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Microbiological quality assessment of meat and dairy products from small-scale factories in european Side of Turkey

Untersuchungen zur mikrobiologischen Qualität von Fleisch- und Milchproduktion aus Kleinbetrieben der Türkei

Bayram Çetin[1]), André Becker[2]), Amir Abdulmawjood[2])

Summary

The present study was conducted to investigate the microbiological quality and safety of 67 meat and 112 dairy product samples manufactured by small-scale factories at the European side of Turkey. The results revealed that the bacteriological quality of the meat and dairy products tested was poor as *Enterobacteriaceae, Escherichia coli, Staphylococcus aureus* and mold counts were found in 76.1 %, 47.8 %, 25.4 % and 50.7 % meat product samples, respectively, and in 67.0 %, 50.0 %, 2.8 % and 54.5 % dairy product samples respectively. Concerning food safety, *E. coli* O157 and *Listeria monocytogenes* were detected by real time PCR assay in 10.5 % and 10.5 % meat product samples, and 5.4 % and 6.3 % dairy product samples, respectively. These results indicated a generally poor microbiological quality of a broad variety of products and the existence of foodborne pathogens in these products highlighted serious health issues. Therefore, in order to increase microbiological safety and quality of products, manufactured in small-scale factories of Turkey, we recommend improving and questioning existing HACCP concepts and conducting a monitoring system as a control of success.

Keywords: Quality, small-scale factories, dairy products, meat products, foodborne pathogens

Zusammenfassung

Die vorliegende Studie beschäftigte sich mit Untersuchungen zur mikrobiologischen Qualität und Sicherheit von 67 Fleisch- und 112 Milchprodukten aus Kleinbetrieben der Türkei. Die bakteriologische Qualität der getesteten Produkte stellte sich als mäßig dar, da *Enterobacteriaceae, Escherichia coli, Staphylococcus aureus* und Hefen aus 76.1 %, 47.8 %, 25.4 % and 50.7 % aller Fleischprodukte bzw. aus 67.0 %, 50.0 %, 2.8 % und 54.5 % aller Milchprodukte isoliert wurden. Als lebensmittelsicherheitsrelevante Keime wurden *E. coli* O157 sowie *Listeria monocytogenes* in jeweils 10.5 % der Fleischprodukte und in 5.4 % bzw. 6.3 % der Milchprodukte gefunden. Die Ergebnisse zeigten eine allgemein dürftige mikrobiologische Qualität einer großen Vielfalt an Produkten und das Vorkommen von lebensmittelassoziierten Krankheitserregern hebt mit Gesundheitsfragen einhergehende Probleme hervor. Um die mikrobiologische Sicherheit und Qualität von Produkten aus Kleinbetrieben der Türkei zu erhöhen, empfehlen wir bestehende HACCP Konzepte zu überdenken und zu verbessern sowie ein Monitoring System zur Erfolgskontrolle zu etablieren.

Schlüsselwörter: Qualität, Kleinbetriebe, Milchprodukte, Fleischprodukte, lebensmittelbedingte Krankheitserreger

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Introduction

National regulations of food hygiene in Turkey which have been adapted to European Union food regulations previous years before are also applied for small-scale food processing plants in many regions including the European side of Turkey called Trakya. The production and consumption of traditionally produced dairy and meat food products have a long tradition in Turkey and the diversity of such products is widely known. The majority of them still offer such traditional meat and dairy products to consumers directly or through weekly markets, farm markets or via retail shops (Arici et al. 1999; Gülmez and Güven 2001; Güner and Telli 2011; Dinkçi et al. 2012). Due to unhygienic conditions there is a possibility of microbial contamination, particularly of pathogen microorganisms which may have serious impacts on the health of consumers (Schuchat et al. 1991; Madden et al. 2001; Lunden et al. 2004; Elizaquivel et al. 2011). Nevertheless, the majority of the small-scale dairy factories still produce under comparatively poor hygienic practices, coupled with ineffective production management as well as unhygienic vending conditions (open packages, not sufficient cooling etc.) thereby making it difficult to preserve the quality of such products.

One of the relevant problems in this area is still the lack of adequate data and information on public health concern of foodborne or other zoonotic bacteria (Beuchat and Ryu 1997; Bohaychuk et al. 2006). For instance, there is currently limited information regarding the occurrence and frequency of hygienic and pathogenic microorganisms in various traditional and commercial meat and dairy products from small-scale producers, predominantly, those sold via such traditional distribution paths (Bostan et al. 2011; Cetin 2017). It has been put forth as a result of determining the microbiological quality of food products in Turkey that there are some complex issues regarding the food safety and hygiene of these products (Elmalili et al. 2005; Yucel and Ulusoy 2006; Çetin et al. 2015). Although the majority of these studies are related with industrial production and few regions of Turkey (Kayisoglu et al. 2003; Öksüz et al. 2004), there is actually limited data on the microbiological quality of dairy and meat products from small scale plants. Therefore, this study was carried out in order to assess the microbiological quality among the traditional food products from small scale dairy and meat factories at the European Side of Turkey and to strategize possible hygiene practices that can be implemented to improve and upgrade the quality and hygiene of meat and dairy production process in the region. The presence of foodborne pathogens such as *Salmonella, Listeria (L.) monocytogenes* and *Escherichia (E.). coli* O157 was investigated, as suggested by the legislation on national food security, but also the numbers of bacteria belonging to the families *Enterobacteriaceae E. coli, Staphylococcus (S.) aureus* and mold were considered as indicators for quality and hygiene of the production process.

Materials and Methods

In this study, a total of 67 meat products samples including Turkish sausage (n=19), minced meatball (n=22), minced meat (n=26) and 112 dairy food samples including soft cheese (n=82), hart cheese (n=15), butter (n=7), and raw milk (n=8) were analyzed. The samples were collected randomly from small-scale producers on the basis of direct farming in the European side of Turkey (the Trakya region) during December 2015 – April 2016. The samples were immediately transported to the laboratory in a refrigerated container at 4 °C until sample preparation and analysis.

Microbiological analysis carried out during this study included the determination of *Enterobacteriaceae, E. coli, S. aureus,* molds and aerobic mesophilic bacteria counts (only for minced meat samples) using conventional cultural methods as expressed in the Turkish food codex regulation on microbiological criteria (Anonymous 2011). A 10 g sample was transferred to 90 mL 0.1 % peptone water (Oxoid, Basingstoke, Hampshire, England) and was homogenized using a Stomacher Lab-Blender 400 (Seward Medical, London, UK). Appropriate 10-fold dilutions of the samples were prepared in peptone water. The enumeration of *Enterobacteriaceae* was performed by Violet Red Bile Dextrose Agar (VRBDA, Oxoid, Basingstoke, Hampshire, England) by using pour plating method and plates were incubated at 37 °C for 24 h according to the standard procedure (ISO 21528-2:2004). *E. coli* was quantified on Tryptone Bile X-Glucuronide (TBX) Agar (Oxoid), followed firstly by 4 h and then 24 h of incubation at 30 °C and 44 °C, respectively, according to the standard procedure (ISO 16649-2:2001). Total mold-yeast count was determined in Rose Bengal Chloramphenicol Agar (Oxoid) by using surface plating method and plates were incubated at 25 °C for 5–7 days (ISO 21527-2:2008). *S. aureus* was determined by surface plating on Baird Parker agar (Oxoid) and incubating plates at 37 °C for 30–48 h (ISO 6888-1:2003). Coagulase test was also applied for verification of *S. aureus* colonies. In addition to the coagulase tube test; gram staining and a panel of phenotypic traits were used to confirm the identity of *S. aureus,* including, clumping factor and/or protein A (latex agglutination, Staphaurex-Plus, Murex Diagnostika, Burgwedel, Germany). Furthermore, all *S. aureus* isolates were analyzed for the presence of SE genes for *sea* to *seo* (Aydin et al. 2011). Total count of aerobic mesophilic bacteria in minced meat samples was determined via pour plate method on plate count agar (Oxoid) and by incubating the plates at a temperature of 30 °C for 24–48 h. All plates were incubated under aerobic conditions (ISO 4833-2:2013). The analyses were carried out as duplicate and the results were expressed as CFU/g.

Detection of foodborne pathogens using real-time PCR

The presence of food borne pathogens such *Salmonella* spp., *L. monocytogenes* and *E. coli* O157 was detected after pre-enrichment using real time PCR assay by the commercial available foodproof® *Salmonella, L. monocytogenes,* and *E. coli* O157 Detection Kit (each Biotecon Diagnostic). For pre-enrichment, each 25 g sample was blended in a stomacher (Seward Stomacher 400 Lab System, Norfolk, UK) with 225 mL of buffered peptone water for Salmonella, with 225 ml of half Fraser Broth (Oxoid, Hampshire, UK) for *L. monoctyogenes,* and for pre-enrichment of *E. coli* O157 with 225 ml of modified tryptone soya broth (Oxoid) supplemented with novobiocin supplement and incubated at 30 °C, 37 °C and 37 °C for 24 h, respectively. A 1.5 mL aliquot of enrichment samples was used for DNA extraction performed according to kit procedure. Subsequently, DNA was measured using a nano-drop spectrophotometer (Thermo Scientific NanoDrop 2000C, USA). Extracted samples were stored at –20 °C until Real Time

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PCR application. Amplifications were carried out via Light Cycler Nano System (Roche Diagnostics, Germany). 5–150 ng/µL DNA concentration was performed in Real Time PCR application.

Result and Discussion

The microbiological qualities of meat and dairy product samples were determined by enumeration of the following microorganisms: *Enterobacteriaceae, E. coli, S. aureus,* molds and aerobic mesophilic bacteria counts (only for minced meat samples) and by analysis of food pathogens *Salmonella, L. monocytogenes,* and *E. coli* O157 by real-time PCR. The microbial analyses of samples have been summarized in Table 1.

In this study, *Enterobacteriaceae* and *E. coli* count were detected in 126 (70.4 %) and 88 (49.2 %) samples, respectively, and majority of the samples showed a count of Enterobacteriaceae (53.6 %) and *E. coli* (27.9 %) more than 10^3 CFU/g (Tab. 1). *Enterobacteriaceae* count has been reported to vary between 10^1 to 10^3 CFU/g in several studies carried out in Turkey on several types of cheeses (Aksu et al. 1999; Tekinşen and Özdemir 2006; Aygun and Pehlivanlar 2006; Vural et al. 2010). Several of the samples, tested in the present study, contained total *E. coli* counts ranging above 10^3 CFU/g (Tab. 1), although, according to the Turkish food codex regulation on microbiological criteria, *E. coli* counts have to be maximum 10^2 CFU/g in white and kashar cheese, butter and milk cream. High levels of *E. coli* may be observed due to improper handling or storage practices, microbiologically poor quality of raw materials and cross-contamination after processing, for example (Elmalili et al. 2005; Elmacioglu et al. 2010; Anonymous 2011). Higher levels of *Enterobacteriaceae* and *E. coli* counts may be considered to deserve special attention, particularly during manufacturing.

Aaerobic mesophilic bacteria count of minced meat samples varied between 10^4 and 10^7 CFU/g, resulting in a low microbiological quality. The present results overlapped with those from previous studies in other regions indicating that the minced meat was found to be mostly contaminated with several pathogenic microorganisms and therefore possesses high risks of health hazards (Gökmen and Alişarli 2003; Gundogan et al. 2005; Çetin et al. 2010).

Regarding the distribution of mold, 95 (53.1 %) of the samples were found to have a mold counts above 10^2 CFU/g and most of those samples had a count ranging from 10^3 to 10^5 CFU/g. Mold counts of the 47 samples (26.3 %) were higher than the permitted level of dairy product according to Turkish Food Standard (10^3 CFU/g or mL). In Turkey, high mold and yeast counts are reported to be caused by not following the hygiene rules in the period from the production to the marketing of the cheese (Yucel and Ulusoy 2006). The levels of mold count obtained in this investigation were comparable to those found by Aksu et al. (1999), Turkoglu et al. (2003) for manufactured dairy products in Turkey. In general mold counts are useful for indicating the shelf-life duration and microbial quality of foods; high level may also be a health hazard due their potentially mycotoxin production.

Overall, about 10.6 % of samples (17 meat product and 2 dairy product samples) were found to be contaminated with *S. aureus,* which in most cases showed higher values than the maximum level (10^2 CFU/g), suggested as a pro-

TABLE 1: *Microbial counts of bacteria in 179 meat and dairy product samples.*

Product category	No. of samples (n=179)	*Enterobacteriaceae*					*Escherichia coli*				Count interval[a, b, c] *Staphylococcus aureus*			Molds			Aerobic mesophilic bacteria			Foodborne pathogens	
		<10	$10–10^2$	$10^2–10^3$	$10^3–10^4$	$>10^5$	<10^2	$10^2–10^3$	$>10^3$	$>10^4$	<10^2	$10^2–10^3$	$>10^4$	<10^2	$10^2–10^3$	$>10^4$	<10^4	$10^4–10^5$	$>10^6$	*E. coli* O157	*L. monocytogenes*
Meat products (n=67)																					
Turkish sausage (Sucuk)	19	13(68.4)	4(21.1)	2(10.5)	0	0	16(84.2)	3(15.8)	0	0	17(89.5)	1(5.3)	1(5.3)	14(73.7)	5(26.3)	0	–	–	–	1(5.3)	2(10.5)
Minced meatball (Köfte)	22	2(9.1)	1(4.5)	8(36.4)	11(50.0)	0	7(31.8)	12(54.5)	3(13.6)	1(4.5)	14(63.6)	7(31.8)	1(4.5)	10(45.5)	12(54.5)	0	–	–	–	3(13.6)	2(9.1)
Minced meat	26	1(3.8)	4(15.4)	12(46.2)	9(34.6)	0	12(46.2)	8(30.8)	6(23.1)	0	19(73.1)	7(26.9)	0	9(34.6)	17(65.4)	0	2(7.7)	11(42.3)	13(50.0)	3(11.5)	3(11.5)
Dairy products (n=112)																					
Soft cheese Beyaz Peynir	50	23(46.0)	12(24.0)	6(12.0)	9(18.0)	0	33(66.0)	9(18.0)	8(16.0)	0	50(100.0)	0	0	32(64.0)	17(34.0)	1(2.0)	–	–	–	2(4.0)	3(6.0)
Lor Peynir	29	0	0	4(13.8)	25(86.2)	0	1(3.4)	4(13.8)	24(82.8)	0	27(93.1)	1(3.4)	1(3.4)	4(13.8)	18(62.1)	7(24.1)	–	–	–	3(10.3)	3(10.3)
Dil Peyniri	3	0	2(66.7)	1(33.3)	0	0	1(33.3)	2(66.7)	0	0	3(100.0)	0	0	1(33.3)	2(66.7)	0	–	–	–	n. d.	n. d.
Hart cheese (Kasar)	15	8(53.3)	4(26.7)	1(6.7)	2 (13.3)	0	12(80.0)	1(6.7)	2(13.3)	0	15(100.0)	0	0	6(40.0)	8(53.3)	1(6.7)	–	–	–	n. d.	1(6.7)
Butter (Tereyagi)	7	3(42.9)	2(28.6)	2(28.6)	0	0	5(71.4)	1(14.3)	1(14.3)	0	7(100.0)	0	0	2(28.6)	3(42.9)	2(28.6)	–	–	–	n. d.	n. d.
Raw milk	8	3(37.5)	1(12.5)	4(50.0)	0	0	4(50.0)	3(37.5)	1(12.5)	0	8(100.0)	0	0	6(75.0)	2(25.0)	0	–	–	–	1(12.5)	n. d.

a: Range in CFU/g; b: detection limit at >10 CFU/g; c: percentage given in parentheses; n. d.: not detected; –: not analyzed; all samples were negative for *Salmonella* spp. by real time PCR

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cess hygiene criteria for cheese products in accordance with the Regulation on Turkish Food Codex Microbiological Criteria (Anonymous 2011). One of each sample of lor cheese, sucuk and minced meat ball samples even showed values above 10^5 CFU/g, which is considered to introduce a significant risk of production of enterotoxins. The moderate occurrence of *S. aureus* in meat and dairy food samples detected in this study is in accordance with the rates reported by several studies from Turkey reported by Günsen and Büyükyörük (2003) (3.2 % in brined cheese the samples from west Turkey), Tekinsen and Özdemir (2006) (5.0 % in Van otlu (Herb) cheese samples from east Turkey), Can and Çelik (2012) (5% of different cheese samples in middle region of Turkey).

A total of 19 coagulase positive isolates, each representing one isolate per sample, were further analyzed. According to the cultural, coagulase tube test, clumping factor, staphaurex latex agglutination test and hemolytic properties the isolates could be identified as *S. aureus.* This was confirmed by PCR amplification of a species specific part of the gene encoding staphylococcal thermonuclease *(nuc)*. The amplification of the thermonuclease gene *(nuc)* had a uniform size of approximately 375 bp (Fig 1). The species-specific part of this target gene has usually been used to identify this species (Brakstad et al. 1992). In addition by PCR amplification, isolates from eight samples were positive for one or more enterotoxin genes (five for SEA; one for SEA, SED and SEJ; one for SED and SEJ and one for SEH and *egc*-gene complex *seg, sei, sem, sen, seo*) (Fig. 1). The presence of *S. aureus* strains harboring enterotoxin genes (SEA and SED) and trans-SEE genes raises concerns about the potential impact of the products from small-scale producers on public health.

Salmonella was not found in any of the samples in this study, but *L. monocytogenes* and *E. coli* O157 were detected using real time PCR in several food samples; *L. monocytogenes* was positive for 7 (6.3 %) milk product samples (3 white cheeses, 3 lor cheses and 1 kashar cheese) and 7 (10.5 %) meat product samples (3 minced meat, 2 meat ball and 2 Turkish sausage), and *E. coli* O157 was positive for 6 (5.4 %) milk product samples (2 white cheeses, 3 lor cheses and 1) raw milk), and 7 (10.5 %) meat product samples (3 minced meat, 3 meat ball and 1 sucuk). Insufficient non-hygienic production should have role, for examples, in *L. monoctyogenes* contamination particularly from production environment and equipment during handling, storage and distribution (Walker et al. 1991; Menendez et al. 1997, Mehmetoglu et al. 2011).

The results of this study showed the presence of pathogenic and enterotoxigenic bacteria in meat and dairy products from small food manufacturing in Trakya region and highlighted their potential hazard for public health and food safety. Currently, small-scale meat and dairy production and direct marketing in weekly markets and farms are still important for consumers in different regions of Turkey. The major problem for this method of production and marketing is insufficient hygiene and food safety standardization. Based on the results of the present study, it can be concluded that there is a need for emphasis on quality control during processing and handling for meat and dairy products quality assessment. It is recommended that hygienic measures are necessary for reducing or eliminating relevant microorganisms and pathogens in small food manufacturing plants by way of safer handling of raw materials, proper cleaning-disinfection and sanitation of critical control points to prevent contamination of traditional products.

Conflict of interest

The authors declare no conflict of interest.

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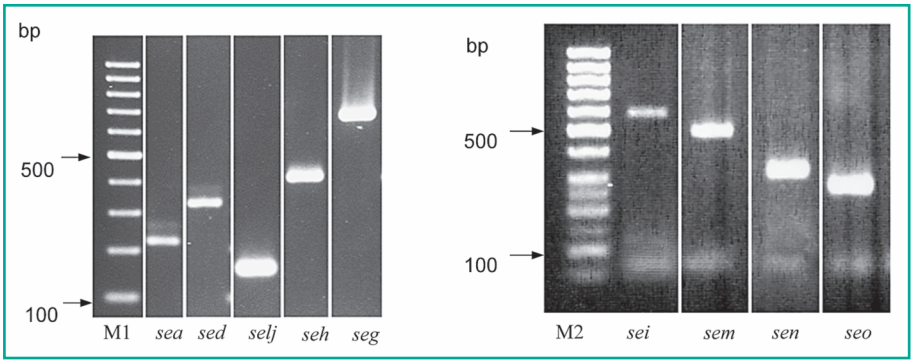

FIGURE 1: *Typical amplicons of detected S. aureus enterotoxin genes for sea (219 bp), sed (317 Bp), selj (142 Bp), seg (642 bp), she (375 bp), sei (576 bp), sem (471 bp), sen (292 bp) and seo (249 bp). M1: Gene Ruler 100 bp and M2 50 bp DNA Ladder (MBI Fermentas, Germany).*

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