DOI 10.7251/VETJEN2301143G UDK 539.16:579.67

Original Scientific Paper

MICROBIOLOGICAL PURITY IN FOOD CHAIN

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Summary

In addition to microbiological criteria for food (food safety criteria and hygiene criteria in the production process), microbiological purity criteria are a very important link in the microbiology of the food chain. Swab samples of equipment, devices, accessories, work surfaces, work clothes and hands of workers engaged in production, processing and traffic in facilities and vehicles that come into contact with food, and where there is a risk of the appearance and spread of an infectious disease, were used for testing. The examined samples originated from facilities for the production, processing and distribution of food, restaurants and other catering facilities where food is served, educational and social protection facilities (accommodation facilities) and vehicles that come into contact with food. The study was carried out during year 2020 and included 2,958 samples. Laboratory analysis of swab samples was performed using BAS EN ISO 4833-1:2014, BAS EN ISO 21528-2:2018, BAS EN ISO 11290-1:2018 and BAS EN ISO 6579-1:2018 methods. The aim of the study is to assess the microbiological purity in the food chain. Of the total number of analyzed swab samples, 94.22% were satisfactory, and 5.78% were unsatisfactory. Observed in relation to the total number of analyzed samples, 77.19% of the samples were unsatisfactory due to the increased number of aerobic microorganisms at 30°C, and 22.81% due to the increased number of *Enterobacteriaceae*. Pathogens *Salmonella* spp. and *Listeria monocytogenes* were not isolated in any swab sample. The obtained results indicate that the general level of hygiene in the production and distribution of food is at a high level, especially due

to the absence of pathogens *Salmonella* spp. and *Listeria monocytogenes*, but that there is still a risk of contamination by *Enterobacteriaceae*, which are indicators of the hygiene of the production process.

Key words: microbiological purity, food chain, number of microorganisms, *Enterobacteriacae*, *Salmonella*, *Listeria monocytogenes*

INTRODUCTION

In addition to microbiological criteria for food (food safety criteria and hygiene criteria in the production process), microbiological purity criteria are a very important link in the microbiology of the food chain. Food handling areas, vehicles, items, utensils and equipment that come into contact with food must be regularly maintained (mechanical cleaning and washing) and disinfected to avoid any risk of contamination, while food and personnel must be constantly monitored (Rulebook, 2004). Food handlers must maintain a high level of personal hygiene and wear appropriate protective clothing, and be trained in food hygiene requirements (Gill and Jones, 1999).

Improper cleaning and disinfection are directly related to the occurrence of various foodborne diseases (Johns, 1991). Training of food handling personnel about basic concept and requirements of personal hygiene is an integral part of measures to obtain a safe product for the consumer (Adams and Moss, 1997).

In the microbiological examination of swabs, the assessment of surface hygiene is mainly based on the determination of the number of aerobic microorganisms at 30°C and *Enterobacteriaceae* per cm^2 (Aarnisalo et al., 2006). In addition to these, analysis is sometimes performed for the presence of other microorganisms, as it has been proven that *Salmonella* spp., *Shigella* spp., *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and faecal streptococci originate from infected persons who handle food (Lawrie, 1998).

Surfaces that come into contact with ready-to-eat food should be analyzed for the presence of *Listeria monocytogenes* as the cause of listeriosis, a serious infectious disease of humans and animals (Aguado et al., 2001; Lundén et al., 2002; Lundén et al., 2003; Suihko et al., 2002; Fonnesbech-Vogel et al., 2001). Study done by Samelis and Metaxopoulos (1999) indicates that, in the food production process, the environment is a more common source of *Listeria monocytogenes* than live animals and carcasses, so special attention should be paid to the cleaning and disinfection process.

Due to the risk to public health, mandatory tests for *Listeria monocytogenes* in ready-to-eat foods and for *Salmonella* spp. in meat, semi-finished products and meat products are conducted in production, processing and trade (Rulebook, 2012; Rulebook, 2019)

The current regulation defines the criteria of microbiological purity and the frequency of control of equipment, devices, accessories, work surfaces, work clothes and hands of workers in production and traffic in facilities and vehicles that come into contact with food, in which there is a risk of the appearance and spread of an infectious disease (FSA, 2013; Rulebook, 2018; Rulebook, 2019).

The aim of the study is to assess the state of microbiological cleanliness in the food chain.

MATERIALS AND METHODS

Swabs samples of equipment, devices, accessories, work surfaces, work clothes and hands of workers in production, processing and traffic in facilities and vehicles that come into contact with food, where there is a risk of the appearance and spread of an infectious disease, were used for analysis. The samples originated from facilities for the production, processing and distribution of food, restaurants and other catering facilities where food is served, educational and social protection facilities (accommodation facilities) and vehicles that come into contact with food. The study was carried out during year 2020 and included 2,958 samples.

Sample collection and transport was performed according to BAS ISO 18593 (ISBIH, 2008).

The assessment of the samples in relation to the number of aerobic microorganisms at 30°C and *Enterobacteriaceae* (Rulebook, 2012; FSA, 2013; Rulebook, 2018), *Salmonella* spp. (Rulebook, 2019) and *Listeria monocytogenes* (Rulebook, 2012; Rulebook, 2019) was performed based on the categories of analyzed samples and microbiological criteria as shown in Table 1.

Table 1 Categories of samples and microbiological purity criteria

Category of samples	Number of aerobic microorganisms at 30°C	*Enterobacteriaceae*	*Listeria monocytogenes*	*Salmonella* spp.
Porcelain, glass, smooth metal surfaces cfu/cm^2	≤ 10	0 - 1	Absence in 100cm^2/swab	Absence in 100cm^2/swab
Other surfaces (wooden, plastic, stone) cfu/cm2	≤ 30	0 - 1	Absence in 100cm^2/swab	Absence in 100cm^2/swab
Plates, bowls, cutlery and smaller dishes; dishes and utensils that come into contact with food cfu/ml (cm^2)	≤ 100	0 - 1	Absence in 100cm^2/swab	Absence in 100cm^2/swab
Hands of persons in contact with food cfu/ml (cm^2)	≤ 200	0 - 1	Absence in 100cm^2/swab	Absence in 100cm^2/swab
Bottles or packaging for liquid products cfu/ml	0 - 1	0 - 1	Absence in the swab	Absence in the swab

cfu: *colony forming units*

Laboratory analysis of swab samples was performed using the following methods:

- detection of *Salmonella* spp. according to BAS EN ISO 6579-1 (ISBIH, 2018a),
- detection of *Listeria monocytogenes* according to BAS EN ISO 11290-1 (ISBIH, 2018b),
- the number of aerobic microorganisms at 30°C according to BAS EN ISO 4833-1 (ISBIH, 2006),

- number of *Enterobacteriaceae* according to BAS EN ISO 21528-2 (ISBIH, 2018c).

In the statistical analysis of the results obtained in our study descriptive statistical parameters were used. The results of the study are presented in tables and graphs.

RESULTS AND DISCUSION

One of the main risks of food contamination originate from the work process of food handlers and microorganisms, pathogens, that are present in or on the staff, which are then transferred from the staff to the food during the handling process (Gordon-Davis, 1998).

It is common that the facilities hygiene control is obtained on surfaces that are most often in contact with food (Watnick and Kolter, 2000).

The structure of the analyzed swab samples by category is shown in Figure 1.

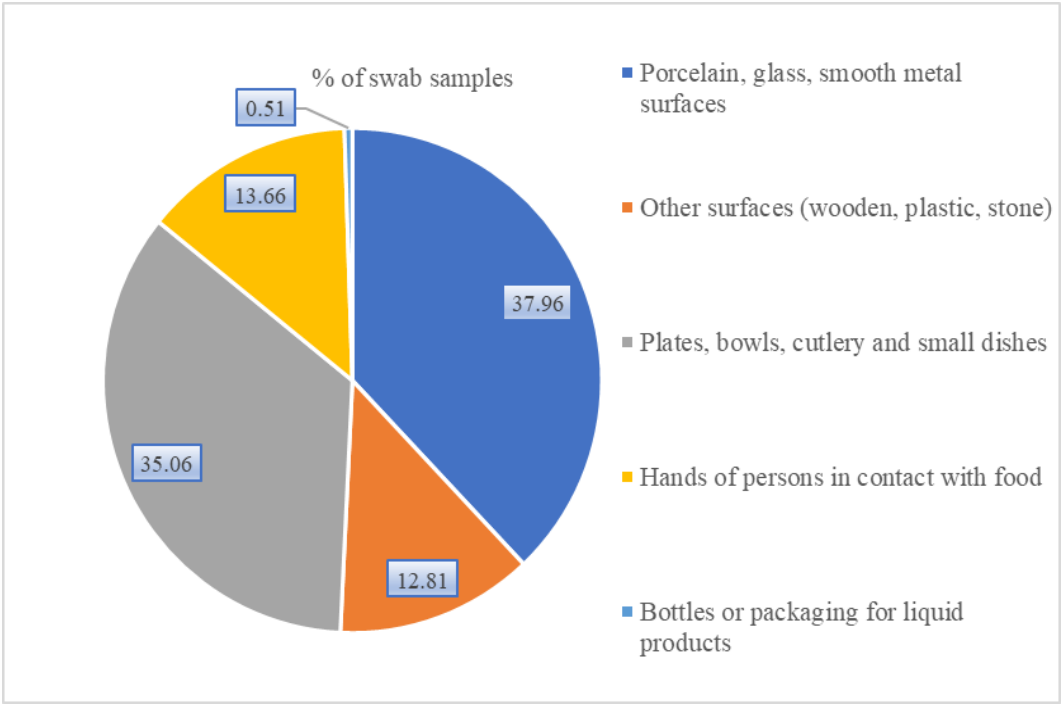

Figure 1 Swab samples by category

The structure of the analyzed samples in relation to the category was similar to the study done by Golić et al. (2019). A negligibly small number of swab samples refers

to the category "bottles or packaging for liquid products" due to the fact that very few subjects in the food business included in this study use bottles or packaging for liquid products in food production. Therefore, the test results of these samples are presented, but not taken into account for the discussion, because we believe that the number of samples is not representative in relation to the total number of samples included in the study.

The structure of the analyzed swab samples in relation to the test parameter is shown in Figure 2.

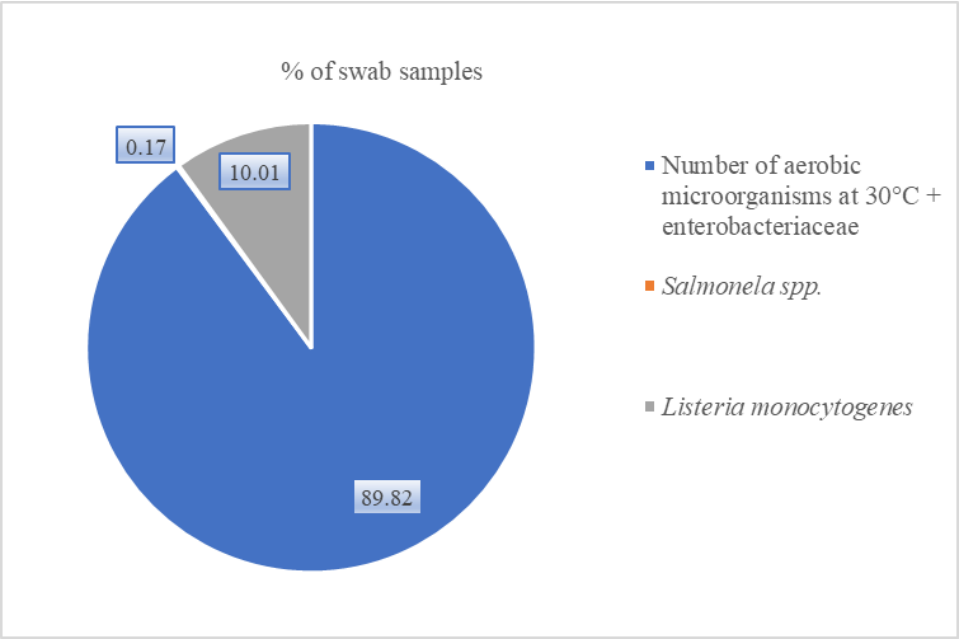

Figure 2 Swab samples according to the test parameter

The structure of the analyzed samples according to the test parameter was similar to the study by Golić et al. (2019) for the number of aerobic microorganisms at 30°C, enterobacteria and *Salmonella* spp. The number of samples analyzed for the presence of *Listeria monocytogenes* is almost double, because this study included a significant number of subjects in the ready-to-eat food business.

Figure 3 shows the dynamics of sampling, i.e. examination of swab samples by month.

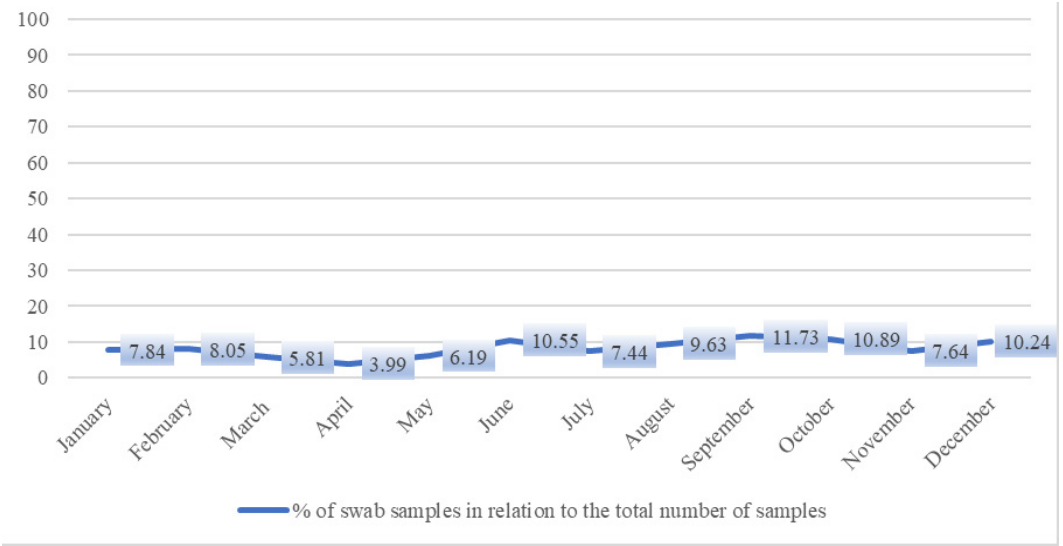

Figure 3 Dynamics of examination of swab samples by month

The samples were taken during all twelve months of year 2020, and the dynamics depended on the availability of subjects in the food business.

By comparing the obtained results with the microbiological purity criteria, the acceptability of the microbiological purity of the sample is evaluated. A sample is of acceptable microbiological purity if it meets the microbiological criteria. If it contains up to 50% more microorganisms, the sample can be of acceptable microbiological purity if it does not contain pathogenic bacteria (Rulebook, 2018; Rulebook, 2019)

Of the total number of tested swab samples, 94.22% were satisfactory, and 5.78% were unsatisfactory. Of the unsatisfactory samples, 77.19% are due to an increased number of aerobic microorganisms at 30°C, and 22.81% are due to an increased number of *Enterobacteriaceae*. Observed in relation to the total number of analyzed samples, 4.46% of the samples were unsatisfactory due to an increased number of aerobic microorganisms at 30°C, and 1.32% due to an increased number of enterobacteria.

In 81% of cases, the occurrence of food-borne diseases is the result of food contamination during the production of which the principles of good production practice were not respected (Rašeta et al., 2012). The absence of *Listeria monocytogenes* indicates an efficient cleaning, washing and disinfection program (Samelis and Metaxopoulos, 1999). In our study, the pathogens *Salmonella* spp. and *Listeria monocytogenes* were not isolated in any swab sample. These results are

consistent with the results obtained by Kalaba et al. (2017) and Golić et al. (2019). The share of unsatisfactory swab samples by category is shown in Figure 4.

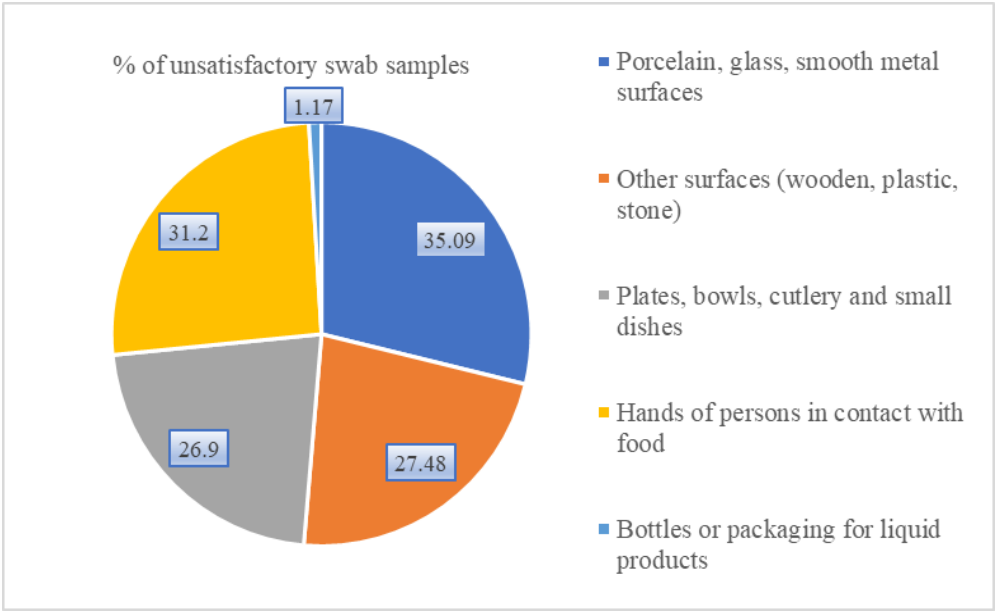

Figure 4 The share of unsatisfactory swab samples by category

The most unsatisfactory samples were from the categories "porcelain, glass, smooth metal surfaces" and "hands of persons in contact with food", which is in accordance with the study done by Golić et al. (2019). This indicates that the hygiene of persons who come into contact with food is a major risk, which is in line with the conclusions of Gordon-Davis (1998).

Figure 5 shows the test results of swab samples by category.

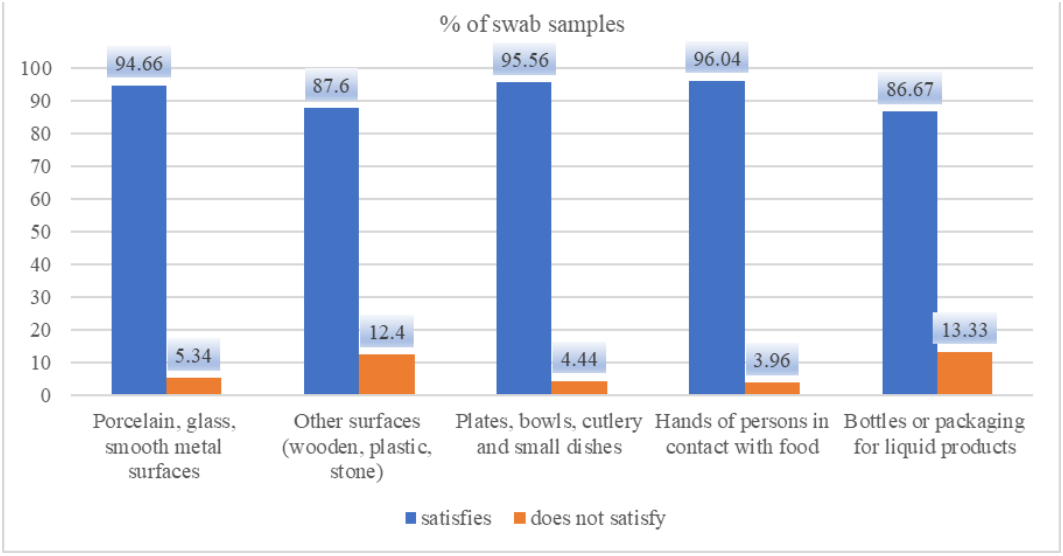

Figure 5 Results of swab samples by category

The obtained results for the category "hands of persons in contact with food" are in accordance with the results obtained by Ivanović et al. (2013) and for this category are significantly more favorable than the results of Golić et al. (2019) and Kalaba et al. (2017). For the categories "plates, bowls, cutlery and small dishes; dishes and utensils that come into contact with food" and "porcelain, glass, smooth metal surfaces" the results are in accordance with the results of Golić et al. (2019), while the percentage of unsatisfactory samples from the "other surfaces (wooden, plastic, stone)" category is significantly higher, probaly due to the specificity of these surfaces, which are more difficult to clean and maintain.

Figure 6 shows the results of unsatisfactory swab samples by category according to the tested parameter.

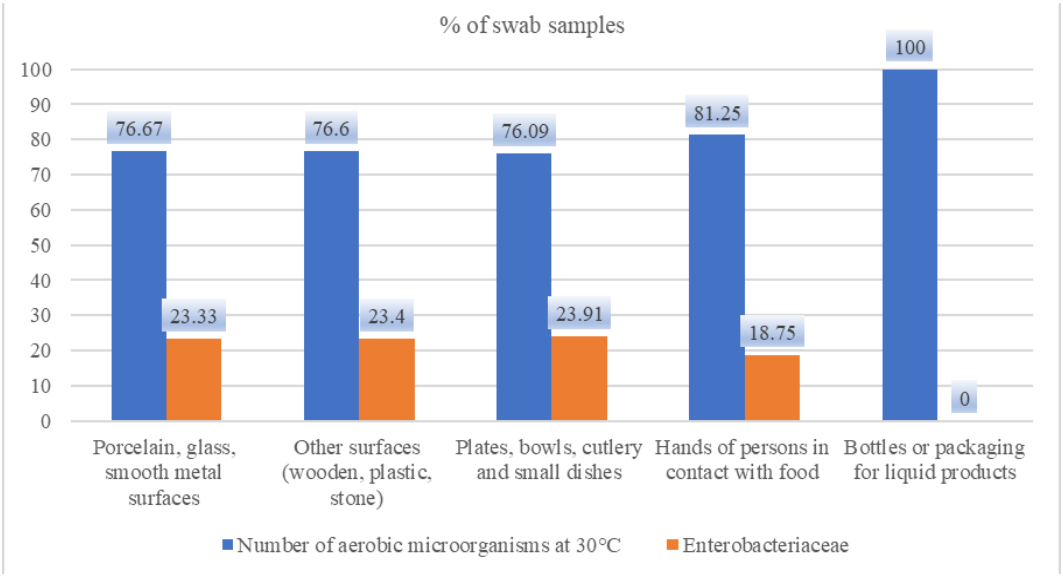

Figure 6 Results of unsatisfactory swab samples by category according to the tested parameter

When analyzing the results of unsatisfactory swab samples by category in accordance to the tested parameter, it is observed that most samples were unsatisfactory due to an increased number of aerobic microorganisms at 30°C, while significantly fewer unsatisfactory samples were due to an increased number of enterobacteria, which is in accordance with the results of Golić et al. (2019). However, completely contrary to the mentioned study, we found that the category "hands of people in contact with food" had the most unsatisfactory samples due to the increased number of aerobic microorganisms at 30°C (81.25%), but also the least unsatisfactory samples due to the increased number of enterobacteria (18.75%), which is a favorable circumstance because the presence of enterobacteria in swab samples decreased, and pathogenic bacteria, some of which are enterobacteria, were not detected at all.

CONCLUSION

The obtained results indicate that the general level of hygiene in the production and distribution of food is at a high level, especially due to the absence of pathogens *Salmonella* spp. and *Listeria monocytogenes*, but that there is still a risk of contamination by *Enterobacteriaceae*, which are indicators of the hygiene of the production process. Certainly, the greatest risk is from saprophytic bacteria,

which can significantly affect the microbiological, physical-chemical and sensory characteristics of the final product in the food chain. Because of this, it is necessary to constantly maintain a high level of awareness of the staff in terms of hygiene, and to implement regular measures, to carry out staff training and testing in the field of minimum hygiene. Also, the process of continuous self-control significantly affects the high level of microbiological purity in the food chain.

Conflict of interest statement: The authors declare that there is no conflict of interest.

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Paper received: 16.05.2023.
Paper accepted: 21.07.2023.