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Prevalence of Escherichia coli in retail poultry meat, ground beef and beef

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Summary

The aim of the study was to determine the prevalence and the level of E. coli contamination in retail poultry meat, ground beef and beef, and to evaluate the compliance of retail meat and meat preparations to requirements of the Turkish Food Codex and health risks for consumers.

A total of 168 retail meat samples were examined for the prevalence and counts of Escherichia coli. Determination of the level of contamination of E. coli was performed using Lauryl Sulphate Tryptose (LST) broth with 4-methylumbelliferyl-β-D-glucuronide (MUG) according to three tubes with the Most Probable Number (MPN) method. Out of the total 168 samples tested, including poultry meat, ground beef and beef (each 56 samples), 90 (53.6%) were contaminated with E. coli. Overall, E. coli was detected in 49 (87.5%) of the poultry meat samples, 27 (48.2%) of the ground beef and 14 (25%) of the beef samples. The contamination level of all retail meats with E. coli was 1.9×10^3 MPN/g. Average counts of E. coli in each group meat were 3.7×10^3 MPN/g in poultry meats, 1.4×10^3 MPN/g in ground beef, and 6.4×10^2 MPN/g in beef samples. E. coli counts in all of the contaminated meats exceeded the established values in the microbiological criteria for retail meats consulted. The results have established retail meats represent hazards to human health and can be a threat to public health. On account of this, it is necessary that the consumers adopt the basic instructions regarding good hygienic practices, good cooking of meat, adequate storage temperature and cross-contamination.

Keywords: Escherichia coli, poultry meat, ground beef, beef, prevalence

Food safety is an increasingly important public health issue. Unsafe food causes many acute and life-long diseases, ranging from diarrheal diseases to various forms of cancer. Foodborne and waterborne diarrheal diseases kill approximately 2.2 million people annually; 1.9 million of them children. Therefore, foodborne diseases impose a significant burden on society in both developed and developing countries (26). Meat has traditionally been viewed as a vehicle for a significant proportion of human foodborne disease. Meat can be contaminated with a variety of pathogens and spoilage bacteria and it would be difficult to monitor each of these organisms in a meaningful way. Indicator organisms such as *Escherichia coli* are groups of bacteria that indicate the possible presence of organisms of concern, and may point to the origins of microbial contamination (5).

E. coli is a normal inhabitant of the intestinal tract of humans and warm-blooded animals. Its presence in raw foods is considered an indication of direct or indirect fecal contamination. Thus, it is used as an indicator organism for possible presence of enteric pathogens in food and water (4, 17). *E. coli* may contaminate foods in a variety of ways, including bowel rupture during evisceration, indirect contamination with sewage and polluted water, and handling and packaging of finished products (19). Meats are an especially common source of *E. coli* contamination, which may be acquired during slaughter through fecal contact (4). Food contamination with pathogen bacteria may also occur at multiple steps along the food chain, including production, processing, distribution, retail marketing, and handling or preparation (28).

Furthermore, some strains of *E. coli* are pathogens for humans and animals. Pathogenic *E. coli* strains are responsible for enteric and diarrheal diseases, urinary tract infections, and sepsis and meningitis. They are capable of causing disease under certain conditions when the immune system is compromised or disease may result from an environmental exposure (17). Foodborne *E. coli* may constitute – indirectly via host fecal flora – an important cause of urinary tract infections (19). They have been increasingly recognized as the most important causes of foodborne diseases and outbreaks all over the world (10, 24).

A standardized method for the detection and enumeration of *E. coli* comprises the most probable number (MPN) technique using Lauryl Sulphate Tryptose

(LST) broth containing a substrate for β-D-glucuronidase (GUD) as an indicator of *E. coli*. The three-tube MPN technique for the enumeration of *E. coli* in foods is a sensitive, but laborious procedure (3). The use of microbial enzyme profiles to detect *E. coli* as a valuable indicator of fecal pollution provides a rapid, reliable, convenient, differential test (18). A medium containing the fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronide (MUG) is used for the detection of enzyme GUD activity. *E. coli* metabolizes MUG, and the substrate is broken down by the enzyme GUD to release 4-methylumbelliferone, which fluoresces under longwave UV light. Over 97% of *E. coli* strains, but also some *Salmonella*, *Shigella* and *Yersinia* spp., are positive for GUD, and these strains are responsible for false positive results. On the other hand, *E. coli* O157:H7 serotype does not have the enzyme GUD and gives false negative result. These false positive reactions may be eliminated easily by an indole test. While *E. coli* is indole positive, others are indole negative. The test is also simple to apply and can be used directly in LST broth cultures with MUG (3, 9).

The aim of the study was to determine the prevalence and the level of *E. coli* contamination in retail poultry meat, ground beef and beef, and to evaluate the compliance of retail meat and meat preparations to the requirements of the Turkish Food Codex and health risks for consumers.

Material and methods

Sample collection. A total of 168 retail meat samples including poultry meat (chicken carcass) (n = 56), ground beef (n = 56) and beef (n = 56) were collected from randomly selected butcher shops and delicatessens in the city of Bolu (Northwest Turkey). The samples were placed into cool boxes (at 4°C) and transported to the laboratory. Microbiological tests were carried out immediately.

Microbiological analysis. Determination of the level of contamination of *E. coli* in meat samples was performed using Lauryl Sulphate Tryptose (LST) broth with 4-methylumbelliferyl-β-D-glucuronide (MUG) (Oxoid) according to three tubes of the Most Probable Number (MPN) method. A 25 g sample of each product was aseptically added to 225 ml of buffered peptone water (Merck), and homogenized for 2 min by stomacher (Bagmixer 400, Interscience, Paris, France). Then serial 10 fold dilutions were prepared with sterile buffered peptone water. From three consecutive dilutions (10^{-1} to 10^{-3}), 1 ml was inoculated into triplicate tubes containing LST+MUG broth with Durham vial and incubated at 37°C for 24-48 h. After incubation, the tubes with turbidity and gas production were examined for fluorescence by a UV lamp (UVP, Inc, USA). Fluorescence of the broth under UV light caused by the hydrolysis of MUG indicates the presence of *E. coli*, which can be further confirmed by testing for indole production after adding Kovacs' reagent (Merck). Finally, the results (positive tubes or negative tubes) were recorded for MPN enumeration (9).

Statistical evaluation. All statistical calculations were undertaken using the SPSS 12.0 for Windows statistical software (SPSS Inc., Chicago, IL, USA). Comparisons within and among groups were made by using a one-way ANOVA test, followed by Duncan's multiple comparison test. Differences were considered to be statistically significant for $p < 0.05$.

Tab. 1. Distribution of the contamination levels of *E. coli* in 168 raw meat samples

Type of meat samples	Cell count (cfu/g or MPN/g)	No. of samples (n = 168)	Percentage (%)
Poultry meat	< 3.0	7	12.5
	3.6×10^2 - 9.2×10^2	19	33.9
	1.5×10^3 - 9.3×10^3	27	48.2
	1.5×10^4 - 4.6×10^4	3	5.4
Mean	3.7×10^3 (3.57 $\log_{10}$ cfu/g)		
Ground beef	< 3.0	29	51.8
	3.6×10^2 - 9.2×10^2	14	25.0
	1.1×10^3 - 9.3×10^3	11	9.6
	1.5×10^4 - 2.4×10^4	2	3.6
Mean	1.4×10^3 (3.11 $\log_{10}$ cfu/g)		
Beef	< 3.0	42	75.0
	3.6×10^2 - 9.2×10^2	11	19.6
	2.3×10^3 - 4.3×10^3	2	3.6
	2.4×10^4	1	1.8
Mean	6.4×10^2 (2.81 $\log_{10}$ cfu/g)		
E. coli positive samples	> 3.0	90	53.6
E. coli negative samples	< 3.0	78	46.4

Explanation: n – number of samples tested

Results and discussion

The results of microbiological analysis of retail meat samples relative to the contamination levels of *Escherichia coli* are represented in Tab. 1. In the study, out of the total 168 samples analyzed, which include poultry meat, ground beef and beef (each 56 samples), 90 (53.6%) were contaminated with *E. coli*. Overall, *E. coli* was detected in 49 (87.5%) of the poultry meat samples, 27 (48.2%) of the ground beef and 14 (25%) of the beef samples.

The contamination level of all retail meats with *E. coli* was 1.9×10^3 MPN/g (3.28 $\log_{10}$ cfu/g). Average counts of *E. coli* in each group of meat were 3.7×10^3 MPN/g (3.57 $\log_{10}$ cfu/g) in poultry meats, 1.4×10^3 MPN/g (3.11 $\log_{10}$ cfu/g) in ground beef, and 6.4×10^2 MPN/g (2.81 $\log_{10}$ cfu/g) in beef samples. All of the contamina-

ted meat samples exceeded the maximum limits of *E. coli* (1.0×10^2 cfu/g) set in Turkish Food Codex (23).

According to the results of statistical analysis, the contamination levels of *E. coli* were significantly different in three different meat groups ($p < 0.05$). In fact, throughout the experiment the frequencies of *E. coli* in ground beef and beef were similar; only *E. coli* in poultry meats demonstrated a higher frequency ($p < 0.05$).

Escherichia coli and fecal coliforms are considered to be the most important and compulsory measure of microbiological quality of food and food related products in terms of hygiene. Their presence is used as indicators of fecal pollution. Among these, *E. coli* is often preferred as a more specific indicator of fecal contamination because it is specific and most reliably reflects fecal origin (6, 9).

According to our results, the contamination rate (53.6%) with *E. coli* in retail meat samples is relatively low in comparison with a report from Iceland, showing a very high contamination rate of various meats with *E. coli* ranging from 73% to 100% (22). In contrast, compared to our result authors in a previous study from Korea found the rather low prevalence of *E. coli* in different meat types ranged from 4.1% to 14.9% (12).

Following slaughter and dressing, the carcasses of animals and birds can be contaminated with predominantly enteric bacteria, including *E. coli*, coming from the skin, hair, feathers, gastrointestinal tract and the environment at the slaughtering facilities (17). During preparation and sale, meat and meat products can also be contaminated by *E. coli* (28). There have been a number of studies on meat hygiene in different countries. In Australia, *E. coli* was detected on 10.3% of carcasses and 5.1% of boneless beef samples (16). In a study reported by Sumner et al. (21), *E. coli* was isolated from 18.8% of beef carcasses for which the mean log of the positives was 0.34. In Croatia, *E. coli* was found in 6% of the beef samples tested (15). Compared to the results mentioned above our results related to beef samples, which were 25% positive for *E. coli* with a mean 2.81 $\log_{10}$ cfu/g, show the high level of contamination of *E. coli*, consistent with a risk to human health due to bacterial hazards in retail beef.

Ground beef can contain microorganisms coming from the carcasses as well as from different equipment used during processing, personnel, air, and water (17). While the bacterial contamination of meat is generally limited to the surface, the mincing process spreads the microbiota to the mass inside. Furthermore, mincing releases meat fluids, which are an excellent medium for bacterial growth (13). Indeed, we stated the high values of *E. coli* with an average of 1.4×10^3 MPN/g (3.11 $\log_{10}$ cfu/g) in 48.2% of ground beef samples in our experiments. In a report from Turkey, *E. coli* was counted at an average of 4.6×10^1 cfu/g in ground beef samples (8). Also, Sırıken (20) found that *E. coli* was positive in 30% of ground beef samples and 20% of them were above 9.44 MPN/g. In a survey conducted by Heredia et al. (11), *E. coli* was detected in 76% of 88 retail ground meat samples in Mexico. Eisel et al. (7) reported that average $\log_{10}$ *E. coli* count ranged from < 1-2 cfu/g and *E. coli* count was usually highest in finished ground beef. The most important factor contributing to the microbiological quality and level of microbial contamination of ground beef was mostly the quality of the raw material obtained from different suppliers of beef (7).

In the present study, contamination levels and frequency of isolation of *E. coli* in poultry meat samples were higher than those in ground beef and beef samples (tab. 1). Poultry meats also differed statistically from the other two by showing a higher frequency of *E. coli* ($p < 0.05$). In 87.5% of poultry meat samples the values with a mean of 3.57 $\log_{10}$ cfu/g were considerably high and exceed the maximum limit of *E. coli* according to Turkish Food Codex (23). Because of the relatively high frequency of contamination of poultry with potential pathogens, raw poultry meats are often known to be sources of infections in man (2, 17). Bacterial contamination of poultry meat depends on the intestine content, which may come in contact with carcasses already in the broiler house, and during transport and slaughter through a vehicle such as transport and processing equipment. High levels of cross-contamination occur especially during defeathering and chilling. Contamination levels may also increase during evisceration of the carcasses, washing and processing due to contamination by personnel (17, 27). Therefore, the variation in the levels of contamination by microbiological indicators over the processing indicates the significance of control procedures adopted by slaughterhouses for the microbiological quality of chicken carcasses (14). A study by Yashoda et al. (27) suggested that while the evisceration process resulted in a significant increase in microbial fecal contamination, lower microbial counts were observed in hygienically processed carcasses as compared with market carcasses. Moreover, *E. coli* was completely absent in hygienically processed carcasses. Alvarez-Astargo et al. (1) reported high microbiological contamination levels and high incidence rates with *E. coli* in retail chicken. They found that average counts for *E. coli* were 2.60 $\log_{10}$ cfu/g in chicken legs and 3.68 $\log_{10}$ cfu/g in chicken wings. Cohen et al. (4) noted that *E. coli* was detected in 43% of the chicken samples, with a mean 2.9 $\log_{10}$ cfu/g in the hot season. In a report from Turkey, Vural et al. (25) showed that *E. coli* was found in all of the retail chicken carcasses and their products tested. Also, the contamination levels of *E. coli* detected were relatively high with a mean of 2.91 $\log_{10}$ cfu/g in chicken carcasses, 5.54 $\log_{10}$ cfu/g in legs, 5.50 $\log_{10}$ cfu/g in wings, 4.05 $\log_{10}$ cfu/g in chicken breast, and 2.77 $\log_{10}$ cfu/g in giblets.

The microbiological quality of the final product depends on the microbiological quality of the raw meat,

other ingredients, personal hygiene and any contamination during the process. To prevent an undesirable level of contamination, mandatory quality assurance practices should be implemented, including Hazard Analysis Critical Control Point (HACCP) and Good Manufacturing Practices (GMP) (17, 21).

Conclusion

Retail meats may be notable hazards to humans because they are not acceptable from the sanitary point of view, as well as handling and processing under unhygienic and poor sanitary conditions. The non-hygienic retail meats may be a major public health issue. To limit this issue, consumers, must adopt the basic instructions regarding good hygienic practices, good cooking of meat, adequate storage temperature and cross-contamination.

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