

Wioletta ADAMUS-BIALEK^{1*} and Monika WAWSZCZAK¹

MICROBIOLOGICAL CONTAMINATION OF FOOD

MIKROBIOLOGICZNE ZANIECZYSZCZENIA ŻYWNOSCI

Abstract: The aim of the study was the statistical analysis of available data of microbiological investigation of food products. Data for the analysis were provided by the sanitary-epidemiological stations in Świętokrzyskie voivodship in 2008–2011. The different types of food products (meat, dairy products, grains products, fish, vegetables, fruit, water, soft drinks, vegetable fats, herbs, coffee, tea, cocoa, foodstuffs intended for particular nutritional uses and nutritional supplements) were studied. The research material was obtained from the products of national origin, food imported with distinguishing between the products originating from the European Union. The presence of *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* spp. and *Yersinia enterocolitica* was analyzed. *Escherichia coli* and *Yersinia enterocolitica* were detected sporadically. *Listeria monocytogenes* was detected the most frequently in confectionery products, convenience foods, milk and milk products, most rarely in fruits and vegetables. It has been shown that the most frequent pathogens in food samples were *Salmonella* spp., also responsible for the largest number of food poisoning in Poland. *Salmonella* spp. were detected primarily in domestic products. The increase of the prevalence of those bacteria was observed during next years (2008–2011). It should be emphasized, that all of the analyzed samples contained at least one of the studied species. Food contamination may cause an increase of food poisoning incidents as well as others diseases caused by these pathogenic bacteria. It is important to observe rules of hygiene during the production, preparation and consumption of food products, but this problem is more complex.

Keywords: food contamination, epidemiology

Introduction

The presence of microorganisms in food products may have an beneficial or adverse effect for human health. For a long time, bacteria are used for food production, eg milk products, bread, beer and other alcohols. Bacteria are present in almost every sold food product. In the present study we focus on an assessment of microbial contaminants in food. In the early seventies of the last century, food contamination has significantly increased. The main reason was the development of industry and agriculture. It also has

¹ Department of Environment Protection and Modelling, Jan Kochanowski University, Świętokrzyska 15, 25–406 Kielce, Poland.

* Corresponding author: wioletta.adamus-bialek@ujk.edu.pl

an impact on the epidemiological situation in Poland [1]. The climatic conditions in Poland are conducive to the development of agriculture. This development is associated with overproduction of sewage, excessive use of organic fertilizers and organic-mineral fertilizers [2]. Food is monitored primarily in terms of heavy metal concentration, the amount of pesticides and nitrates, the presence of antibiotics and microbial contamination. Consumption of contaminated food poses a high risk of serious food poisoning which may lead to death [3]. Food poisoning can be divided into chemical and biological origin. Poisoning of chemical basis are usually caused by preservatives, dyes, antibiotics and pesticides. Chemical contamination poses a threat to the entire society, while microbiological contaminants threaten on groups of persons belonging to young, old, pregnancy and ill (YOPI). People belonging to YOPI have to pay particular attention to the quality of nutrition and consumed products. Poisoning of biological origin are caused by viruses, parasites, protozoa, fungi, bacteria and bacterial toxins [4]. In Poland, food poisoning is a serious epidemiological problem especially during the summer and early autumn. High temperatures and the touristic migration of people makes it difficult to preserve food hygiene, storage and food production. In spite of different etiologies, bacterial poisoning are characterized by common symptoms: diarrhea, abdominal pain, nausea, vomiting, dizziness, increased body temperature, weakness and dehydration [5]. The most important etiological factors include endotoxin of *Salmonella* spp., *Staphylococcus* sp., botulinum toxin produced by *Clostridium botulinum*, less frequently *Clostridium perfringens* type A toxins. Bacteria classified as opportunistic pathogens as *Escherichia coli*, *Proteus vulgaris*, *Bacillus cereus* and other are also important etiological factor of food contamination [6]. Food is the optimal environment for the growth of microorganisms. The number and species composition of microorganisms depend on the environmental conditions that stimulate or inhibit their growth. The basic agents include water activity, temperature, air relative humidity, pH of environment, residual pressure, the availability of oxygen, the presence of salt (cations and anions) [7]. Due to the specific conditions of growth of pathogenic microorganisms, the microbiological food analysis is carried out by several methods. The analyzes are conducted in accordance with the guidelines described in the standard PN-ISO 4832:2007. Full screening of food needs to determine the essential etiological factors: the number of mesophilic aerobic microorganisms grown at 30 °C (PN-EN ISO 4833:2004+A1:2005), the number of coagulase-positive staphylococci grown in 37 °C (PN-EN ISO 6888-1:2001+A1:2004), the number of presumptive *Bacillus cereus* grown at 30 °C (PN-EN ISO 7932:2005), the presence of *Listeria monocytogenes* (PN-EN ISO 11290-1:1999+ A1:2005; PN-EN ISO 11290-2:2000+A1:2005+A1:2006+Ap2:2007), the number of beta-glucuronidase-positive *Escherichia coli* growing in 44 °C (PN-ISO 16649-2:2004), the presence of *Enterobacteriaceae* growing at 37 °C (PN-ISO 21528-1:2005 P.4.1; PN-ISO 21528-2:2005), the number of mold and yeast cultured at 25 °C (PN-ISO 21527-1:2009; PN-ISO 21527-2:2009). Due to the widespread occurrence of microorganisms in food, food poisoning caused by biological factors will be always recorded. Proper storage and ensure hygiene during the production may reduce the risk of infection to a minimum. Control of food processing facilities should be done

as often as possible to minimize the risk of getting contaminated food products to consumer.

Materials and methods

The research materials were the data available from three sanitary-epidemiological stations in Kielce, Busko-Zdroj and Sandomierz in Swietokrzyskie voivodship. The food analysis presence of *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* and *Yerania enterocolitica*. All samples were collected in accordance with the standards in force in Poland. In all stations, analyzed samples belonged to meat products, dairy products, grains products, fish, vegetables, fruit, water, soft drinks, vegetable fats, herbs, coffee, tea, cocoa, foodstuffs intended for particular nutritional uses and nutritional supplements. The statistical analyzes were performed by GraphPad Prism v.6.

Results and discussion

The aim of this study was the investigation of food products based on the presence of pathogenic bacteria. Analysis of food samples was carried out by three sanitary-epidemiological stations in Swietokrzyskie voivodship. This allows to illustrate the local problem of infections of food products over several years.

The available results of microbiological analysis represent prevalence of *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* in food samples, in 2009 and 2011. The data were made available by sanitary-epidemiological station in Busko-Zdroj in Swietokrzyskie voivodship. Analyzing the data carefully, the number of positive samples with bacteria *Salmonella* spp., and *Listeria monocytogenes* decreased by 27 % and 32 % respectively. The presence of *Escherichia coli* in food increased by 1.45 fold in 2011. The frequency of identified bacteria in the food was comparable or diverse between these two years. It was depended on the type of food (Fig. 1).

Salmonella spp. was identified in almost all types of products. Exemption was water and soft drinks. The highest number of samples (more than 50) were tested from the groups of product such as: milk and dairy products, confectionery and vegetables. According to data confectionery products were contaminated by *Salmonella* spp. in 100 % of studied samples. *Salmonella* spp. was found in total number of samples of poultry, eggs and eggs products, grain and grain products, coffee, tea cocoa, delicatessen products and nutritional supplements. It is known that *Salmonella* spp. are commonly found in chicken eggs, chicken and pork meats [8]. All product groups in which *Salmonella* was found seem to be associated with these typical sources of infection [9].

In this study, the number of pathogens detected in food in three sanitary stations in Swietokrzyskie voivodship was analyzed. In all cases it was found, that the most contaminated products included confectionery, milk and milk products. As mentioned above, the most frequently identified species was *Salmonella* spp. The frequent occurrence of this species in milk and milk products was also observed in other countries. For example, *Salmonella* spp. was identified in 27 % of fresh cheese in

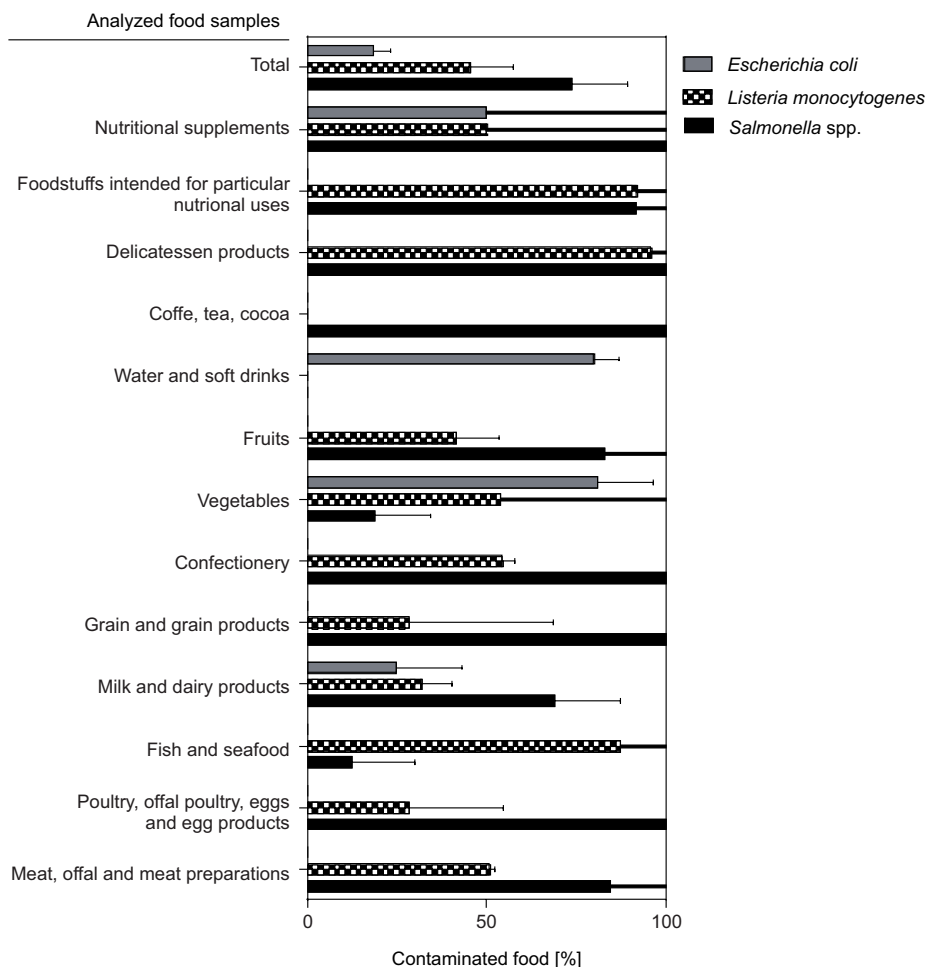


Fig. 1. Percent of food contaminated by particular bacteria (*Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp.) in Busko Zdroj (Swietokrzyskie voivodship) in 2009 and 2011 compared to total analyzed food samples

Mexico. In the same cheese, *Escherichia coli* was identified in 10 % of samples [10]. In the case of milk and milk product samples tested in Swietokrzyskie voivodship, *Salmonella* spp. was detected in 82 % of analyzed products. Such a large percentage of occurrence may result from the diversity of milk products examined in Swietokrzyskie voivodship. *Salmonella* spp. was also found in 12 % of vegetables samples. It is three times less than in the case of vegetables tested for the presence of *Salmonella* spp. in Malaysia [11]. This could be due to different climatic conditions and the sanitary state of individual countries. Traditional physical process of food decontamination (Thermal pasteurization) can eliminate most of vegetative microorganisms (eg *Salmonella*) causing food-borne diseases [12]. Therefore, despite the high prevalence of *Salmonella*

in a food product the risk of infection can be easily reduced by the appropriate preparation of the food. Another pathogen detected in food is *Escherichia coli*. In this study, the presence of *E. coli* was the most frequent detected in vegetables and fruits. It could result from fecal contamination of domestic or wild animals during cultivation or handling [13]. The results presented in this work show also that the most contaminated products were animal origin (meat, milk and milk products). *E. coli* can adapt to adverse environmental conditions. Data prove that *Escherichia coli* can grow in the presence of Biocides which are compounds commonly used in the food system as poultry decontaminants (eg trisodium phosphate) to reduce or eliminate both pathogenic and spoilage micro-organisms [14]. *Salmonella* spp. and *E. coli* were the most frequently detected bacteria in a food product, what could generate a serious risk of epidemics.

We also examined the incidence of *Listeria monocytogenes* in food products. The presence of *L. monocytogenes* was found in all product groups except water and soft drinks, coffee, tea, cocoa. The largest amount was found in processed foods as well as in fish. According to the literature, *L. monocytogenes* in relatively large quantities is present in certain food groups, such as soft cheese, unpasteurized milk, ice cream, various types of meat such as: fermented meats, sausages, hot dogs, turkey, ham RTE (ready-to-eat) and seafood products, among others, smoked cold or warm salmon, shrimp, clams, pickles, fish salads. *L. monocytogenes* is also found in many types of vegetables, fruits and fruit juices [8, 15–17].

Another source of data came from Sanitary-Epidemiological Station in Kielce operates on the whole territory of province. The results provide the overall epidemiological status in Swietokrzyskie voivodship in 2010. The research material was obtained from the products of national origin, food imported with distinguishing between the products originating from the European Union. The data show, that the most polluted were the products of national origin. The most frequently detected bacteria was *Salmonella* spp. The presence of *Salmonella* spp. was detected in 1320 analyzed samples, *Escherichia coli* in 277 samples, *Yersinia enterocolitica* in 51 samples. Based on data from the sanitary – epidemiological station, the frequency of food poisoning incidents caused by bacteria in 2010 was analyzed (Fig. 2). The most common food poisoning was caused by *Escherichia coli*. This pathogen caused illnesses in 18 % of cases. *Yersinia enterocolitica* caused poisoning in 15 % of cases and *Salmonella* spp. – 5 %. According to literature, the pathogenicity of *Y. enterocolitica* and *E. coli* is characterized by a presence of virulence factors, which give a high morbidity in humans, even in small concentration of bacteria in the body [18]. In this study, we observed that *Salmonella* spp. caused infection three times less than *E. coli* and *Y. enterocolitica*. This can be due to the fact, that emergence of infection requires high infective dose of cells, what amounts to 10^6 – 10^8 cells [18].

Similar analysis were made available from Sanitary – Epidemiological Station in Sandomierz. The presence of microbiological indicators were studied in food products in 2008–2010. Samples were collected from all over the city. The increase of number of poisoning caused by *Listeria monocytogenes* was observed in 2009 and 2010. Despite the absence of data of mortality or poisoning caused by *L. monocytogenes* in Swietokrzyskie voivodship, the high number of detected pathogens was observed in

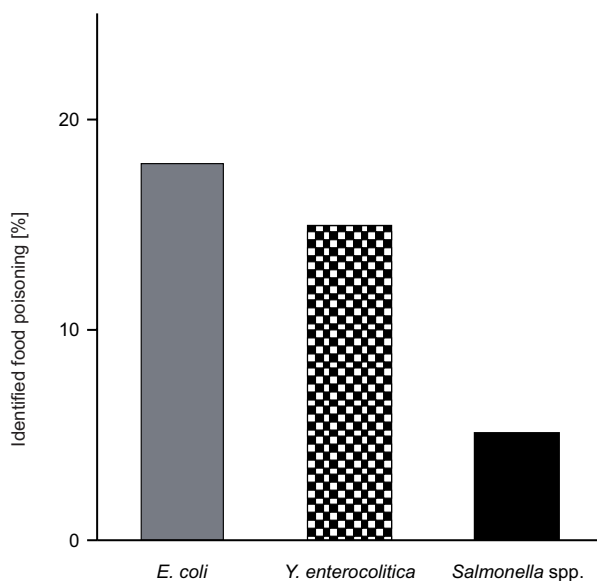


Fig. 2. Percent of food poisoning cases induced by bacteria detected in examined food samples in 2010 in Swietokrzyskie voivodship

2009 (Fig. 1). In 2011, the number of cases has decreased. This phenomenon is observed also in all over the world in recent years [19, 20]. Currently, infections caused by *Listeria monocytogenes* are regarded as a serious threat, because it is characterized by a high mortality rate 20–30 % [21]. In the EU, the proportion of patients with listeriosis has increased by 19.1 % in 2009 in comparison to 2008. In 2009, 1,601 positive cases were recorded, this level remained also in 2010. In 2009, *L. monocytogenes* led to 270 deaths. Meanwhile in the United States microorganism was the cause of 2,500 cases, 2,289 hospitalizations and 449 deaths. Listeriosis is therefore one of the deadly food-borne illness reported in the United States or countries of European Union [8, 22, 23].

It was found, that all analyzed food samples possessed at least one of analyzed bacterial pathogen. It should be emphasized, that the presence of pathogenic bacteria in food is not necessary to illness after consumption. Every organism has defense mechanisms which prevent bacterial infection. Ability to defend is individual feature of the species it and determines the occurrence of disease [24–28].

Conclusions

The most common pathogen detected in food was *Salmonella* spp., also responsible for the largest of the number of cases in Swietokrzyskie voivodship. *Escherichia coli* was identified in meat, milk and dairy products, also in water and soft drinks. In 2009 and 2011, an increase of the *Listeria monocytogenes* positive samples detected in food product were observed.

Acknowledgements

We thank sanitary – epidemiological stations in Swietokrzyskie voivodship for providing source material for the analysis. We would like to thank also Diana Berlinska, Wioleta Nowaczek and Ewelina Sokolowska for technical support.

This study was supported by Jan Kochanowski University statutory research No. s612488.

References

- [1] Nikonorow M. Nadzór sanitarny nad żywnością i przedmiotami użytku Podręcznik instruktora higieny. (Sanitary supervision of food and objects to be used. Guide for hygiene instructor). Warszawa: PWZL; 1985.
- [2] Kłapeć T, Cholewa A. Zagrożenia dla zdrowia związane ze stosowaniem nawozów organicznych i organiczno-mineralnych. (Health hazards associated with the use of organic and organo-mineral fertilizers). *Medycyna Ogólna i Nauki o Zdrowiu*, 2012;18(2):131-136. www.monz.pl.
- [3] Mead PS, Slutsker L, Dietz V, McCaig L F, Bresee J S, Sharpio C, Griffin P M, Tauxe R V. Food-Related Illness and Death in the United States. *Emerging Infections Diseases*. 1999;5(5):607-625. <http://www.ncbi.nlm.nih.gov/pubmed/10511517>.
- [4] Thomas MK, Murray R, Flockhart L, Pintar K, Pollari F, Fazil A. et al. Estimates of the Burden Foodborn Illnes in Canada for 30 Specified. *Pathogens Unspecified Agents*. 2015;12(10):820-827. DOI: 10.1089/fpd.2015.1966.
- [5] Kunachowicz H, Czarnowska-Misztal E, Turlejska H. *Zasady żywienia człowieka (Principles of human nutrition)*. Warszawa: WSiP; 2000.
- [6] Maćkiw E, Ścieżyńska H, Pawłowska K, Mąka Ł. Ocena jakości mikrobiologicznej żywności w Unii Europejskiej w oparciu o doniesienia RASFF. (Evaluation of microbiological quality of food in European Union including RASFF notification). *Bromat Chem Toksykol*. 2012;XLV(3):1046-1049. <http://www.ptfarm.pl/pub/File/Bromatologia/2012/3/1046-1049.pdf>.
- [7] Muller G. *Podstawy mikrobiologii żywności.(Fundamentals of food microbiology)*. Warszawa: WNT; 1990.
- [8] Muskalska KB, Szymczak B. Postępy badań nad bakteriami rodzaju *Listeria*.(Research progress on the genus *Listeria*). *Post Mikrobiol*. 2015;54:123-132. <http://www.pm.microbiology.pl>.
- [9] Padungtod P, Kaneene J.B. *Salmonella* in food animals and humans in northern Thailand. *Internat J Food Microbiol*. 2006;108:346–354. DOI: 10.1016/j.ijfoodmicro.2006.01.025 Source: PubMed.
- [10] Torres-Vitela MP, Mendoza-Bernardo M, Castro-Rosas J, Gomez-Aldapa CA, Garay-Martinez LE, Navarro-Hidalgo V. Incidence of *Salmonella*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Staphylococcal Enterotoxin* in Two Types of Mexican Fresh Cheeses. *J Food Protect*. 2012;75(1):79-84. DOI:10.4315/0362-028X.JFP-11-258.
- [11] Salleh NA, Rusul G, Hassan Z, Reezal A, Isa SH, Nishibuchi M, Radu S. Incidence of *Salmonella* spp. in raw vegetables in Selangor, Malaysia. *Food Control*. 2003;14:475-479. DOI: 10.1016/S0956-7135(02)00105-6.
- [12] Silva FVM, Gibbs PA. Thermal pasteurization requirements for the inactivation of *Salmonella* in foods. *Food Res Int*. 2012;45(2):695-699. DOI:10.1016/j.foodres.2011.06.018.
- [13] Williams JA, Cooper WM, Summage-West CV, Sims LM, Woodruff R, Christman J, et al. Level 2 validation of a flow cytometric method for detection of *Escherichia coli* O127:H7 in raw spinach. *Inter J Food Microbiol*. 2015;215:1-6. DOI:10.1016/j.ijfoodmicro.2015.08.011.
- [14] Alonso-Calleja C, Guerro-Ramos E, Alonso-Hernando A, Capita R. Adaptation and cross-adaptation of *Escherichia coli* ATCC 12806 to several food-grade biocides. *Food Control*. 2015;56:86-94. DOI:10.1016/j.foodcont.2015.03.012.
- [15] Parihar VS, Barbuddhe SB, Danielsson-Tham ML, Tham W. Isolation and characterization of *Listeria* species from tropical seafoods. *Food Control*. 2008;19:566-569. DOI:10.1016/j.foodcont.2007.06.009.
- [16] Medrala D, Dabrowski W, Czekajto-Kołodziejka U, Daczkowska-Kozon E, Koronkiewicz A, Augustynowicz E, Manzano M. Persistence of *Listeria monocytogenes* strains isolated from products in a Polish fish-processing plant over a 1-year period. *Food Microbiol*. 2003;20:715-724. DOI: 10.1016/S0740-0020(02)00173-9.

- [17] Kwiatek K. Occurrence of *Listeria monocytogenes* in selected food of animal origin. Bull Vet Inst Pulawy. 2004;48:269-272.
<http://www.piwet.pulawy.pl/jvetres/images/stories/pdf/20043/20043269272.pdf>.
- [18] Szewczyk EM. Diagnostyka bakteriologiczna. (Microbiological diagnostics). Warszawa: Wyd Nauk PWN;2013.
- [19] Zaremba ML, Borowski J. Mikrobiologia lekarska. (Medical microbiology). Warszawa: Wyd Lekarskie PZWL;2014.
- [20] Kramarenko T, Roasto M, Meremäe K, Kuningas M, Põltsama P, Elias T. *Listeria monocytogenes* prevalence and serotype diversity in various foods. Food Control. 2013;30:24-29.
DOI:10.1016/j.foodcont.2012.06.047.
- [21] Esteban JI, Oporto B, Aduriz G, Juste RA, Hurtado A. Faecal shedding and strain diversity of *Listeria monocytogenes* in healthy ruminants and swine in Northern Spain. BMC Vet Res. 2009;5:1746-1748.
DOI:10.1186/1746-6148-5-2.
- [22] Adak GK, Meakins SM, Yip H, Lopman BA, O'Brien SJ. Disease Risks from Foods, England and Wales, 1996-2000. Emerging Infectious Diseases. 2005;11(3):365-372. www.cdc.gov/eid
- [23] Soon JM, Singh H, Baines R. Foodborne diseases in Malaysia: A review. Food Control. 2011;22:823-830. DOI:10.1016/j.foodcont.2010.12.011.
- [24] Sochocka M, Błach-Olszewska Z. Mechanisms of innate immunity. Postepy Hig Med Dośw. 2005;59:250-258. www.phmd.pl.
- [25] Kopp E, Medzhitov R. Recognition of microbial infection by Toll-like receptors. Curr Opin Immunol. 2003;15:396-401. DOI 10.1016/S0952-7915(03)00080-3.
- [26] Wardemann H, Boehm T, Dear N, Carsetti R. B-1a B cells that link the innate and adaptive immune responses are lacking in the absence of the spleen. J Exp Med. 2002;195:771-780.
DOI: 10.1084/jem.20011140.
- [27] Dempsey PW, Vaidya SA, Cheng G. The art of war: Innate and adaptive immune responses. Cell Mol Life Sci. 2003;60:2604-2621. DOI: 10.1007/s00018-003-3180-y.
- [28] Ferlazzo G, Tsang M.L, Moretta L, Melioli G, Steinman R.M, Munz C. Human dendritic cells activate resting natural killer (NK) cells and recognized via the NKp30 receptor by activated NK cells. J Exp Med. 2002;195:343-351. DOI: 10.1084/jem.20011149.

MIKROBIOLOGICZNE ZANIECZYSZCZENIA ŻYWNOŚCI

Katedra Ochrony i Kształtowania Środowiska
Uniwersytet Jana Kochanowskiego w Kielcach

Abstrakt: Celem pracy była analiza produktów żywnościowych na podstawie obecności patogennych mikroorganizmów. Dane do analizy zostały dostarczone przez stacje sanitarno-epidemiologiczne w województwie świętokrzyskim w latach 2008–2011. Badano różne rodzaje produktów spożywczych (mięso, produkty mleczne, produkty zboża, ryby, warzywa, owoce, woda butelkowana, napoje bezalkoholowe, tłuszcze roślinne, ziola, kawa, herbata, kakao, środki spożywcze specjalnego przeznaczenia żywieniowego oraz suplementy diety). Materiał badawczy pochodził z produktów pochodzenia krajowego oraz z importu z rozróżnieniem produktów pochodzących z Unii Europejskiej. Analizowano obecność takich bakterii, jak: *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* spp. i *Yersinia enterocolitica*. *Escherichia coli* i *Yersinia enterocolitica* były wykrywane sporadycznie. Wykazano, że najczęściej występującym patogenem w próbkach żywnościowych pochodzenia krajowego były bakterie z grupy *Salmonella* spp., które są odpowiedzialne za największą liczbę zatruć pokarmowych w Polsce. Równie często wykrywano *Listeria monocytogenes*, szczególnie w produktach cukierniczych, garmażeryjnych, mleku i produktach mlecznych, a najrzadziej w owocach i warzywach. Nieistety zaobserwowano wzrost częstości występowania tych bakterii w kolejnych latach (2008–2011). Należy podkreślić, że wszystkie analizowane próbki zawierały co najmniej jeden z badanych gatunków. Zanieczyszczenie żywności może spowodować wzrost przypadków zatruć pokarmowych, a także innych chorób wywołanych przez te bakterie chorobotwórcze. Ważne jest, aby przestrzegać zasad higieny podczas produkcji, przygotowania i konsumpcji produktów żywnościowych. Problem ten jest jednak bardziej złożony.

Słowa kluczowe: skażenie żywności, epidemiologia