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Effect of Good Hygiene Practices on *E. coli* O157:H7 Contamination in Some Al-Karkh Area Restaurants, Baghdad, Iraq

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ABSTRACT

Background: In general, dealing with healthy food reduces the risk of opportunistic pathogens, and due to the increasing growth in the restaurant industry in Iraq, with the scarcity of previous evaluations in this subject, this study comes at the right time. **Objective:** This study aimed to assessment the efficacy of training on good hygiene practices (GHP) to reduce potential restaurants contamination by *E. coli* O157:H7.

Methodology: A total of 160 samples were collected from ten different restaurants in Al-Kharkh area, Baghdad, in which 80 samples were taken before implementing GHP and 80 samples after applying GHP. These samples consisted of the following categories: beef burger products before and after grilling, knives, refrigerators, food cutting boards, tables, and workers (hands, nails, and clothes).

Results: There was *E. coli* O157:H7 in 40% (4/10) of restaurants before GHP was implemented. Cultural, biochemical, and molecular-based methods confirmed the presence of *E. coli* O157:H7 in five of eighty samples (6.25%), including raw beef burger (3/10, 30%), knives (1/10, 10%), and cutting boards (1/10, 10%). There was no evidence of *E. coli* O157:H7 in 80 samples after GHP training, demonstrating its effectiveness.

Conclusion: Poor restaurant hygiene aids spreading *E. coli* O157:H7. GHP, in addition to personal hygiene, are crucial; otherwise, *E. coli* O157:H7 strain could contaminate equipment and food.

Keywords: Beef burger, *Escherichia coli* O157:H7, Good hygiene practices.

INTRODUCTION

The most efficient methods for managing and reducing the burdens of foodborne diseases in any community include limiting the causal agent's access, enhancing food handler understanding of food safety, and implementing basic hygienic procedures.

In fact, restaurants have been identified as significant sites for microbial pathogens to get access to food, potentially resulting in food poisoning epidemics. Therefore, restaurants have gotten special attention in many food poisoning outbreaks in industrialized nations, among other probable entrance points for germs, and have been widely linked to numerous poisoning cases in many developed countries [(1)] .

The standard hygiene and contamination indicator, Enterobacteriaceae, was counted in various studies to evaluate the hygiene quality of food handling services and restaurants [(2)]. Microbial food safety is a growing global public health hazard [(3)]. Poorly controlled or inadequate cooking can lead to the persistence of pathogens in foods, which can cause foodborne illness. Lab-confirmed diseases from numerous foodborne pathogens, such as *Campylobacter, Salmonella, Cryptosporidium, Shigella, Shiga* toxin-producing *Escherichia coli* (*E. coli*) O157, and *Yersinia pestis*, are most common. *E. coli* O157:H7 has become the leading food-borne pathogen for humans. *E. coli* O157:H7 is considered as one of the .significant human pathogens *E. coli* O157:H7 is amongst the most common foodborne pathogens with *Salmonella*, Campylobacter which affect millions of the individuals each year, in some of the cases with severe and fatal outcomes, and isassociated with undercooked meat, unpasteurized milk, andfresh fruits ,and vegetables. However*E. coli* O157:H7 could also be directly transmitted to people through stool of adult cattle and young calves. Additionally, *E. coli* O157 might spread between individuals, especially in settings like daycare centers where people come into contact with one another frequently [(4)].

Food hygiene could be compromised by contamination at any point in process, from manufacture to consumptions, and depending on the environment it is stored in (humidity, temperature, and pH foods). Many strategies have been applied for the prevention of foodborne illnesses, which include the regular inspections and surveillance, adoptinga management system for food safety, and certification training mandatory program [(5)].

The food handlers' ability to adapt their practices and improve their abilities is a major factor in reducing the likelihood of food contamination, hence training in this area is highly valued. Effective training is provided when trainees are given pedagogically sound opportunities to learn the necessary knowledge, abilities, and attitudes through instruction, demonstration, practice, and prompt diagnosis on their performances [(6)].

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Although Foodborne outbreaks are common in Iraq, they are rarely reported, investigated, or recorded. In Iraq, several food poisoning (FP)outbreaks have been reported at military camps, colleges, and other settings in recent years, but most have gone uninvestigated or unpublished [(7)].

MATERIALS AND METHODS

1. Sample Collection and Processing

All of study samples were collected from ten different restaurants in Al-Karkh area, Baghdad, Iraq, between November 2021 and April 2022.

A total of 160 samples were collected, eighty samples were taken before and after applying good hygiene practices. These samples were separated into the following, beef burger products before grilling, samples from beef burger products after grilling, knives, refrigerators, food cutting boards, tables, hands and nails of workers, and samples from clothes of workers in restaurants. The same sampling procedure was used after good hygiene practices was implemented [(8)]. Samples were identified accurately by collection date, sample type, and source. The samples were then transported in a sterile ice box with freeze packs to the Central Public Health Laboratory of the Iraqi Ministry of Health in Baghdad, Iraq for microbiological testing.

2. Training

A training course was used to establish the good hygiene practices program. In each establishment, the training extended for a week. The training included theoretical part extend for three days including lectures on food safety, microbiology (bacteria multiplication, and bacterial elimination techniques). Also, practical training, included team trained on controlling temperatures, food corruption, personal hygiene, cleaning and sterilization, control of insects, and rodents [(9)]. Samples were collected for bacteriological examination after one week of practice application with restaurant workers and the application of good hygiene practices.

3. *E. coli* O157:H7 Isolation and Identification

Sample swabs were collected using standard bacteriological techniques and the incubated broth was distributed across the surface of the Sorbitol-MacConkey agar and incubated for 24 hours at 37°C to enhance the level of bacteria (SMAC). Also, Eosin Methylene Blue (EMB) agar was used as *an E. coli* medium that is selective and differential [(10)].

4. IMVIC, VITEK 2® and API 20E tests

A traditional method known as IMVIC tests used to identify *E. coli* isolates biochemically. A collection of IMVIC includes Indole, Methyl Red, Voges-Proskauer, and Citrate Utilization. Those tests used to differentiate between Enterobacteriaceae members. The VITEK 2 compact system employs a 64 unique card identification number for organism identification and a turbid metric approach for susceptibility testing. API 20E test strip is used to diagnose enteric gram-negative rods.

5. PCR

Further confirmation was conducted by PCR to detect the presence of attaching and effacing gene (*eaeA*) and *Shiga* toxin producing genes (stx_1 and stx_2) virulence genes. The PCR procedure in terms of DNA extraction (ZR Fungal/Yeast/Bacterial DNA MiniPrep ™, ZYMO Researcher, USA purity measurements (gel ,(electrophoresis). PCR conditions were carried out according to the manufacturer's protocols.

6. Ethical Approval:

The study was reviewed and approved by the Scientific Committee of the Department of Veterinary Public Health in its season held on September 5th 2021 as well as the Ethics Committee of the College of Veterinary Medicine, University of Baghdad (Ethics No. BU. VM#4172/ 27-10-2021). Samples were taken from restaurants after obtaining the approval of the Iraqi Ministry of Health and Environment, Public Health Directorate (PHD#1981/ 25-10-2021).

Also, the approval was taken from the owners of the restaurants and samples were taken voluntarily without financial compensation and a pledge not to disclose the names of restaurants and employees or their photos with a commitment to scientific integrity. All owners and workers of the restaurants have signed the ethics approval and written agreement to participate in the study.

7. Statistical Analysis

Collected data were subjected to Chi-squared test to determine statistically significant percentages at the 0.05 and 0.01 probability.

RESULTS AND DISCUSSION

1. Prevalence of *E. coli* O157:H7 in Al-Karkh Restaurants: *E. coli* O157:H7 bacteria were screened in ten different restaurants in Al-Karkh areas, Baghdad city. Prior to GHP application, 40% of restaurants (4/10) had *E. coli* O157:H7. The results revealed that there were five isolates from 80 (6.25%) analyzed samples that collected from ten restaurants before GHP. *E. coli* O157:H7. The number isolated from ten different restaurants before GHP from different regions in Al-Karkh area were 3 out of 10 sample (30%) in beef burger before grilling and 1

out of 10 (10%) distrusted into beef burger before grilling, knives and food cutting boards, respectively as detailed in Table (1). *E. coli* O157:H7 was not detected in samples of refrigerators, tables, workers (cloths, hands, and nails ,(and beef burger after grilling. The results of this study showed that training on good hygiene practices was effective in reduction*E. coli* O157:H7, where no detection was observed after training in all samples.

Figure (1) showed the percentages of isolated *E. coli* O157: H7 were significantly higher before good hygiene practices 1.66% compared with the percentage after good hygiene practices from ten different restaurants in Al-Karkh areas. While the percentages of *E. coli* O157: H7 organisms subsequent to good hygiene practices were 0.00 %.

Table (1): Numbers and percentages of samples collected before and after good hygiene practices in ten different restaurants in Al-Karkh areas

Sampling (N= 80)	Positive samples before GHP	Positive samples before GHP %	Positive samples after GHP	Positive samples after GHP %
Knives	1	10	0	0
Refrigerators	0	0	0	0
Food cutting boards	1	10	0	0
Tables	0	0	0	0
Cloths (Workers)	0	0	0	0
Hands & nails (Workers)	0	0	0	0
beef burger before grilling	3	30	0	0
beef burger after grilling	0	0	0	0
Total	**5**	**6.25**	**0**	**0**

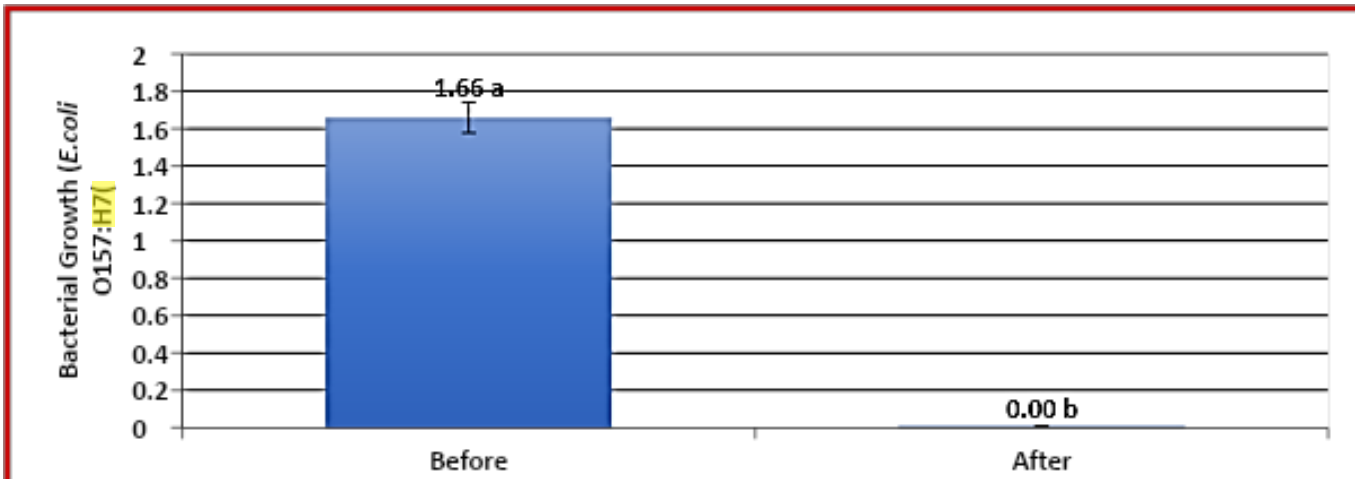

Figure (1): Isolated *E. coli* O157:H7 percentages from collected samples before and after good hygiene practices from ten different restaurants in AL-Karkh areas.

Although foodborne outbreaks caused by *E. coli* O157: H7 are common in Iraq, they are rarely reported, investigated, or recorded. There are also a few studies in Iraq that focus on collecting samples from restaurant utensils, equipment, workers and determining the causes of food poisoning. In a study investigation the contamination of bacteria in restaurants of Technical Institute located at Mosul province, Iraq by sampling workers, swabs, surfaces, and utensils, as well as prepared food showed that the percentage ofGram-negative bacteria had a rate of 40.7%, in which the *E. coli* was the most frequently isolated (18.5%) [(11)].

The foodborne illness can be caused by improper food storage, whereas the contamination of cooked and raw foods before eating can be caused by contacting with pathogen-carrying food or tools and equipments, and insufficient or bad management of food cooking that can allow bacteria to survive [(12)]. Also, the researcher found that food hygiene is affected by many reasons, including the mixing of raw and cooked foods in unsanitary storage and the preparation of large quantities of food in unsanitary environments, negatively impact food hygiene [(13)]. Furthermore, Inadequate knowledge and methods in agriculture, unsanitary conditions throughout the food production chain, a lack of safety checks and balances during processing and preparation, the use of harmful chemicals, polluted sources for ingredients, and improper storage all pose risks to consumer health and safety [(14)].

On the other hand, it was found in current study that raw beef burgers contained the highest proportion of *E. coli* O157:H7, suggesting that this strain could be transmitted via bovine meat. It is likely that E. coli O157:H7, which is found in bovine feces contaminates healthy bovine meat during slaughter and processing [(15)]. Furthermore, the surface of the carcass can be contaminated mainly at slaughter and during skinning, washing of carcass, and grinding of the meat. In addition, the cross-contamination may present during preparation and handling of the food [(16)].

Many researchers surveyed and identified *E. coli* O157:H7 in uncooked beef meat, which supported our results. In the Netherlands [(17)], 1.1% of 571 raw minced beef samples were contaminated with *E. coli* O157:H7, while in India [(18)], 9% (2/22) of minced beef samples contained *E. coli* O157:H7. In Turkey [(19)], three samples of ground beef contained *E. coli* O157:H7, representing 6% of the total. Two Egyptian minced beef samples (6.7%) tested positive for *E. coli* O157:H7 [(20)]. In line with the findings of numerous studies, the pathogen E. coli O157:H7 was isolated from burger minced meat samples despite the fact that ground beef has been widely regarded as a potential vector for the disease. Despite the fact that minced meat has been widely linked to spreading E. coli O157:H7, the pathogen was isolated from burger minced meat samples [(21)].

2. Laboratory Identification of Positive *E. Coli* O157:H7

2.1. Cultural and Microscopic Characteristics of *E. Coli* O157:H7

The isolates showed various colonies on selective culture media grown at 37°C. *E. coli* O157: H7 colonies appeared on media, SMAC small, circular, and colorless, as shown in figure (2A), whereas other *E. coli* strains appeared as pink circular colonies after incubation at the same conditions as *E. coli* O157:H7 on (SMAC-CT) (Figure 2B). The colonies of *E. coli* O157:H7 on this media are the specific media that distinguishes strains of non-fermented sugar sorbitol. While on eosin methylene blue agar (EMB) the bacteria had a green metallic shiny appearance after 24 hours of incubation at 37°C, which is regarded a rapid and accurate technique of distinguishing *E. coli* from other gram-negative pathogens (Figure 3). The results of the microscopic examination of the tested bacterium after Gram staining showed ram-negative, rod-shaped bacterium arranged as singular or pair (Figure 4).

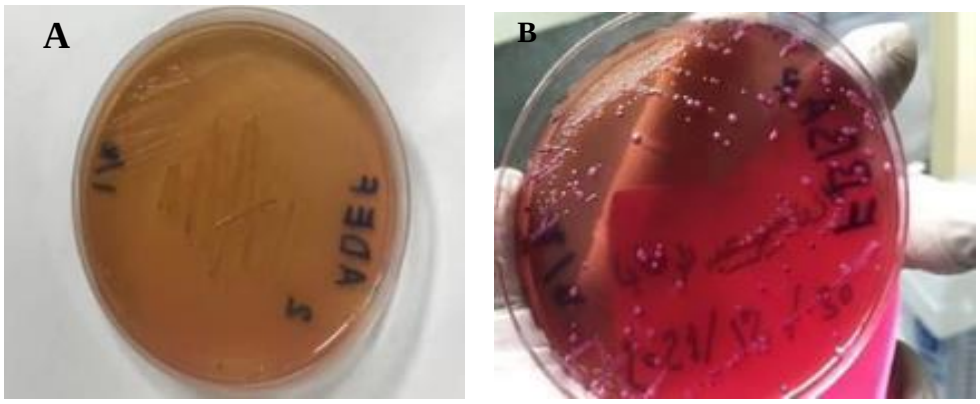

Figure (2): Characteristics of the culture A: After 18 – 20 hours, *E. coli* O157:H7 colonies are colorless. B: non-O157 *E. coli* on (SMAC-CT) agar at 37°C; colonies are dark pink in color due to Sorbitol fermentation

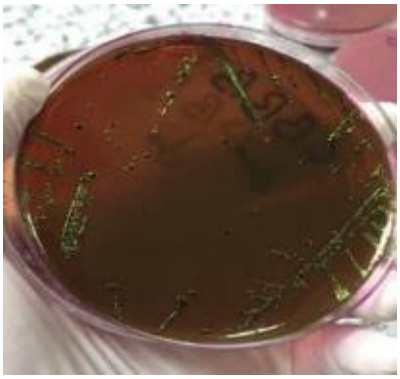

Figure (3): *E. coli* O157:H7 colonies on EMB agar have a green metallic sheen

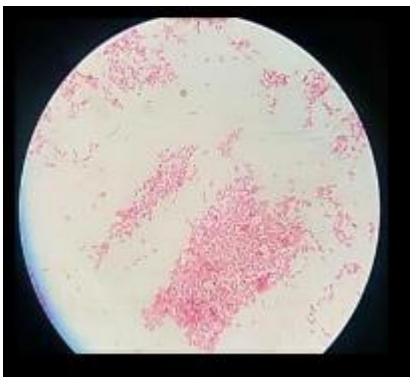

Figure (4): *E. coli* O157:H7 under the light microscope (100×).

2.2. Biochemical Tests (IMVIC and API 20E)

Conventional biochemical tests were used in this study which included IMVIC pattern (Indole, Methyl Red, Voges-Proskauer, and Simmons Citrate test). This test showed typical IMVIC pattern and other biochemical tests of the isolated bacteria gave different results. The IMVIC test was used to distinguish between the Enterobacteriaceae families. The results of the tests showed that motility (Figure 5), catalase, indole, and methyl red were all positive (Figure 5 A & B), whereas oxidase (Figure 5 C & D), Voges-Proskauer, urease, and Simmons citrate were all negative. The TSR agar yielded acid-acid (yellow–yellow) on both the slant and bottom, with gas bubbles but no H_2S, these results of biochemical tests were shown in table (2). Also, API 20E was used to confirm the probable isolates, and the results are shown in table (3).

Table (2): Results of biochemical tests of *E. coli* O157:H7

No.	Biochemical Test	
	Tests	**Results**
1	Catalase	+Ve
2	Oxidase	-Ve
3	Indole	+Ve
4	Methyl red	+Ve
5	Voges-Proskauer	-Ve
6	Simmons citrate	-Ve
7	Urease	-Ve
8	Motility test	+Ve

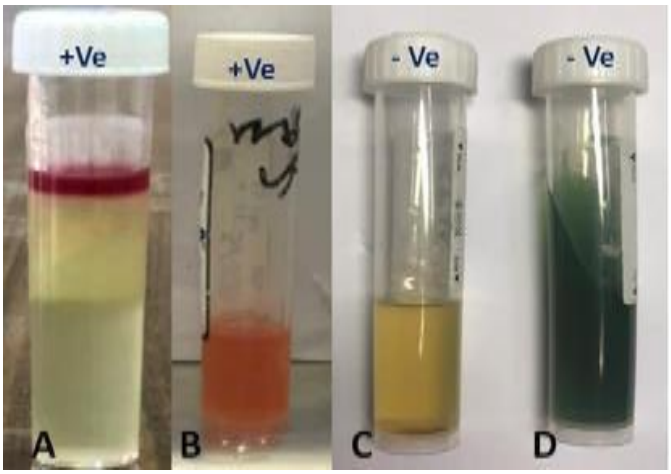

Figure (5): Photographs of IMVIC results (A) Indole, (B) Methyl red, (C) Voges-Proskauer, and (D) Simmons citrate

Table (3): Results of API 20 E test for *E. coli* O157:H7

No.	Test	Results
1	β-galactosidase (ONPG)	+ ve
2	Arginine dehydrolase (ADH)	- ve
3	Lysine decarboxylase (LDC)	+ ve
4	Ornithine decarboxylase (ODC)	+ve
5	Citrate utilization (CIT)	- ve
6	H_2S production (H_2S)	- ve
7	Urease (URE)	- ve
8	Tryptophane deaminase (TDA)	- ve
9	Indole production (IND)	+ ve
10	Acrtoin production (AP)	- ve
11	Gelatinase (GEL)	- ve
12	Glucose (GLU)	+ ve
13	Mannitol (MAN)	+ ve
14	Inositol (ION)	- ve
15	Sorbitol (SOR)	- ve
16	Rhaminose (RHA)	+ ve
17	Sucrose (SAC)	- ve
18	Melibiose (MEL)	+ ve
19	Amygdaline (AMY) and cytochrome oxidase (OX)	-ve
20	Arabinose (ARA)	+ ve

2.3. Biochemical Confirmatory Identification Using VITEK 2® System Technique

The VITEK 2 compact system was used for this test (BioMérieux, France). The field of bacterial examination has been improved by this system technology, which provides more dependable technology, high speed, and high sensitivity for bacterial identification, with findings as high as accuracy. The results were highly accurate as shown in table (4), which indicated positivity of *E. coli* O157:H7, this study found a 96% chance of *E. coli* O157:H7.

Table (4): Isolation percent of *E. coli* O157:H7 isolates according to VITEK 2 Compact system

No	Sources of samples	No. samples	% Positive *E. coli* O157:H7
1.	samples before GHP	80	6.25
2.	samples after GHP	80	0.0
3.	**Total**	**160**	**(3.125)**

Molecular Identification of *E. Coli* O157:H7 Isolates

1. Genomic DNA Extraction of *E. Coli* O157:H7

Table (5) showed Confirmatory diagnosis results as performed by PCR technique for all isolated samples from the restaurants. And as seen in figure (6), results of the amplification of using rfb_{O157} gene, that amplified from knives, food cutting boards and beef burger before grilling are isolated by using conventional PCR with an approximate size of the product was 497 bp and using *16S rRNA* gene with approximate size of the product was 1250-1500 bp (Figure 7). (Figure 8) also used *eaeA* gene, which amplified a product size of approximately 384 bp. In addition, the use of stx1 gene with an approximate size of the product was 180 bp (Figure 9) and using stx_2 gene with an approximate size of the product was 255 bp (Figure 10).

Table (5): The results of PCR examination Genes

		PCR of *E. coli* O157:H7 genes				
Sources of samples	No. of samples	*rfb*$_{O157}$	*eaeA*	*16S rRNA*	*stx*$_1$	*stx*$_2$
Before GHP	80	5	5	5	0	0
After GHP	80	0	0	0	0	0
Total	160	5 (2.5%)	5 (2.5%)	5 (2.5%)	0 (0%)	0 (0%)

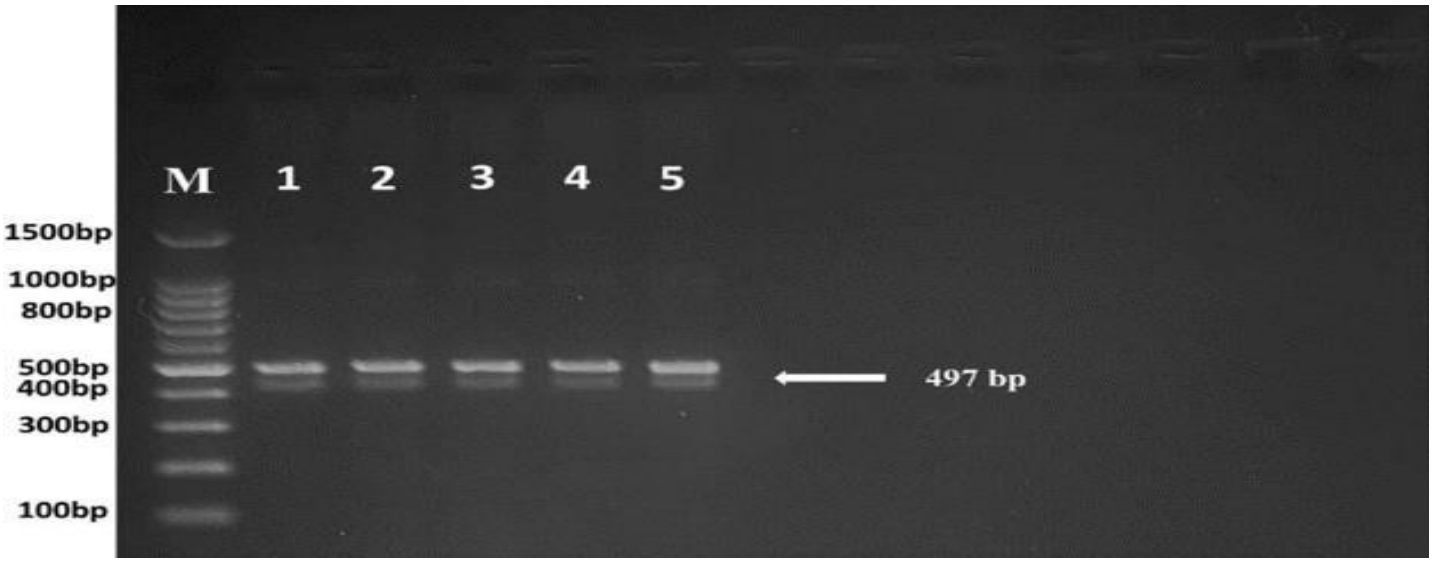

Figure (6): Electrophoresis of a 497 bp fragment of *E. coli* O157:H7 *rfb*$_{O157}$ gene as electrophoresed for 1 hour on ethidium bromide-stained agarose (2%) gel at 70 volt/cm^2 and 1× TBE buffer. Lane M: DNA ladder (100 bp). Lane 1: knives, Lane 2: food cutting boards. Lanes 3 to 5: beef burger before grilling.

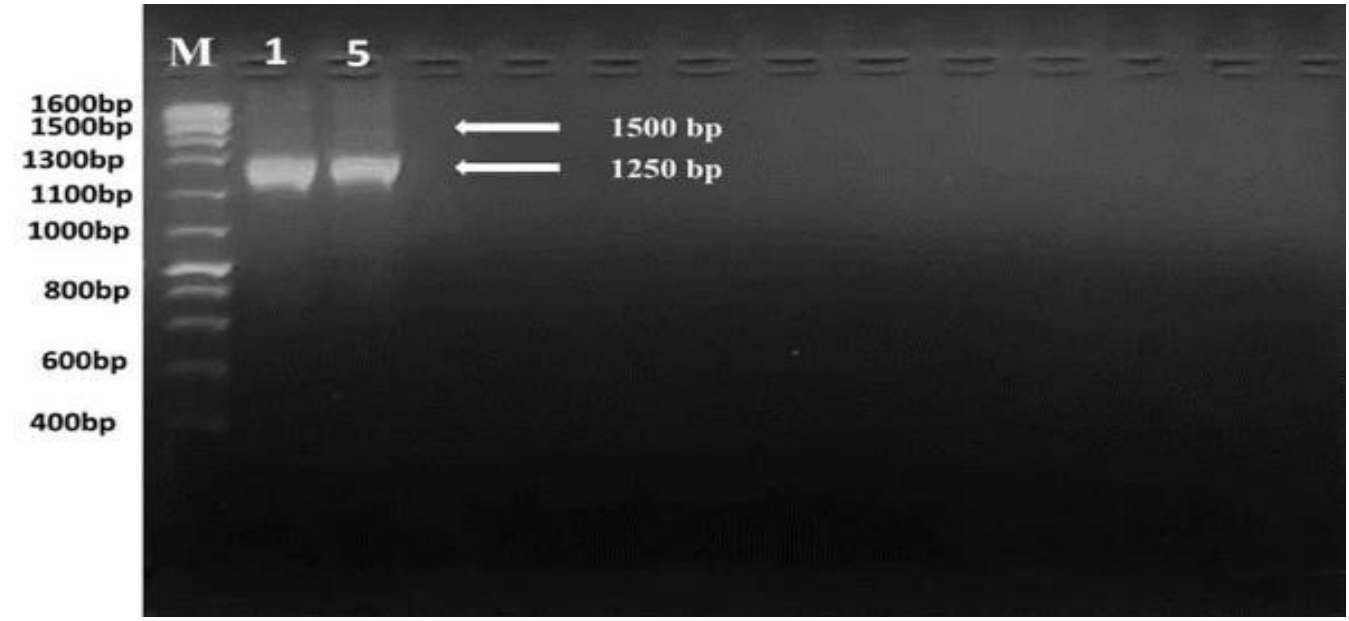

Figure (7): Electrophoresis of a 1250- 1500 bp fragment of *E. coli* O157:H7 16S rRNA gene as electrophoresed for 1 hour on ethidium bromide-stained agarose (2%) gel at 70 volt/cm^2 and 1× TBE. Lane M: DNA ladder (100 bp). Lane 1: knives. Line 5: beef burger before grilling.

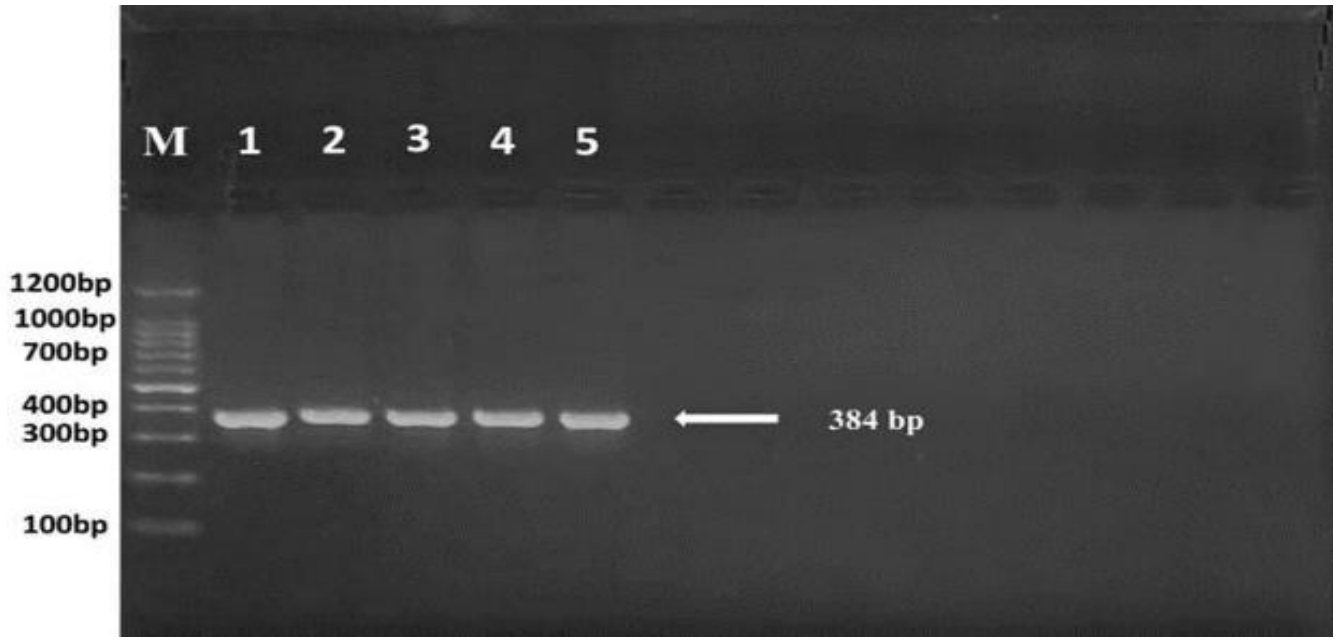

Figure (8): Electrophoresis of a 384 bp fragment of *E. coli* O157:H7 *eaeA* gene as electrophoresed for 1 hour on ethidium bromide-stained agarose (2%) gel at 70 volt/cm^2 and 1× TBE. Lane M: 100 bp DNA ladder. Lane 1: knives. Lane 2: food cutting boards. Lanes 3 to 5: beef burger before grilling

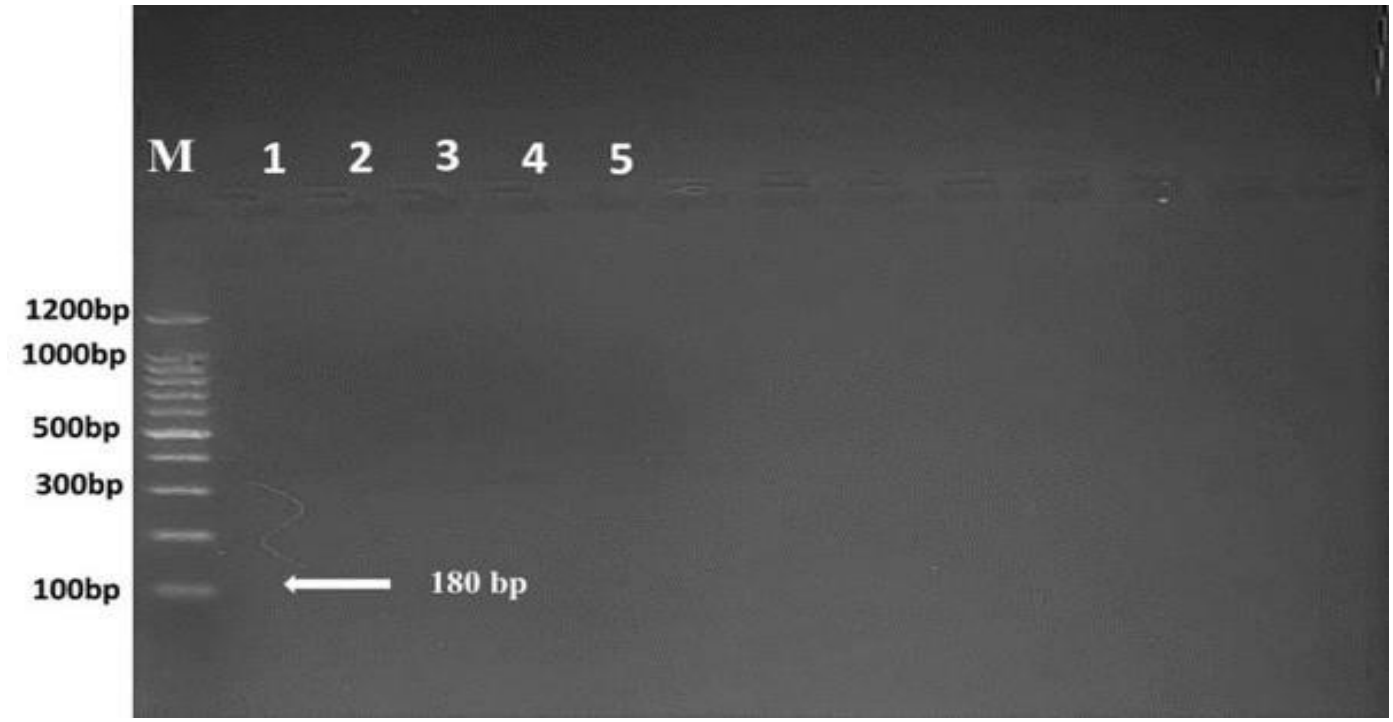

Figure (9): Electrophoresis of a 180 bp fragment of *E. coli* O157:H7 *stx$_1$* gene as electrophoresed for 1 hour on ethidium bromide-stained agarose (2%) gel at 70 volt/cm^2 and 1× TBE. Lane M: DNA ladder (100 bp). Amplification of PCR product was found.

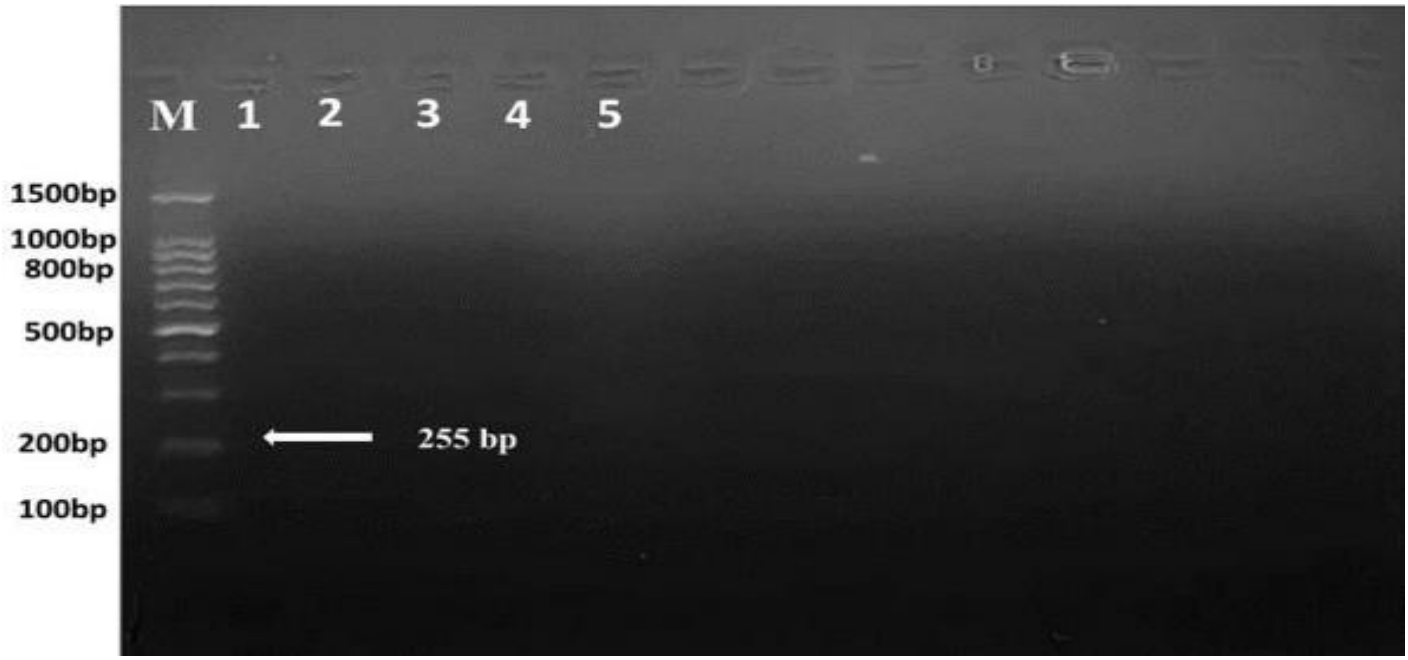

Figure (10): Electrophoresis of a 180 bp fragment of *E. coli* O157:H7 *stx*$_2$ gene as electrophoresed for 1 hour on ethidium bromide-stained agarose (2%) gel at 70 volt/cm^2 and 1× TBE. Lane M: DNA ladder (100 bp). Amplification of PCR product was found.

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DISCUSSION

Several techniques were used to identify this microorganism in the current study. They were confirmed by biochemical test, IMVIC, API 20, Vitek2, and final diagnosis by PCR. The PCR is a powerful molecular biology tool for revealing target DNA in a range of diagnostic samples and for identifying a variety of infections. Diarrheagenic *E. coli* could be diagnosed and differentiated effectively from normal flora attributable to the sensitivity and specificity, in addition to time efficient and accuracy of PCR results [(22)].

Another method used to identify the serotype of *E. coli* is using PCR for detection of *wzx* and *wzy-coding* O-antigen genes and *fliC*-coding H antigen gene [(23)]. Not all of the hundreds of *E. coli* O157:H7 serotypes known have been connected to human illnesses. Many of the virulence genes of STEC are transferable, thus various strains of *E. coli* O157:H7 within a serotype may or not share the same virulence genes, and hence pose varied levels of risk [(23)]. Moreover, the role of international food trade in the spread of these pathogens and the potential for virulence gene transfer between organisms, including between STEC and other pathogenic *E. coli* (which are often more frequently identified as a problem in developing countries) [(24)].

On the other hand, researchers showed that STEC has been identified or isolated using a range of methods available including culture-based methods (selective and enriched media), cell culture, immunological-based methods (immunomagnetic separation), and molecular-based methods PCR and real-time PCR) [(25)].

Yet, there is still no gold-standard method for separating *E. coli* O157:H7. This is attributed to the fact that *E. coli* is a bacterium which often exchanges genetic information with other bacteria (*Salmonella* spp, strains of *E. coli*, and *Shigella* spp) in its environment via horizontal gene transfer pathways. This is due to the mobile genetic elements present in the STEC such as bacteriophages and plasmids. Strains of *E. coli*, therefore, may display different combinations of acquired features which require new diagnostic method [(26)]. The suitable methods to detect STEC are using of PCR, which is useful for differentiation between STEC and another *E. coli* by *stx* gene(s) detection, which is considered the only reliable gene to differentiate between them. Further, the amplification of O-antigen producing genes (*wzx* and *wzy*) can be used to identify bacterial serogroups [(25)]. In addition, the detection of accessory virulence elements such as the *eae* gene, which is used as an indicator of the presence of STEC strains that cause severe illness in humans [(23)]. Nevertheless, the presence or absence of bacteriophages (free bacteriophages) that are incorporated into the STEC genome can affect the PCR sensitivity and accuracy of STEC detection [(27)]. Additionally, the *stx*-coding phage can be eliminated from STEC through repeated subculturing as early as the first subculture [(28)]. As an added complication, a PCR-based

screen for *stx* and *eae* genes, as pathotype-specific genetic markers, may incorrectly conclude the presence of an *E. coli* containing *stx* and *eae* when in fact both two genes may have originated from different organisms [(29)].

The significant decrease in the percentage of the contamination after the application of good hygienic practices due to increase the hygiene to eliminate bacteria and another contaminant. Evaluated knowledge of food safety and hygiene standards among 18 eateries in three distinct areas of the Governorate of Muscat, Oman. The expertise of food handlers in a few restaurants was evaluated using a questionnaire including food poisoning, hygiene, cooking, food handling, and regional rules and regulations. A small yet important inverse correlation between the total knowledge scores and hygiene practices has been found [(30)]. Workers at a wholesale vegetable market in Doha, Qatar were surveyed for a study to assess their knowledge with food safety standards and their own hygienic practices [(31)]. While 77% of food handlers reported washing their hands 4 times daily, the microbiological assessment of their hands, which included coliform and aerobic bacterial counts two log CFU/cm^2, did not support this claim. *Bacillus circulans* (40%) was the most common bacterium found on the hands of produce handlers, followed by *Klebsiella pneumoniae* (17%) and *Staphylococcus sciuri* (25%).

CONCLUSION

In conclusion, the presence of the *E. coli* O157:H7 in the restaurants in Al-Karkh areas, Baghdad city during samples collection was considered to be a proof of contamination, which may cause food poisoning linked to consuming contaminated food. Findings from this study highlight the consequences of weak points in the program to control food handlers, such as the widespread absence of rigorous hygienic procedures in food service establishments. Continuous and consistent GHP training, particularly in the preparation area, such as hand washing and wearing protective clothing and gloves can help lower food contamination risks.

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REFERENCES

1. **Nkhebenyane J, Lues R (2020):** The knowledge, attitude, and practices of food handlers in central South African hospices. Food Science & Nutrition, 8 (6): 2598-2607.
2. **Rosiane C, Elizandra M, Jackline F *et al.* (2018):** Good hygiene practices and microbiological contamination in commercial restaurants. African Journal of Microbiology Research,12(16):362–369.
3. **Meng J, Doyle M (1998):** Emerging and evolving microbial food-borne pathogens. Bulletin de L'Institut Pasteur, 96: 151–163.
4. **FAO (2018):** Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterization, and monitoring. Microbiological Risk Assessment Series No. 31. Rome, FAO. https://www.fao.org/3/ca0032en/ca0032en.pdf
5. **Salas E, Tannenbaum SI, Kraiger K *et al.* (2012):** The science of training and development in organizations: What matters in practice. Psychological Science in the Public Interest, 13 (2): 74-101.
6. **Howton J, Keifer E, Murphy C *et al.* (2016):** A comparison of food safety programs using the customizable tool for online training evaluation. Food Control, 59: 82–87.
7. **Asaad M, Al Lami F, Abdullatif B *et al.* (2014):** Food poisoning outbreak in Tikrit city, Iraq, 2013: *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Salmonella* Typhimurium were the incriminated pathogens. The Iraqi Postgraduate Medical Journal, 13 (2): 169-175.
8. **Abdulabbas S, Ibrahim Z, Muhammed H *et al.* (2022):** Determination of Fractional Inhibitory Concentration (FIC) Index as A Measure of Synergy of Antibiotics in E. coli O157:H7, The Egyptian Journal of Hospital Medicine, 89(2): 7571-7575.
9. **Food and Agriculture Organization of the United Nations (2008):** Risk-based food inspection manual. Rome, FAO Food and Nutrition Paper. Available at http://www.fao.org/3/a-i0096e.pdf
10. **Mohammed A, Mohammed A, Mahmoud J (2013):** Isolation of *Escherichia coli* O157:H7 strain from fecal samples of zoo animal. https://doi.org/10.1155/2013/843968
11. **Haitham A, Jamal N, Fathil W (2020):** Investigation study for detection of bacterial contamination in restaurant's, of Technical Institute of Mosul. EurAsia Journal of BioSciences, 14: 5127-5130.
12. **Codex Alimentarius (2001):** Food Hygiene (Basic Texts), 2nd ed. Food and Drug Administration of The United Nations, Rome. Available at https://www.fao.org/3/y1579e/y1579e00.htm#Contents
13. **WHO (2015):** WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. World Health Organization. file:///C:/Users/MA/Downloads/fg4bg5us.pdf
14. **WHO (2013):** Assuring food safety and quality: guidelines for strengthening national food control systems.https://pesquisa.bvsalud.org/portal/resource/pt/mis-20328
15. **Wells G, Shipmand L, Greene K (1991):** Isolation of *Escherichia coli* serotype O157:H7 and other *Shiga*-like toxin-producing *E. coli* from dairy cattle. Journal of Clinical Microbiology, 29 (5): 985-989.
16. **Duffy L, Blackall P, Cobbold R *et al.* (2014):** Quantitative effects of in-line operations on *Campylobacter* and *Escherichia coli* through two Australian broiler processing plants. International Journal of Food Microbiology, 188: 128-134.
17. **Heuvelink A, Zwartkruis-Nahuis J, Beumer B *et al.* (1999):** Occurrence and survival of verocytotoxin-producing

Escherichia coli O157 in meat obtained from retail outlets in the Netherlands. Journal of Food Protection, 62 (10): 1115-1122.

18. **Shanta D, Deb A, Chattopadhyay U *et al.* (2000):** Isolation of *Shiga* toxin-producing *Escherichia coli* including O157:H7 strains from dairy cattle and beef samples marketed in Calcutta, India. Journal of Medical Microbiology, 49 (8): 765–767.
19. **Baran F, Gulmez M (2000):** The occurrence of *Escherichia coli* O157:H7 in the ground beef and chicken drumsticks. International Journal of Food Safety, 2: 13-15.
20. **Tanios A, Shaaban A, Abd EL-Atty I *et al.* (2002)**: Serological features of pathogenic *E. coli* in meat and meat products. The Journal of Egyptian Veterinary Medical Association, 62 (3): 245-253.
21. **Dontorou C, Papadopoulou C, Filioussis G *et al.* (2003):** Isolation of *Escherichia coli* O157:H7 from foods in Greece. International Journal of Food Microbiology,82 (3): 273-279.
22. **Kohansal M, Asad A (2018):** Molecular analysis of *Shiga* toxin producing *Escherichia coli* O157:H7 and non-O157 strains isolated from calves. Onderstepoort Journal of Veterinary Research, 85 (1): 1-7.
23. **Wang L, Rothemund D, Curd H *et al.* (2003):** Species-wide variation in the *Escherichia coli* flagellin (H-antigen) gene. Journal of Bacteriology, 185 (9): 2936 –2943.
24. **FAO and WHO (2019):** Attributing Illness caused by *Shiga* toxin-producing *Escherichia coli* (STEC) to specific foods: Report. Microbiological Risk Assessment Series. Rome, FAO., Pp:74. :///C:/Users/MA/Downloads/9789241516396-eng.pdf
25. **De Castro E, Sigrist C, Gattiker A *et al.* (2006):** ScanProsite: detection of PROSITE signature matches and ProRule-associated functional and structural residues in proteins. Nucleic Acids Research, 34 (2): W362–W365.
26. **EFSA Biohaz Panel, Koutsoumanis K, Allende A *et al.* (2020):** Pathogenicity assessment of *Shiga* toxin-producing *Escherichia coli* (STEC) and the public health risk posed by contamination of food with STEC. Efsa Journal, 18 (1): e05967.
27. **Macori G, McCarthy S, Burgess C *et al.* (2020):** Investigation of the causes of *Shiga*-toxigenic *Escherichia coli* PCR positive and culture negative samples. Microorganisms, 8 (4): 587.
28. **Joris M, Verstraete K, De Reu K *et al.* (2011):** Loss of *vtx* genes after the first sub-cultivation step of verocytoxigenic *Escherichia coli* O157 and non-O157 during isolation from naturally contaminated fecal samples. Toxins, 3: 672–677.
29. **Capobianco J, Clark M, Cariou A *et al.* (2020):** Detection of *Shiga* toxin-producing *Escherichia coli* (STEC) in beef products using droplet digital PCR. International Journal of Food Microbiology, 319: 108499.
30. **Al-Ghazali M, Al-Bulushi I, Al-Subhi L *et al.* (2020):** Food safety knowledge and hygienic practices among different groups of restaurants in Muscat, Oman. https://doi.org/10.1155/2020/8872981.
31. **El-Nemr I, Mushtaha M, Irungu P *et al.* (2019):** Assessment of food safety knowledge, self-reported practices, and microbiological hand hygiene levels of produce handlers in Qatar. Journal of Food Protection, 82: 561-569.