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ORIGINAL ARTICLE

Microbiological Quality of Minced Meat Samples Marketed in Istanbul

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SUMMARY

Foods can be contaminated with pathogenic microorganisms. To evaluate the microbiological content of minced meat, sixty samples collected from various butcher shops and supermarkets in Istanbul were studied. Following microbiological parameters were determined: total coliform, *Escherichia coli* and the total viable counts and pathogenic microorganisms (*Staphylococcus aureus* and *Pseudomonas* spp.) and yeasts/molds. Results indicated that aerobic plate counts and the number of fecal coliforms such as *E. coli and S. aureus* were particularly high in almost all of the samples analyzed. It was found that the microbiological quality of all the minced meat samples was inadequate and they exhibited high potential for food poisoning if consumed.

Key Words *Pathogen, Staphylococcus aureus, Coliforms, E. coli, Pseudomonas spp.*

İstanbul'da Satılan Kıyma Örneklerinin Mikrobiyolojik Kalitesi

ÖZET

Gıdalar patojenik mikroorganizmalar ile kontamine olabilir. Kıyma mikrobiyolojik içeriğini değerlendirmek için, İstanbul'da çeşitli kasap ve marketlerden toplanan altmış örnek; total koliform, *Escherichia coli* ve total aerobik canlı sayıları, patojen mikroorganizmalar (*Staphylococcus aureus* ve *Pseudomonas* spp.) ve maya/küf gibi mikrobiyolojik parametreler açısından incelenmiştir. Sonuçlar, aerobik canlı bakteri, *E.coli* ve *S. aureus* sayısının hemen hemen incelenen tüm örneklerde yüksek olduğunu göstermiştir. Tüm kıyma örneklerinin mikrobiyolojik kalitesinin yetersiz olduğu ve tüketildiği takdirde gıda zehirlenmeleri bakımından potansiyel bir tehlike oluşturabileceği sonucuna varılmıştır.

Anahtar Kelimeler *Patojen, Staphylococcus aureus, Koliform, E. coli, Pseudomonas spp.*

INTRODUCTION

Poultry, egg, red meat and meat products are the most common food sources responsible of the outbreaks of gastroenteritis (Jalali et al. 2008). Studies on the microbiological quality of food shows that minced meat is a medium rich in nutrients required for the growth of pathogenic microorganisms (Elmalı and Yaman 2005; Norman and Gravani 2006). Microbiological quality of meat is determined by the hygienic quality. High concentrations of microorganisms including pathogens are obviously devastating on the hygienic quality of meat**.** Unhygienic food handling results in contamination of food by pathogens (Tachbele et al. 2006). Microorganisms introduced from environmental exposure, lack of sanitation in slaughtering premises, equipment and outfit, and operators' hands contaminate the meat product (Sachindra et al. 2005; Kozačinski et al. 2006). Food consumers also comprise a link in the chain of food-borne bacterial illnesses with inappropriate storage and cooking of meat and meat products (Sachindra et al. 2005; Kozačinski et al. 2006; Tachbele et al. 2006). Although many bacteria encountered in foods are harmless, some may be potentially pathogenic. Food-borne infections and intoxications can occur due to the presence of certain bacteria (e.g. *E. coli*, *S. aureus, Salmonella* spp. and sulphite reducing anaerobs) (Elmalı and Yaman 2005; Tachbele et al. 2006)**.** Therefore, detection and identification of pathogenic bacteria in food as well as assessing the general microbial load of the food is of great significance (Kozačinski et al. 2006).

Use of indicator bacteria is a common practice to evaluate the hygienic condition of foods and the possible presence of pathogens. Total number of bacteria, coliform bacteria and *E. coli* obtained via total viable counts may, in some instances, reflect the sanitary quality of food. Among all microorganisms, *E. coli* is most frequently the contaminating organism, and is generally a reliable indicator of fecal pollution in water, food, milk and other dairy products (Soomro et al. 2002). In addition to pathogenic bacteria, total count of aerobic mesophilic bacteria, *Pseudomonas* spp., yeasts and molds are also used as general indicators of processing hygiene, storage conditions and spoilage in meat and poultry industries (ICMSF 2005; Kozačinski et al. 2006).

The aim of the current study is to determine the microbiological quality of minced meat products obtained from local butcher shops and supermarkets in Istanbul.

MATERIALS and METHODS

Sampling

Sixty minced meat samples were purchased from various butcher shops and supermarkets in Istanbul and were examined microbiologically to determine their

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microbiological quality and eligibility for safe consumption.

All the samples were transported to laboratory in an icebox and tested immediately upon arrival or stored at 4 °C for a maximum of 24 h until they were analyzed.

Microbiological analysis

The parameters of study were determination of counts of indicator bacteria (total coliform, *E.coli* and the total viable count), pathogenic organisms (*S. aureus* and *Pseudomonas* spp.) and yeasts/molds.

For microorganism counting, 10 g of each sample was transferred aseptically to a sterile stomacher bag containing 90 mL of peptone water and homogenized in a stomacher (IUL Instrument, Spain) for up to 2 min. Subsequent serial decimal dilutions of the samples were made using peptone water (HiMedia, India), as required. Selective agar plates were inoculated with 0.1 mL of the appropriate dilutions (Başkaya et al. 2004; Siriken 2004; Direkel et al. 2010).

Egg-yolk telluride (Oxoid SR54) enriched Baird-Parker Agar (Oxoid, CM275) was used to count *S. aureus*. Samples of 0.1 mL from each one of the serial dilutions were spread on Baird Parker agar (Oxoid). Plates were incubated at 37°C for 24 h and subsequently examined for typical *S. aureus* colonies with typical black appearance (telluride reaction) surrounded by clear zone (lecitinase reaction). The colonies were counted, transferred to slants of Nutrient Agar and incubated overnight at 37°C. Each strain was examined microscopically following Gram staining, then tested for production of catalase, coagulase, thermonuclease (TNase), DNAase and oxidation and fermentation of mannitol. Egg yolk positive, coagulase-positive, and Gram-positive isolates were classified as *S. aureus* (Food and Drug Administration 1992; Başkaya et al. 2004; Çetin et al. 2010; Direkel et al. 2010).

Coliforms and *E. coli* were grown on Violet Red Bile Agar (Oxoid, CM0978) with 4-methylumbelliferyl-β-D-glucuronide (MUG) and incubated under aerobic conditions at 37°C for 24–48 h. Colonies were visualized under UV light at 360 nm and counted. Identification of the colonies were performed with the help of Gram staining and IMVIC tests (Başkaya et al. 2004; Siriken 2004).

Pseudomonas spp. were grown in aerobic conditions on *Pseudomonas* Agar Base (Oxoid, CM0559) with *Pseudomonas* CFC Selective Agar Supplement (Oxoid, SR0103) at 25–30°C for 48 h. Colonies grown on plates were investigated under ultraviolet light. All strains were characterized by the following test: Gram staining, cytochrome oxidase and catalase production, fluorescence production under ultraviolet light and growth on McConkey Agar and 42 °C (Siriken 2004).

Total viable counts (TVC) were taken in quadruplicates using the spread-plate method in Plate count agar (Oxoid, CM0325) following the incubations at 37 °C for 24 h and 48 h (Başkaya et al. 2004).

Yeasts and molds were grown and counted on Potato Dextrose agar (Oxoid, CM0139) acidified with sterile lactic acid (10%, pH 3.5), incubated at 21°C for 5 days (Çetin et al. 2010).

Results were calculated as CFU per gram.

Stock cultures were frozen with 10% glycerol and stored at -20 °C until use.

RESULTS

Among the 60 meat samples, only two did not produce any microorganisms when incubated at 35°C (Table 1). Of the samples, 58 contained coliform bacteria, 58 contained *S.aureus*, 56 showed *Pseudomonas* growth, and 38 of them contained *E. coli*. All of the 60 samples appeared to be contaminated with molds and yeasts (Table 1 and Table 2).

In the samples, the number of TVC ranged between $2.7x10^4$ – $2x10^8$ cfu/g, while the numbers of total coliform bacteria, *E. coli, S. aureus, Pseudomonas,* and molds/yeasts ranged between 3.5 $x10^2$ – $4.5x10^7$ cfu/g, 10^1 – 8.5 $x10^4$ cfu/g, $6.5x10^2$ – $3.7x10^6$ cfu/g, 10^2 – $2.8x10^7$ cfu/g and $7x10^3$ – 4 $x10^8$ cfu/g, respectively.

Among the samples, 32 out of the 58 samples that were *S. aureus*–positive, exhibited high potential for toxin production (Table 2).

Table 1. The profile of the microorganisms detected in the minced meat samples

Microorganisms	n	Positive samples	Minimum amount (cfu/g)	Maximum amount (cfu/g)	Average amount (cfu/g)
TVC 37°C	60	58	$2.7x10^4$	$2x10^8$	9 $x10^6$
Total coliform	60	58	$3.5x10^2$	$4.5x10^7$	$1x10^6$
E.coli	60	38	10^1	8.5 $x10^4$	$2x10^4$
S. aureus	60	58	$6.5x10^2$	3.7 $x10^6$	$6x10^5$
Pseudomonas	60	56	10^2	2.8 $x10^7$	1.4 $x10^6$
Mold and yeast	60	60	$7x10^3$	4 $x10^8$	2.7 $x10^7$

Table 2. The distribution of microorganisms observed in the minced meat samples

Microorganisms	n	$0\text{-}10^1$ Positive samples		$10^1\text{-}10^2$ Positive samples		$10^2\text{-}10^3$ Positive samples		$10^3\text{-}10^4$ Positive samples		$10^4\text{-}10^5$ Positive samples		$10^5\text{-}10^6$ Positive samples		$10^6\text{-}10^7$ Positive samples		$10^7\text{-}10^8$ Positive samples		$10^8\text{-}10^9$ Positive samples	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
TVC 37 °C	60	2	3	0	0	0	0	0	0	12	20	26	43	16	27	0	0	2	3
Total coliform	60	0	0	0	0	6	10	18	30	12	20	20	33	2	3	0	0	0	0
E.coli	60	22	37	0	0	6	10	10	17	0	0	0	0	0	0	0	0	0	0
S. aureus	60	0	0	0	0	2	3	4	7	20	33	24	40	8	13	0	0	0	0
Pseudomonas	60	0	0	0	0	8	13	8	13	20	33	12	20	6	10	2	3	0	0
Mold and yeast	60	0	0	0	0	0	0	2	3	1	0	24	40	12	20	18	30	4	7

DISCUSSION and CONCLUSION

Minced meat is an excellent medium for microorganism growth. The microorganisms normally encountered on meat surface are distributed thoroughly into the meat product and start reproducing when the conditions are favorable during grounding, mixing, storing and packaging, causing loss of product quality and creating potential health hazards (Gökmen and Alişarlı 2003; Başkaya et al. 2004).

In Turkey, marketing of pre-packed minced meat was recently placed under more strict governmental regulations. Research results demonstrate that the minced meat sold in Turkey is found to be heavily contaminated with a variety of microorganisms and they contain pathogens that may cause infection and intoxications that could be seriously hazardous for public health (Sancak et al. 1993; Gökmen and Alişarlı 2003; Gönülalan and Köse 2003; Başkaya et al. 2004; Gündoğan et al. 2005).

Siriken (2004) demonstrated that 21.4% of 70 meat samples hosted coagulase-positive *Staphylococcus* growth, 5.7% of which being above 10^3 cfu/g. In another study conducted in Ankara-Turkey, Gündoğan et al. (2005) isolated *S. aureus* from 14 out of 30 ground beef samples. When the number of *S. aureus* bacteria exceeds 10^5 cfu/g sample, toxin production potential also increases (Bergdoll 1990). In the current study, 58 out of 60 minced meat samples contained *S. aureus*, and 32 of the 58 isolates possessed high potential of toxin production. These results support the findings of previous study (Gündoğan et al. 2005).

Although the number of contaminating *S. aureus* may be very low, under the favorable conditions, particularly at storage conditions above 6°C, bacteria quickly reproduce and begin synthesizing enterotoxins, causing possible food poisonings. In a study by Minematsu et al. (2006), minced beef, chicken and pork samples from 40 grocery stores were investigated and it was found that higher levels of *S. aureus* contaminations were detected in samples collected from supermarkets than those from butcher shops. This was probably because the meat in the supermarkets was grounded and stored at temperatures favoring growth, while in butcher shops the meat was freshly grounded and sold. The current study was conducted from January through August. The importance of the seasonal differences was evident, as the number of *S. aureus* capable of producing toxins were higher in the samples studied in June and July. Thus, in summer months where temperatures are high, cutting, transporting and packaging processes of meat must be more carefully conducted and good manufacturing/good hygienic practices must be benevolently exercised (Öztürk 2007).

Various researchers studied the microbiological quality of minced meat in Turkey. The results showed that pre-packed/stored minced meat possesses higher risks of health hazards.

Tekinşen et al. (1980) reported that the numbers of total bacteria, Staphylococci, coliform bacteria and *E. coli* in the pre-packed minced meat samples obtained in Ankara were $8.4x10^7$ cfu/g, $9.9x10^5$ cfu/g, $8.5\ x10^6$ cfu/g and $4.2x10^6$ cfu/g, respectively.

Sancak et al. (1993) performed a study on meat samples in Van, where they detected the number of total aerobic bacteria, Staphylococci, coliform bacteria and *E. coli* in the ranges of $2.3x10^5$ – $1.4x10^{10}$ cfu/g, 0 – $9.2x10^6$ cfu/g, about $4.0x10^6$ cfu/g and $4.1x10^5$ cfu/g, respectively.

Gönülalan and Köse (2003) conducted another study in Kayseri, where bacterial contamination profile in minced meat samples was as follows; Total aerobic organisms: $7.4x10^5$ – $5.3x10^9$, coliform bacteria: $8.6x10^1$ – $4.5x10^8$ cfu/g, *E. coli*: $<1.0x10^1$ – $5.2x10^5$ cfu/g, and coagulase-positive Staphylococci: $<1.0x10^1$ – $6.7x10^6$ cfu/g.

Başkaya and colleagues (2004) analyzed minced meat samples in a similar study, and found that the numbers of the bacteria were in the range of $3.1x10^4$ – $6.3x10^7$ cfu/g for total aerobic bacteria, $3.3x10^3$ – $6.2x10^4$ cfu/g for coliforms, $<1.0x10^4$ – $1.4x10^4$ cfu/g for *E. coli* and $8.0x10^2$ – $8.2x10^3$ cfu/g for coagulase-positive Staphylococci. These numbers showed that the microbiological quality of the samples did not meet the criteria of the Institute of Turkish Standards.

It was reported by Öztürk (2007) that 79% of the 70 minced meat samples collected in Aydin and Afyon provinces were contaminated with more than 10^5 aerobic mesophilic bacteria, 21.4% contained coagulase-positive Staphylococci, 64% contained more than 1100 cfu/g, and 5.7% contained *E. coli* at concentrations above10^3 cfu/g.

Direkel et al. (2010) found that total mesophilic bacteria, *E. coli*, *S. aureus*, yeast and molds were detected in the meat samples in the amounts of $4.7x10^4$ cfu/g, $6.0\ x10^2$ cfu/g, $3.2x10^5$ cfu/g, $5.8\ x10^4$ cfu/g, $4.8x10^4$ cfu/g and $2.3x10^3$ cfu/g, respectively.

Daly et al. (1976) demonstrated that meat samples contained total aerobic bacteria in the range of $3.5x10^5$ – $1.3x10^9$ cfu/g while all the samples were contaminated with coliform bacteria at the level of $1.9x10^3$ – $5.3x10^6$ cfu/g. Out of the 16 samples, 10 contained coagulase-

positive Staphylococci, in the range of $1.0x10^2$ – $2.5x10^3$ cfu/g.

A research group studied microbial contamination of meat samples in Italy, and found that the average aerobic microorganism content was higher than 10^8 cfu/g. Average numbers of coliforms, fecal coliforms and *S. aureus* were $7.0x10^5$ cfu/g, $5.2x10^2$ cfu/g and $1.0x10^2$ cfu/g (Patano and Caserio 1980).

Vorster and colleagues (1994), in another study with minced meat samples, found that 74.5% of the samples contained *E. coli,* 23.4% contained *S. aureus,* and the number of total aerobic microorganisms was $\log_{10}$ 12.1 cfu/g.

Upon evaluation of the studies focusing on the number of bacteria observed in minced meat samples, our results coincided with those from numerous studies, with some bias towards slightly higher or lower. The differences among the results could be due to the differences of analysis methods employed, the number of sampling, the differences in sampling locations/climate, and the quality of hygienic conditions in the processing plant.

Turkish Food Codex published by Turkish Ministry of Agriculture and Rural Affairs regulates the quality of food to be marketed for human consumption (Turkish Food Codex, 2010). According to the microbiological criteria of this codex, minced meat samples should not contain *E. coli,* however, 64% of the samples harbored this microorganism. In terms of *S. aureus* content, only 10% of the samples were acceptable by Turkish Food Codex.

The current study demonstrates that the bacteriological quality of the marketed minced meats in Istanbul is not acceptable for safe consumption and they contain various bacteria that can trigger serious health issues. Therefore, it is proposed that periodical training and education of the sales personal as well as efficient controlling and inspections must be practiced in the meat processing plants and markets.

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