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Microbiological condition of chicken doner kebab sold in Vienna, Austria

Mikrobiologische Beschaffenheit von Döner Kebab aus Hühnerfleisch in Wien

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Summary

Chicken doner kebabs (n = 71), taken from 71 retail shops along the major public transportation routes in the city of Vienna (subway), were analysed for selected pathogens and hygiene indicator bacteria. *Salmonella* sp. was detected in one, and *Listeria monocytogenes* in three kebabs (25 g sample aliquots). Other *Listeria* species were detected in seven samples. *Campylobacter* sp. was not found. The numbers of *Staphylococcus aureus* in the meat component were <4 log cfu/g, which is below the minimum infectious dose commonly associated with foodborne illness. Total aerobic count (TAC) in the meat component ranged from 2.6 to 7.6 log cfu/g, with a mean of 4.4 ± 1.2 log cfu/g, which was ca. 1.26 log units lower than that of the vegetable component (P<0.05). *E. coli* was recovered from 23 and 18 of the meat and vegetable components (25 g aliquots), respectively. With exception of TAC, no significant differences were found between meat and vegetable component. This might be attributed to cross-contamination when heat treated meat and raw vegetables are assembled to form a ready-to-eat product. Thus, the analysis of the meat component taken directly from the cone or heating plate (as done in several other studies) will most likely underestimate the actual hygiene condition and exposure of the consumer to foodborne pathogens by a complete retail-ready doner kebab.

Keywords: Chicken meat, vegetables, *Salmonella, Listeria, E. coli*

Zusammenfassung

Döner Kebab aus Hühnerfleisch (n = 71) wurde von 71 Geschäften, die in oder nahe bei Stationen der Wiener U-Bahn Linien gelegen waren, bezogen. Die Proben wurden hinsichtlich ausgewählter pathogener und Hygieneindikator-Bakterien untersucht. *Salmonella* sp. wurde in einem, *Listeria monocytogenes* in drei und andere Listerienspezies in sieben Proben nachgewiesen (je 25 g). *Campylobacter* sp. wurde in keiner Probe gefunden. Die Konzentration von *Staphylococcus aureus* im Fleischanteil war <4 $\log_{10}$ kbE/g, und damit unter der für Lebensmittelintoxikationen angenommenen Infektionsdosis. Die aerobe mesophile Keimzahl lag im Fleischanteil zwischen 2,6 bis 7,6 $\log_{10}$ kbE/g, mit einem Mittelwert von 4,4 ± 1,2 (Standardabweichung) $\log_{10}$ kbE/g, was um etwa 1,26 $\log_{10}$ niedriger war als im Gemüseanteil (P<0.05). *E. coli* wurden im Fleisch- und Gemüseanteil bei 23 bzw. 18 Proben nachgewiesen (in je 25 g). Mit Ausnahme der aeroben mesophilen Keimzahl bestanden keine statistisch signifikanten Unterschiede in der Mikroflora zwischen Fleisch- und Gemüseanteil. Dies könnte durch die bei der Zusammenstellung des Kebab erfolgte Kreuzkontamination (hitzebehandeltes Fleisch und rohes Gemüse) bedingt sein. Die Untersuchung des frisch vom Spieß geschnittenen, oder von einer Heißhalteplatte genommenen Fleischanteils alleine (wie in anderen Studien erfolgt), würde somit den tatsächlichen Hygienestatus und die Exposition des Konsumenten hinsichtlich pathogener Bakterien eher unterschätzen.

Schlüsselwörter: Hühnerfleisch, Gemüseanteil, *Salmonella, Listeria, E. coli*

Introduction

Take away foods have been identified as an important source of human foodborne infections (NSWFA, 2011). Among take away foods, doner kebab has become increasingly popular also outside of Turkey (ACM/702, 2004; Currie et al., 2007; Kilic, 2009). Different meat animal species are used for production of this food item, e. g. mutton, beef, chicken, and turkey meat. Only recently doner kebab (doner kebap), gyros has been included in the Austrian Food Codex, chapter B14. Three categories of Döner Kebab (Döner Kebap) Gyros are described: 1. Döner Kebab; 2. Döner Kebab from chicken meat; 3. Minced meat on spit in Döner-Kebab-style („Faschiertesdrehspieß nach Döner-Kebab-Art"). Dependent on the category, also ingredients other than meat and minced meat can be part of the doner Kebab, e. g. salt, spices, onion, edible oil, milk, milk protein, yoghurt, eggs, egg powder, phosphate, soy protein. Thus, due to the mode of production, it is a sensible product. The meat component is cut from a cone, composed of several layers, which is grilled under rotation. Considering the increasingly popular use of chicken meat (Kilic, 2009; Cebirbay and Nizamlioglu, 2010) which might be contaminated with enteric pathogens, such as *Salmonella*, *L. monocytogenes*, *C. perfringens* and *Campylobacter* (NZFSA, 2011), doner kebab might bear the risk of foodborne infection. The duration of grilling is not standardized, i. e. when there is a high consumer demand for this food, it is possible, that the meat cut from the cone has not been properly heated (Cebirbay, 2007). Finally, a serving of doner kebab also includes raw vegetables, which could have been contaminated with pathogens either during primary production and harvest (Chai et al., 2007; Verhoeff-Bakkenes et al., 2011; EFSA, 2011) or has been cross contaminated during food preparation (Evans et al., 1999; Baumgartner et al., 2011).

Thus, not surprisingly, food poisoning outbreaks related with consumption of doner kebab are regularly reported, caused by e. g. *Salmonella mikawasima* (Synnot et al., 1993); VTEC O157 PT2, *Campylobacter*, *S. typhimurium* and *S. enteritidis* (ACM/702, 2004); *E. coli* (Currie et al., 2007); *Staphylococcus aureus* (Baumgartner et al., 2011). It has also been suggested that doner kebab is a risk product as regards to *Clostridium perfringens* (Nichols, 1995; Elmali et al., 2005). But there are no reports on food poisoning due to the presence of *Clostridium perfringens* toxins in doner kebab.

The literature also suggested that doner kebab, especially when made from chicken meat and obtained from take away shops, has a higher microbial contamination than that sold in restaurants (Nichols, 1995).

The aim of the present study was to assess the microbiological condition of doner kebab produced and sold under conditions of high consumer demand – i. e. at places with a high frequency of people and around lunch time. Such conditions would allow a "worst case" estimate of the microbiological safety of such composed foods, as there would be a risk for both underheated meat and deficiencies in hygiene of the personnel during assembly of the kebab. As most studies consider only the meat component, we decided to analyse a "ready to eat" doner kebab including both meat and vegetable component separately, in order to assess if such an approach would result in additional information on food quality and safety characteristics.

The study focused on vegetative bacteria. Spore-forming organisms may find a suitable ecological niche in doner kebab, as indicated by Kayisoglu et al. (2003), Elmali et al. (2005) and in the ACM report (ACM/702, 2004), but we decided to deal with this type of bacteria in a following study.

Materials and Methods

Sampling plan

Sampling sites were defined as doner kebab shops located at or near to (i. e. in a walking distance around 200 m) major nodal points of public transportation in Vienna. All 90 stations of the subway lines were considered as such nodal points. However, for only 42 stations, one or more shops offering doner kebab were either located directly in the station (3 stations) or nearby (35 stations) or both (4 stations), resulting in a total of 71 shops to be included in our study. Based on previous observations, "times of high demand" were around 11 h a. m. to 13 h, on Tuesday through Thursday. Consequently, samples were taken in these periods, in February 2012. From each shop, one retail portion was taken. The portion comprised bread filled with grilled chicken doner and salad (i. e. onion, lettuce, tomato and/or cucumber) but without spice and yoghurt dressing. During sample collection we observed if the doner cone was set as a fresh raw spit in the morning or an already cut spit from yesterday. The wrapped samples were placed in a refrigerated box and arrived within 30 min in the laboratory, where they were analyzed immediately.

Sample examination

Upon arrival, samples were divided in bread, doner kebab (meat and ingredients as stated in the Austrian Food Codex) and vegetable component under sterile conditions. Meat and vegetable component were weighed and then subjected to microbiological analysis. In brief, serial tenfold dilutions were prepared in Maximum Recovery Diluent (Oxoid CM0733) and aliquots spread onto selective agars to assess Total Aerobic Count (TAC), and numbers of *E. coli*, *Enterococcus*, *Staphylococcus* (Tab. 1). In addition, sample portions of 25 g meat were tested for the presence of *E. coli*, *Salmonella*, *Listeria* and *Campylobacter* spp. Likewise, 25 g aliquots of vegetables were tested for *Salmonella* and *Listeria* sp. (Tab. 1).

Statistical analysis

TAC was transformed into log units and statistics were carried out with MS Excel. Possible relationship of the microflora found on the meat and vegetable component was analyzed using the t-test and Chi-square test. Statistical significance was established at $P < 0.05$.

Results and Discussion

Physical characteristics

The weight of the meat component was in the range of 73.2 to 192.3 g, with a mean value of 137.2 ± 28.9 g. This is in the range as reported for doner kebab from retail shops in Austria (50 to 190 g; Bauer, 2006), but slightly less than the standard portion (150 g) generally accepted in Turkey (Anonymous, 2011). The vegetable component had an average weight of 61.2 ± 21.2 g (range 26.3 to 141.5 g).

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TABLE 1: *Media used for microbiological analysis of chicken doner kebab.*

Parameter	Method	Component tested*)
TAC	Colony counting on plate count agar (ISO 4833:2003, 2003).	M, V
E. coli	Enrichment in Buffered Peptone Water (OXOID CM0509), incubation 37 °C, 24 h; streaking onto Coli ID agar (Biomerieux 42017) incubation 42°C, 24h, aerobic condition.	M, V
Staphylococcus sp.	Baird-Parker Agar (Merck Nr.1.05406) mixed with egg yolk emulsion (Merck Nr. 1.03784) to differentiate *Staph. aureus* and *Staph. epidermidis*, incubation 37 °C, 48 h, aerobic condition.	M
Enterococcus sp.	Chromocult Enterococci Agar (Merck Nr.1.00950), incubation 42 °C, 48 h, aerobic condition.	M, V
Salmonella sp.	Enrichment in Buffered Peptone Water (OXOID CM0509), incubation 37 °C, 24 h; inoculation onto the MSRV motility agar (OXOID CM0910) incubation 42 °C, 24 h; streaking suspected sample onto XLD-agar (Merck Nr.1.05287), incubation 37 °C, 24 h; testing the colonies with typical morphology by agglutination (polyvalent I serum, Dade Behring).	M, V
Listeria sp.	Enrichment in $^1/_2$ Fraser Broth [Fraser Broth Base (OXOID CM0895), Fraser Listeria selective supplement (Merck Nr.1.00093), 1.0 g/l Ammonium-ferric(III)-citrate (MERCK 3762)] incubation 30 °C, 48 h aerobic condition. Enrichment culture is streaked onto ALOA Agar (OXOID CM1084, supplements SR0228E and SR0227E); incubation at 37 °C, 48 h aerobic condition.	M, V
Campylobacter sp.	Enrichment in Bolton Broth (OXOID CM0983, supplement: OXOID SR0208E), incubation 42 °C, 48 h microaerobic condition. Enriched culture is streaked onto m-CCDA plate (OXOID CM0739, supplement OXOID SR0155E), incubation 42 °C, 48 h microaerobic condition.	M

*): * M .. meat; V .. vegetable

Microflora of the meat component

TAC ranged from 2.6 to 7.6 log cfu/g, with a mean of 4.4 ± 1.2 log cfu/g. This mean is close to the value reported for chicken doner in Turkey (Kayisoglu et al., 2003; Vazgecer et al., 2004; Yuksek et al., 2009; Bostan et al., 2011). Similar results have been reported in studies from various other countries (note: with unidentified meat animal species), e.g. maximum count >7 log cfu/g and with a mean of 6 log (Nichols et al., 1995; Williamson et al., 2001). However, in one study in 20 out of 71 samples, TAC exceeded 5 log limits as suggested by PHLS (Gilbert et al., 2000). Admittedly, in our study, the meat component had come into the contact with raw vegetable on the ready to serve portion, which may have contributed to the magnitude of total aerobic bacteria.

Other hygiene indicator bacteria were above the limit of detection in less than 50 % of the 71 samples, and the median was <1 log cfu/g (see Table 2). *E. coli* in 25 g aliquots was recovered only from 23 of the 71 samples, and only one out of 71 samples (1.4 %) exceeded the critical limit of 2 log cfu/g (NSWFA, 2009; Klein and Schütze, 2011), with a value of 3.5 log cfu/g. Our results are comparable to those reported for the UK (1.03 %; Williamson et al., 2001), but clearly lower than those reported in studies from Turkey, with 8 % (Vazgecer et al., 2004) and 21 % (Bostan et al., 2011) of doner kebab samples exceeding the 2 log cfu/g limit for *E. coli*. *Enterococcus* was found in the range of <1 log to 6 log cfu/g. In the studies conducted in Turkey there are differences in count of *Enterococcus*, i. e. <2 log cfu/g in chicken meat (Yuksek et al., 2009), <1 log to 3.3 log cfu/g mutton/beef (Cebirbay and Nizamlioglu, 2010). *Staphylococcus* sp. were detected (i.e. ≥1 log cfu/g) in 19 of the 71 samples, with a maximum of 3.6 log cfu/g. This is in the range as reported for chicken doner in Turkey by Vazgecer et al. (2004) and Yuksek et al. (2009).

With respect to bacterial pathogens, *Campylobacter* was detected in none, and *Salmonella* sp. (*Salmonella enterica* group C), and *Listeria monocytogenes* were detected in one and two samples, respectively. Other *Listeria* species were found in six samples (see Tab. 3). According to the renewed guideline of NSWFA (2009) for ready-to-eat foods, presence of *Salmonella* in 25 g of food and ≥2 log cfu/g *Listeria monocytogenes* is identified as potentially hazardous. *Staphylococcus aureus* levels were below the limit of detection in 62 samples (87.3 %), and only three samples exceeded the 2 log cfu/g limit proposed by Gilbert et al. (2000). The range of results (<1 to 4 log cfu/g) corresponded to those reported in other studies (Elmali et al., 2005; Williamson et al., 2001), and it seems that *Staphylococcus aureus* levels >4 log cfu/g are an exceptional finding (Nichols et al., 1995; Jansson et al., 2008). However, *Staphylococcus aureus* concentrations >8 log cfu/g in doner

TABLE 2: *Microbiology results of doner kebabs in log cfu/g.*

Microorganism	Meat (M) or Vegetable (V)	n	Number of samples per log cfu/g – category							
			<1	1–2	2–3	3–4	4–5	5–6	6–7	7–8
Total aerobic count	M	71	0	0	8	20	23	12	4	4
	V	71	0	0	1	3	19	16	24	8
E. coli	M	71	67	3	0	1				
	V	71	70	0	1					
Enterococcus sp.	M	71	36	20	6	7	1	1		
	V	65	38	18	4	5				
Staphylococcus aureus	M	71	62	6	1	2				
S. epidermidis		71	63	5	3					
Other *Staphylococcus* sp.		71	52	13	4	2				
Staphylococcus sp. total	M	71	45	14	8	4				

kebab have been identified as causing foodborne disease by staphylococcal enterotoxins (Baumgartner et al., 2011).

During sample collection we observed that many doner spits were already cut beyond half of the cone which means that it was a leftover from a previous day. This keeping and reheating can easily promote microbial growth inside of a doner cone during shop closure time (ACM/702, 2004) and also even during cooking, as the inner temperature of a cone has an optimal temperature for growth of many bacteria (Cebirbay and Nizamlioglu, 2010).

Microflora of the vegetable component

TAC ranged from 2.6 to 7.7 log cfu/g, with a mean of 5.7 ± 1.2 log cfu/g. Other hygiene indicator bacteria were above the limit of detection in less than 50 % of the 71 samples, see Table 2. *E. coli* was recovered from 25 g aliquots only from 18 of the 71 samples and only in one sample, *E. coli* concentration was above the quantification limit of 1 log cfu/g, with 2.85 log cfu/g. Although *Enterococcus* was found in a lower range than meat (<1 to 4 log cfu/g), median values are the same.

Salmonella sp. were not detected in any sample, whereas *Listeria monocytogenes* and other *Listeria* species were found in one sample each.

Relation of the microflora of the meat to that of the vegetable component

TACs of the meat component were, on an average, 1.26 log units lower than that in the vegetable component ($P<0.05$; t-test), but the correlation between the magnitude of microbial numbers on meat and on vegetables was low ($r = 0.54$).

Regarding the frequency of detection of pathogenic bacteria and *E. coli* in 25 sample aliquots, no statistically significant differences were found ($P>0.1$, chi-square test). The same was the case when *E. coli* and Enterococci in meat and vegetable were compared on a "below/above limit of detection" basis.

Only in one kebab sandwich sample non-*monocytogenes Listeria* sp. was detected both in the vegetable and in the meat component. Im sum, the benefit of examinating of vegetables in addition to the meat component could be considered marginal. By the same token, the absence of significant differences might also indicate that cross-contamination contributes substantially to the overall microbiological condition of the ready-to-eat portion.

Significance of results for food hygiene and safety

Among 71 doner kebabs obtained in retail shops along the major public transportation routes in Vienna city, *Salmonella* sp. was detected in one, and *Listeria monocytogenes* in three kebabs (25 g sample aliquots). Other *Listeria* species were detected in seven samples.

The numbers of *Staphylococcus aureus* in the meat component were <4 log cfu/g, which is below the minimum infectious dose range commonly associated with foodborne illness.

Most of the studies on the microbiological condition of doner kebab are conducted by collecting and analysing only the meat component (usually mutton/beef and less chicken meat) under sterile conditions, and, therefore, do not take into account cross- or recontamination events occurring when the retail-ready portion is assembled. Our data indicate that a separate analysis of meat and vegetable, which had already been in contact, will not bring much additional information. This is, of course, not unexpected. But it is important that the meat component is taken from the retail-ready portion (i. e. that it has already been in contact with the vegetables) and not directly sampled from the grilled meat cone.

TABLE 3: *Microbiology results of doner kebab, presence/absence testing in 25 g doner kebab (meat) and vegetable component.*

Microorganism	Meat (M) or Vegetable (V)	n	Positive samples in 25 g
E. coli	M	71	23
	V	71	18
Salmonella sp.	M	71	1
	V	71	0
Listeria monocytogenes	M	71	2
	V	42	1
Other *Listeria* spp.	M	71	6
	V	42	1
Campylobacter sp.	M	71	0

In terms of microbiological safety of doner kebab, the heating regimen of the meat cone has been identified as a critical point (Cebirbay, 2007), and improvement of preparation techniques, such as monitoring of internal temperature or an additionally heat treatment before serving has been suggested (FPT, 2007). However, heat treatment does not suffice to kill all of the microorganisms in meat at the serving stage. The latter is confirmed in another study in Turkey, where *Listeria monocytogenes* was recovered from already cooked chicken doner kebab (Topcu, 2006). Likewise, Kayisoglu et al. (2003) compared raw and cooked chicken doner meat and reported that, with one exception, all of the cooked samples, were positive for *Salmonella* spp. In cooked beef doner kebab in Turkey *Salmonella* spp. was reported with a frequency 14 % (Elmali et al., 2005). This, again, shows the need to assess food safety on the consumer-ready level rather than to solely concentrate on studying single food components.

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