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Evaluation of microbial contamination of ready-to-eat foods (pizza, frankfurters, sausages) in the city of Ilam

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Abstract

Background: Today in the world, disease resulting from food is considered one of the most important problems in public health. This study aimed to determine the bacterial contamination of ready-to-eat foods, i.e. fast food, in Ilam city.

Methods: In this cross-sectional, analytical study, 270 samples of ready-to-eat food, including pizza, frankfurters, and sausages, were randomly collected and tested for contamination with *Staphylococcus aureus, Escherichia coli, Shigella sonnei, Salmonella arizonae*, and *Enterococcus faecalis*. After examination, the collected data was analyzed using SPSS 20 software and logistic regression.

Results: From a total of 270 samples of ready-to-eat food, 27.77% was contaminated with E. coli, 21.48% with *S. aureus*, 13.33% with *S. sonnei*, 14.44% with *S. arizonae,* and 5.9% with *E. faecalis.* The results showed higher rates of *E. coli* and *S. aureus* contamination in pizza, frankfurters, and sausages. Also, a higher percentage of frankfurters were contaminated with microbial species than pizza or sausages. There were significant differences in microbial contamination rates ($P<0.05$) among the three groups of food. In addition, factors such as indicators (health, sanitation, and lack of hygiene), age, gender, and education level of the operating staff had no effect on the results.

Conclusion: Based on the results, it can be concluded that bacterial contamination of ready-to-eat foods is significantly high in the city of Ilam; therefore, it is suggested that the examination of food in various stages of production and distribution can help reduce bacterial contamination, and training for the operators of shopping centers' ready-to-eat food shops and controlling pathogens are essential.

Keywords: Microbial contamination, Food, Pizza, Frankfurters, Sausages

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Introduction

Foodborne diseases are a major problem affecting people in poor societies (1). Microbial food contamination affects the life of people in developing countries and has a high mortality rate (2). A large variety of micro-organisms or their toxins with different mechanisms are involved in causing foodborne illness (3,4). It is estimated that 30% of people in industrialized countries suffer from diseases caused by food at least once a year (5).National health agencies have reported that the average prevalence of foodborne diseases in the EU and third world countries is 3.38 and 8.915 cases per 100 000 people respectively (6-8). The annual cost of foodborne diseases, including direct medical costs and reduced efficiency, is $5-6 billion (9). Most cases of disease are caused by bacteria, viruses, or parasites (10). The main foodborne pathogens that can contaminate food are *Salmonella* spp., *S. aureus*, *Clostridium botulinum*, *Bacillus* spp., *Acinetobacter* spp., *Escherichia coli*, *Pseudomonas* spp., and the hepatitis A virus. Intestinal parasites include *Giardia lamblia*, *Entamoeba hystolytica*, *Ascaris lumbricoides,* and *Hookworm* (11,12). From 1990 to date, several major groups of foodborne bacteria (*E. coli*, *S. aureus*, *S. arizonae*, and *E. faecalis*), have been considered in a lot of research and have attracted the attention of the food industry (13). The incidence of food-based diseases

in developed countries has had a growing trend. In developing countries such as Iran, although there are no statistics on the incidence of infections and food poisoning, infection and food poisoning occurs far more often than in developed countries because of poor conditions of production, storage, and distribution and due to the low level of health education (14). In a microbiological study, Christison et al examined 4 food distribution centers in South Africa for contamination and reported that 16% and 4% were contaminated with *Salmonella* spp. and *Listeria,* respectively (15). In a 2006 study of bacterial contamination in foods conducted in centers affiliated with Baqiyatallah University, contamination rates of *E. coli* and *Staph* were reported as 12.5% and 13.8%, respectively (16). In a 2000 study of contamination in vegetables, dairy products, and ice cream conducted in Kashan, *E. coli* infection was reported in 15% of samples (17). In a survey conducted on the bacterial contamination of raw milk in Shahre Kord, 70% of samples were contaminated with *E. coli* and 80% with coliform (18). Generally, various studies have been conducted in the field of bacterial contamination of food. In most of these studies, a specific type of bacteria in a food group was studied; therefore, a lot of information on a range of food is not available. The results of studies conducted in Iran show that the quality of ready-to-eat foods is not desirable, and the microbial contamination of these foods is higher than the standard (19,20). A lack of attention to health issues at all stages of pizza and sandwich production decreases the quality of food products. Therefore, to protect the health of consumers, this study aimed to determine the microbial quality of pizza, frankfurters, and sausages in the distribution centers of these products in the city of Ilam.

Materials and Methods

Field study

In this cross-sectional study, a wide range of bacteria (*S. aureus*, *S. sonnei*, *S. arizonae, E. coli* and *E. faecalis*) was evaluated in three groups of foods, pizza (90 samples), frankfurters (90 samples), and sausages (90 samples), in the city of Ilam. Different diagnostic tests were done on each sample. A total of 270 food samples were randomly collected by health experts from fast food within 3 months during 2015 in the city of Ilam. Under sterile conditions, the samples were sent to the microbiology laboratory of the Environmental Health Department where they were refrigerated. Experiments were performed less than 24 hours later. The experiments, designed to determine contamination in food products and identify and confirm the presence of bacteria, were performed according to the Iranian national standard 2461-1 method (21).

Bacterial identification

To identify and enumerate *S. aureus,* the surface culture method for *Staphylococcu*s in a specific medium of Baird Parker agar was used. Black colonies that were colorless and encircled with a clear halo were counted using the coagulase test (22). To identify *E. coli,* the Iranian national standard 2946 method was used. In this technique, after preparation, the sample was cultured in brilliant green broth and peptone water. After 24 hours of incubation, a few drops of Kovacs indole reagent were added to the tube with a peptone water medium. If a red circle formed on the top of the tube, the sample was considered positive for contamination with *E. coli*. Each diagnosis was confirmed by IMVIC tests (23). To detect *Salmonella*, after enrichment in non-selective and selective media such as lactose broth and Tetrathionate broth, the samples were cultured linearly in a selective solid medium of Brilliant Green agar (24). Then, the suspect colonies were transferred to a Triple Sugar Iron agar (TSI) differential medium and investigated for the presence or absence of Salmonella (24). To detect and isolate *Shigella*, after enrichment, the samples in the enrichment medium were used for Xylose lysine deoxycholate agar (XLD) differential culture media (2627) (25). To isolate the bacteria *Enterococcus* the selective medium of Kanamycin aesculin azide (KAA) Agar (Merck, Germany) was used. In this medium kanamycin was the selective agent. *Enterococcus* was determined using esculin hydrolyze, the fermentation of glucose and hydrolysis of esculin, which, in the presence of ferric citrate creates a brown color. Then, from the colonies grown, a few colonies were selected and cultured again on the same specific medium (26). To evaluate the contamination of the three studied groups of food, other factors such as indicators (health, sanitation, lack of hygiene), age, gender, and education level of operators in food supply centers were evaluated using a questionnaire.

Statistical analyses

The results of tests on various food groups were analyzed using statistical software SPSS 20 and regression test, and a level of 5% ($P<0.05$) was considered significant. To comply with ethical issues in research, the names of the food supply centers sampled in this study have been withheld.

Results

In this study, contamination with *E. coli* and *S. aureus* was greater in the three studied food groups (pizza, frankfurters, sausages) than other microbial species. From the 270 food samples tested, 75 samples (27.77%), were contaminated with *E. coli*, 58 samples (21.48%) with *S. aureus*, 39 samples (14.44%) with *S. arizonae*, 36 samples (13.33%) with *S. sonnei*, and 16 samples (5.9%) with *E. faecalis*. From total samples of pizza, 12.22% were infected with *S. aureus*, 5.55% to *S. sonnei* 8.88% to *S. arizonae* 23.33% to *E. coli* and 1.11% to *E. faecalis* (Table1).

The number and percentage of frankfurters contaminated with the microbial species are shown in Table 2.

From all frankfurter samples, 27.77% were contaminated with *S. aureus*, 17.77% with *S. sonnei*, 24.44% with *S. arizonae*, 31.11% with *E. coli,* and 8.88% with *E. faecalis*. The number and percentage of sausages contaminated

Table 1. The frequency of bacteria contamination in pizza

Bacteria	Positive cases		Negative cases	
	Number	Percent	Number	Percent
S. aureus	11	12.22	79	87.78
S. sonnei	5	5.55	85	94.45
S. arizonae	8	8.88	82	91.12
E. coli	21	23.33	69	76.67
E. faecalis	1	1.11	89	98.89

Table 2. The frequency of bacteria contamination in frankfurter

Bacteria	Positive cases		Negative cases	
	Number	Percent	Number	Percent
S. aureus	25	27.77	65	72.23
S. sonnei	16	17.77	74	82.23
S. arizonae	22	24.44	68	75.56
E. coli	28	31.11	62	68.89
E. faecalis	8	8.88	82	91.12

Table 3. The frequency of bacteria contamination in sausages

Bacteria	Positive cases		Negative cases	
	Number	Percent	Number	Percent
S. aureus	22	24.44	68	75.56
S. sonnei	15	16.66	75	83.34
S. arizonae	19	21.11	71	78.89
E. coli	26	28.88	64	71.12
E. faecalis	7	7.77	83	92.23

with the microbial species are shown in Table 3.

From the total samples of sausage, 24.44% was contaminated with *S. aureus*, 16.66% to *S. sonnei*, 21.11% to *S. arizonae*, 28.88% to *E. coli* and 7.77% to *E. faecalis*. The results showed that the percentage of contamination with microbial species was higher in frankfurters than in pizza or sausages. The high contamination of frankfurters (greater than pizza or sausages) is reasonable. In relation to the contamination in the three groups of food with *S. sonnei*, and *S. arizonae*, there is a significant difference; however, the *E. coli* and E. *faecalis* contamination rates were not significant ($P>0.05$). The chance of becoming contaminated with *S. aureus*, *S. sonnei*, *S. arizonae*, *E. coli*, and *E. faecalis* was higher for frankfurters and sausages than for pizza (Table 4).

Also, the chances of contamination in three types of ready-for-consumption food were adjusted for the impact of factors such as location, age, gender, and education level, and it was found that these factors did not influence the outcome (Table 5).

Discussion

The food industry and available technologies and the preparation, distribution, and sale of food (including raw materials or ready-to-eat foods) in health centers are both important issues and require special attention (18). Because of people's interest in using ready-to-eat foods and fast food such as pizza and meat products (sausages, frankfurters) to resolve a lack of protein and since such meat products are cheaper than pure meat, their use in different countries has increased several times in the past decade (14). Due to the importance of contamination of ready-to-eat foods and fast food, pathogenic microbes, and their role in causing human infection, this study examined 270 samples from 3 groups of food (pizza, frankfurters, and sausages). The results showed that among the three groups of foods tested, frankfurters and pizza, respectively, have the highest and lowest bacterial contamination. Contamination was greater with *E. coli* and *S. aureus* in the three food studied groups than with the other microbial species. Also, the percentages of contamination with *E. faecalis* in pizza, frankfurters, and sausages are low. It is noteworthy that among the three

Table 4. Univariate logistic regression analysis of Fast food contamination by a variety of bacteria in Ilam

Bacteria	Univariable			
	Fast food	*P*	Odd ratio	95% CI for OR
S. aureus	Pizza	0.033	-	-
	Frankfurter	0.011	2.762	1.264–6.024
	Sausages	0.037	2.325	1.051–5.128
S. sonnei	Pizza	0.041	-	-
	Frankfurter	0.015	3.676	1.283–10.526
	Sausages	0.023	3.40	1.179–9.803
S. arizonae	Pizza	0.023	-	-
	Frankfurter	0.007	3.311	1.388–7.936
	Sausages	0.025	2.739	1.200–6.666
E. coli	Pizza	0.487	-	-
	Frankfurter	0.272	1.483	0.765–2.873
	Sausages	0.397	1.333	0.684–2.604
E. faecalis	Pizza	0.128	-	-
	Frankfurter	0.044	8.695	1.062–71.428
	Sausages	0.062	7.518	0.904–62.5

Table 5. Multivariable logistic regression analysis of Fast food contamination by a variety of bacteria in Ilam

Bacteria	Multivariable			
	Fast food	*P*	Odd ratio	95% CI for OR
S. aureus	Pizza	0.029	-	-
	Frankfurter	0.009	2.873	1.295–6.369
	Sausages	0.034	2.398	0.186–1.069
S. sonnei	Pizza	0.037	-	-
	Frankfurter	0.014	3.787	1.310–10.989
	Sausages	0.022	3.496	1.200–10.204
S. arizonae	Pizza	0.021	-	-
	Frankfurter	0.007	3.378	1.406–8.130
	Sausages	0.024	2.785	1.141–6.802
E. coli	Pizza	0.464	-	-
	Frankfurter	0.226	1.526	0.769–3.030
	Sausages	0.381	1.362	0.682–2.659
E. faecalis	Pizza	0.114	-	-
	Frankfurter	0.038	9.433	1.127–76.923
	Sausages	0.056	8.064	0.948–66.666

groups of foods a significant difference was observed in contamination with the microbial species. In addition, as shown in Table 4, the contamination of frankfurters and sausages by the microbial species is greater than that of pizza. It is noteworthy that factors such as indicators (health, sanitation, lack of hygiene), age, gender, and education level did not impact the outcome. In studies conducted in Iran and other countries, hygiene and food contamination by pathogenic bacteria has been evaluated. In a study conducted by Mirzabeygi et al in 2006 in western Tehran, the levels of contamination with *S. aureus* in dairy products and sweetmeats were reported as 16% (27). In a study by Sultan Dallal et al in 2007 surveying the microbiological quality of chicken and red meat, 47.8% of chicken samples and 28.8% of meat samples were contaminated with *Salmonella,* which is less than *S. arizonae* contamination in the present study (28). A study by Tavakoli et al was carried out in 4 restaurants affiliated with a military facility in Tehran. A total of 288 samples of ready-to-eat food and salad were examined. In 5 samples (1.73%), *E. coli* was found. The samples did not confirm *Salmonella* contamination. Also, the mean number of coliforms and *S. aureus* in some foods was found to be more than the standard. However, in this study, the rates of contamination with *E. coli* and *S. arizonae* were higher (29). In a study by Faramarzi et al, 642 food samples (58.33% salads and 9.84% dairy products) were found to be contaminated with *E. coli*, and 4.8% of sweetmeats were contaminated with *S. aureus*, which is consistent with the current study (30). Aycicek et al examined the rate of *S. aureus* contamination of ready-to-eat salads and foods in restaurants at military centers in Ankara, Turkey. They tested 512 samples of salads, pizzas, and a variety of meat foods. Their results indicated that 48 samples were contaminated with *S. aureus*, and in meat foods and salads, *S. aureus* contamination was significantly higher than in the other samples, which is consistent with the current study (31). In a study performed by Meldrum et al conducted in the United Kingdom on 1213 salad and 1208 sauce samples, results showed that 4.7% of salad samples and 5% of sauce samples were of lower than acceptable quality, and the presence of microbial *E. coli* and *S. aureus* were confirmed in them (32). In the current study, 27.77% of food samples were contaminated with *E. coli*. In the study of Sagoo et al, 3% of salad samples had confirmed contamination with *E. coli* (33). The presence of bacterial species (*S. aureus, S. sonnei, S. arizonae, E. coli, and E. faecalis*) in the three food groups studied indicates the lack of sufficient attention paid to food hygiene standards in their procurement centers. This may be due to the staff's lack of personal hygiene, contamination of raw materials, or secondary contamination. *E. coli* and *S aureus* contamination in the studied samples is a serious warning to health officials. *S aureus* in raw food is not a good competitor for other bacteria, but in cooked foods in which other microorganisms are destroyed, it is easily grown and creates contamination (34). The tendency of a lot of people to use ready-to-eat foods, the lack of proper observance of environmental health and food processing equipment, the improper washing of hands and the long-term contact of these hands with food are the most important causes of food contamination and disease. The results of this study can be used for operators of fast food centers and also for healthcare providers in health centers. Making the staff of fast food centers aware of the dangers of these bacteria can allow them to act to reduce the bacterial contamination of ready-to-eat foods. The implementation of an HACCP system, another standard certification, and the training of operators at fast food centers can be very effective in preventing or reducing bacterial contamination. Also, a complementary study of bacterial contamination in food preparation, dining room, utensils and equipment used in processing and cooking and personal hygiene operators can be useful.

Conclusion

Based on the results of the current study, the prevalence of bacterial contamination of ready-to-eat foods can have an important role in infection and food poisoning. The spread of foodborne diseases, problems in the field of food hygiene and its importance in health and economics, a lack of awareness, and a lack of respect for the basic principles of health could be the main reasons for contamination and could endanger public health. A lot of people tend to consume fast food, and the bacterial contamination of these foods is a serious problem that needs further investigation. Training for personnel working in fast food preparation centers on the proper observance of health issues, and the overseeing of the preparation, transportation, storage, and supply of ready-to-eat foods seems necessary to prevent the transmission of microbial contamination. The amount of contamination in food must be reduced by taking appropriate actions to ensure the health of the community.

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Ethical issues

The authors certify that all data collected during the study is presented in this manuscript, and no data from the study has been or will be published separately.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MA conceived and designed the study. MTY, MD, MM, FS, and ZG performed the literature search and wrote the manuscript. All authors participated in data acquisition, analysis, and interpretation. All authors critically

reviewed, refined, and approved the manuscript.

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