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Food safety inspection of tas kebab and salad processing line in a catering company

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Abstract: This study was conducted to evaluate the sufficiency of food safety practices in a catering company. The presence of some pathogenic and indicator bacteria was monitored in the samples collected from raw materials, food, food contact surfaces and workers' hands and various steps of the tas kebab (a Turkish meat stew) and salad processing lines. *Bacillus cereus* was found in ready-to-eat (RTE) tas kebab and RTE salad, while *Listeria monocytogenes* was isolated from RTE salad. Furthermore, it was observed that RTE salad contained coagulase-positive *Staphylococcus aureus* without staphylococcal enterotoxin production. The swab samples obtained from cutting board surfaces, knives and workers' hands contained high counts of total aerobic bacteria and some samples were contaminated with coliforms and coagulase-positive/negative staphylococci. The presence of *B. cereus* and *L. monocytogenes* in RTE foods is a serious threat to public health, especially in the catering business. Preventing the presence of toxin-producing bacteria in RTE food is a fundamental action. Also, the occurrence of *L. monocytogenes* in RTE salad and *B. cereus* in RTE tas kebab/salad samples showed insufficient cleaning/disinfection practices. As a result, hygiene practices and regular monitoring in the catering business are necessary for food safety.

Keywords: food business; *B. cereus*; *L. monocytogenes*; ready-to-eat food

The catering industry provides meals that are gastronomically acceptable, providing sufficient nutritional components and designed for convenience at a given price. These companies must ensure safe food that does not pose a risk to consumer health, especially considering that catering businesses serve food to hospital patients, children in schools and elderly residents in nursing homes. Catering business in Turkey as well as in other countries has systems like Hazard Analysis and Critical Control Points (HACCP) to ensure food safety (Dogan and Tekiner 2020), but there are several reasons that lead to failure in HACCP systems, such as the wide range of dishes, part-time workers, and inadequate knowledge. Therefore, reported recurrent problems in the catering business include insufficient hand washing, particularly in incorrect food preparation, cross-contamination between raw and cooked materials, and improper storage and/or heating of food. These problems can contribute to foodborne diseases (Garayoa et al. 2017). Prepared foods in the catering systems can be contaminated by saprophytic bacteria such as total mesophilic aerobes, spoilage and patho-

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gens such as *Salmonella*, *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*. These microorganisms may spread from raw materials and also failures of good manufacturing practices by the employee cause contamination (Petruzzelli et al. 2018).

The ability of foodborne pathogens to attach to food contact surfaces is well known. Thus, their elimination from food processing environments and utensils including cutting boards, slicers, knives, and plates, is essential. These surfaces pose a potential risk in the transfer of bacterial foodborne pathogens to foods via cross-contamination (Djekic et al. 2016). In food processing plants, the sufficiency of sanitation on food contact surfaces is monitored by traditional microbiological analyses (viable counts of total bacteria, coliforms, and *E. coli*). For these analyses, the use of hygiene swabs is an important means of measuring the effectiveness of sanitation, because sampling of a food contact surface that is irregular and/or difficult to clean is performed with swabs (Losito et al. 2017).

The objective of this work was to evaluate the food safety of two selected foods prepared in catering service in Bursa (Turkey), by surveillance of the following parameters: the presence of several pathogens (*Clostridium perfringens*, *Bacillus cereus*, *Salmonella* spp., *L. monocytogenes* and *E. coli* O157), hygiene indicator microorganisms (aerobic colony count, coliforms, *E. coli* and staphylococci) and staphylococcal enterotoxins in food samples, various surfaces in contact with food and food handlers' hands in tas kebab and salad processing lines.

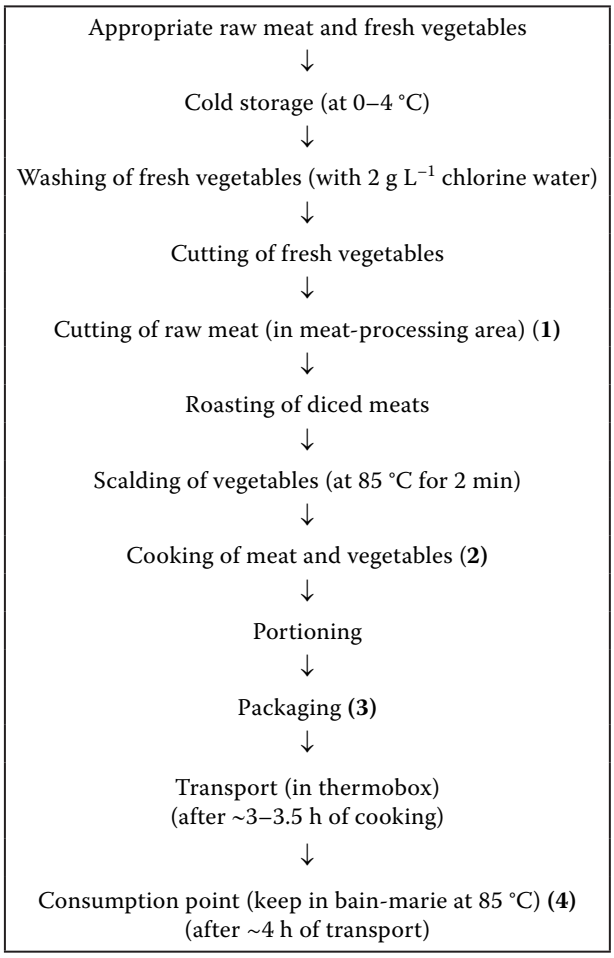

Figure 1. Process flow diagram of the tas kebab production line

MATERIAL AND METHODS

Sampling procedure. This study was carried out in a catering company in Bursa Province of Turkey. From December to March, the company was visited six separate times for sampling of different critical process stages and food contact surfaces from each of the tas kebab and salad production lines. Figures 1 and 2 represent the flow diagrams of the production processes of tas kebab and salad, respectively. In the figures, the sampled points are shown by the numbers. On each visit, one sample of food and food contact surface swabs belonging to both processing lines was obtained. So that, totally 6 samples of each food and swabs from tas kebab and salad lines were collected after the 6 sampling visits. At least 250 g portions of food samples were taken. From each production line, the food contact surfaces (cutting board and knife) after normal cleaning procedures and food handlers' hands before working in food preparation were sampled using sterile swabs (LP Italıana, Italy). The surfaces and hands were swabbed once in a 10 cm^2 area. All samples were aseptically transported to the laboratory under cold chain conditions (Tribeca, Turkey) for further analysis.

Microbiological analyses. The presence of pathogenic bacteria was investigated in food samples from tas kebab and salad processing lines while the analysis of contamination with hygiene indicator microorganisms was performed in both food and swab samples. Aerobic colony, coliform, *E. coli, Staphylococcus* spp., *C. perfringens*, and *B. cereus* counts were enumerated according to ISO 4833-1 (2013), ISO 4832 (2006), ISO 16649-1 (2001), ISO 6888-1 (1999), ISO 7937 (2004), and ISO 7932 (2004) methods, respectively. To confirm presumptive *B. cereus* strains haemolytic activity was tested on blood agar (146559; Merck, US) and positive results were assumed as presumptive

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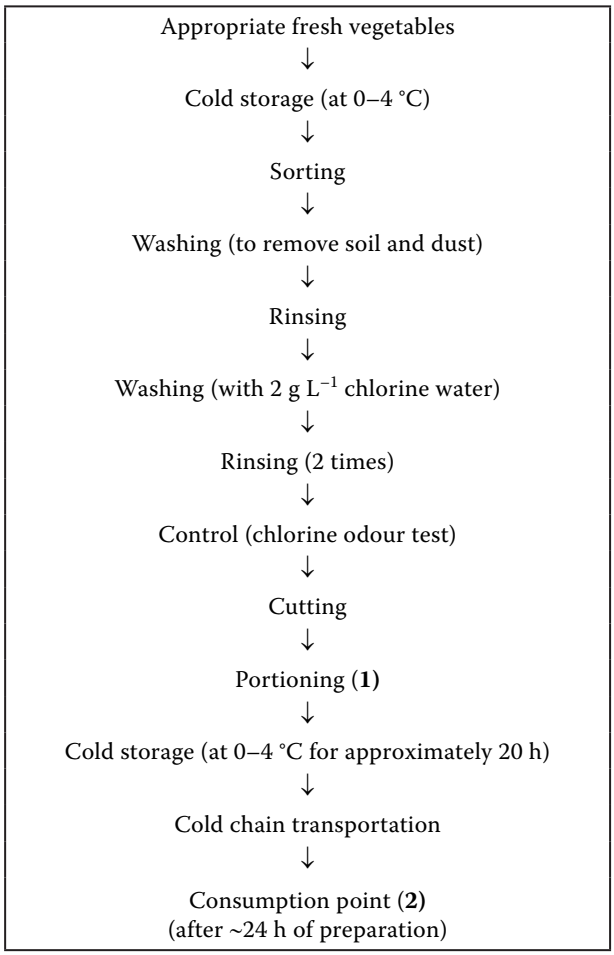

Figure 2. Process flow diagram of the salad production line

B. cereus. To detect coagulase-positive staphylococci, the coagulase test was performed on colonies with typical growth characteristics on Baird-Parker agar (105406; Merck, US). The presence of *Salmonella*, *L. monocytogenes,* and *E. coli* O157 was estimated according to ISO 6579 (2002), ISO 11290 (2004), and ISO 16654 (2001) methods, respectively.

Molecular identification and multiplex polymerase chain reaction (PCR) serotyping of *L. monocytogenes* strains. Chromosomal deoxyribonucleic acid (DNA) of *L. monocytogenes* strains was extracted with spin column filtration kits according to the manufacturer's instructions (QIAamp DNA Mini Kit; Qiagen, Germany). The strains were identified by analysing the 16S ribosomal ribonucleic acid (rRNA) gene derived from DNA extracts using the bacterial universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Lane 1991). The identification results were confirmed by DNA sequence analysis. The sequences obtained were compared against the sequences available in the National Centre for Biotechnology Information database using the Basic Local Alignment Search Tool (BLAST) program 2.5.0.

For differentiation of 1/2a, 1/2b, 1/2c, and 4b serovars in the isolated *L. monocytogenes* strains, a multiplex polymerase chain reaction (PCR) assay was performed as described by Doumith et al. (2004). Amplified PCR products were analysed on 1.5% agarose gels stained with ethidium bromide and visualized using a Bio-Rad Gel Doc 2000TM imaging system (Bio-Rad, US).

Staphylococcal enterotoxin determination. Detection of staphylococcal enterotoxins was done using the VIDAS Staphylococcal Enterotoxins Set II (BioMerieux, France) according to the manufacturer's instructions.

RESULTS AND DISCUSSION

Hygiene indicator microorganisms in processing lines. The mean aerobic colony, coliform and staphylococci counts in raw meat samples from the tas kebab processing line were 4.30, 2.63, and 3.32 log colony-forming units (CFU) g^{-1}, respectively. After the cooking process, bacterial counts were reduced by 2.80, 2.60, and 3.30 log, respectively. The samples of ready-to-eat (RTE) tas kebab contained aerobic colony (mean 1.30 log CFU g^{-1}) and staphylococci (mean 2.00 log CFU g^{-1}), but not coliform bacteria. The detection of coagulase-positive/negative staphylococci in raw meat was acceptable but staphylococcus contamination of RTE tas kebab sample indicates cross-contamination at the transport and/or service point. To prevent cross-contamination in the catering industry is one of the most important public health topics.

The mean microbial load of prepared salad and RTE salad was 3.37 log CFU g^{-1} and 3.11 log CFU g^{-1} for an aerobic colony, 1.74 log CFU g^{-1} and 1.62 log CFU g^{-1} for coliforms, and 2.80 log CFU g^{-1} and 2.60 log CFU g^{-1} for staphylococci, respectively. There were no significant differences between bacterial counts from prepared salad and RTE salad. On the other hand, the presence of *E. coli* was not observed in any of the analysed food samples. To decrease bacterial counts in RTE salad, it is necessary to update the washing procedure of raw materials. The microbial counts in analysed foods are shown in Table 1.

Pathogenic bacteria in processing lines. The incidence of potentially pathogenic bacteria in food from both processing lines is presented in Table 2. No *C. perfringens*, *Salmonella* spp., *L. monocytogenes,* and *E. coli* 0157 were detected in any sample from sampling steps

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Table 1. Microbial counts in food samples from production steps (log CFU g^{-1}) (n = 6)

Sample	Microorganisms								
	aerobic colony count			coliforms			*Staphylococcus* spp.		
	min.	mean ± SD	max.	min.	mean ± SD	max.	min.	mean ± SD	max.
Tas kebab production line									
Raw meat	3.60	4.30 ± 0.41	4.85	< 1.00	2.63 ± 0.35	3.00	2.60	3.32 ± 0.61	4.18
Cooked tas kebab	< 1.00	1.46 ± 0.28	1.30	< 1.00	–	< 1.00	< 2.00	–	< 2.00
Tas kebab before transport	< 1.00	1.30 ± 0.01	1.30	< 1.00	–	< 1.00	< 2.00	–	< 2.00
RTE tas kebab	< 1.00	1.30 ± 0.01	1.30	< 1.00	–	< 1.00	< 2.00	2.00 ± 0.01	2.00
Salad production line									
Prepared salad	2.30	3.37 ± 0.70	4.26	< 1.00	1.74 ± 0.70	2.48	< 2.00	2.80 ± 1.13	3.60
RTE salad	1.48	3.11 ± 0.82	3.67	< 1.00	1.62 ± 0.23	1.95	< 2.00	2.60 ± 0.01	2.60

CFU – colony-forming units; SD – standard deviation; RTE – ready-to-eat

and food contact surfaces of the tas kebab production line.

However, *B. cereus* was isolated only from raw meat samples taken during sampling 2 and sampling 4. We also observed that all food samples obtained at the time of sampling 3 from the tas kebab processing line contained *B. cereus*. The counts of the pathogen were found to be 2.78 log CFU g^{-1} in raw meat and 2.00 log CFU g^{-1} in cooked tas kebab sample by the destruction of 0.80 log after the cooking process. *B. cereus* counts in RTE tas kebab were 2.30 log CFU g^{-1} and did not exceed the acceptable limit (10^3 CFU g^{-1}) of the Regulation on Turkish Food Codex (TFC) Microbiological Criteria (Law of Authorization: 5996). A study undertaken by Tewari et al. (2015) indicated that the contamination rate with *B. cereus* in cooked meat samples (35%) was higher than in raw meat samples (27.8%). Other study also reported *B. cereus* contamination of raw beef burger in Iran (Soleimani et al. 2017).

During sampling 2 from the salad production line of the company, *B. cereus* was detected in one sample of RTE salad and counted as 3.60 log CFU g^{-1}. From sampling 6 from this line, one prepared salad and one RTE salad were contaminated with *L. monocytogenes*, which was confirmed by 16S rDNA sequence analysis. This could be due to contaminated raw vegetables used in salad preparation. Raw vegetables can harbour potential foodborne pathogens through irrigation waters contaminated with faecal material and sewage or organic fertilizers applied to agricultural land (Kemajou et al. 2017). In the current work at a catering company, salad vegetables prior to being cut were washed with chlorinated water, but some factors, such as the types and numbers of microorganisms, the organic matter load of water, contact time, pH, and temperature during application, can affect the degree or efficiency of the washing method used for microbial decontamination of vegetables. Furthermore, the detection of *L. monocy-*

Table 2. Distribution of pathogenic bacteria from production lines

Sample	Pathogens		
	L. monocytogenes	*B. cereus*	coagulase-positive staphylococci
Tas kebab production line			
Raw meat	–	+ sampling 2, 3, 4	+ sampling 6
Cooked tas kebab	–	+ sampling 3	–
Tas kebab before transport	–	+ sampling 3	–
RTE tas kebab	–	+ sampling 3	–
Salad production line			
Prepared salad	+ sampling 6	–	+ sampling 6
RTE salad	+ sampling 6	+ sampling 2	+ sampling 2

RTE – ready-to-eat; *L. monocytogenes* – *Listeria monocytogenes*; *B. cereus* – *Bacillus cereus*

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togenes in salad samples from sampling 6 did not comply with the Regulation on TFC Microbiological Criteria (Law of Authorization: 5996) for this pathogen in RTE salads. Some previously published data also reported the presence of *L. monocytogenes* (Byrne et al. 2016; Kara et al. 2019) and *B. cereus*/*Bacillus* spp. (Chau et al. 2017; Abakari et al. 2018) in raw salad vegetables and/or RTE salads.

Additionally, the coagulase-positive staphylococci were identified in one raw meat sample from sampling 6 from the tas kebab processing line, and in one prepared salad sample from the sixth visit and in one RTE salad sample from sampling 2 from salad processing stages. The coagulase-positive staphylococci counts in these samples were 3.70, 4.60, and 2.00 log CFU g^{-1}, respectively. Other researchers also documented the presence of coagulase-positive/negative staphylococci in raw meat (Guven et al. 2010; Hanson et al. 2011) and in RTE salads (Pamuk et al. 2013; Saifullah et al. 2018).

Serotyping of *L. monocytogenes* strains. The results of the multiplex PCR assay for differentiating the major *L. monocytogenes* serotypes 1/2a, 1/2b, 1/2c, and 4b revealed that two strains isolated in the present study by using the PCR technique did not belong to any of the tested serotypes. However, Braga et al. (2017) evaluated the serotype distribution of *L. monocytogenes* strains isolated from food samples and found that the most prevalent serotypes were 1/2b and 4b. A study carried out by Terzi et al. (2015) in Turkey revealed that one *L. monocytogenes* strain isolated from salad samples was serotyped as 4b. PCR serotyping in the present work did not show the most often potentially pathogenic serovars to humans, because of the contamination of salad samples by only two *L. monocytogenes* strains.

Staphylococcal enterotoxins. In this study we identified coagulase-positive staphylococci at levels of 3.70 log CFU g^{-1} and 4.60 log CFU g^{-1} in raw meat and prepared salad samples, respectively (data not shown), but not staphylococcal enterotoxins in any sample from tas kebab and salad processing steps. Contrary to our findings, Azeez et al. (2016) found that *S. aureus* strains isolated from meat products and shellfish were able to produce enterotoxins. Ma et al. (2018) reported that 14 strains isolated from raw meat of *S. aureus* and 8 strains from cooked meat were positive for staphylococcal enterotoxins/enterotoxin-like genes (se/sel).

According to Regulation on TFC Microbiological Criteria (Law of Authorization: 5996), in 25 g of meat dishes *Salmonella* spp. and staphylococcal enterotoxins should not be detected, and *B. cereus* counts must be lower than 10^2 CFU g^{-1} in a 10-g sample. During one of the six visits, *B. cereus* was detected in RTE tas kebab as 2×10^2 log CFU g^{-1}. The risk of toxin-forming bacteria in food is very important in the catering industry. In order to prevent *B. cereus* contamination and possible intoxication risks in meat dishes, soil contamination should be prevented during slaughter. Regulation on TFC Microbiological Criteria (Law of Authorization: 5996) requires the absence of *E. coli* in 10 g and *Salmonella* spp., staphylococcal enterotoxins, *L. monocytogenes* in 25 g of RTE salad sample. Inconvenience results were determined at a single visit by the presence of *L. monocytogenes*. Raw vegetables may contain pathogens and they should be decontaminated by chlorine or ozone washing. Despite chlorine washing, *L. monocytogenes* was detected in a salad sample.

Hygiene indicator microorganisms on food contact surfaces and workers' hands. Table 3 summarizes

Table 3. Microbial counts taken from swab samples (log CFU cm^{-2}) (n = 6)

Microorganisms		Swabbing the surfaces					
		tas kebab production line			salad production line		
		cutting board	knife	hands	cutting board	knife	hands
Aerobic colony count	min.	2.30	2.00	1.00	3.30	3.00	5.48
	mean ± SD	4.39 ± 1.03	3.86 ± 1.40	2.82 ± 1.57	4.51 ± 0.88	4.47 ± 1.21	3.82 ± 1.18
	max.	5.46	6.04	4.34	5.90	6.11	1.90
Coliforms	min.	1.00	1.00	< 1.00	< 1.00	< 1.00	< 1.00
	mean ± SD	2.08 ± 0.91	1.25 ± 0.85	1.40 ± –	2.47 ± 1.20	2.12 ± 1.30	2.21 ± 1.18
	max.	3.77	2.60	1.40	4.48	3.48	3.00
Coagulase-positive staphylococci	min.	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00
	mean ± SD	2.36 ± 1.41	1.71 ± 0.93	2.02 ± 0.92	1.95 ± 0.95	2.31 ± 1.19	2.33 ± 0.45
	max.	1.00	3.25	2.74	3.08	3.78	3.00

CFU – colony-forming units; SD – standard deviation

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the microbial counts on tas kebab and salad preparation surfaces. The mean aerobic colony counts were assigned as 4.39–3.86 log CFU cm^{-2} (tas kebab making area and 4.51–4.47 log CFU cm^{-2} (salad preparation area) in swab samples taken from cutting boards and knives, respectively. Microbiological criteria for food contact surfaces were established as ≤ 0.60 log CFU cm^{-2} by Garayoa et al. (2017). According to these criteria, all results of knife and cutting board swab samples were considered inadequate (≥ 2log CFU cm^{-2}). In tas kebab making and salad preparation areas total bacteria counts for swab samples from food handlers' hands were recovered as 2.82 log CFU cm^{-2} and 3.82 log CFU cm^{-2}, respectively. The study focused on hygiene quality in several catering establishments revealed 38.2% of unacceptable total aerobic bacterial counts on tools, surfaces, and also, unlike the present research none of the food handlers' hands was contaminated with aerobic bacteria (Dogan and Tekiner 2020). Petruzzelli et al. (2018) revealed total mesophilic aerobes (minimum < 1 log CFU cm^{-2} and maximum 1.51 log CFU cm^{-2}) on work surfaces in the school catering system.

The surface of one cutting board from sampling 5 from tas kebab preparation areas was found to be contaminated with *E. coli* (2.78 log CFU cm^{-2}). Additionally, sampling 3 from the salad processing line showed that one cutting board surface contained coagulase-positive staphylococci (1.60 log CFU cm^{-2}). Similarly, Fernandes et al. (2017) isolated *E. coli* in 6.7% of processing surface samples, collected after the equipment sanitation process from a beef jerky production line. Gutierrez et al. (2012) reported an incidence rate of 3.2% for *S. aureus* on meat industry contact surfaces.

As a result, in the salad processing line coliform bacteria (isolated from a knife and a cutting board) and coagulase-negative staphylococci (isolated from a knife, workers' hands) were detected in sampling 2 and 6, at the same time pathogens (*L. monocytogenes*, *B. cereus*, and coagulase-positive staphylococci) were also isolated in RTE salad. Similarly, in sampling 3 of the tas kebab processing line, *B. cereus* was isolated from RTE tas kebab, and also, from swabs collected from a knife, while workers' hands were contaminated with coliforms and coagulase-negative staphylococci. A failure in the cleaning and disinfection process or unmindful behaviours of workers in salad and tas kebab processing lines can lead to pathogen contamination of RTE foods. On the other hand, a cutting board was contaminated with *E. coli* in sampling 5 from the tas kebab production line but RTE tas kebab samples did not contain any pathogens.

CONCLUSION

The chosen catering company has ISO 22000 certificate and regular audit, and also, the employees completed hygiene training. Nevertheless, pathogens and hygiene indicator bacteria were found in food samples from some production steps and utensils. In the tas kebab production line, a cross-contamination risk was very high depending on raw meat. However, the soil on vegetables and a failure in chlorine washing can be sources of contamination in salad samples. Observations of employee behaviours showed that the kitchen staff go to break time in kitchen clothes and there is no staff to control glove changing frequency. All these facts can clarify the presence of pathogens and some indicator bacteria in analysed samples. Catering foods distributed to several places are consumed by excessively many people, so the presence of toxin-producing bacteria and *Listeria* spp. in RTE food is vital for public health. Regularly training employees by hygiene practices and monitoring the results should be ensured to avoid future risks. As a result, good hygienic and manufacturing practices in the catering industry need to be implemented to prevent microbiological contamination and to ensure food safety for consumers.

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