

Examination of Food Samples Taken From Catering Enterprises in İstanbul in Terms of *Bacillus cereus*, *Salmonella spp.* and *Escherichia coli* O157:H7

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ABSTRACT— Catering sector is a growing sector that has taken its place in everyday life with technological developments that play an active role in accelerating the transition period from agriculture society to industrial society. Therefore, food hygiene and safety are important in catering sector serving the community. When the environment conditions (ph, temperature, water activity etc.) are suitable for the development of microorganisms, pathogenic microorganisms develop and cause infection and intoxication.

In this study; *Bacillus cereus*, *Salmonella spp.* and *Escherichia coli* O157:H7 samples were taken from the establishments producing bulk meals in İstanbul. For this purpose; 100 samples including 15 pieces of soup, 16 pieces of rice, 6 pieces of pasta, 20 pieces of meat meal, 6 pieces of chicken meal, 9 pieces of meatless food, 14 pieces of raw meat and 7 pieces of raw chicken it was collected. Cooked meals samples (79) were analyzed for *Bacillus cereus*, *Salmonella spp.* and *Escherichia coli* O157: H7 bacteria, and raw samples (21) were analyzed for *Salmonella spp.* and *Escherichia coli* O157: H7. The results of the analyzes were evaluated according to Turkish Food Codex Microbiological Criteria Regulation. None of *Escherichia coli* O157:H7 was detected in any of the samples examined, whereas *Salmonella spp.* was detected in 1 sample (1%) *Bacillus cereus* was detected in 12 samples (15.2%). Total of 3 (3%) food samples were found to be unsuitable according to the Turkish Food Codex Microbiological Criteria Regulation, posing a risk for public health.

Keywords— Catering, *Bacillus cereus*, *Salmonella spp.*, *Escherichia coli* O157:H7.

1. INTRODUCTION

The basic needs of people are nutrition, dressing and shelter. At the beginning of the basic needs, they should be able to sustain their lives in a healthy and strong way, so first of all they should be fed adequately and balanced [12, 13].

The reasons such as increasing industrialization on the basis of changes in today's living conditions, increasing urbanization, increasing working population, changing socio-economic factors, increasing the level of welfare and inclusion of women in the working life have led people to the food offered by the companies that provide food services outside the home [13, 27].

According to 2017's data of Federation Of Food Manufacturers Associations, the catering sector, which is growing every day, is second in service sector with its turnover of 22 billion dollars per year, with employment created directly to 400 thousand people and indirect to 2 million people in Turkey.

The food safety expert committee of the World Health Organization (WHO) and the Food Agriculture Organization (FAO) point out that foodborne diseases resulting from contaminated food consumption are the most common health problem in the world. Food poisoning is known to be a general definition of infection or intoxication caused by the consumption of any food or drink [5]. Foodborne health problems can be caused directly by contaminated food items, as well as by adverse environmental conditions, inadequate information on hygiene, negative attitudes and behaviors of producers and consumers, the presence of foodborne disease carriers in society, zoonotic diseases and various sources

of development [6].

Microbiological diseases caused by food are basically divided into 2 main groups; infections and intoxication [20]. Today, there are 27 pathogens cause these diseases. The most important bacteria are *Campylobacter*, *Salmonella*, *Clostridium*, *S. aureus*, *E.coli* O157:H7, *B. cereus* and *L. monocytogenes* [15]. Food infection occurs by an increase in the number of microorganisms in the intestines or breakdown of bacteria in the intestine resulting in the disintegration of endo toxins. The toxin affects only the person who consumes it, and there is no epidemic [20].

In this study, it is aimed to screen the occurrence *E.coli* O157:H7, *B. cereus* and *Salmonella spp.*, in meal and raw food (such as meat, chicken) which produce in caterings, in Istanbul. The results of the screening were evaluated according to the criteria specified in the Turkish Food Codex Microbiological Criteria Regulation.

2. MATERIALS AND METHODS

2.1 Sampling

A total of 100 samples were collected from 15 catering companies in Istanbul; including 22 soup, 16 pilaf, 6 pasta, 20 meat meals, 6 chicken meals, 9 meatless meals samples and 14 raw meat samples (meat, meatballs dough, piece of meat), 7 raw chicken (baguette, chest, wing) samples. The food samples were taken to gamma sterile sample bags with the help of sterile disposable spoons to be 250 grams. The samples were transported to the laboratory. Analyzes of samples taken to avoid increasing microbial load were made on the day they were taken.

2.2 Methods

B. cereus analysis, ISO 7932 (2004); *E. coli* O157:H7, ISO 16654 (2001); *Salmonella spp.* ISO 6579 (2017) were made according to methods.

2.2.1. *Escherichia coli* O157:H7 Analysis

Positive control *E. coli* O157 ATCC 43894 reference strain was used for the *E. coli* O157: H7 analysis. 25 g weighed samples were homogenized for 2 minutes after adding 225 ml Novobiocin modified Trypton broth (mTSB with Novobiocin) for pre-enrichment. Homogenized samples were incubated at 41.5 °C for 12-18 hours. Immunomagnetic separation process was applied. After this, 200 ul were taken and added onto selective medium (Cefixime Tellurite Sorbitol MacConkey Agar) and left for incubation for 24 hours at 37°C. Open yellowish-brownish colonies formed on the incubation medium were inoculated to Nutrient agar and colonies were incubated for 24 hours at 37°C [21].

2.2.2. *Escherichia coli* O157 Latex Agglutination Test

It was done biochemical test to suspected colonies. For this purpose, commercial agglutination test was used (*Escherichia coli* O157 Latex Reagent) [21].

2.2.3. *Salmonella spp.* analysis

Salmonella enteridis ATCC 13076 reference strain was used as positive control for the *Salmonella spp.* analysis. 225 ml Buffered Peptone Water was added to 25 g weighing samples. Homogenous suspension was left to incubate 37 °C 24 hours for pre-enrichment [23].

2.2.4. *Salmonella spp.*'s Selective Enrichment

Transfer 1ml of the broth to 10ml of Muller-Kauffmann Tetrathionate with Novobiocin Broth (MKTTn) and 0.1ml into 10ml of Rappaport-Vassiliadis Soya Peptone Broth (RSV Broth). Incubate the MKTTn Broth at 37°C for 24 hours and the RVS Broth at 41.5°C for 24 hours [23].

2.2.5. Cultivation of *Salmonella spp.* on Selective Media

Subculture the incubated MKTTn and RVS broths were plated onto Xylose Lysine Deoxycholate (XLD) Agar and *Salmonella* ABC (Chromogenic *Salmonella* Medium) and incubate for a further 24 hours at 37°C ± 1°C. *Salmonella spp.* forms black colonies in XLD agar, while in ABC Agar medium it forms a colony in light green tones [23].

2.2.6. Biochemical tests

Agglutination test was performed on samples with catalase (+) and oxidase (-) [23].

2.2.7. *Salmonella spp.* Latex Agglutination Test

In this test, a drop of 0.85% salt was dripped on the Black plate. For identification of *Salmonella spp.*, latex agglutination test (Microgen) was used according to the manufacturer instruction. 'Agglutination (+)' for sediment-forming colonies, 'agglutination (-)' for sediment-forming colonies were obtained [23].

2.2.8. Cultivation of Biochemical Test Kit

Catalase positive, oxidase negative and agglutination positive colonies were transplanted to the biochemical test kit with Microgen GNA-ID panel. These panels were incubated for 24 hours at 37 °C. The results were obtained by coding with Microgen program [23].

2.2.9. *Bacillus cereus* Analysis

225 ml buffered Peptone water was added to 25 g sample and homogenized for 2 min. *B. cereus* ATCC 14579 (CECT) strain was used as positive control [22].

2.2.10. Isolation of *Bacillus cereus*

1 ml of dilution was taken and Mannitol-Egg-yolk-Polymyxine Agar (MYP) was inoculated. It left to incubation for 24-48 hours at 30°C. Each dilution was inoculated to parallel petri dishes in *B. cereus* analysis. [22].

2.2.11. Identification of *Bacillus cereus*

The designated colonies were inoculated to Sheep Blood Agar for biochemical testing and left at 30 ° C for 24 hours for incubation. Hemolytic reactions recorded [22].

3. RESULTS

According to the intensity of production, food samples produced on the days, allowed by the authorities of the food enterprises were taken without paying money. The numbers of different samples taken on different days allowed by the authorities have been completed to 100 samples. The samples were analyzed in terms of three bacteria. The collected samples were examined in the accredited laboratory without waiting.

100 samples were collected from food producing enterprises in Istanbul. In these samples, *E. coli* O157:H7 was searched. For selection of pure form of *E. coli* O157, immunomagnetic separation process was performed. Immunomagnetic separation (IMS) is an easy, rapid and reliable assay, which has been widely used for epidemiological studies of *E. coli* O157:H7 and has provided to accomplish efficient recovering microorganisms from heterogeneous samples [38]. As a result of the investigation, *E. coli* O157:H7 serotype could not found in 100 samples according to agglutination test. An example of a bacteria agglutination test is as in the Figure 1.

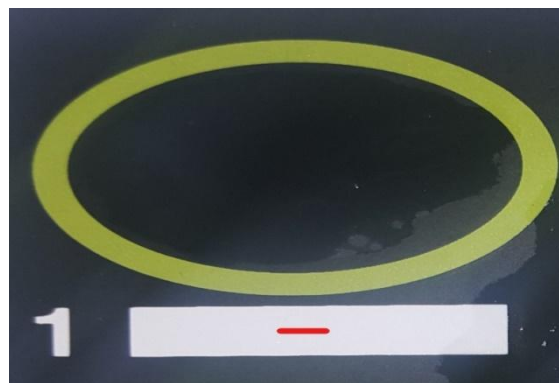


Figure 1: Negative result of the agglutination test of serotype *E. coli* O157:H7

Salmonella spp. analysis was also performed with the same samples. The bacteria was not isolated in the samples of cooked food. The raw sample, which was catalase positive and oxidase negative, was subjected to agglutination test and the test result was positive. However, *Salmonella spp.* was found in 1 (%1) of raw chicken samples. In Figure 1 shows the images of the isolated pathogen in selective media, and in table 1 shows the details of the test results for example. The sediments in the agglutination test are as shown in Figure 3.

Table 1: Biochemical Test Results

SALMONELLA SPP.		OXIDASE	CATALASE	AGGLUTINATION	BIOCHEMICAL TEST
SAMPLE NO. 98	RAW CHICKEN PIECE	-	+	+	95%

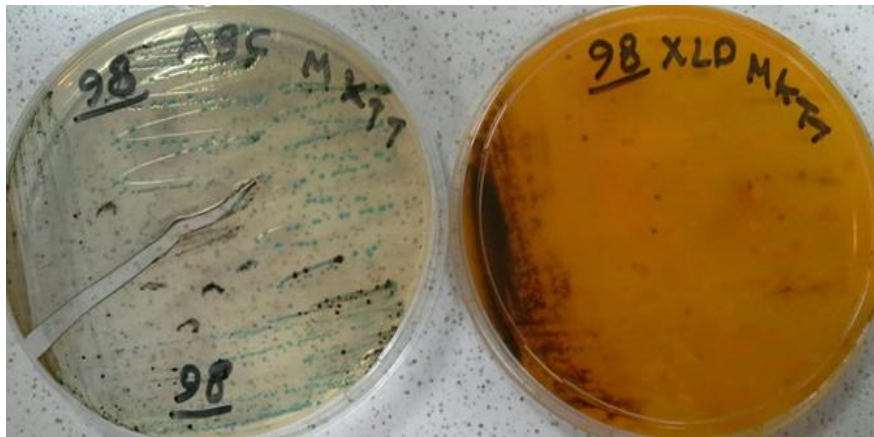


Figure 2: *Salmonella spp.* Colonies

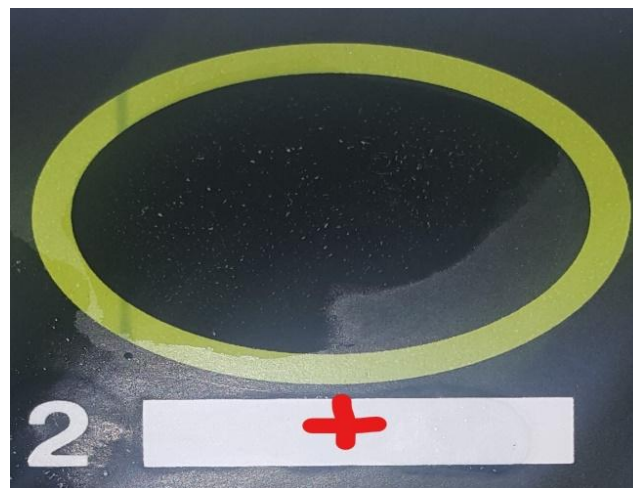


Figure 3: Sediments of *Salmonella spp.* colonies.

Bacillus cereus analysis was done only on cooked food samples (79 samples). The bacteria in these samples were evaluated according to the Turkish Food Codex Microbiological Criteria Regulation and 2 samples were determined above the specified limits. Results of the soup samples were as follows: 1×10^1 colonies in 1 vegetable soup, $6,3 \times 10^1$ colonies in 1 noodle soup, 7×10^1 colonies in 1 carrot soup, $1,3 \times 10^2$ colonies in 1 lentil soup, $2,6 \times 10^3$ colonies in 1 lentil soup and $4,5 \times 10^3$ colonies in 1 wheat soup. Table 2 provides details about food samples and the number of microorganisms. 10^1 colonies in 1 meat potato sample, 4×10^2 colonies in 1 meat meatball sample and were determined. $1,1 \times 10^1$ colonies were isolated in 1 meatless green beans sample. In rice samples, there were 3×10^1 colonies in 1 bulgur rice samples, 10^1 colonies in 1 rice samples. $5,2 \times 10^4$ colonies were found in 1 pasta samples.

Table 2: Examples of *Bacillus cereus* and Colonies Number Distribution

Samples	Number of Samples	<i>Bacillus cereus</i>			
		10 ⁰ -10 ¹	10 ¹ -10 ²	10 ² -10 ³	>10 ³
Soup	6	-	2	3	1
Meat Meals	2	-	1	1	-
Meatless Meals	1	-	1	-	-
Rice	2	-	2	-	-
Pasta	1	-	-	-	1

Sample numbers and percentages in which microorganisms are detected as a result of the research are given in Table 3. As shown in the above table, *B. cereus* is above the limits in two samples (>10³).

Table 3: Sample numbers and percentages of microorganisms detected and not detected

Microorganism	Number of Positive Samples (number/25 gr-mL)	Percent of Positive Samples (%)	Number of Negative Samples (number/25 gr-mL)	Percent of Negative Samples (%)
<i>E.coli</i> O157:H7	0	0	100	100
<i>Salmonella</i> spp.	1	1	99	99
<i>B. cereus</i>	2	2,53	77	97,47

4. DISCUSSION & CONCLUSION

Faults in the production phase pose risks to consumer health in food businesses. There is a high risk of contamination in erroneous stages such as the use of cooked meals, waiting for meals and delayed service [18, 26]. Foodborne diseases occur as a result of these contaminations.

According to statistical data obtained in the USA, UK and the Netherlands, more than 70% of food-borne diseases are associated with food or service sectors [34]. In addition, in developed countries, more than 30% of the population is infected with this type of disease each year, and in developing countries, more than two million people per year are reported to have died due to these reasons [36].

In Australia, of the approximately 5.4 million foodborne cases that occur every year, 15,000 have been hospitalized and 120 have been found to be death cases [30]. As a matter of fact, the risk factors for the emergence of foodborne illnesses are revealed by the fact that the factors leading to these diseases are mostly caused by misinformation and practices in the processing of food such as improper cooking / preparation and faulty temperature control [4, 28 31].

In this study, total of 100 samples including 79 cooked meals and 21 raw meat and chicken samples were collected from the companies that produce in institutional catering in Istanbul and the collected samples were examined in terms of pathogenic microorganisms of *Salmonella* spp., *E. coli* O157:H7 and *B. cereus*.

In their study of Doyle and Schoeni (1987); a total of 164 ground beef, 264 pork, 263 poultry, and 205 lamb samples were tested for *E. coli* O157: H7 in fresh meat and chickens served on sale. The organism was isolated in the case of 6 beef (3.7%), 4 pork (1.5%), 4 poultry (1.5%) and 4 lamb meat (2.0%) and showed that the bacterium was associated with animal derived foods but not only beef. Bacterial counts between 2 x 10⁹ and 5 x 10⁹ cfu / ml were determined; *E. coli* O157:H7 has been found to be important from the dominant microflora of enrichment cultures.

In their study of Alişarlı and Akman (2004); the presence of *E. coli* O157 was investigated in ready minced meat samples sold at retail in Van, and as a result of the analyses, *E. coli* O157 was isolated in 4.66% of the cow mince

samples and 2% of the sheep mince samples.

Mercanoğlu et al. (2006) examined 57 raw chicken samples presented for sale in the market in terms of *E. coli* O157:H7. According to the results of *E. coli* O157: H7 analysis made with the samples; *E. coli* O157:H7 was detected in 1 sample (1.8%) when using classical culture method and 2 samples (3.5%) when immunomagnetic separation method was used.

Dursun (2008)'s study of *E. coli* O157:H7 pathogen microorganism wasn't found in cooked food [16].

In this study, no pathogenic microorganisms of *E. coli* O157: H7, which is present in the warm-blooded animal and in the intestines of humans as natural flora, were detected in the 100 samples examined as a result of the research conducted.

Eleftheriadou et al. (2002); conducted a comprehensive survey in Cyprus in 1991-2000, and 1382 samples of ready to consume food were taken and 4 samples of *Salmonella spp.* were isolated.

Ildız and Çiftçiöğlü (1997) reported that there were no *Salmonella spp.* in the samples of 52 soups and 53 meat dishes studied.

In addition, Ayçiçek et al. (2005) reported that *Salmonella spp.* was not isolated in 130 soups, 232 main meals samples.

Jordan et al. (2006) found *Salmonella spp.* in 1% of the raw meat samples they studied in their studies, while Little et al. (2008) reported that 2,4% of the 3959 raw meat samples were infected with *Salmonella spp.*

Çolak et al. (2007), total of 152 samples (60 soups and 92 ready meals) were analyzed in relation to *Salmonella spp.* and no pathogenic microorganisms of *Salmonella spp.* were found.

Özkan (2009) *Salmonella spp.* was detected in 2 meat dishes in microbiological quality analysis on 794 samples from meals and salads offered for consumption.

Akbulut (2010) in study, a total of 109 samples were analyzed, including 14 soup, 52 meat dishes, 25 rice and 18 pasta, which were served to consumption in 50 catering plants in Istanbul. *Salmonella spp.* was not isolated in any of the samples examined.

In the study conducted by Süzme (2012), 120 poultry meat samples of chickens, wings, baguette, buttocks and chest meats from various poultry sales and markets in Edirne province and districts between February 2011 and January 2012 were investigated for pathogenic bacteria, *Salmonella spp.* was detected in 36 cases.

Şenses et al. (2015), *Salmonella spp.* was not isolated in any of the 666 food samples analyzed.

In this study, *Salmonella spp.* was detected in 1 (1%) of the 21 raw meats and chicken samples while *Salmonella spp.* was not found in 79 cooked foods.

Eleftheriadou et al. (2002) analyses revealed *Bacillus cereus* ($>10^4$ cfu/g) in 5 samples of 1382 ready to consume food samples.

Çolak (2007), *B. cereus* (10^2 - 10^5 cfu / g) was detected in 5 of the 52 samples in which *B. cereus* analysis was performed. Aksu (2010) *B. cereus* was isolated in 2 of the rice samples.

Özkan (2009)'s study, 158 chicken meat and 89 red meat meal *B. cereus*, 3 (1.9%) in sample 10^1 - 10^2 cfu/g range, 1 (0.6%) in sample 10^2 - 10^3 cfu/g range was determined. *Bacillus cereus* was found in 10^1 - 10^2 cfu/g in 2 samples and 10^2 - 10^3 cfu/g in 1 sample in pasta samples. In rice samples, 4 (6.2%) samples were found between 10^1 - 10^2 cfu/g and 10^2 - 10^3 cfu/g in 7 (10.9%) samples. The results do not exceed the specified limits.

Akbulut (2010) in study, *B. cereus* was detected on 1 of the 109 ready to eat daily meals (1.8%) above the limits. *B. cereus* was found in 10^1 - 10^2 cfu/g in 3 (3.4%) samples and in 10^2 - 10^3 cfu/g in 6 (6.7%) samples in red meat. *B. cereus* 3 (3.4%) was detected in meals samples at a concentration of 10^1 - 10^2 cfu/g and in the sample of 10^2 - 10^3 cfu /g in 6 (6.7%) samples. *B. cereus* was not detected in 23 (92%) sample and in 1 Sample (4%), 10^2 - 10^3 was defined as cfu/g and in 1 (%4) was defined as $>10^3$ cfu/g.

Şenses et al. (2015) found that 14 samples of meat and vegetable dishes were found to be unsuitable for the *B. cereus* parameter in microbiological analyzes on various, followed by samples of pasta (2 pieces) and rice (1 piece). The number of *B. cereus* in samples that are not suitable for consumption varies between 1×10^3 - 5.7×10^4 cfu/g.

In this study, 79 samples of cooked meals were studied in terms of *B. cereus*. As a result; 2 soup, 1 meatless dishes, 1 meat dishes, 2 rice were determined in the range of 10^1 - 10^2 , 3 soup, 1 meat dishes was determined in the range of 10^2 - 10^3 . Above the values specified in the TFC Microbiological Criteria Regulation ($> 10^3$); 1 soup and 1 rice sample were found.

B. cereus sports are widely found in baked foods. Endospores are resistant to cooking temperatures. As a result of the conversion of sports into vegetative microorganisms and food poisoning can cause the level of toxin is possible to occur. This situation constitutes important problems in consumption-ready meals and mass consumption places. In order to prevent food poisoning caused by *B. cereus*; if the food is not consumed immediately after being fed, it should be chilled rapidly. If it is to be served hot, it should be kept at the appropriate temperature and consumed in a short time [10].

Salmonella spp. is one of the first causes of foodborne infections and is a dangerous effect of zoonotic character in terms of public health. *Salmonella spp.* is one of the leading pathogens causing foodborne illnesses due to its high resistance to environmental conditions, its long-term viability in food, and its multi-resistance mechanism against antibiotics [35]. Military units have had "food poisoning" suspicious events four times in 27 days in May-June 2017 in Manisa [19]. These events have shown the importance of this pathogen. For the prevention of food poisoning caused by *Salmonella spp.*; hygienic measures should be taken from production to consumption at every stage, especially cross contamination should be avoided.

As a result, some dishes prepared for consumption were examined. While *E. coli* 0157:H7 was not found in the samples, *Salmonella spp.* was determined in 1 raw chicken sample and *B. cereus* bacteria were determined over the limits in 1 soup and 1 pilaf sample. Although the number of studies conducted on the issue directly affecting public health is small, the results show the size of the risk. For this reason, further studies should be done on the subject.

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