

Microbiological evaluation of ready-to-eat sandwiches served near hospitals and schools

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Summary: The aim of this work was to evaluate the microbiological quality of ready-to-eat sandwiches made with cheese (CS, n=80), with fermented Turkish sausage (soudjouk) (SS, n=80), and with both cheese and soudjouk with salad (Mix⁺, n=60) and without salad (Mix⁻, n=50) from five different street vendors located near school and hospitals. Samples were examined for total aerobic mesophilic counts (AMC), yeast and moulds (YM), coliforms, *Escherichia coli* (*E. coli*), presumptive *Bacillus cereus* (*B. cereus*), coagulase positive staphylococci, *Salmonella* spp., and *Listeria* spp., especially *Listeria monocytogenes* (*L. monocytogenes*). The results of the microbiological examinations showed that 45 (56.25%) and 2 (2.5%) of the SS samples; 55 (68.75%) and 6 (7.5%) of the CS samples; 55 (91.6%) and 14 (23.3%) of the Mix⁺ samples, and 36 (72%) and 4 (8%) of the Mix⁻ samples were unsuitable for consumption due to high levels of *Staphylococcus aureus* (*S. aureus*) and presumptive *B. cereus*, respectively. The results also showed that out of a total of 270 samples, 8 (2.96%) were found to be contaminated with *Listeria* species; *L. monocytogenes* (0.37%), *Listeria ivanovii* (1.85%), *Listeria grayi* (0.37%), *Listeria seeligeri* (0.37%). *E. coli* contamination was found in the CS and Mix⁻ groups at incidences of 7.5% and 4.0%, respectively. *Salmonella* spp. were not detected in any of the sandwich samples investigated. The high numbers of coliforms and AMC (up to $\geq 10^6$ cfu g⁻¹) obtained from the examinations also indicated that hygienic conditions of the processed sandwiches were very poor. The results show that the sandwiches examined in this study were of great public health concern.

Key words: Microbiological quality, public health, ready to eat food, sandwich.

Hastane ve okul çevresinde sunulan tüketime hazır sandviçlerin mikrobiyolojik değerlendirmesi

Özet: Bu çalışmanın amacı, hastane ve okullar bölgesi etrafında bulunan beş farklı kafeteryadan temin edilen tüketime hazır peynirli sandviç (PS, n=80), sucuklu sandviç (SS, n=80), peynirli ve sucuklu karışık (yeşillikli, Kar⁺ n=60), ve peynirli ve sucuklu karışık (yeşilliksiz, Kar⁻ n=50) sandviçlerin mikrobiyolojik kalitelerini değerlendirmektir. Örnekler, aerobik mezofilik canlı (AMC), küf ve maya (KM), koliformlar, *Escherichia coli* (*E. coli*), olası *Bacillus cereus* (*B. cereus*), koagülaz pozitif stafilkoklar, *Salmonella* spp., ve *Listeria* spp. özellikle *Listeria monocytogenes* (*L. monocytogenes*) bakımından incelendi. Mikrobiyolojik incelemelerin sonuçları SS örneklerinin 45 (%56.25) ve 2 (%2.5)'sinin, PS örneklerinin 55 (%68.75) ve 6 (%7.5)'sının, Kar⁺ örneklerinin 55 (%91.6) ve 14 (%23.3)'ünün ile Kar⁻ örneklerinin 36 (%72) ve 4 (%8)'inin sırasıyla *Staphylococcus aureus* (*S. aureus*) ve olası *B. cereus*'un yüksek düzeylerinden dolayı tüketime uygun olmadığını gösterdi. Aynı zamanda sonuçlar, toplam 270 örneğin 8 (%2.96)'inin *Listeria* türleri ile; *L. monocytogenes* (%0.37), *Listeria ivanovii* (%1.85), *Listeria grayi* (%0.37), *Listeria seeligeri* (%0.37) ile kontamine olduğunu göstermiştir. *E. coli* kontaminasyonu, PS ve Kar⁻ gruplarında sırası ile %7.5 ve %4 oranında tespit edilmiştir. *Salmonella* spp. incelenen sandviç örneklerinin hiçbirinde tespit edilmemiştir. Bunun yanı sıra incelemelerden elde edilen koliformların ve AMC ($\geq 10^6$ kob g⁻¹)'nın yüksek sayıları da işlenmiş sıcak sandviçlerin hijyenik durumlarının oldukça kötü olduğunu gösterdi. Sonuçlar, bu çalışmada incelenen sıcak sandviçlerin büyük halk sağlığı endişesi yarattığını göstermektedir.

Anahtar sözcükler: Halk sağlığı, mikrobiyolojik kalite, sandviç, tüketime hazır gıda.

Introduction

The consumption of foods contaminated with foodborne pathogenic microorganisms and microbial toxins are responsible for deaths, illnesses, hospitalization, and economic losses (38). Due to their widespread nature, foodborne diseases (FBD), especially gastrointestinal infections, have negative effects on human health. However the symptoms are often mild and self-limiting,

and therefore many patients do not consult a doctor, fecal samples are rarely examined, and most cases are sporadic rather than part of an outbreak (19). Eating out has increased in popularity over recent years, with two-thirds of the population rarely or routinely consuming takeaways in 2000 (3). Other than well-known restaurants and restaurants chains, consumers can find many alternatives, such as ready to eat (RTE) sandwiches and

takeaways, in order to eat out. However, such prepared foods are considered to be susceptible to post-preparation contamination by pathogenic bacteria (37). The country's economic circumstances, social difficulties, life styles and presence of urban characteristics, among other factors, contribute to the growth of the informal sector of the economy, including street food vending. Street food is defined as "ready-to eat foods and beverages manufactured and/or sold by vendors and peddlers especially in street and other similar public places" by the Food and Agriculture Organization (2). The street-vended food industry provides employment and cheap RTE meals to a large proportion of the population in developing countries like Turkey, yet little is known about its role in the transmission of FBD. The immunocompromised people, undergoing steroid treatments and young children are more susceptible to FBD. Therefore, especially street-vended foods sold nearby hospitals and schools are of high health concern.

This study aimed to investigate the microbiological quality and the safety of RTE sandwiches available from street vendors located around hospitals and schools.

Materials and Methods

Sampling: The samples were collected in packages after preparation in the same manner as how a consumer would buy them. On reaching the laboratory, product type, purchase date and place of the samples were recorded and the samples kept in their original packages, not in a cooling box so as to replicate consumer behavior, until beginning of the analysis. The time elapsed between preparation of the sandwiches and analysis was 20 min. CS (n=80 samples), SS (n=80 samples), Mix⁺ (n=60 samples) and Mix⁻ (n=50 samples) investigated in this study were periodically obtained from vendors between the period of July and September 2012.

Microbiological examinations: In order to provide a homogeneous mixture of bread and ingredients, twenty-five grams from each composite sample was weighed and homogenized with 225 ml saline peptone solution (8.5% saline + 1% peptone) in a Bag Mixer (Interscience, France) for two min. The homogenates were then submitted to serial 10-fold dilutions and the following procedures were performed. All media and chemicals used in this study were supplied from Oxoid, Hampshire, UK.

Aerobic mesophilic counts (AMC), yeast and moulds (YM): The enumerations of AMC were determined from duplicate 0.1 ml samples of appropriate dilutions (10^{-3} and 10^{-4}) on Plate Count Agar (Oxoid CM325) at 30°C for 3 days (8). The levels of YM were determined in duplicate 0.1 ml samples from appropriate dilutions (10^{-1} and 10^{-2}) on Potato Dextrose Agar (Oxoid CM139, desirable acidified with 10% tartaric acid) at 21°C for 5 days (26).

Coliform counts and E. coli: The numbers of coliforms were determined using The Most Probable Number (MPN) method with three series of dilutions (10^{-1} to 10^{-3}). One ml of each dilution was transferred into tubes containing Lauryl Sulphate Tryptose broth (Oxoid CM451) and to Durham tubes by using a sterile pipette. The tubes were incubated at 37°C for 48 h. All tubes became turbid; those that produced gas were selected and one loop full from each selected dilution was transferred into tubes containing EC Broth (Oxoid CM0853), then incubated at 45°C for 48 h. In order to determine presumptive *E. coli*, one loop full from the samples that gave positive reactions in EC Broth was transferred into tubes containing tryptone water (Oxoid CM87) and incubated at 45°C for 48 h. After the incubation period, the tubes were examined for indole production (6).

Staphylococcus aureus: In order to isolate *S. aureus*, 0.1 ml homogenates from appropriate dilutions (10^{-1} to 10^{-3}) were plated out in duplicate on Baird Parker Agar (Oxoid CM275) with egg yolk tellurite emulsion (Oxoid SR0054C, 50 ml L⁻¹) by spreading with a Drigalsky handle and incubated at 37°C for 48 h (8). The diagnostic black colonies with halo were chosen and confirmed by a Staphaurex rapid test kit (20). *S. aureus* ATCC 33592 was used as positive control.

Bacillus cereus: Samples (0.1 ml) from the 10^{-1} to 10^{-3} dilutions were taken and spread on duplicate Petri dishes containing Mannitol Egg Yolk Polymyxin Agar (Oxoid CM0929) supplemented with egg yolk emulsion (Oxoid SR0047, 10% v/v) and polymyxin B (Oxoid SR00991, vial/500 ml). The Petri dishes were then incubated at 30°C for 24 h. After the incubation period, typical *B. cereus* colonies were selected, from which at least five were used in order to define presumptive *B. cereus* (5). The result was expressed in Colony Forming Units per gram of sandwich (cfu g⁻¹), then indicated as log₁₀ g⁻¹ counts.

Salmonella spp.: Homogenization of the samples (25 g each) was carried out in a stomacher bag containing 225 ml buffered peptone water (Oxoid CM509). The homogenized samples were incubated at 37°C for 24 h. Then, 0.1 ml from the dilutions for each sample were transferred into Rappaport Vassiliadis Enrichment Broth (Oxoid CM866) and Selenite Cystine broths (Oxoid CM0699) and incubated at 42°C and 37°C for 24 h respectively. One loopful from each enrichment broth was taken and spread on Brilliant Green Agar (Oxoid CM0263) and Xylose Lysine Desoxycholate Agar (Oxoid CM0469). The plates were then incubated at 37°C for 24 h. Presumptive *Salmonella* colonies were identified by a salmonella latex test kit (Oxoid FT0203A) (4). *Salmonella* Enteritidis ATCC 13076 was used as positive control.

Listeria monocytogenes: In order to determine the presence of *L. monocytogenes*, examinations were

performed using a two-step enrichment procedure (1); 25 g sample was diluted and homogenized in 225 ml of Half Fraser broth (Oxoid CM0895) with Half Fraser supplement (Oxoid SR166) and incubated at 30°C for 48 h. A subset of 0.1 ml was then inoculated in tubes containing 10 ml Fraser broth (Oxoid CM0895) with Fraser supplement (Oxoid SR156) and incubated at 37°C for 24 h. Subsequently, one loopful from each tube was streaked onto Oxford Agar (Oxoid CM856, added with oxford supplement, 1 vial/500 ml, Oxoid SR140) and incubated at 37°C for 48 h. Colonies showing *Listeria* spp. characteristic were taken and identified using the Microbact 12L (*Listeria* Identification System, Oxoid MB1128) system according to the manufacturer's instructions. *L. monocytogenes* ATCC 13932 (serotype 4b) and ATCC 19111 (serotype 1/2a) were used as positive controls.

Results

The incidence and levels of microorganisms isolated from the samples are shown in Table 1 and Table 2. Overall, the data indicated that although no *Salmonella* spp. was found in any of the samples analyzed, the microbiological quality of the sandwiches investigated was very poor. Of particular concern was the isolation of *L. monocytogenes* in 0.37% (1 out of 270) of the sandwiches made with Mix⁺ sample (Table 3). *L. ivanovii* was found in 1.85% of the samples (5 out of 270), in particular in CS and Mix⁺ samples. In one Mix⁻, *L. ivanovii* was the most common *Listeria* spp. For all samples, high AMC (100%), total coliforms (11.1%, 30 out of 270), *E. coli* (2.96%, 8 out of 270), presumptive *B. cereus* (9.6%, 26 out of 270) and *S. aureus* (70.7%, 191 out of 270), were found (Tables 1 and 2). The results of this study showed that the numbers of *E. coli*, *B. cereus*

Table 1. Incidence (%) and counts of bacteria found in sandwiches sold around hospitals and schools.

Tablo 1. Hastane ve okullar çevresinde satılan sandviçlerde bulunan bakteri sayıları ve oranları (%).

Type of sandwich	No. of samples tested ⁿ	AMC ^a Log ₁₀ cfu/g	Yeast ^a		Mould ^a		Coliforms ^b		<i>E. coli</i> ^b	
			Log ₁₀ cfu/g (no of positive samples, %)	Log ₁₀ cfu/g (no of positive samples, %)	MPN (no of positive samples, %)	MPN (no of positive samples, %)				
CS	80	4.0-6.64	2.0-3.0 27 (33.75%)	2.0-3.39 30 (37.5%)	10-630 10 (12.5%)	10-350 6 (7.5%)				
SS	80	3.0-5.90	2.0-3.32 37 (46.25%)	2.0-3.65 18 (22.5%)	100±0 3 (3.75%)	ND				
Mix ⁺	60	3.30-7.04	2.0-3.84 28 (35.0%)	2.07-3.74 39 (65%)	> 1100 13 (21.6%)	ND				
Mix ⁻	50	3.0-6.69	2.0-3.60 12 (24.0%)	2.0-3.49 16 (32%)	> 1100 4 (8.0%)	> 1100 2 (4.0%)				
Totally	270	3.0-7.04	2.0-3.84 104 (38.52%)	2.0-3.74 103 (38.15%)	10->1100 30 (11.11%)	10->1100 8 (2.96%)				

^a: Log₁₀ cfu g⁻¹

^b: Most Probable Number

ⁿ: All samples were negative for *Salmonella* spp.

Table 2. The presence and incidence (%) of some pathogenic bacteria in the tested sandwiches.

Tablo 2. Test edilen sandviçlerdeki bazı patojen bakteri varlığı ve oranları (%).

Type of sandwich	No. of samples tested ⁿ	<i>S. aureus</i> ^a		<i>B. cereus</i> * ^a		<i>Salmonella</i> spp ⁿ
		Log ₁₀ cfu/g (no of positive samples, %)	Log ₁₀ cfu/g (no of positive samples, %)	Log ₁₀ cfu/g (no of positive samples, %)	Log ₁₀ cfu/g (no of positive samples, %)	
CS	80	2.50-5.07 55 (68.75%)	2.0-2.93 6 (7.5%)	2.0-2.32 2 (2.5%)	ND	
SS	80	2.0-4.63 45 (56.25%)	2.0-3.20 14 (23.3%)	2.0-2.35 4 (8.0%)	ND	
Mix ⁺	60	2.0-4.90 55 (91.6%)	2.0-3.20 14 (23.3%)	2.0-2.35 4 (8.0%)	ND	
Mix ⁻	50	2.07-5.74 36 (72.0%)	2.0-3.20 14 (23.3%)	2.0-2.35 4 (8.0%)	ND	
Totally	270	2.0-5.74 191 (70.74%)	2.0-3.20 26 (9.62%)			

^a: Log₁₀ cfu g⁻¹

^b: Most Probable Number

*: Presumptive *B. cereus*

ⁿ: All samples were negative for *Salmonella* spp.

ND: Not Detected

Table 3. Types of *Listeria* spp. in sandwiches.
Tablo 3. Sandviçlerdeki *Listeria* spp. Tipleri.

Type of sandwich	No. of samples tested ^c	<i>L. monocytogenes</i>	<i>L. ivanovii</i>	<i>L. grayi</i>	<i>L. seeligeri</i>
		No. and % of positive samples			
CS	80	ND	2 (2.5%)	1 (1.25%)	ND
SS	80	ND	ND	ND	ND
Mix ⁺	60	1 (1.6%)	2 (3.3%)	ND	1 (1.6%)
Mix ⁻	50	ND	1 (2%)	ND	ND
Totally	270	1 (0.37%)	5 (1.85%)	1 (0.37%)	1 (0.37%)

^c: For the presence/absence test
ND: Not Detected

and *S. aureus* exceeded the safety levels stated in the Turkish Food Codex (7).

Discussion and Conclusion

The high microbiological contamination could be due to post-contamination, between preparation and consumption. These findings suggest that some changes in production practices should be made to enhance the microbiological quality of these foods, in particular with regard to the AMC and *S. aureus* levels. Higher-than-desirable numbers of mould and yeast in tested samples may have arisen from nonfresh bread and/or ingredients with low microbiological quality. Coliforms and *E. coli* may contaminate sandwiches during processing through contamination and/or faecal material as a result of poor sanitary practices, improper handling and improper hygiene conditions. Bostan and et al. (13) and Fang et al. (21) detected *E. coli* in RTE foods at rates of 71.9% (n=96) and 88% (n=50), respectively. In our study, we found the rate of *E. coli* to be relatively low, 2.96%. The main reason for this difference might be the high temperature application used by the vendors in this study to make sandwiches just before collecting samples. Şireli and Gücükoğlu (36) identified *Listeria* spp. in 13% of RTE foods (n=100), 10% of which was *L. monocytogenes*. We detected 3% (n=270) and 0.37% *Listeria* spp. and *L. monocytogenes*, respectively with the same method. This result is also similar with Shen et al. (33), Little et al. (24), Christison et al. (16), and Bouayad and Hamdi (14), who isolated *L. monocytogenes* in 2.97%, 2.7%, 4% and 2.6% of RTE food samples respectively. However, Meloni et al. (28) found that 9.5% of RTE products were contaminated with *L. monocytogenes*. Mouspye and Von Holly (29), Cho et al. (17), Rodriguez et al (31), and Balzaretto and Marzano (10) found neither *Listeria* spp. nor *Salmonella* spp. in any RTE products. However, salmonellosis was associated with the consumption of sandwiches by Boxall et al. (15).

Similarly, *L. monocytogenes* was found in sandwiches that have been associated with outbreaks in the United Kingdom (18, 24, 25, 30, 34) and in Switzerland (12). Of

the RTE sandwiches collected and analyzed in this study, 1 (0.37%) sample out of 270 was found to be contaminated with *L. monocytogenes*. This *L. monocytogenes* contamination likely resulted from (i) Insufficient heat treatment to kill the organism or (ii) Crosscontamination through air and equipment (14). Christison et al. (16) and Garcia et al. (22) determined *Salmonella* spp. in 11 out 70 and 5 out 103 samples tested, respectively. Aycicek et al. (9) detected of *S. aureus* in RTE meals from military cafeterias in 48 (9.4%) samples. Bezerra et al. (11) pointed out that 33 (31.4%) of hamburgers tested were unsuitable for human consumption, testing positive for coliforms and *Staphylococcus* at unacceptably high levels; high levels of microbiological contamination were also detected on the hands of the food handlers. According to Meldrum et al. (27) one of the food types with the poorest microbiological quality is egg mayonnaise sandwiches; when 475 were tested for *S. aureus* and *L. monocytogenes*, 4 (0.8%) and 2 (0.4%) respectively were found to be contaminated. While Hanashiro et al. (23) did not detect any *S. aureus* strains, they found *B. cereus* in 5 of 33 RTE sandwiches.

In a questionnaire conducted by Sert and Kapusuz (32), it was reported that although the overall opinions of students regarding foods sold by street vendors were generally negative, they preferred these foods because of the low cost and fast service. Government and the food industry each has an important role to play in identifying, assessing and managing risks associated with the consumption of foods (35). The large number of contamination index microorganisms (*E. coli* and coliforms) and pathogens (*S. aureus*, *L. monocytogenes* and *B. cereus*) found in the sandwich samples tested in this research suggests that these RTE foods pose a potential health hazard to consumers, especially considering that they were being sold near schools and hospitals. The results show that these sandwiches were produced under low hygienic conditions and with poor ingredient storage conditions. Improper handling and improper hygiene might lead to the contamination of

food. Training in personal hygiene, good manufacturing practices (GMPs), cleaning and sanitation procedures could improve the microbial quality of the sandwiches served. It has been clearly understood from the above-mentioned studies that almost all street-vended food is of poor microbiological quality. With the development of modern transportation techniques and vehicles, foodborne infection/intoxication agents may meet with humans who have not encountered these agents before and are consequently vulnerable to infection. This might be in two ways; first for touristic reasons and second due to imported food and food ingredients. Therefore consumption of uncontrolled street-vended foods could have serious implications on public health.

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