

The Microbiological Quality of Grilled Meats (Kebabs) and Salads Consumed in Sanliurfa Restaurants

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Abstract

The aim of this study was to investigate the microbiological quality of grilled meats (kebabs) products and salads consumed in the restaurants in Sanliurfa, Turkey. A total of 150 samples of grilled meats and 25 mixed salad samples were examined for total aerobic bacteria, coliforms, enterococci, coagulase positive staphylococci, *Salmonella* spp., *Listeria* spp., yeasts and molds. Total bacteria were detected in all of the grilled meat samples, except Urfa kebab samples. Except for *Salmonella* and *Listeria* spp., all of the tested microorganisms were found in all of salad samples. According to the results of this study, the microbiological quality of the grilled meats served in Sanliurfa restaurants may be evaluated as tolerable. However, the results of the microbiological analysis of salad samples served with the grilled meats show a potential risk for human health. The high number of total bacteria of shish kebabs and the high numbers of total bacteria, coliforms and coagulase positive staphylococci of salad samples indicated the poor hygienic conditions during food preparation and serving.

Keywords: Grilled meat, Salad, Microbiological quality, Restaurant

1. Introduction

A hygienic manufacturing and consumption of foods is necessary for human nutrition and public health. While the majority of cases of foodborne diseases are of unknown cause, bacteria and viruses are the most likely causative agents (McCabe-Sellers and Beattie 2004). Food contamination with microorganisms during manufacturing, processing, marketing, storage and consumption caused outbreaks of foodborne diseases and economical losses (Todd 1985; Török et al. 1997). Institutions or restaurants, where foods are prepared for consuming by many people are considered to be the primary source of foodborne outbreaks, due to epidemiological selection (outbreaks involving several people are more likely to be traced back to the source than are individual cases), lack of quality assurance in food services, and failure of employees to follow critical behaviors that mitigate the potential for foodborne illness (McCabe-Sellers and Beattie 2004; Török et al. 1997; Meldrum et al. 2009). In England and Wales, several outbreaks of foodborne diseases have been reported associated with kebabs prepared by take-away restaurants (Meldrum et al. 2009).

Grilled meats (kebabs) such as Adana kebab, shish kebab (called kushbashi in Turkish), grilled chicken and doner kebab are commonly consumed in almost every restaurant in Turkey, including Sanliurfa, a historical, touristic and most populous city in southeastern region of Turkey. The kebabs are generally served with salad or raw vegetables such as lettuce, parsley, peppermint. Different special grilled meat products such as liver and heart kebab (spicy grilled liver or heart meat divided into small morsel sized pieces on skewers), Urfa kebab (approx. 5 cm long, spicy grilled meatball on skewers), Adana kebab (approx. 20 cm long, spicy grilled meatball on skewers), shish kebab (spicy grilled meat divided into

morsel-sized pieces on skewers) and chicken shish kebab (spicy grilled chicken meat divided into morsel-sized pieces on skewers) are served and consumed in the restaurants.

Because of common consumption of grilled meat products in the restaurants and no study carried out on the microbiological aspects of these products in southeastern area of Turkey, determining their hygienic quality and to inform authorities for public health is necessary. The purpose of this study was to determine the microbiological quality of grilled meat products and salads consumed commonly by indigenous peoples as well as by domestic and foreign tourists in Sanliurfa restaurants.

2. Materials and Methods

2.1 Sampling

In total 150 grilled meat products and 25 salad samples were randomly collected from different restaurants in Sanliurfa, Turkey, for microbiological examination. The analysed samples consisted of 25 heart kebabs, 25 liver kebabs, 25 Urfa kebabs, 25 Adana kebabs, 25 shish kebabs (kushbashi), 25 chicken shish kebabs and 25 mixed salads samples consisted of peppermint and parsley. At least 200 g of grilled meat was placed in sterile plastic bags and transported to the laboratory under aseptic and refrigerated conditions. The samples were microbiologically analyzed within 2 h.

2.2 Microbiological analysis

In order to determine the microbiological changes of samples, except for *Listeria* and *Salmonella* spp., 10 g of samples were diluted in 90 ml of a sterile 0.1% (w/v) peptone water solution (Merck, 107228) and homogenized in a Colworth Stomacher Lab-Blender 400 (London, UK) for 2 min. The homogenate was decimally diluted in the same solution and each dilution was plated in duplicate on the media required for the different microbial groups to be examined (Messer et al. 1992). The spread plate technique (0.1 ml) was used for total aerobic bacteria, coagulase positive staphylococci, total coliforms, yeasts and molds (Swanson et al. 1992).

The total aerobic bacteria were counted on standard plate count agar (PCA, Oxoid, CM325) after incubation at 30 °C for 72 h. Yeasts and molds were determined on potato dextrose agar (PDA, Oxoid, CM139) acidified with 10% tartaric acid incubated at 21 °C for 5 days. Coagulase positive staphylococci were counted on Baird Parker agar (BPA, Oxoid, CM275) supplemented with egg yolk tellurite emulsion (Oxoid SR54) at 37 °C for 48 h and coagulase test was conducted. Total coliforms were enumerated on violet red bile agar (VRBA, Oxoid, CM107) after incubation at 30 °C for 24 h. Plates with 30-300 colonies were used for counting and the results expressed as logarithm of colony forming units per gram (\log_{10} cfu/g). Enterococci were counted on Slanetz-Bartley agar (SB, Oxoid) at 35 °C for 48 h.

The presence of *Listeria* spp. was investigated by using the following procedure: A 25 g of sample was homogenized with 225 ml Modified University of Vermont broth (UVM, Oxoid CM863) in a stomacher and incubated at 30 °C for 48 h. After incubation 0.1 ml of the pre-enrichment samples were added to 10 ml Fraser Broth (FB, Oxoid CM895) and incubated for 24 h at 35 °C. Following enrichment, a loopful of FB cultures were streaked onto Modified Oxford agar (MOX, Oxoid CM856) and the plates were then incubated at 35 °C for 48 h. The plates were examined for colonies with *Listeria* spp. characteristics. Presumptive five *Listeria* colonies, which were brown coloured by aesculin hydrolysis, were streaked onto Tryptic Soy Agar-Yeast Extract (TSA-YE, Difco 0370) plates for purity and biochemical tests. These colonies were confirmed by Gram staining, catalase test, β -hemolysis, CAMP test, MR-VP reaction, nitrate reduction test and fermentation of dextrose, maltose, rhamnose, xylose and mannitol as suggested by APHA (Donnelly et al. 1992).

The presence of *Salmonella* spp. was investigated by the following procedure: 25 g sample was aseptically transferred to stomacher bag containing 225 ml of buffered peptone water (BPW, Oxoid CM509) and homogenized for 2 min. After incubation for 24 h at 37 °C, 0.1 ml of the pre-enrichment sample were added to 10 ml Rappaport-Vassiliadis soya peptone broth (RVS, Oxoid CM866) and incubated for 24 h at 43 °C. Following enrichment, a loopful of RVS culture was streaked onto brilliant-green phenol-red lactose sucrose agar (BPLS, Merck 7237). The media was incubated 24-48 h at 37 °C. The plates were examined for colonies with *Salmonella* characteristics. Presumptive *Salmonella* colonies, which exhibited pink color surrounded by a reddish pink or red zone, were streaked onto tryptic soy agar plates for purity. These isolates were then tested biochemically by inoculating into urea broth

(Oxoid), O-nitrophenyl- β -D-galacto-pyranoside (ONPG discs, Oxoid DD13), lysine iron agar (Oxoid CM381) and triple sugar iron agar (Oxoid CM277). Isolates showing typical *Salmonella* biochemical reactions were confirmed by slide agglutination test using polyvalent O antiserum (Flowers et al. 1992).

2.3 Statistical analysis

The numerical results are given as means and standard deviations (SD), with n being the number of samples. The statistical analyses were performed using SPSS software package (SPSS for Windows, 9.05 program).

3. Results

Total aerobic mesophilic bacteria were detected in all of grilled meat products except Urfa kebab whereas all of the investigated microorganisms in this study except *Salmonella* and *Listeria* spp. was detected in all salad samples used. The results of microbial properties and their frequency distributions in the grilled meat product and salad samples are presented in Table 1, 2 and 3.

Table 1. The microbiological numbers in the grilled meat and salad samples¹ (\log_{10} cfu/g)

	Liver kebab	Heart kebab	Chicken shish kebab	Urfa kebab	Adana kebab	Shish kebab	Salad
Sample Number	25	25	25	25	25	25	25
Total bacteria	3.38 \pm 0.16	2.24 \pm 0.52	4.59 \pm 0.22	ND ²	3.19 \pm 1.63	5.05 \pm 0.20	5.96 \pm 0.45
Coliform	ND ²	ND ²	ND ²	ND ²	ND ²	ND ²	5.61 \pm 0.32
<i>Enterococcus</i> spp.	ND ²	ND ²	ND ²	ND ²	ND ²	ND ²	3.36 \pm 0.25
CPS ³	ND ²	ND ²	ND ²	ND ²	ND ²	ND ²	2.76 \pm 0.32
Yeast/Mold	ND ²	ND ²	ND ²	ND ²	ND ²	ND ²	5.31 \pm 0.17
<i>Listeria</i> spp.	ND ²	ND ²	ND ²	ND ²	ND ²	ND ²	ND ²
<i>Salmonella</i> spp.	ND ²	ND ²	ND ²	ND ²	ND ²	ND ²	ND ²

¹Mean values \pm standard deviation; ²ND: Not detected; ³Coagulase positive staphylococci.

Table 2. Frequency distributions of the total bacteria in the grilled meat samples (n=25)

Grilled meat products	Percentage of samples in different population groups				
	<2	2-3	3-4	4-5	5-6
Liver kebab (n = 25)	-	-	100	-	-
Heart kebab (n = 25)	16	84	-	-	-
Chicken shish kebab (n = 25)	-	-	-	100	-
Urfa kebab (n = 25)	100	-	-	-	-
Adana kebab (n = 25)	20	-	40	40	-
Shish kebab (n = 25)	-	-	-	28	72

Table 3. Frequency distributions of the microorganisms in salad samples (n = 25)

Microorganisms	Percentage of samples in different population groups					
	<2	2-3	3-4	4-5	5-6	6-7
Total bacteria	-	-	-	-	40	60
Coliform	-	-	-	4	96	-
<i>Enterococcus</i> spp.	-	12	88	-	-	-
CPS ¹	-	92	8	-	-	-
Yeasts/molds	-	-	-	-	100	-
<i>Listeria</i> spp.	-	-	-	-	-	-
<i>Salmonella</i> spp.	-	-	-	-	-	-

¹Coagulase positive staphylococci.

4. Discussion and Conclusion

Grilled meat products such as shish kebab, Adana kebab, chicken shish kebab and doner kebab were widely consumed in almost every restaurant in Turkey. These are served with a salad or raw vegetables depending on consumer preference. In this study, the microbiological quality of different grilled meat products and salad samples were analysed, because of common consumption of these products in Sanliurfa restaurants and no study carried out on this subject in this area of Turkey.

The results of microbiological analysis of grilled meats obtained from this study (Table 1) are lower than the results of similar foods produced from grilled meat in different countries for total bacteria by Abdullahi et al. (2006) and Nemati et al. (2008), but in agreement with the results by Küpeli-Gençer and Kaya (2004), Gülmez et al. (2005), Patricia and Azanza (2005) and Ismail (2006). Coliforms, *Enterococcus* spp., coagulase positive staphylococci, yeasts and molds and *Listeria* spp. were not detected in any grilled meat samples in this study in contrast to the studies of Abdullahi et al. (2006) and Nemati et al. (2008) for coliforms and yeast and mold; Küpeli-Gençer and Kaya (2004) and Ismail (2006) for coliforms, coagulase positive staphylococci and *Enterococcus* spp. and Küpeli-Gençer and Kaya (2004) and Gülmez et al. (2005) for coagulase positive staphylococci and *Listeria* spp. In present study, *Salmonella* spp. were not detected in all grilled meat samples in agreement with the studies of Küpeli-Gençer and Kaya (2004), Gülmez et al. (2005), Ismail (2006) and Nemati et al. (2008). Küpeli Gençer and Kaya (2004) have reported that the total bacteria is an important indicator for determining microbiological quality in cooked meat products and should be lower than 10^5 cfu/g. Because it has been reported that none of the ready-to-eat food samples contaminated with $10^5 <$ cfu/g total bacteria implicated in any foodborne disease outbreaks, a total bacteria number of $<10^5$ cfu/g was recommended as guideline value for the ready-to-eat food (Patricia and Azanza 2005). In another study, samples of cooked *hawawshy* sandwiches contaminated with total bacteria numbers less than 10^5 cfu/g were evaluated as tolerable, numbers of $<10^6$ cfu/g as unsatisfactory, while counts ranging from $10^6 - 10^9$ cfu/g as unacceptable (Ismail 2006). According to the these results, the number of total bacteria in shish kebab samples in present study is higher than 10^5 cfu/g and can be evaluated as unsatisfactory, while other grilled meat products contaminated with the microorganism numbers less than 10^5 cfu/g are tolerable (Table 1, 2). The limits of yeasts/molds and coagulase positive staphylococci in heat treated meat products were determined as 10^2 cfu/g, as well as for *Escherichia coli* O157:H7, but there must be no *Salmonella* spp. and *L. monocytogenes* in 25 grams of this products by the Turkish Food Codex (TFC, 2010). However the limits for other microorganisms investigated in this study were not specified by TFC. The grilled meat samples examined in the present study can be evaluated as satisfactory with respect to microbiological quality according to the TFC. Among the important factors contributing to the presence of microorganisms in prepared foods including cooked or heat treated foods were reported to be improper process conditions such as inadequate cooking, postprocess recontamination by handlers, improper storage, cross-contamination, unsafe ingredients used for the preparation, contaminated equipment and poor personal hygiene, in their is poor personal hygiene prominent factor (McCabe-Sellers and Beattie 2004; Abdullahi et al. 2006; Küpeli-Gençer and Kaya 2004; Reij and Den Aantrekker 2004). The absence of the tested microorganisms except total bacteria in grilled meat products might be explained by the decrease of bacteria due to heat used during the grilling process. The presence of total bacteria in the samples used might indicate a contamination after grilling process due to insufficient sanitary conditions of the equipment used by the restaurants and improper personal hygiene during the food serving.

Grilled meat products are generally served with salad or raw vegetables such as lettuce, parsley, peppermint etc. in restaurants. In the salad samples, all tested microorganisms except *Listeria* and *Salmonella* spp. were isolated in this study (Table 1, 3). Gülmez et al. (2005) have detected coliform and *S. aureus* in fewer samples of raw vegetable salads, however *Salmonella* spp. and *L. monocytogenes*. Ponniah et al. (2010) found *Listeria* spp. and *L. monocytogenes* in 102 (33.3%) and 68 (22.5%) of the 306 raw salad vegetables sold at retail level in Malaysia, respectively. In a study (De Sousa et al. 2002), total 30 salads samples obtained from different shops were examined for microbiological quality and were found 5.38 log cfu/g total bacteria, 3.91 log cfu/g yeast and molds and 3.80 log cfu/g coliforms, which are lower than those found in the present study. In the salad samples, *Enterococcus* spp. also were isolated in this study (Table 1, 3). The food chain has been established as an important source of *Enterococcus* spp., which has implications for the contamination of foodstuff, although, it is not a foodborne pathogen per se (Franz et al. 1999; Fisher et al. 2009). *Enterococcus* spp. can be used as indicators of faecal contamination, but also they have been implicated in outbreaks of foodborne illness, and they have been ascribed a beneficial or detrimental role in foods (Franz et al. 1999). Meldrum et al.

(2009) have reported that 4.7% of 1213 salad vegetable samples obtained from kebab take-away restaurants in UK were evaluated as unsatisfactory microbiological quality due to presence of *Escherichia coli* and/or *S. aureus* at levels of $\geq 10^2$ cfu/g, and 0.3% of salad samples as unacceptable quality due to presence of *S. aureus* or *Salmonella* spp. The authors emphasized that the presence of *S. aureus* in ready-to-eat salad vegetables is an indication of poor hygiene practices in kebab take-away restaurants. The results obtained in the present study for coagulase positive staphylococci numbers in salad samples (Table 1) are in agreement with these results mentioned above. Salad samples examined by this study should be considered as unacceptable microbiological quality due to contamination with coagulase positive staphylococci in higher numbers than the limit value of 10^2 cfu/g specified by TFC (2010) (Table 1, 3) and high coliform levels.

In conclusion, this study indicated that the microbiological quality of the grilled meat products except shish kebab may be evaluated as tolerable, but shish kebab as unsatisfactory. However, salad samples served with the grilled meat products in Sanliurfa restaurants were evaluated as unacceptable and should be considered a risk for human health due to high contaminations with the coliforms and coagulase positive staphylococci. The high number of total bacteria of shish kebab samples and the high numbers of total bacteria, coliforms and coagulase positive staphylococci of salad samples obtained from this study indicated the poor hygienic conditions during food preparation and serving. For improving the microbiological quality of foods, the preparation and serving should be carried out in good sanitary conditions and under proper hygienic handling and practices in order to prevent post-process contaminations in restaurants. The results of this study demonstrated that the education of food handlers and strict official controls for restaurants about food, kitchen and personal hygiene are necessary to ensure food safety. In addition, further studies on microbiological analysis of equipment, bench and hands of employees in the restaurants are required.

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