

FOODBORNE OUTBREAK ASSOCIATED WITH STAPHYLOCOCCUS AUREUS ENTEROTOXIN AND BACILLUS CEREUS IN THREE STUDENT DORMITORIES, KAYSERİ PROVINCE, TURKEY, NOVEMBER 2014

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Abstract

On 13–14 November 2014 an outbreak associated with consumption of dinner prepared in the same kitchen resulted in intoxication of 121 persons out of 310 in three student dormitories in Kayseri Province, Turkey. We conducted a retrospective cohort investigation to identify the cause and to implement the control measures. Probable case was defined as “Onset on 13 November 2014 of at least one of the symptoms of nausea, vomiting, abdominal pain, and diarrhoea among persons who ate dinner in three dormitories”. We conducted face-to-face interviews with 285 persons. Nasal swabs from food handlers, drinking water samples and food samples were tested for pathogens. Males were affected two times more than females (RR=1.6, 95%CI:1.3–2.1). We analyzed data by logistic regression in a model controlling chicken drumstick consumption and sex. People who ate full serve of mashed potatoes developed illness four times more (OR_{adj}=3.6; 95% CI:1.6–7.8); and who ate half serve of mashed potatoes developed illness three times more than non-exposed (OR_{adj}=3.4; 95% CI:1.3–8.7). *Staphylococcus aureus* enterotoxin and *Bacillus cereus* was identified from the leftover mashed potatoes. In conclusion this outbreak was likely due to contaminated mashed potatoes. This outbreak is likely to be caused by mashed potatoes contaminated with Staphylococcal enterotoxin and *Bacillus cereus*. As public health actions arrangements in kitchen were done according to the regulations. Money penalty was given to dormitory. Food handlers were trained on certificate of hygiene.

Keywords: Foodborne diseases, *Staphylococcus aureus*, *Bacillus cereus*, enterotoxins.

STAPHYLOCOCCUS AUREUS ENTEROTOKSİNİ ve BACILLUS CEREUS İLİŞKİLİ OLARAK ÜÇ YURTTA GÖRÜLEN GIDA KAYNAKLI SALGIN, KAYSERİ, KASIM 2014

13-14 Kasım 2014 tarihinde Kayseri ilinde üç ayrı öğrenci yurdunda bulunan 310 kişi arasında 121 kişinin zehirlenmesi ile sonuçlanan ve aynı mutfakta hazırlanan akşam yemeğinin tüketimi ile ilişkili bir salgın yaşanmıştır. Bu retrospektif kohort çalışmasını, olası nedenlerin ortaya konulması ve koruma, kontrol önlemlerinin alınması için yürüttük. Olası vaka tanımını, “13 Kasım 2014 tarihinde, üç yurttan akşam yemeği yiyen ve aynı tarihlerde bulantı, kusma, karın ağrısı ve ishal şikayetlerinden bir ya da daha fazlasını yaşayan kişiler” olarak belirledik. 285 kişi ile yüzyüze görüşme sağladık. Gıda elleyicilerinden burun sürüntüsü, içme sularından örnekler ve gıdalardan numuneler alınarak patojenler açısından test edildi. Erkekler kadınlardan iki kat daha fazla etkilenmişti (RR=1.6, 95%CI:1.3–2.1). Tavuk yemeyi ve cinsiyeti kontrol ederek bir lojistik regresyon modeli oluşturduk. Buna göre tam porsiyon patates püresi yiyenler hiç yemeyenlere göre dört kat daha fazla (OR_{adj}3.6; 95% CI:1.6–7.8), yarım porsiyon yiyenler ise yemeyenlere göre üç kat daha fazla (OR_{adj}=3.4; 95% CI:1.3–8.7) hastalık geliştirmişti. Kalan patates püresinde *Staphylococcus aureus* enterotoksini ve *Bacillus cereus* üredi. Dolayısıyla bu salgın, kontamine patates püresinin tüketimi ile ilişkili görünmektedir. Bu salgının Stafilokok enterotoksini ve *Bacillus cereus* ile kontamine patates püresinden kaynaklanmış olduğu düşünülmektedir. Halk Sağlığı eylemi olarak mutfakta yönetmeliklere göre düzenlemeler yapıldı. Yurt yönetimine idari para cezası verildi. Gıda elleyicilerinin hijyen sertifikası eğitimi almaları sağlandı.

Anahtar Kelimeler: Gıda kaynaklı hastalıklar, *Staphylococcus aureus*, *Bacillus cereus*, enterotoksinler.

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Introduction

According to the World Health Organization “disease of infectious or toxic nature caused by or thought to be caused by the consumption of food or water” is called foodborne disease (1). Foodborne diseases including both foodborne intoxications and foodborne infections are frequently called inaccurately as food poisoning (2). These outbreaks are characterized by occurrence of illness within usually short time period such as hours and days. Symptoms start usually after a meal, among individuals who have consumed food/foods in common (3).

Staphylococcal intoxications are common causes of foodborne diseases in the world. Ingestion of coagulase positive Staphylococcal enterotoxins is mainly the cause of staphylococcal intoxications (3). Symptoms, including vomiting, diarrhoea and stomach cramps show a rapid onset. *Staphylococcus aureus* is a significant cause of foodborne diseases with a number of 241.000 cases per year in the United States (4). True incidence can not be known or is underestimated due to lack of reporting of mild cases, misdiagnosis, improper sample collection and laboratory examination, lack of seeking medical attention and lack of routine surveillance of clinical stool specimens for *S. aureus* or its enterotoxins (4).

Bacillus cereus has two types of intoxications. The emetic form and the other is the diarrhoeal form. The emetic form causes vomiting usually after one to five hours after ingestion of contaminated food. In contrast four to sixteen hours of incubation period is needed for presentation of diarrhoeal form of the disease. Both types are usually mild illnesses which last not more than 24 hours (5).

On 13 November, 2014, Provincial Public Health Directorate reported a

cluster of gastroenteritis cases from two student dormitories in Kayseri. According to the first information given by local health authorities 92 patients from two dormitories applied to hospitals in one night. Forty-four of patients were transported by ambulance to emergency services of hospitals. This event was also published online in the following morning. The common exposure was the dinner prepared in the same kitchen, in one of the dormitories. Not only students, but also some of the workers had symptoms. One of the dormitories was a girls' and the other was a boys' dormitory. Main symptoms of the patients were nausea, vomiting, abdominal pain and diarrhoea. Symptomatic treatment was given to all the patients. Only one case was hospitalized. The dinner menu included tomato soup, chicken drumstick, mashed potatoes and salad. We conducted an investigation to identify the cause and to implement control measures. Our hypothesis was that “the outbreak in two dormitories on thirteenth November was associated with food or foods at the dinner prepared in the same kitchen”. When we started field investigation we got the information that another girls' dormitory was also affected from the outbreak. We also included that dormitory into the investigation.

In this paper we will summarize an outbreak investigation due to the pathogens we mentioned above. Our aim is to address importance of these types of outbreaks and make recommendations to manage outbreaks and avoid appearance of new outbreaks due to these conditions. Secondary aim -as being field epidemiologist- is to share field experience with public health professionals.

Material and Method

The study population included all the residents and workers of three dormitories in Kocasinan, Melikgazi and Talas districts of Kayseri which is one of the biggest cities in Central Anatolia.

Since population is composed of closed communities which are easily accessible, retrospective cohort investigation was conducted.

The cohort is defined as people who ate dinner in dormitories on 13 November 2014. List of dormitory records were taken and used as a check list to be sure that all of the students and workers are accessed. The number and conditions were easy to access all residents and workers. No sampling was done; all population was in target. We interviewed 285 persons out of 310 (Response rate: 92%).

Case Reporting by Physicians and Notification by Provincial Directorate

On November 13, increased number of gastroenteritis cases was reported by physicians in emergency departments of the hospitals to Provincial Public Health Directorate in Kayseri. Cases had a common history of being residents of the two dormitories. The dormitory directorates also called ambulances at 10 pm when the number of students who started to suffer from nausea, vomiting and abdominal pain increased. Common exposure of the cases was the dinner served in both dormitories. Cases have started at 5:00 pm and showed a peak at 10 pm. Outbreak was confirmed and finally notified by the local health authorities of province to the Public Health Institution at that night. First case was seen at 5:00 pm, 30 minutes after eating meal which is served between 4:00 pm and 7:00 pm.

Epidemiological Investigation

The study population included all of the residents and workers of three dormitories in Kocasinan, Melikgazi and Talas districts of Kayseri.

In this retrospective cohort investigation we calculated initially the descriptive statistics then we performed univariate analysis (χ^2 tests, relative risk) and multivariate analysis (Logistic regression, adjusted odds ratios). A p value less than 0.05 was considered as statistically significant.

The investigation cohort was defined as people who ate dinner in the dormitories on November 13, 2014. According to the lists of residing students in the dormitories, there were approximately—some of the students registered but not resides in—300 students and 10 workers in the dormitories. Person who reported at least one of the following symptoms: nausea, vomiting, abdominal pain and diarrhoea, was regarded as a case. Face to face interviews with a written questionnaire were conducted to gather information from the cohort on November 17–18. Thirteen students were interviewed by telephone calls on November 21.

In the field investigation, 140 persons out of 144 persons in the first girls' dormitory, 47 persons out of 52 persons in the second girls' dormitory and 98 persons out of 114 persons in the boys' dormitory were interviewed. Response rates were 97%, 90% and 86% respectively (Figure 1).

Laboratory Investigation

According to regulations, food samples are routinely taken in the kitchen. Food samples from soup, main dish –chicken drumstick–, mashed potatoes and salad were taken (n=5).

Nasal swabs were obtained from food handlers (n=6) and the truck driver (n=1) who transports food to other dormitories. These nasal swabs were tested initially by Provincial Public Health Laboratory and positives ones were sent to Microbiology Reference Laboratory in Public Health Institution in Ankara.

Identification of strains were done according to Reverse Passive Latex Agglutination method (SET-RPLA, Denka Seiken, Tokyo, Japan).

Staphylococcal enterotoxin investigation in food was done by Mini VIDAS device which uses Enzyme Linked Fluorescence Assay (ELFA) principles for detection, *Bacillus cereus* and *E.coli* were investigated as number of colony forming unit per gram (cfu/gr) under the standards, and *Salmonella spp.* were

investigated as cfu/gr as well. All analyses were done according to “Regulation On Turkish Food Codex Microbiological Criteria, Annex 1: Food Safety Criteria” (6).

Water samples were obtained from the kitchen and lavatory of the dormitories.

No clinical specimens were taken during the outbreak, which is a limitation for this investigation.

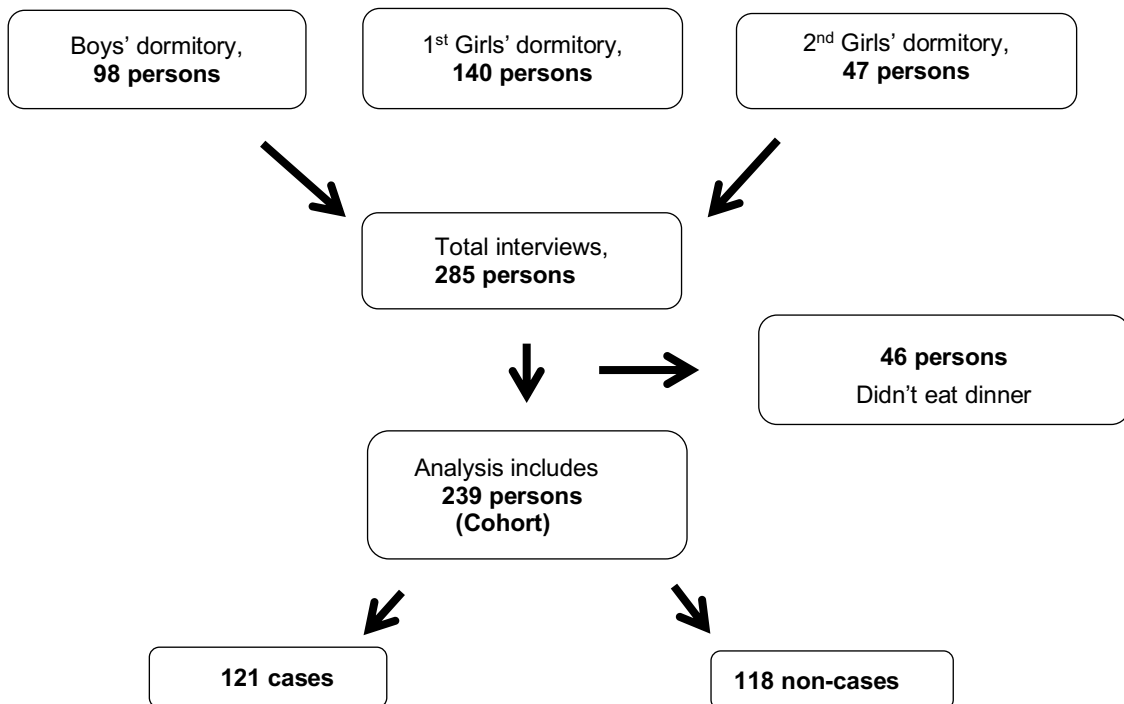


Figure 1: Case finding. Kayseri; 2014

Results

Descriptive Statistics about All Respondents

The respondents' age ranged from 13 to 62 years and the median age was 20 years. Mean age was 20 ± 3.2 years. Ratio of respondents' females to males was 2.01.

Attack rates by sex were 68.4% among males and 41.9% among females.

Attack rates in three dormitories were also different; in the 1st girls'

dormitory 41.6%, in the 2nd girls' dormitory 42.1% and in the boys' dormitory 67.9%.

Descriptive Statistics about Cases

Median age of the cases was 20 years (min-max: 16- 34 years). Mean age of the cases was 20.7 ± 3.2 years. Males were affected two times more than females ($p < 0.001$ RR=1.6 CI 95% 1.3–2.1) (Table 1).

Table 1: Attack rates according to consumption of foods in the menu. Kayseri, November 2014

Foods	Case		Non-case		Total		
	n	AR (%)	n	AR (%)	n	RR	CI (95%)
Soup							
Yes	94	50.8	91	49.2	185	1.01	0.75-1.37
No	27	50.0	27	50.0	54		
Mashed potatoes							
Yes	106	57.3	79	42.7	185	2.06	1.31-3.22
No	15	27.8	39	72.2	54		
Chicken							
Yes	119	52.7	107	47.3	226	3.42	0.95-12.32
No	2	15.4	11	84.6	13		
Salad							
Yes	106	53.3	93	46.7	199	1.42	0.93-2.16
No	15	37.5	25	62.5	40		

AR: Attack rate RR: Relative Risk CI: Confidence Interval

The most common symptoms were nausea, abdominal pain and vomiting in both sexes (Figure 2). The epidemic curve showed a point source

outbreak. The cases increased just after the dinner. The peak was 5 hours after dinner time (Figure 2).

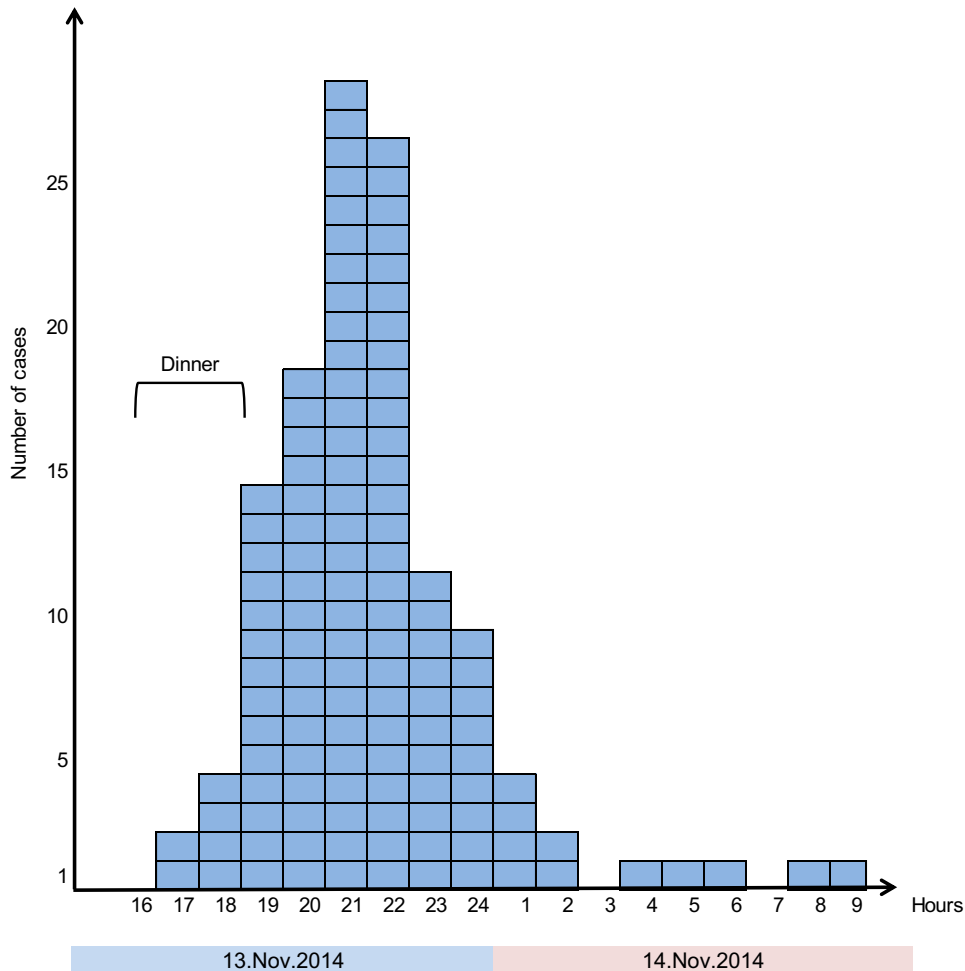


Figure 2: Epi curve. Kayseri; November 2014

Mean incubation period was 4.04 ± 2.8 hours, and the median incubation period was four hours (min-max: 0.5–15 hours).

People who ate mashed potatoes developed the disease two times more than the people who did not (RR:2.06, 95% CI 1.31–3.22) (Table 1). According to the table there are 15 patients who did not consume but developed the disease. Possible explanations will be discussed in the discussion part of the article.

To evaluate the dose response relationship, we categorized respondents into four groups who ate full serve and more, half serve, one spoon and people who consumed none of

these foods as the reference group (Table 2).

According to the information given by the workers who served food at dinner, students had mashed potatoes with chicken in the same plate. As sex was found to be associated with the disease and the chicken consumption, we controlled sex in the logistic model as a confounding factor. The respondents who ate full serve or more mashed potatoes had a fourfold disease risk than who didn't (OR_{adj}=3.61, 95% CI 1.66–7.85) and the respondents who ate half serve of mashed potatoes had a threefold risk (OR_{adj}=3.42, 95% CI 1.34–8.70) (Table 2).

Table 2: Dose response relationship according to amount of consumed mashed potatoes

Mashed potatoes*	Case		Non-case		OR _{adj}	CI (95%)	p
	n	AR (%)	n	AR (%)			
Full serve or more	63	66.3	32	33.7	3.61	1.66-7.85	0.001
Half serve	18	54.5	15	45.5	3.42	1.34-8.70	0.009
One spoon	25	43.9	32	56.1	1.99	0.88-4.48	0.095
None (Reference)	15	27.7	39	72.3	-	-	-

AR:Attack Rate

OR_{adj}:Adjusted Odds Ratio

*Chicken consumption and sex controlled.

Laboratory results

Tomato soup and salad, chicken drumstick samples were tested negative for pathogens; staphylococcal enterotoxin, *Bacillus cereus*, *Salmonella spp.*, and *E.coli* (Salad was not tested for staphylococcal enterotoxin)

Mashed potatoes were contaminated with Staphylococcal enterotoxin, *Bacillus cereus* and *E.coli* (Table 3). Frozen drumstick sample was contaminated with *Listeria monocytogenes*.

Water samples were tested negative for any pathogens.

No environmental sampling has been done by Food, Agriculture and Livestock Provincial Directorate who is the responsible and authorized body of taking these samples.

Nasal swabs taken from food handlers were tested negative except one. But the strain isolated was identified not enterotoxigenic according to the further investigations done in Microbiology Reference Laboratory in Public Health Institution of Turkey in Ankara.

Table 3: Assesment of mashed potatoes sample. Kayseri, 2014

Parameter	Analysis method	Number of specimen	Result
Staphylococcal Enterotoxin	Detection of staphylococcal enterotoxin by Mini VIDAS device (ISO 6888-1,1999)	1	<u>Detected</u>
Bacillus cereus (cfu/gr):	Colony count technique at 30 °C (TS EN ISO 7932, 2009)	1	<u>2x10⁵</u>
Salmonella spp. (/25gr):	Horizontal method for detection of <i>Salmonella</i> spp. (TS EN ISO 6579, 2005)	1	Not detected
E.coli (cfu/gr):	Horizontal method of beta glucuronidase-positive Escherichia coli- colony count at 44 °C (ISO 16649-2-2001)	1	<u>3.8x10³</u>

cfu/gr: colony forming unit per gram

Discussion

This outbreak was likely to be associated with consumption of mashed potatoes that is contaminated with staphylococcal enterotoxin and *B. cereus*.

Staphylococcal food poisoning is usually caused by *Staphylococcus* strains and rarely caused by consumption of foods which were contaminated with preformed staphylococcal enterotoxins (3). Outbreaks has been reported associated with a wide range of food groups like dairy products, meat, creamy cheese, tuna fish, poultry, bakery, ice cream and potatoes (7). But the exact incidence cannot be estimated because the coordination and execution of all activities to confirm an outbreak is not easily carried out, resulting in under reporting of the foodborne outbreaks and lack of recorded information about the outbreak (7).

In foodborne outbreaks it is generally difficult to gather evidence on

the agent or the source of the outbreak. There are some steps to follow in outbreak investigation; notification of outbreak, inspection of food and environment, sampling and analyses of the suspected foods and/or clinical samples, analyses of the data collected, elaboration and publication of final reports (7). In an epidemiological study which reviewed 57 outbreaks, it was found that 74% of outbreaks were confirmed with microbiological tests whereas 26% of the outbreaks were confirmed epidemiologically (7). In our study, both microbiological and epidemiological confirmation could be provided. Staphylococcal food poisoning can be confirmed by detection of 10⁵ cfu/gr bacteria in the food remnants or detection of staphylococcal enterotoxin in the remaining foods (3). We detected Staphylococcal enterotoxin in the mashed potatoes.

Staphylococcal foodborne diseases are types of gastroenteritis

typically presenting merely with vomiting or both vomiting and diarrhoea (8). Dinges et al. give the incubation period changes as half an hour to eight hours (8). In our study the mean incubation period was 4.04 ± 2.8 hours. This is compatible with the incubation period of *Staphylococcus*. There are some cases with longer incubation periods than eight hours in our investigation. These cases may recalled eating time wrong or had foods with themselves in the room and ate later again after keeping them in room temperature. Another explanation might be that these cases are the cases caused by *B.cereus* which has a longer incubation period.

Systemic toxicity symptoms such as fever and hypotension are rarely seen in staphylococcal food poisoning (8). In our investigation self-reported fever was recorded. One quarter of the cases reported that they had fever. We do not know if they really had fever.

Staphylococcal food poisoning is mostly self-limited and patients recover usually in 24 to 48 hours (8). Although these cases should be reported to the health authorities characteristics of the disease cause underreporting. In our investigation, since very high number of patient applied to hospitals all together, the local health authorities, were informed immediately.

Staphylococcal food poisonings are caused by five conditions 1) Raw material contaminated with pathogen or carrier person, 2) Cross contamination by utensils, 3) Food content that is suitable for growth of bacteria and production of toxin, 4) Suitable temperature and time for bacterial growth, 5) Consumption of adequate amount of bacteria or toxin (4).

Most of the staphylococcal food poisonings are caused by poor hygienic conditions during processing steps of the preparation (3). Mucosal carriers among food handlers are one of the important causes of outbreaks. Those people carry *S.aureus* asymptotically

in their nostrils and hands. Besides, kitchen equipment and surfaces are also probable causes of the outbreaks (9). Due to lack of environmental investigation, contamination source could not be revealed in our investigation. But nasal swabs were obtained from all food handlers and food truck driver. Since all nasal swabs except one were tested negative for the agent and the *S.aureus* strain isolated from one of the chef assistants was not enterotoxigenic. In this situation contamination from carrier individuals does not explain the conditions proposed for contamination.

In this investigation another pathogen detected in mashed potatoes was *B.cereus*. Foodborne disease that is caused by emetic exotoxins of *B.cereus* is one of the pathogens that should be considered in differential diagnosis of the staphylococcal disease because of the same characteristics such as rapid onset and similar symptoms (3,7). *Bacillus cereus* presents with two types of disease as diarrhoeal and emetic form. Emetic form is caused by heat-resistant toxins and associated with farinaceous food like bakery, noodle, rice, pasta. Whereas diarrhoeal type of disease is usually caused by heat-labile preformed enterotoxins and associated with meat, soups, vegetables and dairy products (5,10,11). In this outbreak there were 61 cases suffering diarrhoea. Of these, 42 cases had also vomiting. Number of the cases only with diarrhoea was 19.

According to According to the United States Centers for Disease Control and Prevention (CDC), confirmation of *B.cereus* food intoxication is assured by isolation of pathogen in two or more stool samples or detection of a number of 10^5 cfu/gr bacteria in a properly taken food sample with epidemiological association (12).

In our investigation 2×10^5 cfu/gr was detected in mashed potatoes. We also found a strong epidemiological

association with the disease and a dose-response relationship.

In routine practice of foodborne disease algorithm, samples are examined for non- O157:H7 *E.coli* in the laboratory and sub-grouping of the pathogen is not done. *E.coli* O157:H7 is tested in specific conditions. The detected number of *E.coli* was 3.8×10^3 cfu/gr which was less than infectious dose for all types of *E.coli* (13,14). There are some common serogroups of *E.coli*. Those are mentioned below respectively: Enterotoxigenic *E.coli* which is responsible for tourists' diarrhoea has a longer incubation period which changes in range of 15-50 hours. Enterotoxigenic *E.coli* has a higher infectious dose of 10^8 cfu/gr (15,16). Enteropathogenic *E.coli* (EPEC) is one of the most common causes of the infantile diarrhoea (14). High infectious dose of 10^8 - 10^{10} cfu/gr was confirmed by volunteer studies for EPEC (11). Enteroinvasive *E.coli* presents with dysenteric *Shigella* type hemorrhagic diarrhoea which was not seen in our cases (14,15). Enteroinvasive *E.coli* (EIEC) has an infectious dose of 10^6 cfu/gr (13,14). Enteroaggregative *E.coli* is one of the causes of persistent diarrhoea mostly has been seen in children. Enterohemorrhagic *E.coli* (EHEC) typically starts with abdominal cramps and within watery diarrhoea concluding with bloody diarrhoea. Incubation period is 1-8 days and the most common serotype of EHEC, *E.coli* O157:H7 has an infective dose of 1-100 cfu/gr (15). But, this strain is not epidemiologically associated with this outbreak since none of the cases had bloody diarrhoea. In conclusion, the food is contaminated with *E.coli* but the amount of the bacteria is not enough to present the disease and epidemiological characteristics of the subgroups are not compatible with characteristics of susceptible population and the cases (13,14).

Listeria monocytogenesis was detected in raw chicken drumstick. *L. monocytogenesis* is in *Listeriaceae* family, Gram positive, non-spore-forming 0.5–2.0 µm in size aerobe, microaerophilic, facultative anaerobe, coccoid or rod shaped mobile bacteria. *L. Monocytogenesis* is known to be heat labile (17). According to the Food and Agriculture Organisation of United Nations, FAO, *L. Monocytogenesis* is inactivated in chicken by cooking at 60° C for 5-8 minutes and at 70° C 0.1–0.3 minutes (18).

According to the information received from the cook, the chicken was fried in the oven at 180°C for 45-50 minutes, which is enough to eliminate the pathogen. Thus, this bacterium is not regarded as one of the pathogens responsible for this outbreak.

Despite there were 15 patients who did not consume mashed potatoes and developed disease which can be explained by variation in the individual immune status of the respondents or they misremembered if they ate or not. And also there is always a incidence of developing gastroenteritis independent from outbreak which is less than seen during outbreak.

We had some limitations in this study. The major limitation was lack of determination of contamination source. Microbiological investigation was incomplete because environmental and clinical samples could not be obtained. Another limitation is we could start interviews three days after notification.

In conclusion regardless of contamination source, this outbreak is likely due to be associated with mashed potatoes contaminated with *S.aureus* enterotoxin and *B. cereus*.

As public health actions, money penalty was given to the dormitory. Technical and hygienic arrangements were made in the kitchen according to

the regulations. Chef assistants were trained to get a certificate of hygiene which is mandatory in Turkey since 2013. The chef assistant with *S. aureus* positive nasal swab was given treatment by family doctor.

We recommend taking environmental samples routinely in case of an outbreak. We also recommended The Provincial Public Health Directorate to raise awareness and promote taking clinical samples in hospitals.

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