Vol. 1, No. 1 **ISSN 2831- 1795**

Scientific Conference SANUS 2023

Book 2: PROCEEDINGS

Naučna konferencija SANUS 2023

Knjiga 2: Zbornik radova

Prijedor, June 23rd - 24th, 2023

Book 2: Scientific Conference SANUS 2023

Organizers
PI College of Health Sciences Prijedor, Republic of Srpska, Bosnia and Herzegovina
Academy of Applied Studies Belgrade, the College of Health Sciences, Republic of Serbia
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Text editing in English: Dijana Srdić, English Language and Literature Teacher
Responsible person for the organizer: Prof. dr Ilija Stijepić, director
Website: www.vmspd.com
Printing house: Grafički atelje Grafit
Number of books printed: 100 pieces
ISSN: 2831 - 1795

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doi: 10.7251/ZSAN2302261Z **Review paper**

MICROBIOLOGICAL QUALITY AND THE MOST COMMON FOOD CONTAMINANTS

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Abstract: *Considering the importance of the microbiological correctness of food from the aspect of health safety and the most common causes of food contamination (bacteria) – the causes of alimentary intoxications and toxoinfections in humans, whether sporadic or epidemic, this work aims to analyze and sublimate the most common bacterial species isolated from contaminated food and biological samples of individuals with alimentary intoxication or toxoinfection. At the global level and especially based on the cases reported by the European Center for Disease Control and Prevention (ECDC) and the World Health Organization (WHO) for 2022, a trend of increasing epidemics caused by microbiologically contaminated food has been observed. The latest data from the U.S. Food and Drug Administration (FDA) and ECDC show that epidemics caused by Salmonella typhimurium, Escherichia coli, Yersinia spp., and Campylobacter spp. are increasing compared with 2021. The highest reporting rate was among young children aged 0 to 4 years. The most common pathogens are Salmonella Enteritidis and Salmonella Typhimurium, resulting in 61,236 cases of salmonellosis reported in 30 countries, 14% more than in 2020 (53,163 cases). In 2021, there were 6,534 cases of Shiga toxin-producing E. coli infection compared with 4,824 in 2020. Similarly, Yersinia spp. and most commonly Y. enterocolitica caused 6,876 cases of yersiniosis in 2021 compared with 5,747 in 2020, and also deaths. The conclusion is that eggs and egg products remain the most dangerous foods in epidemics, but several major incidents have been linked to contaminated vegetables (especially lettuce), fruits, and sesame seeds. To prove the genetic link, i.e., that the bacteria have the same origin in different sick individuals, the entire genome must be sequenced. In the suppressing epidemics, food storage and preservation conditions, lettuce washing, handling-washing, cleaning and disinfection in food preparation areas, and distribution are of particular importance.*

Key words*: food safety, bacterial contamination, alimentary intoxications, toxoinfections*

Introduction

Food safety and quality management require the application of the Hazard Analysis Critical Control Points (HACCP) system and related standards. HACCP is a systematic preventive approach to protect food and consumers from chemical (allergens, histamine, cyanogenic glycosides, mycotoxins, heavy metals, pesticides), physical (glass, metal, personal items, plastic, stones, wood, bones), and biological (bacteria such as *Salmonella* spp., *Campylobacter* spp., *Brucella* spp., *Escherichia coli*, *Staphylococcus*, *Listeria monocytogenes*, viruses, prions, protozoa, helminths) contaminants [1]. In general, food safety refers to the procedures followed in the handling, processing, and distribution of food to prevent its contamination, which can lead to foodborne illness due to the ingestion of toxins (alimentary intoxications) or the ingestion of viable pathogens and their toxins (toxoinfections) [2]. Hygienic measures must be taken to ensure the microbiological safety of food. These include (1) keeping hands, surfaces, utensils, and equipment clean during food handling, processing, distribution, and storage; (2) avoiding cross-contamination during food preparation and storage; and (3) mandatory risk assessment of chemical, physical, and biological hazards [2-4]. It is estimated that 20% of alimentary intoxications and toxoinfections are acquired at home, most likely through cross-contamination via surfaces (cutting boards, spice containers) [5]. It is also important to store fresh fruits and vegetables on special shelves, separate from meat, and to provide adequate thermal processing of foods, especially eggs, fish, and meat. Frozen foods should not be thawed outside the refrigerator (5±3°C) because bacteria have a short generation time and multiply rapidly at room temperature [3].

According to the report of the World Health Organization (WHO) in 2022, 600 million people fall ill after consuming contaminated food and 420,000 die each year, most of them children under 5 years old (40%) with 125,000 deaths per year [6]. Biological contaminants are the main cause of foodborne illness, with bacteria playing an important role. The results of zoonoses surveillance in 36 European countries in 2020 showed that the most commonly reported human zoonoses were campylobacteriosis, salmonellosis, yersiniosis and, to a lesser extent, Shiga toxin-producing *E. coli* (STEC) and *L. monocytogenes* infections [7, 8]. Diseases caused by *L. monocytogenes* and West Nile virus infections were the most severe zoonotic diseases with the highest number of deaths. In 2020, the 27 EU member states reported 3,086 foodborne disease outbreaks (a 47.0% decrease compared to 2019), mostly caused by Salmonella, and 20,017 human cases (a 61.3% decrease). The pathogen-food pairs of greatest concern were *Salmonella* in "eggs and egg products", norovirus in "crustaceans, shellfish, mollusks, and products containing them", and *L. monocytogenes* in "fish and fish products". In addition, cases of tuberculosis caused by *Mycobacterium bovis* or *M. caprae*, *Brucella*, *Trichinella*, *Echinococcus*, *Toxoplasma*, rabies, *Coxiella burnetii* (Q fever), and tularemia have been reported. This review focuses primarily on bacterial foodborne illnesses (sporadic or epidemic) reported in recent years to analyze and sublimate the most common bacterial species isolated from contaminated food and biological samples of individuals with alimentary intoxication or toxoinfection. A brief overview of cases involving other sources of biological

contamination is also provided to show that even rare and less common contaminants can cause serious problems in people.

Biological foodborne outbreaks

In 2021, an increase in infections caused by *Salmonella*, *E. coli*, and Yersinia was registered compared to 2020 in Europe [7, 8]. Thirty countries reported 61,236 salmonellosis cases, of which 60,494 were laboratory-confirmed, 14% more than in 2020 (53,163), most of them in the Czech Republic and Slovakia [7]. Medical assistance was sought in 31,357 cases, and 38% were hospitalized, mainly in Cyprus, Greece, and Lithuania. At least 73 people died in 2021. Compared to adults, the incidence in children up to 4 years of age was 11 times higher. *S. enteritidis* and *S. typhimurium* were isolated and identified. The highest percentage of travel-related cases was in France, Iceland, Sweden, and Slovenia. *S. enterica* serovar Montevideo caused sporadic cases from January 2021 to January 2022, but the source was not identified. The largest outbreak occurred in June in Finland, where more than 700 patients from daycare centers in three cities became infected with *S. typhimurium*. All facilities were supplied by the same central kitchen, and the infectious strain was detected in the vegetables consumed.

Eggs and egg products remain the most vulnerable foods in *Salmonella* outbreaks, but several major incidents have been associated with contaminated vegetables, fruits, and sesame seeds. Thus, Degaga et al. [9] studied the microbial quality of 100 samples of 5 different types of raw vegetables from two markets in Ethiopia from January to October 2020. The results showed (1) the highest presence of aerobic mesophilic bacteria (5.7 log CFU/g), followed by bacteria from the *Enterobacteriaceae* family (4.7 log CFU/g), and least yeasts and molds; (2) the highest contamination with aerobic mesophilic bacteria of cabbage (6.4 log CFU/g) and the lowest of green pepper (4.7 log CFU/g); (3) *S. aureus* as a common contaminant, especially in cabbage and lettuce; and (4) the presence of *Salmonella* spp. in a large number of samples. The Food and Drug Administration (FDA) is currently investigating an outbreak related to an infection with *S. typhimurium* of unknown origin that has infected 274 people [10]. The outbreak has been declared over, but investigators continue to track and test samples. In early 2023, the FDA and the Center for Disease Control and Prevention (CDC) reported an epidemic caused by contaminated fresh, raw alfalfa sprouts (manufactured in Nebraska) with *Salmonella* in three states [11]. Fifteen people were infected, two of whom were hospitalized. Infants, children, pregnant women, the elderly, and people with weakened immune systems are at higher risk of becoming severely ill or even life-threatening. Because the bacteria in the samples of those who became ill are closely related genetically, it is reasonable to suspect that the people in this epidemic became ill from the same food.

Given the epidemics associated with fresh lettuce and other leafy greens, particularly from California, consumption of spinach contaminated with *E. coli* O157:H7 resulted in more than 200 cases of infected individuals in the United States, many of whom suffered acute renal failure, and five deaths [12]. This led to a recall of spinach in the

United States and a ban on imports in Mexico. In 2022, an investigation of outbreaks of *E. coli* O103, O145 (Shiga toxin-producing *E. coli* (STEC) O145), and O26 associated with farm visits was conducted in the United Kingdom between July and September [13]. There were 11 cases of *Cryptosporidium* and two individuals confirmed to have *E. coli* O26. Patients with *Cryptosporidium* visited an open farm during the incubation period of the disease. An outbreak of *E. coli* O103 with 11 cases was associated with soft cheese made from raw milk from a dairy farm in eastern England. Corrective actions taken included pasteurization in soft cheese production, revision of HACCP procedures, and increased control measures. An outbreak of *E. coli* O145 in 10 patients was traced to the consumption of dairy products from a dairy farm in northwest England, with disease onset from mid-July. Problems with pasteurization, cleaning, and storage of milk crates were identified.

In 2021, 6,876 confirmed patients with yersiniosis were reported, compared with 5,747 in 2020, with the highest levels in Germany (1,912) and France (1,451), with one-third of the 1,649 patients hospitalized but no deaths [14]. *Y. enterocolitica* was the most common, while 115 patients with *Y. pseudotuberculosis* were reported in 11 countries.

The investigation into the outbreak of *L. monocytogenes* infection caused by the consumption of contaminated enoki mushrooms is ongoing, and the product has been recalled from the market [15].

In late 2022, the public learned that infants had become ill and possibly died from consuming infant formula contaminated with *Cronobacter* in the United States [16]. The FDA conducted several inspections, but the contaminant was not found in the manufacturing facility. The problem is that the *Cronobacter* isolates were not genomically analyzed to determine if two cases were from 2021. The recall and temporary closure of the facility contributed significantly to the infant formula shortage that occurred in the United States in 2022.

As of February 16, 2023, 258 cases of *Shigella* infection (221 confirmed and 37 probable cases) have been reported in 10 European countries associated with the recent travel of infected individuals to Cabo Verde (Senegal) [17]. Sequencing of 106 human isolates from the Czech Republic, the Netherlands, Sweden, the United Kingdom, and the United States revealed a genetically compact cluster, suggesting a common source of infection. Cases of coinfection of *Shigella* with other gastrointestinal pathogens (*Salmonella*, *Campylobacter*, *Cryptosporidium*, and strains of diarrheagenic *E. coli*) have also been reported. Information on possible vectors of infection or common exposures has not yet been identified. Multiple routes of transmission are likely; foodborne transmission (including infected food handlers) is most likely, but human-to-human transmission is also possible. The most recent cases were reported in Sweden on January 19, 2023, suggesting that there remains a moderate risk of new infections among travelers to Cabo Verde, particularly those staying in the Santa Maria region.

According to one study, an outbreak of *Clostridium perfringens* reportedly occurred in 30 individuals in Greece in 2021, among students and staff of a high school on the island who had consumed food supplied by a catering company [18]. Of the 11 food

items analyzed, consumption of spaghetti with ground (minced) meat was associated with the occurrence of gastroenteritis that lasted an average of one day and a maximum of three days, with no hospitalized cases. Six samples of spaghetti with ground meat were positive for *C. perfringens* and four samples were also positive for *Bacillus cereus*. *C. perfringens* was detected in two of three stool samples from students. Testing for *C. perfringens* in clinical and food samples was not performed due to a lack of laboratory capacity, so the pathogen could not be confirmed. The investigation revealed deficiencies in the reception and distribution of meals by the catering company and the school's reception committee, staff training, and the implementation of the HACCP concept.

In February 2023, leptospirosis, salmonellosis, and shigellosis were confirmed in Argentina in an epidemic with two deaths [19]. Leptospirosis was confirmed in two investigated cases, while *Salmonella* and *Shigella* were detected in the fatal cases. Initial investigations revealed that all patients had eaten meat and meat products such as offal in the recent past. In two cases there was a connection with a butcher. However, testing of the seized products was negative for *Salmonella*, *E. coli*, and *Shigella*.

Contaminated oysters continue to cause disease in several European countries and Hong Kong [20]. In Helsinki, Finland, at least 170 people became ill in February and March 2023 after eating oysters in various restaurants. Cases have also been reported in other Finnish cities. Norovirus was detected in nine batches of oysters, two of which were from the Netherlands, six from France, and one from Ireland. Norovirus is the most common cause of foodborne illness outbreaks in Finland. Between 2017 and 2021, oysters caused 11 norovirus outbreaks, sickening more than 110 people. In March 2023, norovirus was detected in shellfish in Sweden, infecting 7 people. Spain and Italy also issued The Rapid Alert System for Food and Feed (RASFF) for norovirus in oysters from France. In February, twenty people in Belgium became ill from norovirus in oysters from France. Two outbreaks in Denmark from late 2022 to early 2023 were caused by oysters. The first, with 19 cases, involved oysters from France that originally came from Ireland. Another outbreak with 73 cases is associated with oysters from Norway. Hong Kong authorities have reported several outbreaks linked to raw oysters, including one from Ireland that caused at least 16 illnesses earlier this year. According to the health authority, Ireland has seen an increase in norovirus cases and outbreaks in recent weeks. England has also seen a significant increase in norovirus cases, according to the UK Health Security Agency (UKHSA). Laboratory reports of norovirus are 77% higher than the five-year average for the same period before the coronavirus pandemic. The increase is seen in all age groups, but especially in those over 65 and under 5. Hand washing with soap and water is key to combating the virus. Affected food workers should stay away from work for at least two days after symptoms subside to avoid spreading the infection. Norovirus can be transmitted directly from person to person and indirectly through contaminated surfaces, food, and beverages.

Microbiological contaminants

Hospitals must provide nutritionally adequate and safe food. The microbiological safety of food is of particular concern to patients, staff, and employees in stationary healthcare facilities [21]. Although food safety has undoubtedly improved, hospital-acquired gastroenteritis outbreaks still occur worldwide [22, 23], with a vulnerable population (elderly) having a higher mortality rate than their community-acquired counterparts [24]. In addition, extraintestinal and postinfectious manifestations associated with intestinal pathogens may further contribute to mortality and morbidity, e.g., Guillain-Barré syndrome (*Campylobacter*), hemolytic uremic syndrome (STEC, *S. dysenteriae* serotype 1), intestinal perforation (*Entamoeba histolytica*), etc. [25].

The clinical presentation of infectious gastroenteritis is broad and can occur rapidly, as with toxin (1-24 hours after ingestion), *Vibrio parahaemolyticus* (4-30 hours), astrovirus, and norovirus (12-48 hours). The incubation period of common bacterial foodborne diseases such as *E. coli*, *Salmonella*, *Shigella*, *Yersinia*, *Vibrio*, *Campylobacter*, *Cryptosporidium*, *Cyclospora*, and *Giardia* ranges from 1 day to 14 days, making it difficult to determine the foods involved in transmission without microbiological diagnoses. Others have long incubation periods, such as *Listeria* (2-6 weeks), hepatitis A (15-50 days), and prion-related diseases (5-20 years) [26]. Noroviruses are considered the main cause of epidemic gastroenteritis both in healthcare and other settings Hospital epidemics caused by noroviruses are difficult to prevent and control because of the low infectious dose (18-100 virus particles), the short incubation period (12-48 hours), and resistance to inactivation by freezing, heating to 60°C, routine chlorination of water, low pH, and treatment with ethanol or detergent-based cleaners. Finally, there are multiple routes of transmission, including the fecal-oral route, ingestion of aerosolized vomit, and indirect contact via contaminated surfaces in the environment. *Cryptosporidium* and *Giardia* are resistant to routine chlorination of water. In 1993, *Cryptosporidium* caused the largest documented outbreak of gastrointestinal illness in an industrialized country (an estimated 403,000 cases) due to a contaminated drinking water supply [27].

Outbreaks of moderately infectious doses of pathogenic bacteria (103 to 105 live organisms), such as *Salmonella* and *Campylobacter*, occasionally occur in healthcare facilities. The most common routes of transmission for *Salmonella* were food (59.6%) and human-to-human transmission (13.5%) [23, 28]. Undercooked meat/poultry, eggs, sprouts, and other vegetables are responsible for most salmonellosis. It is mainly associated with eggs, through external contamination of the shell during passage through the chicken's cloaca and internal contamination through bacterial penetration through the eggshell.

There are increasing reports of multiresistant zoonotic pathogens. Emerging resistance of *Salmonella* [29] and *Campylobacter* [30] contributes to increased mortality and morbidity in both outbreaks and sporadic cases. Pathogens in high infectious doses (>10^6 viable organisms), such as enteroinvasive *E. coli* (EAEC),

enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), and *Vibrio cholerae*, usually cause illness through ingestion of contaminated food or water.

E. coli is a fecal pathogen. The main sources of *E. coli* O157 (STEC O157) outbreaks are raw or inadequately cooked meat products, raw milk, and fecal contamination of water, vegetables, fruits, and the environment. Infection with *E. coli* O157 can be asymptomatic or result in symptoms ranging from abdominal cramps, mild diarrhea, and bloody diarrhea to life-threatening illness, including hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP)

L. monocytogenes is a ubiquitous pathogen that has been detected in plants, soils, silage, sewage, slaughterhouse waste, human feces (1-10%), animal feces, food processing environments, and catering facilities. The infectious dose is 10^7 to 10^8 CFU in healthy hosts, while it is 10^5 to 10^7 CFU in individuals at high risk of infection [31]. In high-risk individuals (e.g., pregnant women, elderly, and immunocompromised hosts), the mortality rate is high (20-50%). The organism can multiply at -18 to 10°C [32]. Thermal processing of food at 75°C can destroy *Listeria*.

One of the largest and most common causes of hospital-acquired infections is multiresistant *C. difficile* [33]. The sources of community-acquired *C. difficile* are contaminated food and exposure to spores from the soil [34].

To destroy non-spore-forming microorganisms, food should be cooked so that the internal temperature of the entire food is maintained above 70°C for at least 2 minutes [35].

Conclusion

Foodborne illnesses cannot be completely prevented because of the many potential food contaminants in the environment, but their outbreaks can be suppressed or mitigated by taking appropriate measures to prevent food contamination or to rapidly identify pathogens and eliminate potentially contaminated foods.

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