LISTERIA MONOCYTGENES ISOLATED IN READY-TO-EAT FOOD IN SOUTH BAČKA REGION OF VOJVODINA PROVINCE, SERBIA

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Abstract - Listeria monocytogenes is pathogenic bacterium that can contaminate food products during and after processing. As ready-to-eat food does not undergo any treatment to ensure its safety before consumption, the risk of foodborne disease must be considered if this pathogen is present in the food. As diseases caused by contaminated food are an important public health problem today, the aim of this study was to determine the prevalence of Listeria monocytogenes in different ready-to-eat food products. In the seven-month period from June 1 to December 31, 2011, a total of 1 380 food samples were examined in the Division of Sanitary Bacteriology, Center for Microbiology, Institute of Public Health of Vojvodina in Novi Sad. A total of 912 samples were analyzed for the presence of Listeria monocytogenes according to ISO 11290-2. The identity of suspected Listeria monocytogenes was confirmed using the VITEK 2 Compact system (BioMerieux, France). Out of 912 samples, Listeria monocytogenes was detected in 18 (1.97%). Listeria monocytogenes was mostly found in cooked meals (in 6 samples out of 18), sandwiches (4 samples) and frozen food, such as ice-cream and frozen vegetables (4 samples). It was also found in tofu bread spreads (2 samples), cream cheese (1 sample) and cakes (1 sample). The presence of Listeria monocytogenes in some ready-to-eat food could present a public health hazard, particularly to the high-risk population group, because of the high mortality rate associated with listeriosis and the widespread nature of the organism. Monitoring of listeriosis is essential to prevent foodborne outbreaks, and in assessing human health risk in ready-to-eat foods.

Key words: Listeria monocytogenes, ready-to-eat food

INTRODUCTION

Diseases caused by contaminated food are a very important public health problem today (Shahbazi et al., 2013). Listeria is widely distributed in our environment and the most important species is Listeria monocytogenes, which has long been recognized as the cause of a serious human disease known as listeriosis, causing mild clinical manifestations such as fever, muscle aches and sometimes gastrointestinal symptoms such as nausea and diarrhea in healthy individuals; abortion, stillbirth, premature delivery in pregnant women, and meningoencephalitis and septicemia in immunocompromised persons, proving that the disease has a high fatality rate in susceptible populations (Awaisheh, 2009; Bĕrzins et al., 2009). Transmission is generally by the eating of contaminated food, because Listeria monocytogenes is a pathogenic bacterium that can contaminate food products during and after processing, and ready-to-eat food does not undergo any treatment to ensure its safety before consumption (Van Coillie et al., 2004). The important impact that listeriosis has on public health is not always recognized, particu-
larly since this disease is relatively rare compared to other common foodborne infections (Gormley et al., 2010; Williams et al., 2011) Also, listeriosis is under-reported due to its status as a non-notifiable disease in many countries and because of the absence of adequate surveillance programs (Awaisheh, 2010). Given the considerable public health implication, the monitoring of \textit{Listeria monocytogenes} incidence in foods is important. For this reason, the risk of food-borne disease must be considered if this pathogen is present in the food.

According to the old Serbian Rulebook, food samples were not analyzed for the presence of \textit{Listeria monocytogenes}. However, since June 1 2011, the Regulation on general and specific requirements for food hygiene at all stages of production, processing and circulation (Official Bulletin of Republic Serbia, no. 72/2010) has been implemented and \textit{Listeria} testing has been introduced. The aim of this study was to determine the prevalence of \textit{Listeria monocytogenes} in different ready-to-eat food products.

**MATERIALS AND METHODS**

**Sampling**

Between June 1 and December 31, 2011, a total of 1380 food samples were aseptically sampled on the territory of Vojvodina Province. Among them, 912 were ready-to-eat foods. The samples were placed in sterile bags and taken to the laboratory in a cooled container to 4ºC, where they were analyzed within four hours from sampling.

**Microbiological analysis**

Isolation and identification of \textit{Listeria monocytogenes} was performed following the SRPS EN ISO 11290-2 method, according to the Serbian Rulebook on general and specific requirements for food hygiene in all stages of production, processing and circulation (Official Bulletin of Republic Serbia, no. 72/2010), which is in agreement with European Commission Regulation (EC) No. 2073/2005. According to the Rulebook, ready-to-eat food must not support the growth of \textit{Listeria monocytogenes} within its shelf life; it must not exceed 100 per g at the point of consumption (SRPS EN ISO 11290-2:2010, 2010).

Constituents of each food sample were mixed well, and 10 g of each sample was aseptically weighed using a sterile spatula and homogenized for 1 min with 90 ml of buffered peptone water in a sterile stomacher bag. The initial suspension was left to stand for 1 h at 20ºC± 2ºC, in order to resuscitate the stressed microorganisms. A dilution range was prepared after resuscitation. Thereafter, 0.1 ml of the initial suspension was transferred to each of two dishes of ALOA agar (Agar Listeria, according to Ottaviani and Agosti), and incubated in an inverted position at 35ºC-37ºC for 24-48 h. Typical colonies of \textit{Listeria monocytogenes} are green-blue surrounded by an opaque halo. Five presumptive colonies were picked from each selective agar plate at two successive dilutions, purified using trypticase soy agar and subjected to morphological and confirmation tests. Isolates with non-spore-forming Gram-positive coccobacilli, positive for catalase and umbrella growth in motility test medium at 25ºC, were examined for hemolysin production using tryptose agar with 5% sheep blood. In addition, suspected isolates were tested for fermentation of rhamnose, xylose and mannitol and used in the CAMP test to assess synergistic lysis of red blood cells against \textit{Staphylococcus aureus} ATCC 25923 and \textit{Rhodococcus equi} ATCC 6939. The identity of suspected \textit{Listeria monocytogenes} colonies was confirmed using the VITEK 2 Compact system, BioMerieux, France.

**Statistical analysis**

Laboratory data were entered into a database using SPSS (statistical package for the social science) for Windows. The $\chi^2$ test was used to compare variables. All differences where the probability of the null hypothesis was $p<0.05$ were considered significant.

**RESULTS AND DISCUSSION**

In this study, 912 samples were analyzed for the presence of \textit{Listeria monocytogenes}. 
All *Listeria monocytogenes* positive samples (18 samples out of 912) showed a contamination level higher than 100 cfu/g food.

Products showing the highest contamination rate were cooked meals (33.3%, 6/18), sandwiches (22.2%, 4/18), with unexpectedly high contamination in frozen foods, such as ice cream and frozen vegetables (22.2%, 4/18). Contamination was also found in tofu bread spreads (11.1%, 2/18), cream cheese (5.6%, 1/18) and cakes (5.6%, 1/18).

According to the EFSA Report, in the EU the number of confirmed listeriosis cases has increased from 2004 to 2006 with a slight decrease observed in 2007, increasing once more from 2008 to 2009. The fatality ratio among reported cases was high, round 16.6% (EFSA, 2009; EFSA, 2011).

Even though the pilot investigation performed in Novi Sad in 2007 indicated that 4.4% of examined food samples had been contaminated with *Listeria monocytogenes*, for Serbia there are no continuous data for previous years, since *Listeria monocytogenes* examination in food products has only been obligatory since June 2011 (Trajković-Pavlović et al., 2007).

According to our data, the incidence of *Listeria monocytogenes* observed in ready-to-eat foods in this study was lower than that reported by EFSA: the percentage of *Listeria monocytogenes* observed was about 1.97%, whereas EFSA reported a prevalence rate of 4.4%, (EFSA, 2009).

This study reveals a higher contamination of *Listeria monocytogenes* in sandwiches and cooked meals. The percentage of the pathogen detected in sandwiches was higher than the findings of Little et al. (2009) and Meldrum et al. (2010), who isolated *Listeria monocytogenes* in about 7% and 0.05%, respectively, of sandwiches (p value < 0.05).

For cooked meals, the results are much higher than Wagner's findings in Austria (33.3% vs. 4.5%). This difference was statistically significant (p value < 0.05) (Wagner et al., 2007).

For sandwiches and cooked meal samples, the data revealed an inadequate application of Good Hygienic Practice to prevent post-processing contamination.

The incidence of *Listeria monocytogenes* in frozen food was around 22%. The results observed in this study highlighted a percentage higher than that reported by Cabejo et al. (2008) and Mengesha et al. (2009). Considering that these products are packed at large-scale industrial food processing plants, this high percentage is probably due in part to significantly contaminated raw materials and in part to inadequate application of Good Manufacturing Practices (GMPs). In fact, the manufacturing processes may not ensure elimination of the pathogen, especially if the raw materials are heavily contaminated. Considering that the processes involve a lot of handling by workers as well as the use of technically often complex equipment, post-processing contamination is considerable.

The data highlight the importance of prevention and control of *Listeria monocytogenes*, which could be achieved by the appropriate implementation of Sanitation Standard Operating Procedures, Good Manufacturing Practices and Hazard Analysis and Critical Control Point systems.

In the cream cheese samples, the prevalence of the observed pathogen was in agreement with other authors (Mengesha et al., 2009; Wagner et al., 2007; Gebredsadik et al., 2011). The low incidence of *Listeria monocytogenes* of around 6% may be due to the high-temperature treatment of milk in order to eliminate cells of the pathogens.

Compared with the findings of Mengesha et al. (2009), the cake samples were less contaminated with *Listeria monocytogenes*: Mengesha et al. observed 6.5% positive samples compared to 5.6% in our study. Therefore, this study confirmed that pastries and cakes are considered a risk product for human listeriosis and *Listeria monocytogenes* contamination is of concern for the confectionery industry.
Because of the high mortality rate associated with listeriosis and the widespread nature of the organism, the presence of *Listeria monocytogenes* in some ready-to-eat food items could present a public health hazard, particularly to the high-risk group of the population. Surveillance of listeriosis is essential to prevent foodborne outbreaks and in the assessment of human health risk in ready-to-eat foods.

REFERENCES


