od usitnjenog oblikovanog mesa. Diplomski rad. Sveučilište u Zagrebu. Veterinarski fakultet. Rukopis (strojem), str. 26.

**Kočila, P. (1995).** Bakteriološka ispravnost proizvoda od usitnjenog oblikovanog mesa. Diplomski rad. Sveučilište u Zagrebu. Veterinarski fakultet. Rukopis (strojem), str. 33.

Marinculić, A., B. Habrun, LJ. Barbić, R. Beck (2009): Biološke opasnosti u hrani. Hrvatska agencija za hranu, Osijek.

Paulsen, P., E. Schopf, J. M. Smulders (2006): Enumeration of Total Aerobic Bacteria and Escherichia coli in Minced Meat and on Carcass Surface Samples with an Automated Most-Probable-Number Method Compared with Colony Count Protocols. Journal of Food Protection 69:10, 2500–2503

**Popović, V. (1998):** Higijenska ispravnost proizvoda od usitnjenog mesa. Diplomski rad. Sveučilište u Zagrebu. Veterinarski fakultet. Rukopis (strojem), str. 26.

Varga A., D. Plavšić., B. Kokić., T. Tasić., L. Šarić., J. Gubić, B. Šarić (2012): Assessment of minced and grill meat microbiological safety in year 2012. XV International Feed Technology Symposium. COST-"Feed for Health" joint Workshop, Proceedings. Edts Lević, J.; Sredanović, S.; Đuragić, O. Novi Sad, Serbia, 3-5 October, 2012 2012 pp.273-277

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# d) knjiga:

Njari, B., N. Zdolec (2012): Klaonička obrada i veterinarski pregled / Herak-Perković, Vlasta (ur.). Zagreb; Veterinarski fakultet Sveučilišta u Zagrebu, 2012.

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