



Investigating the Abundance of *Escherichia coli* in Traditional Hamburgers of Qom city and the Isolates Antibiotics Resistance

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Abstract

Background and aim: Hamburger is one of the foods that is made of beef or sheep meat and has its own nutritional value. However, this food item may carry many pathogenic microorganisms and on this basis the purpose of this study is to investigate the amount of *Escherichia coli* in traditional hamburgers of Qom city and the antibiotic resistance of isolates to imipenem and penicillin.

Materials and Methods: In this study, we randomly isolated 80 cases of traditional hamburgers from the supply centers of this product in Qom city and transferred them to the veterinary health laboratory in Qom city on ice, and isolated *Escherichia coli* and investigated the antibiotic resistance of the isolates.

Results: In this study, out of a total of 80 traditional hamburger samples, 20 cases were infected with *Escherichia coli* and the highest resistance of the isolates was related to gentamicin with 10% and tetracycline with 15%, respectively.

Conclusion: Considering that *Escherichia coli* has dangerous strains such as O157H7 and the most consumers of hamburgers are children and teenagers, so it is necessary that the health control of raw materials and tools be evaluated more by regulatory institutions.

Keywords: *Escherichia coli*, Qom, Antibiotic resistance, Street food

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Introduction

Today, due to the problems caused by industrial societies and the job consequences resulting from them and also the tendency of people to use ready meals such as meat and its products like hamburgers, sausages and lunch meat as well as the special condition of these products in eliminating the lack of protein, their consumption has increased. These foods can act as an agent for many infectious and non-infectious microorganisms that their growth is strengthened in favorable conditions and they act as active carriers of disease and humans get foodborne diseases through the food (Sezgin & Şanlıer, 2016). During the last decade, the prevalence and occurrence of microbial diseases caused by food has increased not only in developing countries with poor health, but also in developed countries with high health standards and strict monitoring. Due to the increased use of hamburger by people and also the use of meat in the preparation of this product and the its increased consumption, the possible risk of food poisoning and the possibility of contamination of this product with major bacteria that cause food poisoning is high (Omemu & Aderoju, 2008).

The consumption of red meat and its products has a long history in most cultures. Meat is a source of nutrients such as essential amino acids for the body like histidine, isoleucine, leucine, methionine, tryptophan and fatty acids such as linoleic acid and arachidonic acid. The presence of minerals such as phosphates and vitamins, especially group B vitamins, shows the value and importance of this product in human nutrition and also a sign of wealth in some countries (María, 2008; De Smet & Vossen, 2016).

Meat is one of the main sources of unsaturated fatty acids with a double bond, especially oleic acid with the formula c18:1. About 40% of meat's fatty acid is unsaturated with a double bond. The meat of ruminants also contains trans fatty acids (De Smet and Vossen 2016).

Meat, especially red meat and viscera, is one of the rich sources of iron, such that 100 grams of beef thigh steak has 3 milligrams of iron. 50 to 60% of meat iron is in heme form, which is the product of hemoglobin, and it is more effectively absorbed by the body than non-hemoglobin iron, which is the only source of iron available in plants. In addition, many iron absorption inhibitors such as phytate have no

effect on iron. The most important compounds that produce color in meat are pigments that absorb some wavelengths of light and reflect other lights. The structure and texture of muscles also affect the amount of absorption and reflection of light (Arshad, 2018; Wang *et al.*, 2022).

Usually, during slaughtering, skinning and cutting, microorganisms are mainly transferred from the digestive system and the external surface of the animal to the carcass, but a larger number are transferred to meat through knives, clothes, air, workers, wagons, boxes and equipment in general. The consumers' concern about the freshness of meat has increased continuously. Reliable methods for evaluating the microbiological quality or the freshness of meat are beneficial to consumers and producers, but it is difficult to find these methods in suspension foods (Wang *et al.*, 2022). Common spoilage of meats is divided based on aerobic or anaerobic conditions or the type of spoiling microorganism (bacteria, yeasts or molds). Most of the spoilage microorganisms use sugar, protein and fat for their survival and reproduction. Among the most common compounds used by microorganisms for energy production are carbohydrates and carbons. However, it is possible that carbonaceous compounds such as esters, alcohols, lipids, other organic acids and their salts, as well as fats can be used by microorganisms as a source of energy. Fat should be hydrolyzed to glycerin and fatty acids with the help of lipase, and then microorganisms may use it as an energy food. The products obtained from the breakdown of proteins, such as lipids and amino acids, can be used as an energy source for many proteolytic microorganisms, when a better energy source is not available (Blakstad & Allen, 2018; Hussein *et al.*, 2020).

Escherichia coli is one of the microorganisms of the Enterobacteriaceae family, which is dangerous Gram-negative bacteria that causes disease in humans. The most important strains of this bacteria are EPEC, ETEC, EIEC and EHEC, among which O157H7 has a significant risk. The habitat of this bacterium is the intestines of warm-blooded animals, and its presence in food indicates non-compliance with hygiene principles (Niyonzima *et al.*, 2015).

Hamburger is one of the meat products that is obtained from red meat through the formulation and

mixing of permitted raw materials. This food is available to consumers in a ready form and may cause infections and dangerous food poisoning due to reasons such as insufficient hygiene, lack of cooking time and heat and very high microbial load. Also, in traditional hand-made hamburgers, in addition to the meat itself, contamination may enter this product from the hands of cooks and workers, spices and other ingredients that are added to the meat, and endangers the health of the consumer. Therefore, considering the interest of a large number of children and teenagers to this food and the possibility of contamination with pathogenic bacteria such as *Escherichia coli*, the purpose of the present study is to investigate the contamination of handmade hamburgers in Qom City with *Escherichia coli* (Kano & Kynčl, 2018; Young & Nestle, 2021).

Materials and Methods

Sampling

We randomly selected 80 traditional hamburger samples from the supply centers of this product in Qom city during 5 months and, in order to avoid secondary contamination, we transferred them in ice flask to the specialized veterinary food hygiene laboratory of Qom city for microbial analysis and antibiotic resistance measurement of the isolates.

Escherichia Coli Isolation Method

Twenty five grams of hamburger samples was weighed with a precise digital scale and then was transferred to 225 ml lactose broth culture medium (Mirmedia, Iran) for 24 hours at incubator temperature. Then, 1 cc of the enriched sample medium was cultured on EMB agar medium (Mirmedia, Iran) and after 24 hours of incubation, the

colonies with metallic green polish were selected and cultured in the differential media of Simon Citrate, VP-MR, TSI and SIM (Mirmedia, Iran) and their positive samples were determined (Heidarzadi *et al.*, 2021).

Determination of Antibiotic Resistance

In this study, antibiogram was done by Diffusion Disk method. After preparing the microbial suspension according to McFarland's 0.5 standard solution, it was cultured in Mueller-Hinton agar medium and after that, antibiogram discs containing gentamicin (GE) and tetracycline (TE) were placed on the culture medium and after 24 hours of incubation, at a temperature of 37 degrees, we measured the diameter of the non-growth halo using existing caliper rulers (Heidarzadi *et al.*, 2021).

Data Analysis

A 95% confidence interval was considered for the prevalence of *Escherichia coli* infection. The sensitivity of bacterial isolates to antibiotics was compared with Friedman's nonparametric test. SPSS version 26 was used in this study.

Results

The prevalence of *Escherichia coli* infection in traditional hamburgers of Qom city was 20 infected cases out of a total of 80 samples which indicates a high level of infection (Table 1). Examining the status of antibiotic isolates showed that the highest resistance is related to tetracycline and the lowest resistance is related to gentamicin (Table 2).

Type of food	Escherichia coli infection	No infection with Escherichia coli
Traditional hamburger	20 cases (16%)	60 cases (84%)

Table 1. The status of Escherichia coli infection.

Type of antibiotic	Sensitive	Semi-sensitive (semi-resistant)	Resistant
Gentamicin (GE)	13 (30%)	21 (48%)	10 (23%)
Tetracycline (TE)	17 (39%)	27 (27%)	15 (34%)

Table 2. The status of antibiotic isolates to gentamicin and tetracycline.

Discussion

In the review of Zurita et al.'s study in 2020 on the contamination of sandwiches and ready-to-eat foods with *Escherichia coli*, they reported that 34% of the 20 food samples collected in the study were contaminated with *Escherichia coli* which is different from the results of the present study, which found 16% positive (Zurita et al., 2020).

Sarker et al.'s 2013 study was designed on food contamination with pathogenic *E. coli* isolates from 7 different groups of street foods. In cream, eggs, non-fried dry food and fried dry food, salad, raw and cooked sandwiches, among 400 studied samples, almost all of them were infected with a variety of bacteria that considering the amount of *Escherichia coli* bacteria in cooked sandwiches, they were 100% contaminated and the results of the polymerase chain reaction (PCR) test showed that out of 130 *Escherichia coli* isolates screened from all food categories, 52 (13%) were Enterotoxigenic *E. coli* and 32 (8%) were Shiga. 14 isolates (3.5%) were enteropathogenic and 6 isolates (1.5%) were enteropathogenic, which is much higher than the contamination of the present study (Sarker, Islam et al. 2013). In 2013, Hosseini et al. investigated the level of *E. coli* O157:H7 contamination in processed meat products from two factories in Shiraz and Tehran that out of the total of 400 hamburger samples tested, 136 samples equivalent to 19.9% of *Escherichia coli* contamination were reported, which is somehow consistent with the present study (Hosseini et al., 2011).

In Faramarzi et al., studies on bacterial contamination of food in the supply level of western areas of Tehran in 2013, it was determined that in terms of contamination with mesophilic bacteria in each food group, from the total of 642 food samples, 50% of salads and 6.36% of protein products had the highest and lowest levels of *Escherichia coli* contamination which is different from the results of the above research, because in the sandwiches tested in this study, 9.28% contamination with *Escherichia coli* had been reported (Faramarzi et al., 2012). In the study conducted by Massiha et al., in 2015 on the microbial quality of food samples collected from the eastern region of Gilan, it was indicated that in this study, aerobic mesophilic bacteria, *Escherichia coli* and coliforms are known as the most polluting food substances ($P < 0.001$), such that in total, 11.55% of

contamination was with *Escherichia coli*, 6.97% with aerobic mesophilic bacteria and 6.8% with coliform contamination. Meanwhile, the amount of contamination in meat products in this study, was recorded 12% with *Escherichia coli* out of a total of 104 tested cases, which is somewhat related to the results of the present study (Massiha et al., 2015). In Heydarzadi et al., study on the amount of contamination of samosas sold in Sistan and Baluchistan province with *Salmonella* and *Escherichia coli* and the antibiotic resistance of the isolates, they found that the amount of contamination of this food item with *Escherichia coli* was 12.5%, which is consistent with the results of the present research. Also, in measuring the antibiotic resistance of the isolates, it was determined that the highest resistance was related to imipenem (77%), tetracycline (38%) and ampicillin (46%) that regarding tetracycline, it is equal to the results of this research (Heydarzadi et al., 2021).

In Sohrabi et al.'s research on the comparative study of coli form and *Escherichia coli* contamination in food using different chromogen environments and their evaluation with the reference method in 2019, it was reported that out of 100 food samples, *Escherichia coli* contamination was reported in 66, 80, 84 and 80 cases, respectively (Sohrabi et al., 2020).

Dontorou et al.'s study on six hundred samples of unpasteurized cow's, sheep's and goat's milk, raw ground beef, hamburger, uncooked frozen beef, sandwiches (containing ham or turkey, mixed vegetable salad with mayonnaise and lettuce), sausage and traditional Greek sausage showed that 2% of the sampled hamburgers are contaminated with *Escherichia coli*, which has a high difference with the results of the present study, and the contamination level in this study is lower (Dontorou et al., 2003). In the study of Kalantari et al. in 2012, on the determination of bacterial contamination isolated from sandwiches in Kerman city and their resistance to common antimicrobials, it was reported that *Escherichia coli* was 40.3% of the most common pathogen isolated from the sampled sandwiches and also, antibiotic resistance to gentamicin of *Escherichia coli* isolates was 4.2%, which is different from the results of this study (Kalantari et al., 2012).

Conclusion

The presence of *Escherichia coli* bacteria, with its toxic and dangerous strain, can cause problems for the body, especially for children, teenagers, pregnant women, and even people with weak immune systems and create many dangers for them. Therefore, identifying the risk factors of *Escherichia coli* bacteria, washing hands sufficiently with water and various detergents, cooking sandwich products and especially materials with a hard texture, where the presence of high elasticity is an obstacle for the heat to penetrate deep into the food, all can be important and valuable reasons for reducing the occurrence of this bacteria and the absence of infection and food poisoning caused by this microorganism. The presence of *Escherichia coli* in any foodstuff indicates the low quality of the product in all stages of preparation, also, with the absence of poisoning caused by bacteria in food, we will see a significant reduction in the use of antibiotics and subsequently, antibiotic resistance.

Conflict of interest

Nil.

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بررسی میزان آلودگی به اشرشیاکلای در همبرگرهای سنتی شهرستان قم و مقاومت آنتی بیوتیکی جدایه ها

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چکیده

زمینه و هدف: همبرگر یکی از مواد غذایی است که از گوشت گاو یا گوسفند تشکیل شده و دارای ارزش غذایی خاص خود می-باشد اما با این وجود این ماده غذایی ممکن است حامل بسیاری از میکروارگانیسم-های پاتوژن باشد و بر همین مبنا هدف از مطالعه حاضر بررسی میزان اشرشیاکلای در همبرگرهای سنتی شهرستان قم و مقاومت آنتی بیوتیکی جدایه-ها به امی-پنم و پنی-سیلین است.

مواد و روش‌ها: در این مطالعه تعداد ۸۰ مورد همبرگر سنتی از مراکز عرضه این محصول در شهرستان قم به صورت تصادفی جدا گردید و در کنار یخ به آزمایشگاه بهداشت دامپزشکی شهرستان قم انتقال داده شد و جداسازی اشرشیاکلای و مقاومت آنتی-بیوتیکی جدایه-ها بررسی گردید.

یافته‌ها: در این بررسی از مجموع ۸۰ نمونه همبرگر سنتی، ۲۰ نمونه به اشرشیاکلای آلوده بود و بیشترین مقاومت جدایه-ها به ترتیب مربوط به جنتامایسین با ۱۰ درصد و تتراسایکلین با ۱۵ درصد بود.

نتیجه‌گیری: با توجه به اینکه اشرشیاکلای دارای سویه-های خطرناکی همچون O157H7 می-باشد و بیشترین مخاطب همبرگرها، کودکان و نوجوانان می باشند لذا لازم است که کنترل بهداشتی مواد اولیه و ابزارآلات بیشتر مورد ارزیابی نهادهای نظارتی قرار گیرد.

واژه‌های کلیدی: اشرشیاکلای، قم، مقاومت آنتی بیوتیکی، غذاهای خیابانی

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