



RESEARCH ARTICLE

Identification of *Enterobacteriaceae* and *Staphylococcaceae* at Turkish sucuk

Mukadderat Gökmen¹, Recep Kara², Levent Akkaya¹, Veli Gök³

¹Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Balıkesir University, Balıkesir,
²Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, ³Department of Food Engineering,
Faculty of Engineering, Afyon Kocatepe University, Afyonkarahisar, Türkiye

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*mgokmen@balikesir.edu.tr

Özet

Gökmen M, Kara R, Akkaya L, Gök V. Türk sucuğunda *Enterobacteriaceae* ve *Staphylococcaceae* identifikasyonu. **Eurasian J Vet Sci, 2013, 29, 4, 192-197**

Amaç: Bu çalışmada, Afyonkarahisar'da beş farklı işletmede üretilen Türk sucuğu örneklerinde *Enterobacteriaceae* ve *Staphylococcaceae* familyalarına ait türlerin identifikasyonu yapıldı.

Gereç ve Yöntem: Materyal olarak Afyonkarahisar'ın beş farklı bölgesinde üretilen Türk fermente sucukları kullanıldı. *Enterobacteriaceae* sayımı için Violet Red Bile Glucose Agar ve (ISO 21528-2 2004), *Staphylococcaceae* sayımı için Baird Parker Agar (FDA BAM 2001) kullanıldı. *Enterobacteriaceae* tür düzeyinde API-20E test kiti (bioMérieux Inc API- 20 E 2006) ile *Staphylococcaceae* tür düzeyinde API-Staph test kiti (bioMérieux Inc API-Staph 2009) ile identifikasyonu yapıldı.

Bulgular: Türk sucuklarında *Staphylococcaceae* ve *Enterobacteriaceae* türlerinin izolasyon ve idetifikasyonu yapıldı. Araştırmada 90 *Staphylococcus* izolatı Türk sucuklarından izole edildi. İzole edilen *Staphylococcaceae* izolatları *S. aureus* (%12.94), *S. epidermidis* (%15.29), *S. simulans* (%12.94) ve *S. carnosus* (%11.76) olarak tanımlandı. İzole edilen *Enterobacteriaceae* izolatları ise *Citrobacter diversus* (%5.96), *Enterobacter sazazaki* (%11.91), *Enterobacter gergoviae* (%17.87), *Klebsiella ozaerae* (%6.38), *Salmonella arizonae* (%6.38), *Salmonella spp* (%8.94), *Escherichia coli* (%8.94), *Serratia marcescens* (%6.81), *Serratia liquefaciens* (%6.38) ve *Yersinia enterocolitica* (%4.26) olarak tanımlandı.

Öneri: Türk sucuklarında *Staphylococcaceae* familyasına ait starter kültür olarak kullanılabilen *S. simulans* ilk defa tespit edilmiştir. Ayrıca Türk sucuklarında tespit edilen *Staphylococcaceae* ve *Enterobacteriaceae* familyasına ait patojen bakteri türlerinin gıda güvenliği ve halk sağlığı açısından risk taşıdığını göstermektedir.

Anahtar kelimeler: *Enterobacteriaceae*, *Staphylococcaceae*, Türk Sucuğu, sucuk

Abstract

Gokmen M, Kara R, Akkaya L, Gok V. Identification of *Enterobacteriaceae* and *Staphylococcaceae* at Turkish sucuk. **Eurasian J Vet Sci, 2013, 29, 4, 192-197**

Aim: In this study, the species from *Enterobacteriaceae* and *Staphylococcaceae* families have been identified in Turkish fermented sausages samples of five different corporations which are produced in Afyonkarahisar.

Materials and Methods: Turkish fermented sausages of five different corporations which are produced in Afyonkarahisar were used as the material. In order for the calculation of *Enterobacteriaceae*, inoculation was made into Violet Red Bile Glucose Agar medium (ISO 21528-2 2004) and for the calculation of *Staphylococcaceae* Baird Parker Agar medium (FDA BAM, 2001) was used. *Enterobacteriaceae* were identified at species level with API- 20 E test kit (bioMérieux Inc API-20 E 2006). *Staphylococcaceae* were identified with API-Staph test kit (bioMérieux Inc API-Staph 2009).

Results: Isolation and identification of *Staphylococcaceae* and *Enterobacteriaceae* species in Turkish sausage flora were done. 90 *Staphylococcus* isolates were derived from the Turkish sausage samples in the research. *S. aureus* (12.94%), *S. epidermidis* (15.29%), *S. simulans* (12.94%) and *S. carnosus* (11.76%) from *Staphylococcaceae* family have been identified. 235 *Enterobacteriaceae* isolates from Turkish sausage samples were identified at the species level as *Citrobacter diversus* (5.96%), *Enterobacter sazazaki* (11.91%), *Enterobacter gergoviae* (17.87%), *Klebsiella ozaerae* (6.38%), *Salmonella arizonae* (6.38%), *Salmonella spp* (8.94%), *Escherichia coli* (8.94%), *Serratia marcescens* (6.81%), *Serratia liquefaciens* (6.38%) and *Yersinia enterocolitica* (4.26%).

Conclusion: *S. simulans* from *Staphylococcaceae* family which can be used as a starter culture has been found in Turkish sausages for the first time. Pathogenic bacterium species from *Staphylococcaceae* and *Enterobacteriaceae* families determined in Turkish sausages indicate a risk for food safety and public health.

Keywords: *Enterobacteriaceae*, *Staphylococcaceae*, Turkish sausage, sucuk





Introduction

Turkish sausage is a broadly popular meat product in Turkey (Aksu and Kaya 2004). Turkish sausage which is a fermented meat product is made by adding salt, sugar, garlic, spices and various additives into grinded meat and fat and seasoning the mixture in natural or artificial casing at a certain temperature, relative humidity and air circulation (Gokalp 1995). During the production phase of fermented sausages, some chemical and physico-chemical changes like acidity, color formation, fat lipolysis and proteolysis of proteins occur as a result of fermentation of carbohydrates (Garcia Fontan et al 2007). Rod-shaped and gram-positive microorganisms of the genus *Micrococcus* and *Staphylococcus* play a significant role in the fermentation and maturation of fermented sausages (Gurakan et al 1995). According to the new classification the species of *Staphylococcus* take part in *Staphylococcaceae* family. Most of the coagulase-negative *staphylococcus* species are in the normal microflora of fermented food and it has been long known that some species like *S. carnosus* and *S. xylosus* are used as the starter culture in meat fermentation (Zell et al 2008). Some studies carried out in our country (Sancak et al 1996, Atasever et al 1998, Con et al 2002, Siriken et al 2006) report that Turkish sausages are contaminated especially by *S. aureus*.

Various members of Enterobacteriaceae family (*Salmonella*, *Yersinia*, *Shigella*, and *Escherichia*) are enteropathogenic due to the capacity of enterotoxin production that causes significant gastrointestinal changes (Tornadijo et al 2001). Moreover, as a result of amine decarboxylase activity of Enterobacteriaceae bacteria, biogenic amines like tyramine, histamine, tryptamin, cadaverine, putrescine and spermidine are generated from amino acids during the ripening of the sausage (Papavergou 2011). The number of Enterobacteriaceae in the sausage depends on the contamination of the carcass with the microorganisms in the gastrointestinal systems of the animal (Troeger and Woltersdorf 1989). Thus, some studies on Turkish sausages (Sancak et al 1996, Con and Gokalp 1998, Con et al 2002, Siriken et al 2006, Kok et al 2007, Oksuztepe et al 2011) report such a contamination with Enterobacteriaceae.

In this study, isolation and identification of *Staphylococcaceae* and Enterobacteriaceae species in Turkish sausage flora have been made.

Materials and Methods

In this study, Turkish sucuk of five different corporations which are produced in Afyonkarahisar were used as the material. Turkish sausage samples were put into sterile stomacher bags, were brought to the laboratory under cold chain and they were analyzed the same day.

Microbiological Analysis

Preparing Dilutions and Culture: 10 g of Turkish sucuk samples in sterile stomacher bags were homogenized by addition of 90 mL of sterile peptone physiological saline (0.85% NaCl + 0.1% peptone). Decimal solutions up to 10⁻⁷ were prepared from the homogenization of 1:10 diluted samples. Prepared dilutions were inoculated to Plate Count Agar (PCA, Oxoid CM0325) medium for Total Mesophilic Aerobic Bacteria and incubated at 35 °C for 48 hours (FDA BAM 2001); and for the calculation of Enterobacteriaceae, inoculation was made into Violet Red Bile Glucose Agar (VRBGA, Oxoid CM0485) medium and the culture was incubated at 37±1°C for 24±2 hours (ISO 21528-2:2004). In order for the calculation of *Staphylococcaceae* Baird Parker Agar (BPA; Oxoid CM0275) medium was used and the culture was incubated at 35±1°C for 48 hours (FDA BAM 2001). The Identification of Enterobacteriaceae and *Staphylococcaceae* Isolates: Characteristic red-pink or purple colored colonies with a dark-red zone multiplied in VRBGA medium were counted. Oxidase and fermentation tests were performed for identification. Oxidase negative and glucose positive colonies were acknowledged as Enterobacteriaceae. Enterobacteriaceae positive colonies were identified at species level with API-20 E test kit (bioMérieux Inc: API-20 E 2006). Typical colonies reproduced in BPA medium were identified with API-Staph test kit (bioMérieux Inc: API-Staph 2009).

Chemical analysis

Due to some physico-chemical characteristics of Turkish sausage samples, determination of pH was made according to ISO 2917 (2002), determination of moisture was made according to ISO 1442 (1999), determination of salt was made according to ISO 1841-1 (2001), determination of aw (water activity) was made according to Rödel et al (1975) and nitrate determination was made according to ISO 3091 (1975).

Statistical analysis

In the analysis of the data derived from the study Kruskal-Wallis Test was performed. In statistical evaluation of analysis results, microbiological counting results were used after being transformed into logarithmic units (log 10).

Results

In this study, the isolation and identification of *Staphylococcaceae* and Enterobacteriaceae species in Turkish sausage flora were done. So, Turkish sucuk of five different corporations which are produced in Afyonkarahisar were used as the material. Some microbiological and physico-chemical results of the analyzed samples were shown in Table 1.



Table 1. Microbiological (kob/g) and physico-chemical results of Turkish sausage samples

	aw	pH	Humidity (%)	Salt (%)	Nitrate (ppm)	TAMB	Enterobacteriaceae	Staphylococcaceae
Min.	0.70	5.10	39.21	3.26	40.00	6.04	3.60	3.00
Max.	0,78	5.20	44.26	3,79	70.00	6.26	5.70	4.72
Ort.	0.75	0.10	42.40	3.58	54.00	6.15	4.94	3.93
SD*	0.03		1.93	0.20	11.40	0.09	0.94	0.74

TAMB: Total Aerobic Mesophilic Bacteria, SD* Standard Deviation

Table 2. Isolation and identification of *Staphylococcaceae* from the Turkish sausage samples.

	A		B		C		D		E		Total	
	n	%	n	%	n	%	n	%	n	%	n	%
<i>S. aureus</i>	4	2.35	4	2.35	4	2.35	6	3.53	4	2.35	22	12.94
<i>S. epidermidis</i>	4	2.35	4	2.35	8	4.71	4	2.35	6	3.53	26	15.29
<i>S. simulans</i>	6	3.53	4	2.35	2	1.18	6	3.53	4	2.35	22	12.94
<i>S. carnosus</i>	4	2.35	2	1.18	4	2.35	6	3.53	4	2.35	20	11.76
Total	18	20.00	14	17.65	18	18.82	22	21.18	18	22.35	90	100

Table 3. Isolation and identification of Enterobacteriaceae from the Turkish sausage samples.

	A		B		C		D		E		Total	
	n	%	N	%	n	%	n	%	n	%	n	%
<i>Citrobacter diversus</i>	0	0.00	5	2.13	0	0.00	4	1.70	5	2.13	14	5.96
<i>Enterobacter sazazaki</i>	5	2.13	9	3.83	5	2.13	0	0.00	9	3.83	28	11.91
<i>Enterobacter gergoviae</i>	0	0.00	21	8.94	5	2.13	4	1.70	12	5.11	42	17.87
<i>Klepsiella ozaerae</i>	0	0.00	5	2.13	0	0.00	5	2.13	5	2.13	15	6.38
<i>Salmonella arizonae</i>	0	0.00	5	2.13	4	1.70	0	0.00	6	2.55	15	6.38
<i>Salmonella spp.</i>	0	0.00	5	2.13	8	3.40	3	1.28	5	2.13	21	8.94
<i>Escherichia coli</i>	0	0.00	5	2.13	5	2.13	4	1.70	7	2.98	21	8.94
<i>Serratia marcescens</i>	6	2.55	5	2.13	0	0.00	0	0.00	5	2.13	16	6.81
<i>Serratia liquefaciens</i>	5	2.13	0	0.00	3	1.28	7	2.98	0	0.00	15	6.38
<i>Yersinia enterocolitica</i>	0	0.00	0	0.00	5	2.13	5	2.13	0	0.00	10	4.26
ND*	4	1.70	10	4.26	5	2.13	8	3.40	11	4.68	38	16.17
Total	20	8.51	70	29.79	40	17.02	40	17.02	65	27.66	235	100.00

*ND: Not detected

Ninety *Staphylococcus* isolates were derived from the Turkish sausage samples in the research. Identification of the isolates is as in the following: 12.94% *S. aureus*, 15.29% *S. epidermidis*, 12.94% *S. simulans* and 11.76% *S. carnosus* (Table 2).

Two hundred and thirty-five Enterobacteriaceae isolates from Turkish sausage samples were identified at the species level as 5.96% *Citrobacter diversus*, 11.91% *Enterobacter sazazaki*, 17.87% *Enterobacter gergoviae*, 6.38% *Klepsiella ozaerae*, 6.38% *Salmonella arizonae*, 8.94% *Salmonella spp.*, 8.94% *Escherichia coli*, 6.81% *Serratia marcescens*, 6.38% *Serratia liquefaciens*, and 4.26% *Yersinia enterocolitica* (Table 3).

Discussion

Coagulase negative staphylococci (CNS) are not only existent in natural flora of fermented meat products but also they are at relatively high rates in fermented sausages which are produced without starter culture (Miralles et al 1996). In this study, *S. simulans* of CNS in Turkish sausages produced in Afyon province was determined as the dominant species for the first time and it was followed by *S. carnosus* (Table 2). Since *S. simulans* has nitrate reductase and urease activities (Drosinos et al 2005) it is used as the starter culture in the production of fermented sausages in Italy (Coppola et al 1997). Drosinos et al (2005) identified 25 staphylococci isolates out of 219 and Drosinos et al (2007) 46 Staphylococci





strains out of 300 isolates from traditional fermented sausages as *S. simulans*. *S. carnosus* is used as lipolytic starter culture in fermented sausages (Jessen 1995). Papamanoli et al (2003) reported that *S. saprophyticus* forms dominant flora in naturally fermented sausages and followed by *S. carnosus* and *S. xylosum*, relatively. Aymerich et al (2003) detected *S. carnosus* in chorizos which is a fermented meat product. The studies on the isolation and the identification of starter microorganisms which contain the natural flora of Turkish sausages identified *S. carnosus* (Nazli 1998) and *S. xylosum* (Kaban and Kaya 2008). It has been thought that the differences between the microbiological floras of Turkish sausages in our country stem from non-standardized production methods, various technological and hygienic applications and variety of raw material. In addition, the starter which is not being used is seen as another significant factor in the formation of differences (Con et al 2002).

In this study, among 90 *Staphylococcus* strains, *S. epidermidis* rated the highest with 26 isolates of coagulase-positive staphylococci (Table 2). Today, *S. epidermidis* is generally considered as the opportunistic pathogen (Vuong and Otto 2002). Aymerich et al (2003) found *S. epidermidis* in the 11.8% of fermented meat products (fumets). In their studies at different meat corporations Schlegelova et al (2008) identified *S. epidermidis* in the 5 raw material (meat) samples out of 259, 7 surface samples out of 121 and 22 meat product samples out of 157. Schlegelova et al (2008) reported that contamination of meat products with *S. epidermidis* and such pathogens was inevitable as the circumstances during the preparation of meat products were convenient for the bacteria to survive and multiply. In this study, 22 *S. aureus* isolates were identified. In their study carried out at fermented sausage production facilities in 5 different countries Talon et al (2007) detected *S. aureus* in 6.1% of 314 samples from different surfaces. They reported the highest rates of contamination at grinder, mixer and filling machines. Martin et al (2007) identified 38 (23%) Gram positive cocci out of 166 which they isolated from three different fermented sausage production phase as *S. aureus*. The studies in our country indicate the rates of *S. aureus* in sausage samples as 12% (Kok et al 2007) 6.6% (Erdogru and Ergun 2005) and 10% (Oksuztepe et al 2011). Toxigenic strains of *S. aureus* (due to staphylococcal enterotoxin) result in food based intoxications. People, raw materials, environment and the equipment are among the sources of contamination of the food (Stastkova et al 2011). Because *S. aureus* is salt and nitrite tolerant, it can produce enterotoxin under appropriate circumstances at the beginning phase of fermentation and continue to multiply, as well (Gonzalez-Fandos et al 1999).

In this study, within Enterobacteriaceae family *Enterobacter gergoviae* was identified at the highest rate with 42 isolates and this was followed by *Enterobacter sakazaki* with 28 isolates (Table 3). Enterobacter species are widespread in the

nature and some of them (*E. gergoviae* and *E. sakazaki*) are opportunistic pathogens which cause wounds, inflammation and urinary tract infections at humans (Noveir et al 2000). In this study, 21 of Enterobacteriaceae strains isolated from Turkish sausages were identified as *Salmonella spp.* The national studies on Turkish sausages (Erdogru and Ergun 2005, Siriken et al 2006, Kok et al 2007) reported that *Salmonella spp.* was identified. *Salmonella* species are food-based primary pathogens so they are not allowed in any food (Noveir et al 2000). It has been reported that in case of contamination of traditionally produced Turkish sausages with *S. typhimurium*, raw consumption of the sausage or marketing before the fermentation time and conditions are complete represent a risk for public health (Kara and Akkaya 2010). *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica* species of *Yersinia* genus of Enterobacteriaceae family is important for human health. *Y. enterocolitica* is a significant food pathogen which can develop at low temperatures and multiply at +4°C (Varnam and Evans 1991). In this study, *Y. enterocolitica* was identified in analyzed sausage samples. Many national (Aytac et al 1994, Evrensel et al 2006) and international (Fredriksson-Ahomaa et al 2007, Hudson et al 2008, Damme et al 2010, Fredriksson-Ahomaa et al 2012) studies reported *Y. enterocolitica* in different meat products. It was reported that this microorganism was isolated from dirt, water, animals and various food; it can exist everywhere and generally the food was the main resource of Yersiniosis (Bari et al 2011). In this study, 21 *E. coli* isolates were gathered (Table 3). Since *E. coli* has the capacity of developing and surviving under acidic conditions, this bacteria can be isolated from sausages at ripening phase or even from the final product (Garcia Fontan et al 2007). At national studies on Turkish sausages (Sancak et al 1996, Noveir et al 2000, Erdogru and Ergun 2005, Kok et al 2007) *E. coli* was reported.

Conclusions

Afyonkarahisar is one of the leading provinces which hold the first places in annual sausage production and market share. We strongly believe that *S. simulans* which was identified as the dominant species in Turkish sausages for the first time in our study can be used as the starter culture and also this can offer an insight to new studies on the standardization of starter cultures. Pathogenic bacteria species of Staphylococcaceae and Enterobacteriaceae families identified in Turkish sausages impose a risk for food safety and public health. Thus, good hygiene practices (GHP) and good manufacturing practices (GMP) in Turkish sausages are fundamentally important in terms of food safety and public health. In order to achieve the concept of "from farm to table", HACCP and GMP procedures should be followed at all chains between the production and consumption and facilities which produce sausage with traditional methods should be taken under control and be transformed into places where modern technology is used.



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