How a routine checking of *Escherichia coli* in retailed food of animal origin can protect consumers against exposure to *Campylobacter spp.* and *Listeria monocytogenes*?

Kako rutinska provera hrane životinjskog porekla u prometu na prisustvo *Escherichia coli* može zaštititi potrošača od izloženosti *Campylobacter spp.* i *Listeria monocytogenes*?

Ljiljana Trajković-Pavlović*†, Budimka Novaković*, Mirjana Martinov-Cvejin*†, Vera Gusman*†, Sanja Bijelović†, Nataša Dragnić†, Dragana Balač*†

*University of Novi Sad, School of Medicine, Novi Sad, Srbija, †Institute of Public Health of Vojvodina, Novi Sad, Srbija

Abstract

Background/Aim. According to the literature that has been published over the last two decades *Campylobacter spp.* and *Listeria monocytogenes* can be identified as causes of numerous diseases derived by consuming food of animal origin. The purpose of this paper was to find out how established national microbiological criteria of the Republic of Serbia on food safety in retailed food of animal origin could contribute to consumer’s protection against exposure to foodborne pathogens such as *Campylobacter spp.* and *Listeria monocytogenes*. Methods. During a routine microbiological safety control of randomly selected 60 samples of fresh poultry meat, 30 samples of other fresh meat ready-made for grilling, 30 samples of sausage products, 37 samples of heat-treated meat, 39 samples of toppings for fast food of animal origin and 31 samples of dairy products a national food safety criteria (*Escherichia coli* origin and *Listeria monocytogenes* borne pathogens such as *Staphylococcus*, *Proteus* spp., sulphite-reducing *Clostridia*) were applied and, as well as, testing to *Campylobacter spp.* and *Listeria monocytogenes*. In determination of *Campylobacter spp.* and *Listeria monocytogenes*, food quality control methods of the Food and Agriculture Organization (FAO) were applied, while in determination of the other above motioned bacteria, national provisions on microbiological methods were applied who are adjusted to the FAO ones. Results. Related to the national criteria on microbiological food safety, 88 (38.8%) samples, out of the total 227 tested, were rejected. When to these results, the results of laboratory tests on *Listeria monocytogenes* were added, a terminal number of rejected samples were not changed. When to these results, the results of *Campylobacter spp.* testing were added, 91 (40.1%) out of the 227 samples were unsatisfied. Results of logistic regression model with occurrence of *Escherichia coli* as dependent variable indicated that *Escherichia coli* was 4.5 times likely to occur among samples with *Campylobacter spp.* than among samples without *Campylobacter spp.* (OR = 4.515, 95% CI: 1.019–20.002). Sensitivity of the fitted model (Hosmer-Lemeshow ρ = 0.268) was 76.8% and its specificity was 75.0%. At the same time *Escherichia coli* was confound in all (100%) food samples that were contaminated by *Listeria monocytogenes*. Conclusion. Statistical analysis indicated that *Escherichia coli* was completely sensitive to identify all samples contaminated with *Listeria monocytogenes* and highly sensitive to identify samples contaminated with *Campylobacter spp.* Nevertheless, 3 (1.3%) of the tested samples were not covered with *Escherichia coli*.

Key words: food contamination; food inspection; food microbiology; escherichia coli; campylobacter; listeria monocytogenes.

Apstrakt

Uvod/cilj. Prema podacima publikovanim u poslednje dve decenije *Campylobacter spp.*, i *Listeria monocytogenes* su identifikovani kao uzročnici brojnih oboljenja nasilnih unošenjem namirnica životinjskog porekla. Cilj rada bio je da se utvrdi koliko primena nacionalnih kriterijuma Republike Srbije (RS) za mikrobiološku ispravnost namirnica animalnog porekla u prometu štiti stanovništvo od izloženosti *Campylobacter spp.* i *Listeria monocytogenes* poreklom iz namirnica. Metode. U skladu sa kriterijumima RS za mikrobiološku ispravnost namirnica u prometu, metodom slučajno izbora obavljena je kontrola mikrobiološke ispravnosti 60 uzoraka svežeg pilećeg mesa, 30 uzoraka svežeg mesa prpi-

Correspondence to: Ljiljana Trajković-Pavlović, Ćirpanova 4, 21 000 Novi Sad, Republic of Serbia; Phone: 381 62 86 220 67; E-mail: ljtp@Eunet.rs
Introduction

According to data published in the last two decades, Campylobacter spp. appeared as an emerging causative agent of foodborne diseases. It was identified in various kinds of food of animal origin. Infections of vulnerable population groups caused by Listeria monocytogenes are also a growing problem. Different degrees of severity of foodborne diseases caused by Listeria monocytogenes with systematic manifestation, and even, lethal outcome of foodborne diseases caused by Salmonella spp. are mandatory in food of animal origin but does not apply to consumers protection against exposition to foodborne pathogens such as Campylobacter spp. and Listeria monocytogenes.

Methods

The investigation was performed during a routine microbiological safety control of food of animal origin retailed in Novi Sad, the capital of the PV with approximately 400 000 inhabitants, by the Sanitary Inspectorate of the Secretariat for Health and Social Welfare of the PV. In the period July-October 2004 and July-October 2005, 227 samples of food of animal origin were chosen randomly between 9 am and 2 pm. Samples were trained to perform sampling under sterile circumstances. They did not have any instructions to choose, nor avoid specific shops. The investigation included 97 samples of fresh meat (60 samples of fresh poultry meat, 37 samples of other kinds of fresh meat ready made for grill) and 130 samples of “fast food” toppings and 31 samples of dairy products.

Methods for the laboratory determination of Aerobic plate count, Salmonella spp., Escherichia coli, and Staphylococcus, sulphite-reducting Clostridia, Proteus spp. and Salmonella spp. are mandatory in food of animal origin but does not require mandatory checking of Campylobacter spp. and Listeria monocytogenes in any kind of food. Presence of these bacteria in food can be investigated provided this is related to the actual food safety legislation of the Republic of Serbia, checking of Aerobic plate count, Escherichia coli, coagulasa positive Staphylococcus, sulphite-reducting Clostridia, Proteus spp. and Salmonella spp. are mandatory in food of animal origin but does not require mandatory checking of Campylobacter spp. and Listeria monocytogenes in any kind of food. Presence of these bacteria in food can be investigated provided this is indicated from the epidemiological point of view.

The purpose of this paper was to find out how the established microbiological criteria for food safety in the Republic of Serbia that requires a regular laboratory checking of certain pathogenic bacteria like Salmonella spp. and coagulase positive Staphylococcus and bacteria indicators of food processing hygiene like Escherichia coli spp., Aerobic plate count, Proteus spp, sulphite-reducting Clostridia in retailed food of animal origin could contribute to consumers protection against exposition to foodborne pathogens such as Campylobacter spp. and Listeria monocytogenes.

Methods for the laboratory determination of Aerobic plate count, Salmonella spp., Escherichia coli, coagulasa positive Staphylococcus, sulphite-reducting Clostridia and Proteus spp. were adjusted to the National Provisions on microbiological methods for analysis of food. The food groups were tested as follows:

- Fresh poultry meat: Salmonella spp. in 25 g, coagulasa positive Staphylococcus in 0.01 g, sulphite-reducting Clostridia in 0.1, Proteus and Escherichia coli in 0.001 g and Aerobic plate count 1g no more than 1000;
- Fresh meat ready made for grilling: Salmonella spp. in 25 g, coagulasa positive Staphylococcus and Proteus, sulphite-reducting Clostridia in 0.01 g, Escherichia coli in 0.001 g and total Aerobic plate count in 1g no more than 3 000 000;
- Sausage: Salmonella spp. in 25 g, coagulasa positive Staphylococcus, Escherichia coli and Proteus in 0.01 g and sulphite-reducting Clostridia in 0.1 g;
- Heat-treated meat of “fast food”: Salmonella spp. in 25g, coagulasa positive Staphylococcus, sulphite-reducting Clostridia, Proteus, and Escherichia coli in 0.1 g and Aerobic plate count in 1 g no more than 10 000;
- Topping for fast food: Salmonella spp. in 25 g, coagulasa positive Staphylococcus sulphite-reducting Clostridia;
tridia, Proteus and Escherichia coli in 0.1 g and Aerobic plate count in 1g no more than 10 000; 
- Soft cheese: Salmonella spp. in 25 g, coagulasa positive Staphylococcus, sulphite-reducting Clostridia, Proteus and Escherichia coli in 0.01 g and Aerobic plate count in 1 g no more than 10 000;

The applied method was adjusted to the national criteria on microbiological food safety following the provisions of the general articles and the articles specified for certain food items 12.

All food items were, also, tested on Campylobacter spp. and Listeria monocytogenes per 25 g of aseptically weighted samples, applying methods for microbiological analysis of the FAO/Manuel of Food Quality 20.

Laboratory methods are validated according the Serbian standards SRPS ISO/ IEC 17025:2006 21.

The number of the bacterial colonies were counted manually using a Reichert Darkfield Colony Counter-model 3328.

Serotypization of the tested bacteria was not performed.

The obtained data were presented by the use of a descriptive statistical method including frequency distributions and frequency percent of the identified bacteria. We analyzed logistic regression model with dependent variable occurrence of Escherichia coli (positive = 1, negative = 0) and independent variable occurrence of Campylobacter spp. adjusted for the groups of food. The model was presented by estimated coefficients of model (B), estimated odd standard errors (SE) of the coefficients, $p$-values for Wald test, odds ratio (OR) with 95% confident interval (CI). For the assessment of the model fit, the Hosmer Lemeshow test was applied that included table of classification and area under the ROC curve. The goodness fit test of Hosmer Lemeshow was applied that included table of classification and area under the ROC curve (AUC).

The results of logistic regression model with dependent variable, occurrence of Escherichia coli (positive = 1, negative = 0) indicated that Escherichia coli was 4.5 times likely (positive = 1, negative = 0) to occur among samples with Campylobacter spp. than among samples without Campylobacter spp. (Table 2). The

Results

Escherichia coli was the most frequently identified bacteria as a reason for food samples rejection. Out of 227 samples E. coli was found in 67 (29.5%) ones, followed by coagulasa positive Staphylococcus, Campylobacter spp. and Aerobic plate count above the national microbiological criteria that were found in 16 (7%), 15 (6.6%), and 15 (6.6%) respectively. Listeria monocytogenes was identified in 4 (1.8%) and Salmonella spp. in 2 (0.9%) tested samples. Proteus and sulphite-reducting Clostridia were not identified in any of the tested samples. Campylobacter spp. was identified in 11 (18.3%) samples of fresh poultry meat, in 3 (8.1%) samples of fresh meat readymade for grilling and in 1 (2.5%) sample of toppings of animal origin for grilled meat. Listeria monocytogenes was identified in 3 (5%) samples of fresh poultry meat and in 1 (2.7%) sample of fresh meat readymade for grilling. Out of all tested samples, only Escherichia coli was identified in all three food groups (fresh poultry meat, fresh meat readymade for grilling and toppings for grilled meat of animal origin) as it was for Campylobacter spp. and Listeria monocytogenes. Escherichia coli was identified in 41 (68.3%) samples of fresh meat, in 13 (35.14%) samples of fresh meat readymade for grilling and in 2 (5.13%) samples of toppings of animal origin for grilled meat.

Related to the national criteria on microbiological food safety, 88 (38.8%) samples, out of the total 227 tested, were rejected. When to these results, the results of laboratory tests on Listeria monocytogenes were added, a terminal number of rejected samples were not changed. When to these results, the results of Campylobacter spp. testing were added, 91 (40.1%) out of the 227 samples were unsatisfied.

Out of a total number of the tested samples (n = 227) in 157 (69.2%) Escherichia coli nor Campylobacter spp. were identified. Samples contaminated only by Escherichia coli participated with 24.2%, contaminated by Escherichia coli and Campylobacter spp. participated with 5.3% and contaminated only by Campylobacter spp. participated with 1.3%, in the total number of the tested samples respectively (Figure 1).

![Fig. 1 – Distribution of samples contaminated by Escherichia coli and Campylobacter spp.](image-url)

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli only</td>
<td>69.2%</td>
</tr>
<tr>
<td>Campylobacter spp. only</td>
<td>24.2%</td>
</tr>
<tr>
<td>Both</td>
<td>1.3%</td>
</tr>
<tr>
<td>Not detected</td>
<td>0%</td>
</tr>
</tbody>
</table>

Escherichia coli was confound in 80% of all tested samples that Campylobacter spp. was identified. It was confound with Campylobacter spp. in 81.8% samples of fresh poultry meat, in 66.7% samples of fresh meat readymade for grilling, in 100% samples of toppings of animal origin for grilled meat (Table 1).

The results of logistic regression model with dependent variable, occurrence of Escherichia coli (positive = 1, negative = 0) indicated that Escherichia coli was 4.5 times likely to occur among samples with Campylobacter spp. than among samples without Campylobacter spp. (Table 2). The
Hosmer-Lemeshow goodness of fit test, $C = 5.194$, $df = 4$, $p = 0.268$ indicated that model fitted quite well. The results of classifying the observations of *Escherichia coli* using the fitted model (cut-off 0.50) are presented in Table 3. Sensitivity of the model was 76.8% (correctly classified samples with *Escherichia coli*), specificity was 75%. The overall rate of a correct classification was estimated as 75.7%. Area under Receiver Operating Characteristic (ROC) curve was $0.814$, (95% CI: $0.743–0.884$), so we found good discrimination, mining the likelihood that samples contaminated by *Campylobacter* spp. would have higher expected probability (using model) to be contaminated with *Escherichia coli* than samples without *Campylobacter* spp. (Figure 2).

At the same time *Escherichia coli* was confound in all (100%) food samples that were contaminated by *Listeria monocytogenes*.

### Table 1

<table>
<thead>
<tr>
<th>Bacteria of interest (positive)</th>
<th>Fresh poultry meat (N = 60)</th>
<th>Fresh meat ready-made for grilling (N = 37)</th>
<th>Toppings for grilled meat of animal origin (N = 39)</th>
<th>Total (N = 136)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter</em></td>
<td>11</td>
<td>3</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>41</td>
<td>13</td>
<td>2</td>
<td>56</td>
</tr>
<tr>
<td><em>Campylobacter</em> and <em>Escherichia coli</em></td>
<td>9</td>
<td>2</td>
<td>1</td>
<td>12</td>
</tr>
</tbody>
</table>

| * | Percent are number of positive *Escherichia* and *Campylobacter* / number of positive *Campylobacter* |

### Table 2

Logistic regression model with dependent variables of *Escherichia coli* occurrence

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>SE</th>
<th>$p$</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter</em>-positive</td>
<td>1.513</td>
<td>0.756</td>
<td>0.045</td>
<td>4.542 (1.033, 19.984)</td>
</tr>
<tr>
<td>Groups of food</td>
<td>-1.614</td>
<td>0.305</td>
<td>0.000</td>
<td>0.199 (0.109, 0.362)</td>
</tr>
<tr>
<td>Constant</td>
<td>2.251</td>
<td>0.529</td>
<td>0.000</td>
<td>9.498</td>
</tr>
</tbody>
</table>

*B* – estimated coefficients of logistic regression model; *SE* – estimates of standard errors of the coefficients; *p* – values for Wald test; *OR* – odds ratio; *CI* – confidence interval.

### Discussion

Campylobacteriosis and listeriosis are foodborne infections of a great public health concern. *Campylobacter* spp. was recognized as the most common cause of bacterial gastroenteritis in many countries. Infections can be associated with sequels-like reactive arthritis and Gillian–Bare syndrome. Additional problem is its resistance to some antimicrobial medications. Infections caused by *Listeria monocytogenes* are usually associated with clinical severity, high rate of hospitalization and relatively high fatality rate. The PV official reporting system every year indicates an increasing number of cases of campylobacteriosis. Several cases of human listeriosis were also reported mainly in clinical obstetrics wards. It would be difficult to speculate on the proportion of the reported cases attributable to food because these pathogens can be transmitted by other routes, but there is no doubt that food is a dominate route of transmission. A presence of *Proteus* and sulphite-reducing *Clostridia*, bacteria that are widely presented in the environment, in human and animal intestine were not identified in any of the tested samples. Enzymes produced by these bacteria cause food spoilage. The obtained results indirectly showed that at the time of microbiological analyses, the tested food samples were not under process of spoilage.
and other investigations 4, 15, 16, 39. Coagulasa positive coccus This was expected regarding coagulasa positive presented in food of animal origin than as an enteric pathogen and fecal indicator, was less pre-

demonstrated. In the tested samples Salmonella spp., as an enteric pathogen and fecal indicator, was less pre-

ented in food of animal origin than Campylobacter spp. and Listeria monocytogenes. The results were in line with other investigations 4, 15, 16, 39. Coagulasa positive Staphylo-
coccus, also, was not identified in the same food groups as it was for Campylobacter spp. and Listeria monocytogenes. This was expected regarding coagulasa positive Staphylo-
coccus was not fecal-derived bacteria 40. The results of our investigation showed that only Escherichia coli was found in the same food groups as it was for Campylobacter spp. and Listeria monocytogenes. We were interested how fare Escherichia coli, as an index organism, could contribute to protect population from possible exposure to Campylo-
bacter spp. and Listeria monocytogenes. Statistical analysis indicated that it was completely sensitive to identify all samples contaminated with Listeria monocytogenes and highly sensitive to identify samples contaminated with Campylobacter spp. Nevertheless 3 (1.3%) of the tested samples were not covered with Escherichia coli. The ob-
tained data indirectly supported the results of investigations that confirmed a fecal contamination of meat during pro-
cessing as a main way of transmission of Campylobacter spp. and Listeria monocytogenes 5, 28–30.

These findings were in line with already well docu-
mented practice that the most efficient method for food safety protection was a preventive approach derived throughout the risk assessment method 41, 42. Food business operators, regarding this approach, are required to decide themselves sampling frequency and parameters that should be checked in intermediate products and processing envi-
tronment in order to prevent the presence of pathogenic mi-
Croorganisms and parameters that can help them to control safety of row materials, hygiene and other parameters im-
portant for production process safety. They should conduct studies in order to investigate potential source of pathogenic bacteria and compliance with the established criteria for microbiological safety of food that is placed on the market, as well as, studies concerning shelf life of a product 43, 44. A special, science-based programs should be performed for food that have received minimal processing or precooking and have enhanced, but limited, shelf life like vacuum-
packaging meat, meat salads, soft cheese, dairy producers, some kind of sausages, etc 45, 46. Depending on environ-
mental conditions, some spoilage microorganisms, like Proteus and Clostridia, can grow in deep tissues which checking is of special importance for vacuum packed meats 47. Some spoilage microorganisms have adapted themselves to coexistence with other spoilage microorganisms 47. Some microorganisms can change oxidative stability of the prod-

uct 48, other can inhibit growing of certain pathogenic bacte-

ria 49, 50.

The European Commission (the Regulation No 2073/2005 on microbiological criteria for foodstuffs), recog-
nized Escherichia coli as an index organism of faecal con-
tamination that must be checked on regular bases throughout the whole processing line of fresh mechanically separated, minced meat, meat preparations, cheese made from milk or whey that undergoes heat treatment, butter and cream made from row milk or milk that undergoes a lower heat treatment than pasteurization. Retailed food is not checked for Es-
cherichia coli, except for some sea food that can be eaten row 44. As it was suggested by the EU Committee on Veteri-
nary Measures relating to Public Health, this regulation does not require checking verotoxogenic Escherichia coli in end-
products because it was unlikely to reduce associated risk for consumers. The EU authorities on food safety included man-
datory checking of Listeria monocytogenes in the majority of ready-to-eat food and Salmonella spp. in the majority of food of animal origin. The established microbiological criteria on food safety in EU do not recognize Proteus and sulphite-
reducing Clostridia as indicator organisms for retailed food safety 42. Harmonization of efforts and interdisciplinary communication of veterinary and medical practices are of great importance in the whole process of achieving an ap-
propriate level of protection of the population from zoonotic foodborne diseases, such are campylobacteriosis and listerio-
sis 41, 42, 51. The EU member countries are in charge to imple-
ment that kind of practice throughout national programs for monitoring zoonoses and zoonotic agents thereof. The pro-
grams include mandatory survey of campylobacteriosis, listeriosis, salmonellosis and agents thereof and verotoxogenic Escherichia coli on a regular basis 52. Microbiological testing of retailed food throughout application of the established micro-
obiological criteria for assumption/rejection of food placed on the market by the local authorities remains only a tool of contribution 41, 42.

Conclusion

Our investigation and the performed statistical analysis indicated that Escherichia coli was completely sensitive to identify all tested retailed food samples of animal origin contaminated with Listeria monocytogenes and highly sensi-
tive to identify samples contaminated with Campylobacter spp.

Acknowledgements

This investigation was conducted as a part of the project “Food safety control at green market in Novi Sad” supported by the Department for Environment Health Protection of the Municipality of Novi Sad.
REFERENCES


Received on May 13, 2009.
Revised on June 24, 2009
Accepted on July 24, 2009.