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Professional Paper

Risk Assessment and Adoption of Foodstuffs Sampling Plans for Microbiological Safety Based on the Results of Multiyear Food Sampling

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Abstract: The goal of this paper is to point at the possibility of risk assessment and adoption of foodstuffs sampling plans for microbiological safety based on the results of multiyear food sampling. The results of microbiological food analysis performed in the Public Health Institute of the Republic of Srpska – Regional centre Doboj in the period 2015 – 2019 were used as a sample.

Keywords: foodstuffs, microbiological safety, risk assessment.

INTRODUCTION

In the daily diet, people use various types of food. Many of these foods are a suitable environment for the development and reproduction of microorganisms which, when introduced into the human body, can be the cause of many infectious diseases. Biological contamination of food most often occurs due to improper food storage. Saprophytic microorganisms do not pose a danger to human health, but by breaking down proteins, fats and carbohydrates, they reduce the nutritional value of food. Pathogenic microorganisms present in food are danger to human health.

The primary goal of food hygiene is to eliminate or reduce the risk of exposure to food borne diseases.[1] It is necessary to monitor the hygiene of the food production process, storage, transport, as well as food distribution, which can be achieved by applying the applicable legislation for microbiological cleanliness of the facility.[2] That is, in order to protect food from contamination, it is necessary to implement legal regulations in primary production, which relate to the prevention of infectious diseases transmitted by food, in secondary production and processing, to control hygienic conditions and temperature, in the transport phase to control packaging, means of transport and food storage. The importance of maintaining personal hygiene of employees working on food preparation (wearing jewellery, rings, and bracelets) was confirmed by research which proved the presence of enterobacteria and staphylococci, and even some *E.coli* colonies as indicators of faecal contamination.[3]

Food monitoring calls for microbiological analysis which show which microorganisms are present, i.e. whether food meets microbiological standards that each country adopts individually. Application of applicable legislation in food control should ensure food safety at the time of production and at the time of placing it at market during its declared shelf life. Microbiological analysis and interpretation of the obtained results in Bosnia and Herzegovina are performed according to the standards prescribed by the applicable legislation at the level of Bosnia and Herzegovina, i.e. its entities. It is important to monitor sampling results over several years, to make risk assessments based on them and to adopt a sampling plan or adjust the existing plan for the next period in order to achieve even greater reliability in the production process.[4]

PAPER GOALS

1. To point at the importance of risk assessment and adoption of food sampling plans for microbiological safety based on analysis of the results of multiyear food sampling.
2. To present the results of food analysis for microbiological safety performed at the Public Health Institute of the Republic of Srpska - Regional Centre Doboj in the period 2015-2019.

MATERIALS AND METHODS

The research was conducted as a retrospective study that included the results of food analysis for microbiological safety submitted to the Public Health Institute of the Republic of Srpska - Regional Centre Doboj in the period 2015-2019. The Central Protocol of the Laboratory of the Public Health Institute of the Republic of Srpska - Regional Centre Doboj was used as data source. The results of the analysis of food samples are presented by individual years in relation to 10 food groups: milk and dairy products, meat and meat products, eggs, biscuits and related products, vegetables and vegetable products, fruits and fruit products, grains and grain products, oil, margarine, mayonnaise, non-alcoholic drinks and beer. Determination of microbiological safety of food samples was performed in accordance with the relevant regulations in the field of food safety applicable during the research period (Rulebook on microbiological criteria for food - Official Gazette of RS 109/12, Rulebook on microbiological criteria for food of animal origin - Official Gazette of RS 69/19)). The results of the analysis are presented in the form of tables and graphs. Methods of descriptive (tables and graphs) and inferential (chi-square test) statistics were used in data processing.

RESULTS

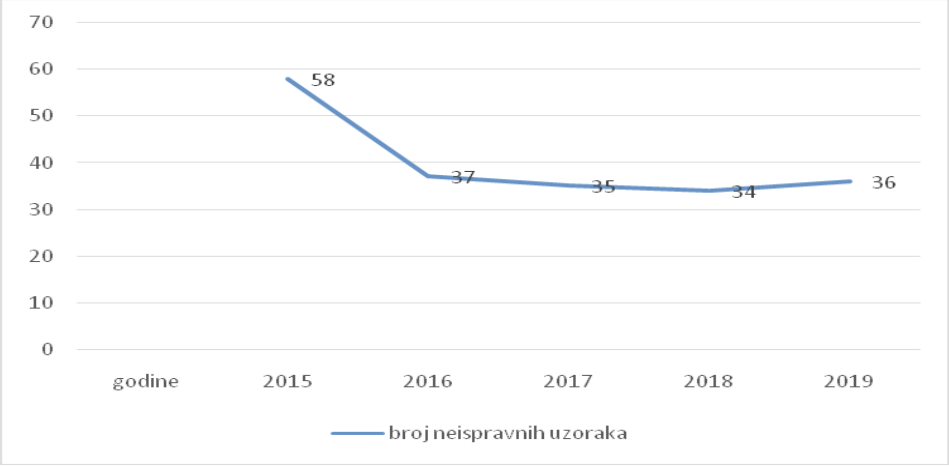
In the period 2015-2019, a total of 14,784 food samples were analysed for microbiological safety at the Public Health Institute of the Republic of Srpska - Regional Centre Doboj with 200 samples or 1.35% marked as contaminated. (Table 1). In relation to the origin of the analysed foods, the lowest percentage of contaminated samples was recorded for imported foods, at a level of high significance (χ^2=17.184; p=0.0017).

Table 1. Review of food analysis for microbiological safety in the period 2015- 2019

		Total	Imported	Off-the-shelf	From production
2015	Total	2645	733	497	1415
	Contaminated	58	2	23	33
	%	2.19	0.27	4.63	2.33
2016	Total	3155	446	469	2240
	Contaminated	37	1	6	30
	%	1.17	0.22	1.28	1.34
2017	Total	2987	270	594	2123
	Contaminated	35	1	9	25
	%	1.17	0.37	1.51	1.18
2018	Total	3062	302	655	2105
	Contaminated	34	0	3	31
	%	1.11	0	0,46	1.47
2019	Total	2935	260	668	2007
	Contaminated	36	0	1	25
	%	1.23	0	1.15	1.25

Total	Total	14.784	2.011	2.883	9.890
	Contaminated	200	4	42	144
	%	1.35	0.20	1.46	1.46

Table 1 shows the total number of analysis, and the number or percentage of contaminated foods by year. The highest level of irregular samples compared to other years was in 2015, and this difference was highly significant. (χ^2 =20.542; p=0.0003). Graph 1, in a more obvious way in the form of a curve, shows previous descriptive difference in terms of contaminated samples in the first year of the observed period compared to other years.



Graph 1. Graphic representation of the number of contaminated food samples by years

In the period 2015-2019 200 samples were found contaminated and most commonly only one cause of contamination was recorded per analysed food sample, more precisely 1.04 on average.

Table 2. Causes of microbiological contamination of analysed foodstuffs in the period 2015-2019

		Total of samples	1	2	3	4	5	6	7	8
2015	No	58	0	0	15	15	3	0	16	9
	%	2.19	0	0	25.86	25.86	5.17	0	27.59	15.52
2016	No	37	0	0	0	5	5	0	24	4
	%	1.17	0	0	0	13.51	13.51	0	64.86	10.81
2017	No	35	2	0	0	4	9	0	15	6
	%	1.17	5.71	0	0	11.42	25.71	0	42.85	17.14
2018	No	34	2	0	0	7	2	0	21	2
	%	1.11	5.88	0	0	20.59	5.88	0	61.76	5.88
2019	No	36	3	0	1	14	0	0	15	10
	%	1.23	8.33	0	2.78	38.89	0	0	41.67	27.78
TOTAL	No	200	7	0	16	45	19	0	91	31
	%	1.35	3.35	0.00	7.65	21.53	9.09	0.00	43.54	14.83

Legend: 1. *Staphylococcus 2.* Staphylococcal enterotoxin *3. Escherichia coli 4.* Yeasts and molds *5. Salmonella 6.Listeria monocitogenes 7. Enterobacteriaceae* 8. Total number of microorganisms

Three most common causes of contamination are *Enetrobacteriaceae* (43.54%), followed by the presence of yeasts and molds (21.53%), and an increased total number of bacteria (14.83%) (Table 2). No malformations for staphylococcus and listeria monocytogenes were found in any of the samples.

Table 3. Overview of the number of analysis and the share of contaminated findings in relation to foodstuffs groups in the period 2015-2019

Foodstuffs		2015	2016	1017	2018	2019	Total
Milk and dairy products	Number of analysis	156	229	204	188	128	905
	% of contaminated	10.90	10.04	6.86	10.64	6.25	9.06
Meat and meat products	Number of analysis	381	305	424	518	576	2204
	% of contaminated	5.77	1.96	1.89	0.39	0.00	1.72
Eggs	Number of analysis	11	15	14	15	13	68
	% of contaminated	0.00	0.00	0.00	0.00	0.00	0.00
Biscuits and related products	Number of analysis	130	196	193	206	164	889
	% of contaminated	1.54	0.00	0.00	0.48	1.22	0.56
Vegetables and vegetable products	Number of analysis	22	41	41	40	29	173
	% of contaminated	0.00	0.00	2.44	5.00	0.00	2.31
Fruits and fruit products	Number of analysis	1	8	13	7	3	32
	% of contaminated	0.00	0.00	0.00	0.00	0.00	0.00
Oils, margarine, mayonnaise	Number of analysis	25	35	36	38	38	172
	% of contaminated	4.00	0.00	0.00	0.00	2.63	1.16
Grains and products	Number of analysis	560	922	818	725	624	3.649
	% of contaminated	0.89	0.65	0.49	0.83	2.56	1.01
Refreshing non-alcoholic drinks	Number of analysis	170	123	101	83	99	576
	% of contaminated	1.76	0.00	4.95	0.00	1.01	1.56
Beer	Number of analysis	532	167	135	142	131	1107
	% of contaminated	0.00	0.00	0.00	0.00	0.00	0.00

Among food groups, microbiological contamination was most often recorded in milk and milk products, i.e. almost every tenth product (9.06%) was microbiologically contaminated (Table 3). During the analysis of eggs, fruits and fruit products, and beer, no positive case was recorded. The percentage of contaminated samples in other food groups ranged from 0.56% (biscuits and related products) to 2.31% (vegetables and vegetable products).

Table 4 Causes of microbiological contamination of analyzed foodstuffs for the period 2015-2019 in relation to food groups

Foodstuffs		1	2	3	4	5	6	7	8
Milk and dairy products	Number of contaminated samples	1	0	1	1	1	0	76	3
	% of contaminated	1.20	0	1.20	1.20	1.20	0	91.57	3.61
Meat and meat products	Number of contaminated samples	0	0	15	0	18	0	0	5
	% of contaminated	0.00	0.00	39.47	0.00	47.37	0.00	0.00	13.16
Biscuits and related products	Number of contaminated samples	1	0	0	2	0	0	2	0
	% of contaminated	20.00	0.00	0.00	40.00	0.00	0.00	40.00	0.00
Vegetables and vegetable products	Number of contaminated samples	0	0	0	2	0	0	0	1
	% of contaminated	0.00	0.00	0.00	66.67	0.00	0	0.00	33.33
Oils, margarine, mayonnaise	Number of contaminated samples	0	0	0	1	0	0	1	1
	% of contaminated	0.00	0.00	0.00	33.33	0.00	0.00	33.33	33.33
Grains and products	Number of contaminated samples	1	0	0	30	0	0	7	1
	% of contaminated	2.56	0.00	0.00	76.92	0.00	0.00	17.95	2.56
Refreshing non-alcoholic drinks	Number of contaminated samples	0	0	0	1	0	0	0	8
	% of contaminated	0.00	0.00	0.00	11.11	0.00	0.00	0.00	88.89
	Total % of contaminated samples	3	0	16	37	19	0	86	19

Legend: 1. Staphylococcus 2. Staphylococcal enterotoxin 3. Escherichia coli 4. Yeasts and molds 5. Salmonella 6.Listeria monocitogenes 7. Enterobacteriaceae 8. Total number of microorganisms

Table 4 shows the causes of microbiological contamination of the analysed foodstuffs by food groups. In the case of milk and dairy products, the total number of bacteria was stated as the cause of the contamination in as much as 91.57%. In meat and meat products, the most common causes of contamination were *Salmonella* (47.37%) and *Escherichia colli* (39.47%), while in grains and products, slightly more than ¾ of contaminated samples (76.02%) were yeasts and molds.

DISCUSSION

As there is no so-called concept of zero risk of contaminants in food, it is a tendency in all developed countries around the world to apply modern scientific methods to identify certain risks and reduce them to a minimum.[2] one way is to analyze the results of multi-year sampling.

In the period of five years, 2015-2019, in the microbiological laboratory of the Regional Centre Doboj a total of 14,784 food samples were analysed for microbiological safety, where in 200 foods or 1.35% some kind of microbiological contamination was detected (Table 1). During the first year of the observed period lowest number of samples was analysed but it resulted in the highest number of contaminated samples. Data for the entire territory of the Republic of Srpska show a steady increase in the number of analysed samples, from 14,498 in 2015 to 17,228 in 2019. [5-9] The total number of samples for microbiologi-

cal safety was 82,661out of which 719 or 0.87% were contaminated, which is evidently a lower percentage of contaminated samples compared to the share of contaminated samples analysed in the Regional Centre Doboj in the same period. Such a 'low' percentage of contaminated samples in the Republic of Srpska was primarily conditioned by the participation of contaminated samples in 2016 (0.61%) and 2017 (0.59%). The causes of this phenomenon should be specifically examined. If we transfer the analysis to the entire territory of Bosnia and Herzegovina, then we notice almost twice as many samples in 2018 (66,035) compared to 2015 (34,469).[11] Percentage of contaminated samples in the period 2015-2018 in Bosnia and Herzegovina was 1.19%.

Highest level of contaminated samples was recorded in 2015. Similar results were obtained based on the collected data on the results of laboratory analysis of food samples for microbiological safety for the entire territory of Bosnia and Herzegovina, where the percentage of contaminated samples in 2015 was 1.50%, and in 2018 (for 2019 no results have been published yet) only 1.20%. The results for the entire territory of the Republic of Srpska show lower level of contaminated samples, with the lowest percentages recorded in the first three years of the observed period, and in 2018 and 2019 the share of contaminated samples was similar to those recorded in the Regional Centre Doboj, i.e. for the whole Bosnia and Herzegovina.

Differences in the share of contaminated food samples in relation to their origin (Table 1), with lowest level of contamination of imported samples compared to off-the-shelf and food from production, can be explained primarily by quality controls in countries of origin. The three most common causes of food contamination in the five-year period were *Enetrobacteriaceae* (43.54%), followed by the presence of yeasts and molds (21.53%), and an increased total number of bacteria (14.83%) (Table 2). If we add that *Escherichia coli* were also a very frequent cause of contamination, we can make a conclusion that causes of contamination were hygienic failures. It is these, the so-called hygiene indicators, especially in the case of their increased number, that should be taken as a warning about possible appearance of pathogens and the need to perform additional analysis to exclude pathogens. Right behind these causes of contamination stands *Salmonella*. Very similar causes of food contamination were recorded for the entire territory of Bosnia and Herzegovina: *Enterobacteriaceae, Escherichia coli*, aerobic mesophilic bacteria, yeasts and molds, *Salmonella*. [10 - 13] The three-year expert report of the Croatian Food Agency registered the presence of an increased number of aerobic mesophilic bacteria in most food categories, followed by enterobacteria, yeasts and molds. Reports from the European Food Safety Authority and Centres for Disease Control from different countries show some similarities, but also differences in terms of the most common food contaminants: *Campylobacter spp., Salmonella spp. (especially Salmonella enteritidis), Listeria monocytogenes, Escherichia coli, Yersinia enterocolitica*. [14,15] While *Listeria monocytogenes* is one of the commonest causes of contamination here, at the same time, for a five-year period, Doboj Regional Centre did not record this type of contamination in any foodstuffs. At the Republic of Srpska level only individual cases have been reported. [5 - 9]

Food of animal origin (meat, poultry, fish, eggs, milk) is most often contaminated, while food of plant origin is less suitable for the reproduction of microorganisms, and thus less dangerous to human health. So, milk and milk products proved to be the ' riskiest' food group followed by meat and meat products (Table 3). Of particular concern is the fact that almost every tenth sample of milk and milk products was contaminated, having 9 out of 10 causes of contamination related to an increased number of total bacteria. The most common causes of contamination in meat and meat products, *Salmonella and Escherichia coli*, indicate poor hygiene, as well as the risk of transmitting salmonella to humans, i.e. causing food poisoning. It is indicative that, for example only in 2019, two major epidemics of food poisoning were registered in the

area under the jurisdiction of the Doboj Regional Centre, where salmonella was undeniably confirmed by laboratory analysis as the causative agent, and the epidemiological survey among patients showed that the route of transmission was through mayonnaise. However, the mayonnaise samples from the places where the incriminated food was consumed were negative because they were probably 'prepared' for sampling in the meantime. In this, but also in similar cases in previous years, it was a 'homemade' mayonnaise which was not properly stored before use, primarily referring to the air temperature during summer months. This indicates the importance of preventive analysis of this type of food, i.e. raw materials (primarily eggs) from which these foods are obtained. However, the number of analysis shows a low level of eggs control, i.e. egg products, primarily mayonnaise. Dominant cause of contamination in the group of grains and grain products was in the form of yeasts and molds. By its activity mold creates compounds that can be useful, e.g. antibiotics, but also harmful, e.g. mycotoxins. And it is the molds in grains that create the fear of the appearance of mycotoxins, i.e. increased control of this group of foods for mycotoxins is required, whether they are imported or domestically produced..[16] An additional problem, a potential danger to human health, is the diet of domestic animals with foods in which mycotoxins are present, which may result in the presence of mycotoxins in milk and meat. In the whole of Bosnia and Herzegovina, meat and meat products, as well as grains, proved to be the riskiest food groups.

CONCLUSION

The lack of so-called zero risk of contaminants in foodstuffs imposes the need for constant monitoring of microbiological food safety. In the five-year period, 2015-2019, in the microbiological laboratory of the Regional Centre Doboj a total of 14,784 food samples were analysed for microbiological safety, where in 200 foods or 1.35% some kind of microbiological contamination was detected. Hygiene indicators, as the most common causes of contamination, indicate the need for higher hygiene control throughout the food production process, from raw materials to the final product, as well as the need to conduct additional analysis to exclude pathogens. Milk and milk products, and meat and meat products, have proven to be groups of foods whose consumption carries the highest risk of transmitting the infection through food. Given that mayonnaise has proven to be the most common way of transmitting infectious diseases through food in the area of jurisdiction of the Regional Centre Doboj in epidemic form, the control of this food, i.e. eggs as a basic raw material for its production, in addition to the already mentioned two groups of foods, should be one of the priorities in food control, especially during summer months. Complete absence of listeria monocytogenes and staphylococci in food indicates the need to additionally examine this and to conduct additional tests.

Based on the multi-year continuous monitoring of test results, it is possible to make a risk assessment and adopt plans for food sampling for microbiological safety. Accordingly, we propose that in Bosnia and Herzegovina, in addition to previous annual reports for local levels, entity levels, level of Bosnia and Herzegovina, which are often stereotypical, analysis of results of foodstuffs control, should be done for a period of several years e.g. three –year period. Application of the proposed method would improve food safety in Bosnia and Herzegovina.

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