

Investigation of Microbiological Quality of Raw and Cooked “Doner Kebab” Consumed in Istanbul

Aylin Kaya^{1,*}, Haydar Özpinar¹, Burcu Çakmak Sancar²

¹Department of Food Safety and Nutrition
Istanbul Aydın University, Turkey

²Department of Nutrition and Dietetics
Essenyurt University, Turkey

*Corresponding author's email: aylinkaya89 [AT] gmail.com

ABSTRACT—Meat and meat products play an important role in foodborne infections and poisonings. Some of the microorganisms that can develop in meat and meat products cause deterioration in different forms without directly affecting human health, others cause infection and poisoning in humans without causing any deterioration of meat and its products. Rapid urbanization in recent years, shortening the time spent on food and improving the economic situation of the community has led to the development and importance of the fast food sector. The development of the ready-to-eat food industry has led to increased consumption of red and poultry food. Fast food foods have meat doner and chicken doner at the head.

The aim of this study was to investigate the prevalence of *Salmonella*, *E.coli* O157, *Listeria monocytogenes* and Coagulase positive *Staphylococcus* in red meat and chicken meat doner sold in Istanbul. In order to perform collection; a total of 60 samples (30 cooked doner samples including 15 chicken doners, 15 red meat doners and 30 raw doner samples including 15 chicken doners, 15 red meat doners) were randomly collected from 60 different restaurants. Raw meat and poultry doner samples were examined for *Salmonella*, *E. coli* O157 and Coagulase positive *Staphylococci* and cooked meat and chicken doner samples were examined for *Salmonella*, *L.monocytogenes* and Coagulase positive *Staphylococci*.

As a result; no *E. coli* O157:H7 were found in any of the raw meat doner and raw chicken doner samples; also no *L. monocytogenes* were found in any of the cooked meat doner and cooked chicken doner samples. However, *Salmonella* spp. was detected in 7 of 60 samples (11,6%). 2 of these samples (3,33%) are raw meat doner, 1 of these samples (1,67%) are cooked meat doner and 4 of these samples (6,67%) are raw chicken doner. No *Salmonella* spp. were found in any of the cooked chicken doner samples. Coagulase-positive *Staphylococci* was found in 4 of 60 samples (6.66%). Detected Coagulase Positive *Staphylococci* were found in 1 of raw meat doner, in 2 of cooked meat doner, in 0 of raw chicken doner and in 1 of cooked chicken doner.

Keywords— Fast Food, Doner, Coagulase positive *Staphylococci*, *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*

1. INTRODUCTION

Doner kebab is defined as a meat dish that is prepared by marinating meat or minced meat; either red (e.g. beef, veal, sheep, lamb, goat) or poultry (e.g. chicken, turkey), with various additives and flavorings for 3-12 hours, placing it on the rotisserie tightly with inner fat, and roasting it in a vertically positioned cooker [18,1,15,20].

Doner kebab is reported to be first made in Bursa by Iskender Bey by roasting boneless lamb and beef meat on fire on the rotisserie; the same technique used for making lamb chawarma, about 150 years ago [1]. On the other hand, it is claimed that it was first made in Kastamonu and spread to other regions over time [22]. The similarity between the technique used in the making of doner kebab and other cooking techniques in Turkish cuisine is one of the factors that makes doner kebab a traditional Turkish food.

Doner kebab is consumed mainly in European countries, and many other countries such as the United States, Canada, Mexico, Iran, Saudi Arabia, with various different names such as "donair, doner, gyros, dona-kebab, donna-kebab, shiwarma, chawarma" [5,14]. There are some minor differences in the making of doner kebab according to the raw material procurement and spices specific to the culinary culture of the country. While beef is preferred over mutton in Germany, it is prepared with pork in Greece and is called "gyros"[13,15,16].

The main raw materials of doner kebab are red meat and poultry. In addition to its high digestibility, meat; as an animal-derived food, is of great importance for body in terms of adequate and balanced nutrition, since it contains high amount of energy, essential amino acids and fatty acids, and high amounts of minerals such as iron, phosphorus, zinc [6,24].

Doner is a part of the fast-food industry in many countries today and has long taken its place among the globally consumed foods.

It is a scientific necessity to determine the possible risks of doner kebab, born in our country and now world-wide popular, in terms of public health. As its raw material is meat, it is important to investigate the process of its microbiological qualities from the raw stage to the final stage of consumption. This study was carried out to evaluate the microbiological quality of doner by detecting the presence of foodborne pathogenic microorganisms such as *Salmonella* spp, *E. coli* O157, *Listeria monocytogenes* and Coagulase Positive Staphylococci in raw and cooked doners consumed in Istanbul.

2. MATERIALS AND METHODS

A total of 60 samples; 30 cooked doner samples including 15 raw chicken doners, 15 raw red meat doners and 15 cooked chicken doners, 15 cooked chicken doners, 15 cooked red meat doners, were collected from 60 different restaurants in Istanbul. The doner samples were taken in gamma sterile sample bags, each including 250 grams of doner, with sterile disposable spoons on aseptic conditions. The samples were placed in a refrigerator with a cool-pack and transported to the laboratory. Analysis of the samples were made on the day they were taken to avoid increasing microbial load. Raw meat and poultry doner samples were examined for *Salmonella*, *E. coli* O157 and Coagulase positive *Staphylococci* and cooked meat and chicken doner samples were examined for *Salmonella*, *L.monocytogenes* and Coagulase positive *Staphylococci*. *L.monocytogenes* analysis ISO 11290-1(2017); *E. coli* O157:H7, ISO 16654 (2017); *Salmonella* spp. ISO 6579-1 (2017), Coagulase Positive Staphylococci ISO 6888-2(2003) were analyzed according to methods.

2.1 *E. coli* O157:H7 Analysis

Positive control *E. coli* O157 ATCC 43894 reference strain was used for the *E. coli* O157: H7 analysis. For pre-enrichment, 225 mL Novobiocin Modified Tryptone Soya Broth were added to the samples each in 25 g sterile Stomacher bags and allowed to incubate at 41.5 ° C for 12-18 h after homogenization.

After incubation, immunomagnetic separation process was applied. After washing operation, which is the last step, 50 uL of wash tampon was added to the tubes, the tubes were shaken thoroughly, and when a pinkish color developed, it was taken with a micropipette and transferred to the Cefixime-Tellurite Sorbitol MacConkey Agar. In addition, enriched culture was also transferred to the CT-SMAC medium by means of loops. The media were left to incubate for 24 hours at 37 ° C. Colorless, weak yellowish-brown (straw yellow) colonies on the medium were evaluated as suspicious, and transferred to Nutrient Agar for purification and allowed to incubate for 24 hours at 37 ° C. For biochemical testing, agglutination was performed with a commercial test kit. The sediment-forming colonies were identified as 'Agglutination (+)' and the sediment-free colonies as 'Agglutination (-)' [9].

2.2 *Salmonella* spp. Analysis

Salmonella enteridis ATCC 13076 reference strain was used as positive control for the *Salmonella* spp. analysis. After 25 g of sample was weighed, 225 mL Buffered Peptone Water was added for pre-enrichment. The homogenized suspension was left to incubate for 24 hours at 37 ° C. For selective enrichment, 0.1 mL of BPW-enriched samples were inoculated to Rappaport Vasiliadis Soya Broth, 1 mL to Muller-Kauffmann Tetrathionate-Novobiocin Broth. The MKTTn Broth was incubated at 37 ° C for 24 hours and the RVS Broth at 41.5 ° C for 24 hours. After incubation, RVS and MKTT Broth were transferred to selective media, XLD Agar and ABC Agar and left to incubation for 24 hours at 37 ° C for development. Black colonies in XLD Agar and pale green tones in ABC Agar media were considered suspicious. Suspected colonies were transferred to Nutrient agar for purification and left to incubate for 24 hours at 37 ° C. Catalase, oxidase and agglutination tests were performed for identification, followed by confirmation with commercial biochemical test kit. Catalase was identified as positive and oxidase was identified as negative for *Salmonella* spp. Agglutination test was performed for the colonies with catalase (+) and oxidase (-). For this, *Salmonella* spp Latex Agglutination Test was used. Colonies that were agglutination positive were cultured on commercial biochemical test kit Microgen GNA-ID Panel. It was left to incubate for 24 hours at 37 ° C. The results were obtained with the Microgen Program [10].

2.3 *Listeria monocytogenes* Analysis

L.monocytogenes ATCC 19155 reference strain was used as positive control for the *L.monocytogenes* analysis. 25 grams of the sample was weighed and homogenized by being added 225 mL of Half Fraser Broth and incubated at 30 ° C for 24 hours for pre-enrichment. After incubation, 100 ul of the pre-enrichment culture was added to the tubes containing 10 ml of Fraser broth and incubated at 37 ° C for 24 hours for selective enrichment. Palcam Agar and *Listeria* Chromogenic Agar (LCA) media from both pre-enrichment and selective enrichment media were inoculated and

incubated at 37 °C for 24 hours. *L. monocytogenes* forms a 1.5-2 mm olive green-gray colored, sometimes black-centered but always black zoned colony on the Palcam Agar medium. On the LCA agar medium, green zone colonies were identified and considered suspicious for the presence of *L. monocytogenes*. Suspected colonies were transferred into Nutrient Agar and incubated for 24 h at 37 °C. The Microgen Listeria Test Kit was used as the biochemical identification kit and the results were evaluated on the database on computer [11].

2.4 Coagulase Positive Staphylococci Analysis

S. aureus ATCC 25923 reference strain was used as positive control for the Coagulase Positive Staphylococci analysis. 25 gr sample was weighed, Buffered Peptone Water was added and homogenized, and diluted to 10⁻⁵. Each dilution was inoculated to Baird Parker (BPA) Agar supplemented with RPF (Rabbit Plasma Fibrinogen) and allowed to incubate for 48 hours at 37 °C. Microgen Staph Test was applied to 3-5 black-colored typical or atypical colonies forming a zone surrounded with a light-colored area [12].

3. RESULTS

A total of 60 samples; 30 cooked doner samples including 15 raw chicken doner, 15 raw red meat doners and 15 cooked chicken doners, 15 cooked red meat doners, were collected from restaurants in Istanbul. The samples were examined for *E. coli* O157:H7, *Salmonella* spp., *L. monocytogenes* and Coagulase positive Staphylococci based on the criteria in the Turkish Food Codex Microbiological Criteria Regulation [18].

3.1 Results of *E.coli* O157:H7

As a result of the inspection made on 15 raw meat doner and 15 raw chicken doner samples, no *E. coli* O157:H7 were found in any of the samples.

3.2 Results of *Salmonella* spp.

Salmonella spp. was detected in 7 of 60 samples (11,6%). 2 of these samples (3,33%) are raw meat doner, 1 of these samples (1,67%) are cooked meat doner and 4 of these samples (6,67%) are raw chicken doner. No *Salmonella* spp. were found in any of the cooked chicken doner samples (Table 1,2).

Table 1: The number and and percentages of samples detected *Salmonella* spp.

Microorganism	Number of positive samples	Percentages of positive samples (%)
<i>Salmonella</i> spp.	7 (detected in 25 g)	11,6%

Table 2: Sample diversity, number and percentage of samples detected *Salmonella* spp.

Microorganism	Meat Doner				Chicken Doner			
	Raw Meat Doner		Cooked Meat Doner		Raw Chicken Doner		Cooked Chicken Doner	
	Number of positive samples	Percentage of positive samples(%)	Number of positive samples	Percentage of positive samples(%)	Number of positive samples	Percentage of positive samples(%)	Number of positive samples	Percentage of positive samples(%)
<i>Salmonella</i> spp.	2	3,33%	1	1,67%	4	6,67%	0	0%

3.3 Results of *L.monocytogenes*

As a result of the inspection made on 15 cooked meat doner and 15 cooked chicken doner samples, no *L. monocytogenes* were found in any of the samples.

3.4 Results of Coagulase positive Staphylococci

According to the research done on raw and cooked meat and chicken samples, Coagulase-positive *Staphylococci* was found in 4 of 60 samples (6.66%). Detected Coagulase Positive Staphylococci were found in 1 of raw meat doner, in 2 of cooked meat doner, in 0 of raw chicken doner and in 1 of cooked chicken doner. The amounts of Coagulase-positive Staphylococci identified in these samples are shown in Table-3 and Table-4.

Table 3: Sample diversity, number and percentage of samples detected Coagulase positive Staphylococci

Microorganism	Meat Doner				Chicken Doner			
	Raw Meat Doner		Cooked Meat Doner		Raw Chicken Doner		Cooked Chicken Doner	
	Number of positive samples	Percentage of positive samples(%)	Number of positive samples	Percentage of positive samples(%)	Number of positive samples	Percentage of positive samples(%)	Number of positive samples	Percentage of positive samples(%)
Coagulase-positive Staphylococci	1	1,67 %	2	3,33%	0	0	1	1,67%

Table 4 : Result of Coagulase positive Staphylococci

Microorganism	Meat Doner				Chicken Doner			
	Raw Meat Doner		Cooked Meat Doner		Raw Chicken Doner		Cooked Chicken Doner	
	Average (cfu/g)	Min–Max (cfu/g)	Average (cfu/g)	Min–Max (cfu/g)	Average (cfu/g)	Min–Max (cfu/g)	Average (cfu/g)	Min–Max (cfu/g)
Coagulase positive Staphylococci	$3,3 \times 10^2$	$3 \times 10^1 - 1 \times 10^5$	$1,4 \times 10^2$	$3 \times 10^1 - 6,4 \times 10^4$	0	0	7×10^2	$3 \times 10^1 - 6,4 \times 10^4$

4 DISCUSSION & CONCLUSION

When the doner is cooked at an effective temperature for a sufficient time, it is regarded as safe in microbiological sense because the pathogenic bacteria in the raw meat are inhibited [2]. The heat treatment only provides safety up to a certain level. When the doner slices are too thick and the cooking time is short, the inner parts are not cooked sufficiently. In this case, the heat in the inner parts of doner can be at a level that allows microorganisms to multiply [8,16].

This study was conducted to identify the presence of foodborne pathogenic microorganisms such as *Salmonella* spp, *E. coli* O157, *Listeria monocytogenes* and Coagulase Positive Staphylococci in raw and cooked doners and evaluate the microbiological quality of doners consumed in restaurants in Istanbul. Raw meat and poultry doner samples were

examined for *Salmonella*, *E.coli* O157:H7 and Coagulase positive Staphylococci and cooked meat and chicken doner samples were examined for *Salmonella*, *L.monocytogenes* and Coagulase positive Staphylococci.

E. coli O157: H7 which is a pathogenic microorganisms and found in human and animal intestinal flora were not detected in examined samples.

Salmonella spp. was detected in raw chicken doner samples more than in raw red meat samples. Since it is a food-borne disease-causing pathogen with zoonotic character, *Salmonella* spp. is dangerous in terms of public health. The high resistance of *Salmonella* spp. to environmental conditions, its ability to maintain long-term viability in food, and the multiresistant mechanism against antibiotics are among the reasons why it is on the top of the list of pathogens leading to foodborne illnesses [17]. In the Bassam (2011) study, the ratio of *Salmonella* spp. in raw meat doner was 8.33% and in raw chicken doner was 2.77%.. Kayisoglu et all. (2003) analyzed a total of 60 cooked chicken doner and 30 cooked meat doner samples. *Salmonella* spp was found in 48 of cooked chicken doner and 12 in cooked meat doner samples[14]. However, *Salmonella* spp. was detected in 7 of 60 samples (11,6%). Among 30 cooked doner samples; *Salmonella* spp. was found in 1(1,67%) of these sample of cooked meat doner. Among 30 raw red meat/chicken doner samples; *Salmonella* spp. was detected in 4 (6,67%) samples of raw chicken doner and 2 (3,33%) samples of raw red meat doner.

Listeria monocytogenes is an important pathogen in terms of public health which can develop in the refrigerator temperature and spread widely and maintain its vitality even under adverse conditions such as cooling, freezing, heating and drying processes [3]. It has been reported that *Listeria* spreads in a cycle from the infected animal to the contamination of soil and green food; and through the green food, to animals that provides milk and meat. It is, then transmitted from contaminated vegetable, fruit, milk and meat to humans [4]. In this study, 30 cooked red meat/chicken doner samples were analyzed but no *Listeria monocytogenes* bacteria was detected.

Staphylococcus aureus is an important pathogen related the combination of toxin-mediated virulence, and antibiotic resistance. This bacterium causes nosocomial infections and community-acquired diseases. The symptoms of staphylococcal food poisoning are abdominal cramps, nausea, vomiting, sometimes followed by diarrhea (20). Coagulase Positive Staphylococci was detected as 10^4 cfu / g in 3 of 30 cooked samples (1 cooked chicken doner, 2 cooked meat doners), of 60 red meat/chicken doner samples examined. 10^5 cfu / g values were obtained from 1 of the 30 raw doner samples (raw red meat).

In a study conducted in the Antakya, a total of 50 doner samples were collected from 15 different regions. Microbiological evaluation was made by counting Coagulase positive Staphylococcus, *Escherichia coli* O157: H7, *Salmonella* spp, and *Listeria monocytogenes*. As the same of our study, coagulase positive Staphylococcus was detected in 2 samples. However, *E. coli* O157: H7 was observed in 12, *Salmonella* spp. in 7 and *L. monocytogenes* in 2 samples. While in our study *E.coli* O157 and *L.monocytogenes* were not detected in any samples.

Bostan et al. identified *S. aureus* in none of the samples [7]. In a study conducted in Elazığ, *S. aureus* were not detected in any of the samples although other microorganisms were found in certain numbers.

Vildan et al. examined the chemical composition and microbiological quality of 40 doner samples (cooked) obtained randomly from eight different restaurants in Erzurum in certain periods. The number of lactic acid bacteria, and Enterobacteriaceae and *S.aureus* was below the detectable limit in 20% and in 60% of the samples, respectively. The number of Coliform group bacteria in 45% of the samples, *E. coli* in 67.5% and *C. perfringens* in 85% was below the detectable limit. While none of the samples contained *Salmonella*, *Listeria* was detected in 8 of the 32 samples examined.

In conclusion, in this study, where the microbiological quality parameters of raw and cooked red meat doner /chicken doner samples were examined, *L. monocytogenes* and *E. coli* O157:H7 bacteria have not been found. However, detection of *Salmonella* spp. and Coagulase Positive Staphylococci, which are food safety indicators, suggests that these products can be considered as risky foods, especially in terms of public health, and that they should be kept under strict control. Inadequate of hygiene practices in manufacturing and also hygiene inspection is a major threat to human health. For this reason, careful checking of the entire process is required such as personnel hygiene, working environment and equipment. Also the risk of cross-contamination should be considered in low hygiene conditions. For this reason, training programs on general hygiene rules, general hygienic practices need to be integrated into food process in order to eliminate food poisoning.

5 ACKNOWLEDGEMENT

The authors express acknowledgement to Istanbul Aydin University for its support in this work during in 2017-2018.

6 REFERENCES

- [1] Acar MS , 'A comparative study of microbiological quality of döner kebabs made from slaughtered animal meat and poultry' , Istanbul University, Institute of Health Sciences, Department of Food Hygiene and Technology, PhD Thesis, Istanbul,Turkey,1996.
- [2] Acar MS, Çiftçioglu G, 'A Study on Microbiological Quality of Döner Kebabs made from Butcher's Animal Meat and Poultry' , Istanbul University, Journal of Veterinary Faculty, vol.23 ,no.2, 395-404,1997.

- [3] Anonymous 2009b, Center for Food Safety and Applied Nutrition (CFSAN) ,Draft Assessment of the Relative Risk to Public Health from Food borne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods, Jan 2001, <http://www.foodsafety.gov/~dms/lmrisksu.html>,2009.
- [4] Arda M , Minbay A , Leloglu N, Aydın N, Kahraman M, Akay Ö, Ilgaz A, İzgür M, Diker, ‘Special Microbiology, Medisan Publishing’, pp.362 Ankara ,Turkey,1999.
- [5] Ayaz M, Othman, FA, Bahareth, TO, Al-Sogair, MA, Sawaya WN, ‘Microbial Quality Of Shawarma in Saudi Arabia’ , Journal of Food Protection, vol.48 , no.9, pp. 811-814,1985.
- [6] Baysal A, ‘Nutrition, Hatipoğlu Publications’, 9th Edition, pp. 250-260, Ankara, 2002.
- [7] Bostan K, Eker F, Muratoglu K, Aydın A, ' A Study on Microbiological Quality and Microbial Development in Cooked Doner Kebabs ' Kafkas Univ Vet Fak Derg, vol.17 no.5, pp. 781-786 , 2011.
- [8] Cebirbay MA , Aktas N, ‘Traditional Food of Turkish Cuisine: Doner Kebap. International Congress of Asian and North African Studies’, 38. ICANAS, 38. ICANAS Material Culture Proceedings Book, vol I, pp.329-339, Ankara,2008.
- [9] ISO 16654. ‘Horizontal method for the detection of *Escherichia coli* O157’. Microbiology of food and animal feeding stuffs, 2001.
- [10] ISO 6579. ‘Horizontal method for the detection, enumeration and serotyping of *Salmonella* Part 1: Detection of *Salmonella* spp.’. Microbiology of the food chain, 2017.
- [11] ISO 11290-1. ‘ Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. – Part 1: Detection method’ , Microbiology of the food chain ,2017.
- [12] ISO 6888-2. ‘Horizontal method for the enumeration of coagulase-Positive staphylococci Part-2:Technique using rabbit plasma fibrinogen agar medium’ , Microbiyology of food and animal feding stuffs, 2003.
- [13] Jöckel S, Stengel G, ‘Doner Kebab Untersuchung und Beurteilung Einer Türkischen Spezialitat’, Fleischwirtschaft, vol. 64, no.5, pp.527-538,1984.
- [14] Kayısoglu S, Yılmaz İ, Demirci M, Yetim H, ‘Chemical Composition And Microbial Quality of Doner Kebabs Sold İn Tekirdag Market’, Food Control, vol.14, pp. 69-474,2003.
- [15] Kupeli V, ‘Chemical Composition and Microbiological Quality of Leaf Doners’, Atatürk University, Institute of Science, Department of Food Engineering, Graduate Thesis, Erzurum,Turkey,1996.
- [16] Stolle A, Eisgruber H, Kerschöfer D, Krausse G, ‘Doner Kebab Investigations on Traffic Perception and Microbiological Hygenic Properties in the Munich Area’, Fleischwirtschaft, vol.73. no.8, pp. 834-837, 1993.
- [17] Sireli UT, Çil-İplikçiöğlü G, Ozansoy G, ‘A Threat in Food Safety: *Salmonella* spp.’. Turkish Clinics J Food Hyg Technol-Special Topics, vol.1, no.2, pp.17-22, 2015.
- [18] TGK (Turkish Food Codex), Communiqué on Microbiological Criteria, Communiqué no. 2009/6, Official Gazette 06.02.2009 / 27133, Ankara,Turkey, 2009.
- [19] TSE, TS 11859, "Doner Meat is not cooked", Turkish Standards Institute, pp. 1-7,2003.
- [20] Tunail N, ‘ Microbial infections and intoxications. Food Microbiology and Applications.’, Ankara University Faculty of Agriculture GI, Turkey, 2000.
- [21] Todd ECD , Szabo R, Spiring F, ‘Donairs (Gyros)- Potential Hazards and Control’, Journal of Food Protection, vol.49, no.5, pp.369-377, 1986.
- [22] Tükel C, Dogan HB, ‘Staphylococci aureus. Food Microbiology and Applications’,Ankara University Faculty of Agriculture Food Engineering Department publication, pp.357-366, Ankara,Turkey,2000.
- [23] Yaman, R, ‘Story of Doner Kebab’, Research on Turkish Cuisine Culture, Publications of Turkish Folk Culture Research and Promotion Foundation, no. 3, pp. 92-101,1993.
- [24] Yıldırım, Y, ‘Meat Industry’, Uludağ University Veterinary Faculty Publications, 4th Edition, Ankara,Turkey,1996.