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Research Article

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Evaluation of *Staphylococcus Aureus*, *Escherichia Coli*, and *Salmonella* Contamination in Sausages and Bologna Sold in Tehran City, Iran

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Abstract

Background and aim: Sausages and bologna are heat-treated products whose chemical composition is based on red meat and chicken. These products are considered ready-to-eat (RTE) foods, and because some of them use chicken paste or meat paste, they are highly susceptible to bacterial contamination. This study aimed to evaluate the contamination of *Staphylococcus aureus*, *Escherichia coli* (*E. coli*), and *Salmonella* in sausages and bologna offered in Tehran city.
Materials and Methods: One hundred samples, including 50 sausages and 50 meats bologna, were sampled from supply centers in a simple random manner and transferred to the food quality control and health laboratory. Different microbial culture tests were used to search for target microorganisms. All experiments were performed under sterile conditions.
Results: The results showed that the level of contamination in sausages and bologna in the present study was higher than the national standard of Iran. In the studied sausages, 14 samples out of 50 samples and in bologna, 20 samples out of 50 samples were found to be contaminated with *E. coli*, *Salmonella*, and *Staphylococcus aureus* microorganisms.
Conclusion: Considering the risks that pathogenic microorganisms cause for humans, it is recommended to minimize the use of sausages and bologna and if it is used, it should be well placed in heat treatments to minimize the risk of microbial contamination.

Keywords: *Sausage and Blonga, Escherichia coli, Salmonella, Staphylococcus aureus, Tehran*

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Introduction

Food safety is a major global public health problem. Foodborne diseases in humans due to bacterial pathogens and their toxins are well known all over the world. Diseases transmitted through food impose economic burden and quality of life on society through acute complications and chronic consequences. According to the World Health Report, approximately one tenth of people around the world become ill each year due to food contamination, and an average of 1.8 million people die annually due to foodborne diseases. For example, in the United States, approximately 31 pathogens cause 9.4 million illnesses, 56,961 hospitalizations, and 1,351 deaths each year (Scallan *et al.,* 2015). Therefore, it is necessary to quickly identify the pathogens responsible for the outbreak of food-borne diseases. Suitable typing methods for searching virulence genes of pathogenic bacteria are usually evaluated according to features such as typing ability, replication speed, speed, simplicity of work and cost-effectiveness. In addition, typing methods should be stable and suitable for use in all outbreak strains. They have high detection ability and high replication speed, repeatability over time and high compatibility with epidemiology, that is, the known isolates related to the outbreak should have the same subgroup, while the isolates not related to the outbreak should have different subgroups. to be Finally, typing technology should be selected with fast typing speed, reasonable cost, simple operation technology, and convenience of laboratory operation in order to minimize epidemics caused by food pathogens (Newell *et al.,* 2010; Gould *et al.,* 2013).

Today, consumers are not only eager for safe and nutritious food products, but also demand that the food they consume should be healthy and natural. The functional food market has grown rapidly in the past decade. The United States of America, Northern Europe and Central European countries are the future markets of the world, as consumers are becoming interested and understanding the idea behind such foods (Chin *et al.,* 2000). Sausages and sausages are among the most common and famous meat food products that are prepared with the main base of meat (chicken/beef). These foods are called Ready-to-Eat (RTE) RTE food products provide a source of accessible and nutritious meals to the consumer (Thienhirun & Chung, 2018).

Ready-to-eat foods refers to a group of foods that are raw, partially or fully cooked, cold or frozen and do not undergo a heat treatment and preparation process before consumption (Rocourt *et al.,* 2003; Razeh *et al.,* 2022). The United States Food and Drug Administration defines ready-to-eat foods as foods that are already cooked and do not need to be cooked and are kept in the kitchen or refrigerator until consumption. Also, the United States Public Health Service defines ready-to-eat foods as foods that are used without additional washing, cooking, and preparation (Safari & Saeidi Asl, 2011). The Codex Committee of the World Health Organization has classified this group of foods in the group of high-risk foods; because they are the most susceptible food group for the growth of microbes that cause food poisoning (McCarthy & Burkhardt, 2012). On the other hand, this group of foods is considered as an important source of intestinal diseases all over the world, which itself can be caused by the lack or absence of written and effective health guidelines in this field. Among the best-selling ready-to-eat foods in Iran, we can mention all kinds of strudel, pizza, sausages, hamburgerd, cold sandwiches, salads and other meat products; Therefore, the most important microorganisms that can enter food and endanger the health of consumers include: *Staphylococcus aureus*, *Escherichia coli* (*E. coli*), *Salmonella*, Clostridium, fecal coliforms and other pathogenic microorganisms. (Zwietering *et al.,* 2015, Holck *et al.,* 2017, Eslami *et al.,* 2017). Staphylococci are everywhere in nature. *Staphylococcus aureus* is the most important species in this group; because some strains are capable of causing human food poisoning. The primary reservoir is on the skin and mucous membranes of mammals and birds. *Staphylococcus aureus* is often isolated from minced meat. Enterotoxigenic strains of *Staphylococcus aureus* in minced meat can grow enough to produce enterotoxin before consuming a toxic dose. Enterotoxin is heat stable and is not destroyed by heat treatment. Due to the thermal stability of enterotoxin, the level of meat contamination must be kept as low as possible during the production process. The primary contamination of meat occurs during slaughter. Hygiene deficiencies cannot be compensated for even by the strictest hygiene measures during the subsequent production process. Microbiological hygiene measures in meat production are carried out with the aim of protecting

the consumer against pathogenic agents. To prevent contamination of meat with *Staphylococcus aureus*, the sources of *Staphylococcus aureus* should be identified and well known (Kadariya *et al.,* 2014).

E. coli is one of the most common causes of several common bacterial infections in humans and animals. *E. coli* is a prominent cause of gastroenteritis, urinary tract infection, septicemia and other clinical infections such as neonatal meningitis. *E. coli* is also prominently associated with diarrhea in pets and domesticated warmbloods. The treatment of *E. coli* infections is threatened by the emergence of antimicrobial resistance. The prevalence of multidrug-resistant *E. coli* strains is increasing worldwide, mainly due to the spread of mobile genetic elements such as plasmids. An increase in multidrug-resistant strains of *E. coli* is also occurring in Europe. Therefore, the spread of resistance in *E. coli* is a growing public health concern in European countries. *E.coli* O157H7 is the most important strain of *E. coli*, which is on the rise with emerging events and different symptoms (Heidarzadi *et al.,* 2021).

The genus *Salmonella*, a member of the Enterobacteriaceae family, includes rod-shaped, motile gram-negative organisms. The cells are oxidase negative and catalase positive, produce gas from diglucose and use citrate as the only carbon source. *Salmonella* have several endotoxins: antigens include O, H and Vi. *Salmonella enterica* and *Salmonella enteritidis* are the most common serovars isolated from humans worldwide (Heidarzadi *et al.,* 2021); Considering the risks mentioned, the purpose of this study is to evaluate the contamination of *Staphylococcus aureus*, *E. coli* and *Salmonella* in sausages and bologna sold in Tehran, Iran.

Materials and Methods

Sampling method

In order to test, 100 sausages and bologna, including 50 meat sausages and 50 meat bologna, were randomly sampled from supply centers in Tehran. The samples were transferred to the food quality control Shahrekord laboratory of Islamic Azad University for testing.

Escherichia coli search method

Twenty-five grams of the samples were weighed and placed in 225 ml of lactose broth (Merck, Germany) for 24 hours at 37°C. 1 ml of the enriched sample medium was cultured on EMB Agar culture medium (MirMedia, Iran) and after 24 hours of incubation, the colonies with metallic green polish were selected and for confirmation in the differential culture medium including Simon Citrate (Merck, Germany), MR_VP, TSI and SIM were cultured and their positive samples were identified (Heidarzadi *et al.,* 2021).

Salmonella detection method

First, 25 grams of the samples was mixed with 225 cc of lactose broth culture medium and kept at 37°C for 24 hours. Then one mml of the enriched sample was transferred to 10 ml of selenite-cystine (Italy, liofilchem) and 1 ml to 10 ml of tetrathionate broth (Italy, liofilchem). After 24 hours of incubation, selenite-cystine medium was cultured linearly on Salmonella-Shigella agar, bismuth sulfite agar, and brilliant green agar (Italy, liofilchem). In the same way, tetrathionate was cultured on the mentioned media. Then, after 24 hours, two or more of the typical plots were transferred to TSI and LIA media (Italy, liofilchem) and the results were interpreted according to the standard guidelines (Heidarzadi et al., 2021).

The method of searching for *Staphylococcus aureus*

To isolate *Staphylococcus aureus*, 5 grams of samples were transferred into a sterile weighing container, and then 45 ml of Ringer's solution was added to it as a solvent to obtain a 10^{-1} dilution. After dissolving the samples and creating a homogeneous solution, 0.5 ml of it was cultured using a sampler on Parker-Baird Agar medium by the surface culture method. The cultivated plates were kept at 37°C for 24 hours.

After the end of the incubation, in case of growth, bacteria with round and black colonies were cultured from the suspicious colonies on the mannitol salt agar medium by means of a sterile loop for confirmation culture. The environments were again placed in the incubator at a temperature of 37°C for 24 hours and after 2 hours Dnase test was performed on the positive mannitol colonies (yellow colonies with a yellow halo) to confirm *Staphylococcus aureus*. Also, the desired bacteria were evaluated with coagulase test, and the result of this test was positive for *Staphylococcus aureus* (Pishadast *et al.,* 2021).

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Statistical analysis

SPSS version 22 software was used for statistical analysis. The one-way variance test was used to compare the average data and the significance level was considered P<0.05.

Results

Table 1 shows the amount of contamination in sausages and bologna sold in Tehran city. According to statistical analysis, the level of contamination in sausages and bologna in the present study was higher than the national standard of Iran. In the studied sausages, out of 50 samples, 14 samples (14%) were contaminated with *E. coli*, *Salmonella*, and *Staphylococcus aureus*, respectively 2, 0, and 26%.

Statistical analysis showed that there is no significant relationship between the type of meat product and the prevalence of microorganisms (P>0.05). Also, statistical analysis showed that in the studied bologna, 20 samples (20%) were contaminated with pathogenic microorganisms, of which 4, 0, and 36 samples were contaminated with *E. coli*, *Salmonella*, and *Staphylococcus aureus*, respectively. Statistical analysis showed that there is no significant relationship between the meat product and the prevalence of microorganisms (P>0.05).

Type	*E. coli*	*Salmonella*	*Staphylococcus aureus*	Total
Sausage	Contamination: 1 sample (2%)	Contamination: 0 sample	Contamination: 13 sample (26%)	Contamination: 14 sample (14%)
	No contamination: 49 samples (98%)	No contamination: 50 samples (100%)	No contamination: 37 samples (74%)	No contamination: 86 samples (86%)
Bologna	Contamination: 2 sample (4%)	Contamination: 0 sample	Contamination: 18 sample (36%)	Contamination: 20 sample (20%)
	No contamination: 48 samples (96%)	No contamination: 50 samples (100%)	No contamination: 32 samples (32%)	No contamination: 80 samples (80%)

Table 1. The amount of contamination in sausage and bologna products.

Discussion

According to the results obtained from the present study, the reason for the high level of contamination of the studied bologna compared to sausages is the high level of moisture in bologna, because high humidity in food stimulates the growth of pathogenic microorganisms, especially *Staphylococcus aureus*. It may also indicate improper formulation in bologna preparation. A lot of salt and phosphate are added to meat products, one of the reasons for which is the reduction of Aw and the opening of actin and myosin filaments and the increase of water retention, which may be the reason for the growth of *Staphylococcus aureus* due to the lack of salt in the standard amount. In the current study, the prevalence of the evaluated pathogenic microorganisms such as *Staphylococcus aureus*, *Salmonella* and *E. coli* was higher than the national standard of Iran, which indicates a weakness in the preparation and possible distribution of the product. The microbiological quality of RTE street food products in Johannesburg, South Africa was investigated by Chiou et al. In this study, 51 samples were taken to determine the microbiological quality. *Bacillus cereus* was observed in 22%, *Clostridium perfringens* in 16%, *Salmonella* species in 2% and *E. coli* in 2% of the samples (Chiou *et al.,* 1996), Which is consistent with the present study regarding the prevalence of *E. coli*. The study of Güngör and Gökoğlu in 1997 on the contamination of meat products showed that the total number of aerobic mesophilic bacteria, *Staphylococcus aureus*, *E. coli*, yeasts and molds in minced meat was 7.02, 3.83, 4.42 and 1.62 log cfu/g, respectively. The number of *E. coli* and yeast-mold in sausage dough reached 3.99 and 1.72 log cfu/g, respectively (Güngör & Gökoğlu, 2010), which is consistent with the results of this study regarding *Staphylococcus aureus* and *E. coli* contamination.

Arthur et al' study on *Salmonella* and *E. coli* contamination of meat products found that *E. coli* O157:H7 was detected in 60% of samples from each discharge area, while *Salmonella* was detected in more than 70% of Samples were identified from each laboratory area (Arthur *et al.,* 2008); While in the present study, there was no *Salmonella* contamination in any of the samples, *E. coli* was also found in 3 samples (3%), which is highly different from these results. In 2004, in the United States, Rivera-Betancourt et al, found that the contamination rate of *E. coli* and *Salmonella* in meat products was 2% and

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2%, respectively (Rivera-Betancourt *et al.,* 2004). In the present study, the rate of contamination with *Salmonella* was zero and the rate of contamination with *E. coli* was observed in 3 samples, which is somewhat consistent with the results obtained from the present study in terms of the prevalence of *E. coli*.

A study in Nigeria by Adeyanju et al, in 2004 on meat products for pathogenic microorganisms reported that *Salmonella* contamination of 99 samples of poultry products (53 chickens and 46 turkeys) obtained from retail markets was at 33% (chicken 32.1% (17/53) and turkey 34.8% (16/46)), while *E. coli* was at 43.4% (chicken 47.2% (25/53) and turkey 39.1% (18/46)). From the processing plant, twelve isolates of *Salmonella* were obtained and the prevalence rate was 22.6% and three *E. coli* isolates were obtained at 5.7% (Adeyanju & Ishola, 2014). In the current study, the type of meat products, sausages and bologna was 40%, which is not aligned with the results of the above study regarding *salmonella*, but it is somewhat consistent regarding *E. coli*.

The results of Sadat-Karimi et al, studies in 2017 on the microbial contamination of chicken dough found that 38% of the 100 samples were positive in the coagulase-positive *Staphylococcus* count factor, 24% in the *E. coli* count factor, and 18% in the total microorganism count factor. (Sadat-Karimi *et al.,* 2010), which is consistent with the results of this study regarding *Staphylococcus aureus* infection and does not agree with *E. coli*. In a study on microbial contamination in sausages and bologna sold in Kermanshah city, Sadeghi et al, reported that the microbial contamination of the samples was 100% mold and yeast, 58% coliform, 53% *staphylococcus*, 17% *E. coli* and 3% was *salmonella*. All microorganisms were present in 3% of the collected samples (Sadeghi *et al.,* 2013), in the present study, the total contamination rate for bologna was 20% and 14% for sausages.

Gibbons et al, study on RTE food samples and ready-to-eat meat products sold in Thailand reported that cooked products and raw products were sampled at the same time, and as a result, in the product the ready-to-use products were negative for the 4 tested pathogens *Listeria*, *Salmonella*, *Campylobacter* and *E. coli*. But in raw products, a different report was presented. In raw products, 54% *E. coli*, 36.4% *Listeria* and 58% *Campylobacter* were identified in the samples (Gibbons *et al.,* 2006). Regarding the contamination of RTE products, it is somewhat consistent with the results of this research. Paton et al, study on the contamination of sausages reported that 19 out of 21 cases were contaminated with enterohemorrhagic *E. coli* (Paton *et al.,* 1996), which is very different from the results of the above study.

A study entitled the antibiotic sensitivity of *Staphylococcus aureus* isolated from sausages in Meknes Province, Morocco was conducted by Ed-Dra et al, who reported that out of a total of 156 sausage samples, *Staphylococcus aureus* was found in 63 samples (40.38%). In addition, the antimicrobial resistance study showed that 84.13% of the isolated *staphylococcus aureus* were resistant to streptomycin, 76.20% to tetracycline, 42.86% to ampicillin, 41.27% to doxycycline, 38.1% to penichlor and 5.5% to penicillin (Ed-Dra *et al.,* 2018). The contamination rate of *Staphylococcus aureus* for the meat products of the present study was 31%, which is lower than the results of the mentioned study.

Hachemi et al' study on the epidemiological situation of sausage in Algeria, evaluation of the quality and antibiotic resistance of *Staphylococcus aureus* isolates and risk factors related to consumer habits on food poisoning showed that the overall prevalence of *Staphylococcus aureus* contamination from sausages and bologna was 22.25%. More than 83.33% of the strains showed resistance to at least one of the tested antibiotics. The most important of them was for tetracycline (58%), followed by fosfomycin (33%), penicillin G (25%), and oxacillin (36%). Out of 440 meat consumers, 22.16% got food poisoning after consuming sausage (Hachemi *et al.,* 2019), which is consistent with the present study from the point of view of abundance of *Staphylococcus aureus*.

Cabedo's study on RTE foods is as follows: To assess the prevalence of these pathogens in RTE food, 140 samples of RTE fish products, 501 samples of RTE meat products, 462 samples of RTE dairy products and 123 samples of RTE food and desserts, totaling 1226 samples of Retail stores and food industries were collected. A total of 1379 samples including 187 RTE fish products and 569 RTE meat products, 484 RTE dairy products and 139 RTE food and desserts were collected and analyzed for the presence of *Salmonella*. *Salmonella* was isolated from 1.2% of smoked salmon samples, 1.5% of frozen chicken croquettes, 2% of cooked ham samples and

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11.1% of dried sausage samples (Cabedo *et al.,* 2008), which in the present study no *salmonella* was found.

The study of Ghazi was conducted with the aim of investigating intestinal bacterial contamination in imported red meat and imported sausage. This study was conducted on 40 samples of imported meat and 30 samples of sausages. The samples were collected from different areas of Basra city, Iraq. Bacterial contamination of imported products (red meat, sausages, and bologna) was investigated. In addition, total bacterial counts were performed for the samples and compared with specific control rates. Extracts of meat samples were cultured in different growth and diagnostic environments in order to screen for bacterial contamination, which is mainly provided by coliform bacteria, *Klebsiella pneumoniae* and Proteus species. The results of the present study showed a high level of bacterial content in imported red meat and imported sausage, in which the number of coliform, *Klebsiella pneumoniae* and *Proteus bacteria* exceeds the limit set by the Iraqi Central Quality and Standardization (Ghazi, 2019), which is The frequency of the standard is consistent with the results of the present study.

Conclusion

Foodborne diseases are a major problem that mainly affects people in poor communities. Microbial contamination of food affects the lives of people in developing countries and has a high mortality rate. Many types of microorganisms or their toxins play a role in causing foodborne diseases with different mechanisms. The results of this study showed that the most pathogenic organisms were *Staphylococcus aureus* and *E. coli*, respectively in sausages and bologna sold in Tehran city, which was higher than the national standard of Iran. Therefore, according to the dangers that pathogenic microorganisms cause to humans, it is recommended to minimize the use of sausages and bologna, and if they are used, they should be well cooked in heat treatments to minimize the risk of microbial contamination.

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Conflict of interest

There is no conflict of interest between the authors.

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ارزیابی آلودگی به *استافیلوکوکوس اورئوس*، *اشریشیاکلی* و *سالمونلا* در سوسیس و کالباس‌های عرضه شده در شهرستان تهران، ایران

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چکیده

زمینه و هدف: سوسیس و کالباس جزو فرآورده‌های حرارت دیده محسوب می شوند که پایه و اساس ترکیبات شیمیایی آن‌ها گوشت قرمز و مرغ می‌باشد. این فرآورده ها جزو غذاهای آماده مصرف (Ready-to-Eat; RTE) محسوب می شوند و به دلیل اینکه در برخی از آن‌ها خمیر مرغ یا خمیر گوشت به کار می رود به مراتب مستعد آلودگی های باکتریایی هستند، لذا هدف از مطالعه حاضر ارزیابی آلودگی به *استافیلوکوکوس اورئوس*، *اشریشیاکلی* و *سالمونلا* در سوسیس و کالباس های عرضه شده در شهرستان تهران بود.

مواد و روش‌ها: تعداد ۱۰۰ نمونه شامل ۵۰ نمونه سوسیس و ۵۰ نمونه کالباس گوشتی را از مراکز عرضه به صورت تصادفی ساده نمونه گیری شد و به آزمایشگاه بهداشت و کنترل کیفی مواد غذایی منتقل گردید. از آزمون های مختلف کشت میکروبی برای جستجوی میکروارگانیسم های هدف استفاده شد. تمامی آزمایش ها در شرایط استریل انجام گرفت.

یافته‌ها: نتایج نشان داد که میزان آلودگی در سوسیس و کالباس های مطالعه حاضر از استاندارد ملی ایران بالاتر بود. در سوسیس های مورد مطالعه از ۵۰ نمونه ۱۴ نمونه و در کالباس ها از ۵۰ نمونه ۲۰ نمونه آلودگی به میکروارگانیسم های *اشریشیاکلای*، *سالمونلا* و *استافیلوکوکوس اورئوس* یافت شد.

نتیجه‌گیری: با توجه به مخاطراتی که میکروارگانیسم های پاتوژن برای انسان به وجود می آورند، توصیه می شود استفاده از سوسیس و کالباس به حداقل رسانده شود و در صورت مصرف، به خوبی در تیمارهای حرارتی پخت قرار گیرد تا ریسک آلودگی های میکروبی به حداقل برسد.

واژه های کلیدی: *سوسیس و کالباس*، *اشریشیاکلی*، *سالمونلا*، *استافیلوکوکوس اورئوس*، *تهران*

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