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Performance assessment of food safety management system in meat processing plants and local market of Tehran: A microbial source tracking study

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ABSTRACT

The implementation of Food Safety Management Systems (FSMS) is crucial for preventing foodborne diseases. This study aimed to assess the performance of FSMS by detecting coliforms and *Escherichia coli* as indicators of foodborne pathogens in hamburger samples and evaluating the main sources of contamination in the final products. Three meat processing plants (A, B, and C) that implemented FSMS were evaluated based on prerequisite program (PRP) parameters in an observational study. A total of 107 samples were collected from raw materials, food handlers' hands, contact surfaces of food processing equipment, and products from the three plants. Additionally, 45 hamburgers were purchased from local markets in Tehran. Polymerase chain reaction analysis was conducted on the positive samples to confirm the presence of *E. coli* O157:H7. The data were described using frequency (percentage) and mean (standard deviation), and a significance level of 5% was considered. Results showed that approximately 38% (41.107) of samples from the three plants were contaminated with coliforms and *E. coli*, with only one sample contaminated with *E. coli* O157:H7 from raw meat in plant C. Moreover, 80% (36.45) of hamburger samples collected from local markets contained coliforms lower than 10^2 CFU/g and E. coli lower than 10 CFU/g, with no contamination of *E. coli* O157:H7. The study found significant differences in the number of coliforms and *E. coli* among the three factories ($p<0.05$), with factory C having the highest and factory B having the lowest values. Implementation of FSMS in the food chain resulted in reduced microbial contamination. The study concluded that there is no safety concern regarding *E. coli* O157:H7 contamination in hamburgers marketed in Tehran.

1. Introduction

Foodborne diseases pose a significant public health concern globally, affecting both developed and developing countries. It is estimated that 420,000 people die each year as a result of consuming contaminated foods (1). This issue becomes more critical when it involves the consumption of nutritious foods containing meat, which provides an ideal environment for the growth of various microorganisms, including *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, and *Shigella* (2, 3). The consumption of ready-to-eat foods like hamburgers, lamb burgers, bacon burgers, and beef burgers as cost-effective alternatives to meat is increasing worldwide (4, 5). In Iran, hamburgers, defined as products containing 30-95% minced meat and categorized into three types, are subject to specific regulations (6). Besides meat, hamburgers contain several ingredients that must be monitored to prevent contamination during processing. Microbial examinations are necessary to

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ensure the safety of meat-based products for human consumption (7). Coliforms, which belong to the Enterobacteriaceae family, are part of the normal flora of domestic animals and are challenging to eliminate from animal-based products. This group of microorganisms includes various strains of *E. coli*, some of which are invasive, such as O157:H7, and pose a high risk. Therefore, monitoring the presence of coliforms in animal-based products is essential. Coliforms are susceptible to high temperatures and sanitation measures in food production systems. As a nutritious food, Hamburgers provide a suitable medium for microbial enumeration. However, the thermal processing of ready-to-eat hamburgers eliminates viable thermos-sensitive microorganisms that can cause severe infections in consumers (8). International standards do not require regular monitoring of coliforms in hamburgers. Consequently, the main objective of this study was to evaluate the performance of FSMS by detecting coliforms and Escherichia coli as indicators of foodborne pathogens in hamburgers obtained from different processing plants and local markets in Tehran, Iran. Additionally, the study aimed to identify the main sources of contamination in the final products.

2. Materials and methods

2.1. Sampling

Initial sampling was conducted from October 2017 to June 2018. The samples were collected from various sources, including (1) hamburger ingredients such as meat, onion, spices, pepper, and additives, (2) food handlers, (3) surfaces, and (4) products from three plants identified as A (n=33), B (n=41), and C (n=33), with each sample collected in triplicate, resulting in a total of 107 samples. In June 2018, an additional 45 hamburgers were purchased from local markets in different regions of Tehran. These hamburgers were obtained from plants A, B, and C, with 15 samples from each plant collected in triplicate. This allowed for a comparison between the hamburgers obtained directly from the plants and those available in the local markets, providing insights into potential differences in microbial contamination levels. This comprehensive sampling approach aimed to assess the presence and levels of microbial contamination in different components of the hamburgers and investigate potential contamination sources throughout the production and distribution processes.

2.2. Microbial analysis

Isolation, enumeration, and detection of *E. coli* were performed according to international standards (9).

2.3. Molecular analysis

Microbial serotyping of *E. coli* was conducted using PCR in a thermocycler apparatus (Bio-RAD, USA). DNA extraction of *E. coli* was performed using a diagnostic kit (CinnaGen, Iran). The PCR reaction was carried out in a total volume of 50 µl, including Taq DNA polymerase, Tris-HCl, MgCl2, dNTPs, KCl, primers, and DNA. The cycling conditions consisted of an initial denaturation at 95°C for 4 minutes, denaturation at 95°C for 40 seconds, annealing at 56°C for 40 seconds, and extension at 72°C for 20 seconds. After amplification, PCR products were analyzed by gel electrophoresis using a 1% agarose gel. Ethidium bromide staining and UV light were used to visualize the separated DNA bands (10).

2.4. Evaluation of the food safety management system

To assess the performance of FSMS, a 37-item checklist prepared by the Iran Food and Drug Administration, based on ISO 22002-1, was utilized. This checklist included all prerequisite programs (PRPs) related to food safety. The score was based on the checklist assessment.

2.5. Statistical analysis

Data were described using frequency (percentage) and mean (standard deviation). Statistical analysis was performed using one-way ANOVA followed by multiple comparisons test (multiple Bonferroni test). The normality of error distribution in quantitative variables was assessed using the Kolmogorov-Smirnov test. The Pearson correlation coefficient was used to examine the linear relationship between the number of coliforms, PRPs, and *E. coli*. Statistical analyses were conducted using SPSS software (version 24), and a significance level of 5% was considered.

3. Results and discussion:

The results of the study showed that approximately 38% (41.107) of the samples collected from the three plants were contaminated with coliforms and *Escherichia coli*. Only one sample was found to be contaminated with *E. coli* O157:H7, which originated from raw meat in plant C. Among the hamburger samples purchased from local markets, 80% (36.45) had coliform counts lower than 10^2 CFU/g and Escherichia coli counts lower than 10 CFU/g. None of the market samples were contaminated with *E. coli* O157:H7. Plant B, which had the highest score (score=360) in terms of prerequisite programs (PRPs), showed the minimum contamination of coliforms and *E. coli*. Based on the PRPs score, plant B had the highest score of 360, followed by plant A with a score of 338, and plant C with a score of 285. Plant B also had the lowest number of coliforms (288) and *E. coli* (40) among the three plants. Plant C had the highest numbers of coliforms (1083) and *E. coli* (125). Therefore, while plant B had the lowest contamination score in terms of coliforms and Escherichia coli, it had the highest PRPs score, which is logical. Biochemical tests confirmed *E. coli* contamination in 20 plant and market samples. These samples were further analyzed using PCR for serotyping. No significant differences in E. coli contamination between the hamburgers purchased from the local market were found. *E. coli* O157:H7 was detected by PCR in one sample from the production plants,

specifically associated with raw meat from plant C. *E. coli* O157:H7 is a highly pathogenic strain that can cause severe intestinal infection in humans, leading to bloody diarrhea. Meat products provide a suitable environment for the growth of bacteria, making it important to monitor the entire food chain to prevent bacterial contamination.

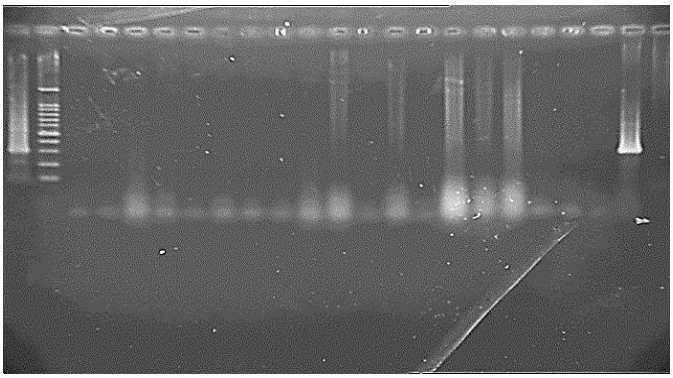

Fig. 1. The agarose gel showing the separated samples containing *E. coli* genes; left to right: column 1 positive control, column 2 marker, column 3 negative control, columns 4 to 23 the samples contaminated with E. coli in preliminary tests. Column 22 is related to the red meat of plant C that was contaminated with *E. coli* O157:H7.

E. coli O157:H7 is a highly concerning serotype of *E. coli* due to its significant health risks and relatively high prevalence worldwide (11). The primary transmission route to humans is through consuming contaminated meals, particularly those containing meat-based foods (12). This strain of *E. coli*, specifically *E. coli* O157:H7, causes severe intestinal infections in humans and can damage the intestinal wall, resulting in bloody diarrhea. It is commonly referred to as enterohemorrhagic E. coli infection. Being rich in protein, meat products provide an ideal environment for bacterial growth. Therefore, monitoring the entire food chain to prevent the exponential increase of bacteria in such nutrient-rich products is crucial. Consistent with our study findings, meat was identified as the main source of contamination in hamburgers, and the microbial load increased in formulations with higher percentages of meat (as observed in Table 1). Similarly, Shahrokhabadi et al. (13) reported a 27.8% contamination rate of *E. coli* O157:H7 in beef meat collected from slaughterhouses, highlighting the potential role of fresh raw meat as a transmission source of *E. coli* O157 in the human body. Additionally, Dass et al. (14) found that the history of bacterial co-habitation in the environment significantly influenced the resistance of *E. coli* O157:H7 to sanitation measures in meat plants. They suggested that a higher diversity

Table 1. Microbial enumeration of the red meats as raw material for preparing hamburgers in three plants A, B, and C.

Raw meat test result	Plant	Coliform (%)	*E. coli* (%)
The first stage of hamburger production- 30%	A	42 a	94.66 a
The second stage of hamburger production- 60%	A	61.66 b	96.33 b
The third stage of hamburger production- 60%	A	61.33 b	98.66 c
The first stage of hamburger production- 30%	B	78.66 a	87.33 a
The second stage of hamburger production- 30%	B	82.33 b	95.66 b
The second stage of hamburger production- 60%	B	84.33 c	95.66 b
The third stage of hamburger production- 30%	B	84.33 c	99.66 c
The third stage of hamburger production- 60%	B	85.33 c	99.66 c
The first stage of hamburger production- 30%	C	40.33 a	77.33 a
The second stage of hamburger production- 60%	C	47.66 b	78.33 b
The third stage of hamburger production- 60%	C	55.33 c	83.33 c

of microorganisms in the environment leads to greater adaptation of *E. coli* O157:H7, potentially contributing to its persistence. This consideration should be considered when implementing food safety management systems in production units to prevent microbial aggregation and enhance control measures against pathogens. The microbial load is a useful indicator for the presence of non-permitted ingredients in the products, such as offal, viscera, gut, cartilaginous tissues, and so on, which are more likely to be contaminated than meat. However, the presence of other ingredients like onion, pepper, and spices in hamburgers can have antimicrobial properties that restrict microbial metabolism and limit the water activity required for bacterial growth. Interestingly, spices were identified as the second source of microbial contamination in the hamburgers from plants A and C. However, plant B demonstrated no contamination of spices as raw materials, likely due to the thorough evaluation of suppliers and the implementation of immediate gamma irradiation of spices before use. Furthermore, the relatively low contamination levels observed in the final products of the plants in our study can be attributed to establishing a robust food safety management system, as indicated by the high scores obtained in the PRPs checklists. This comprehensive system considers all potential sources of contamination and effectively prevents cross-contamination of products. The lower contamination observed on the hands of staff in plant B, as compared to the other plants (as shown in Table 2), directly correlates with the higher PRPs scores documented in Table 3. This finding aligns with the results reported by Oyedele et al. (15) which demonstrated a similar microbial load on the hands of retailers handling fruits. A comparison of the results obtained from the hamburgers sampled from the three plants and those purchased from local markets indicated higher contamination levels in the former group, as shown in Table 4. Two possible hypotheses can be considered to explain these findings. Firstly, manufacturers may add certain additives, such as sodium lactate and sodium chloride, commonly used in commercial food production, to hamburgers (16). These additives may have antimicrobial properties or create an unfavorable environment for microbial growth, thereby suppressing the

growth of microorganisms during the shelf life of the products. This could explain the lower microbial contamination observed in the hamburgers obtained from local markets, where such additives may not have been used to the same extent. Secondly, it is plausible that storing the hamburgers under freezing conditions in the markets may have contributed to reducing microbial contamination. Freezing temperatures can cause the formation of ice crystals, which can physically damage bacterial cells and disrupt the microorganisms in the hamburgers. This freezing process might have occurred during the storage and distribution of the products in the local markets, leading to a decrease in microbial contamination levels compared to the hamburgers sampled directly from the plants (17). Both of these hypotheses explain the lower microbial contamination observed in the hamburgers obtained from local markets compared to those sampled from the plants.

Table 2. Contamination of raw materials, hands of operators, and surfaces of the equipment in three plants of A, B, ad C.

Contamination source	Plant A		Plants B		Plants C	
	Coliforms (%)	*E. coli* (%)	Coliforms (%)	*E. coli* (%)	Coliforms (%)	*E. coli* (%)
Raw meat	55	96	83	96	48	80
Spices	25	0	0	0	20	8
Onion	7	2	12	4	6	3
Pepper	3	0	5	0	2	0
Hands of operators	7	1	0	0	10	5
Surfaces of equipment	3	1	0	0	14	4

Table 3. PRPs scores of plants A, B, and C compared to their contamination with coliforms and *E. coli*.

	PRPs score											
Plant	Design	Personal hygiene	Personal health capabilities	Warehouses, cold storage and heating	Production and processing	Washing, disinfection and cleaning	Raw material suppliers	Product distribution	Certification HACCP and ISO 22000	Total points from 360	Total coliform count CFU/cm^2	*E. coli*
											Mean (SD)	Mean (SD)
A	40	45	20	67	82	29	35	15	5	338	596.66(253.88)[a]*	95(44.44)[e]
B	40	45	20	78	84	33	35	15	10	360	288(63.10)[b]	80.40(24.61)[f]
C	32	45	10	57	78	30	20	13	0	285	1083.33(299.05)[c]	125(47.69)[g]

* Heterogeneous letters in each column indicate a significant difference between the three factories.
* One-way ANOVA with Bonferroni multiple comparison test.

Table 4. Comparing microbial contamination of the samples prepared at the three plants and local markets.

Hamburger	Coliform		*E. coli*	
	Factory A	Local Market	Factory A	Local Market
30%	880	89(24.16)	145	10
60%	390	89(24.16)	60	10
60%	520	89(24.16)	80	10
Mean (SD)	596.66 (145.55)[a]	89 (24.16)[b]	95 (25.65)[c]	10[d]
Hamburger	**Coliform**		***E. coli***	
	Factory B	Local Market	Factory B	Local Market
30%	200	66(10.19)	12	10
30%	260	66(10.19)	27	10
60%	285	66(10.19)	33	10
30%	335	66(10.19)	60	10
60%	360	66(10.19)	72	10
Mean (SD)	288 (28.22) [e]*	66(10.19) [f]	40.8(11) [g]	10[h]
Hamburger	**Coliform**		***E. coli***	
	Factory C	Local Market	Factory C	Local Market
30%	760	119(61.83)	80	10
60%	1140	119(61.83)	120	10
60%	1350	119(61.83)	175	10
Mean (SD)	1083 (172.65) [i]	119(61.83) [j]	125 (27.53) [k]	10[l]

* Heterogeneous letters in each column indicate a significant difference between the three factories.
** One-way ANOVA- Bonferroni post hoc test.

Further investigation and analysis would be necessary to determine the exact factors contributing to the observed differences in contamination levels between the two groups. One of the notable strengths of this study is its novelty, as it is the first of its kind conducted in Iran, with no similar studies conducted previously. Additionally, the study encompassed sampling from two locations, including a factory and a daily market, providing a broader perspective on microbial contamination in hamburgers. However, it is important to acknowledge some limitations of the study. One limitation was

the reluctance of factories to cooperate fully, likely due to concerns about potential consequences. Despite this limitation, the factories eventually agreed to participate after being reassured about the study's objectives and procedures.

4. Conclusion

In conclusion, this study highlights the significance of ensuring the safety of raw materials and implementing hygienic handling practices in food production. Red meat was identified as the primary source of microbial contamination in hamburgers. Implementing PRPs, including good manufacturing practices, good hygienic practices, and proper storage practices, can effectively reduce food safety hazards and the risk of cross-contamination during production. Although the production plants in this study obtained acceptable PRPs scores, there is room for improvement through regulated monitoring based on a comprehensive checklist. Higher PRPs scores were associated with lower contamination levels observed in the samples collected from the plants and those obtained from the local markets. Evaluating meat suppliers and closely monitoring slaughterhouses can ensure the maintenance of acceptable safety levels. Furthermore, implementing best practices in transportation and storage can further enhance the safety of meat products. Continued efforts in implementing and improving food safety management systems, along with rigorous adherence to PRPs, can contribute to the reduction of microbial contamination and the overall enhancement of food safety in the production and distribution of hamburgers.

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