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Isolation and identification of *Escherichia coli* O157:H7 isolated from Veal Meats and Butchers' Shops in Mosul city, Iraq

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ABSTRACT

Escherichia coli (*E. coli*) O157:H7 is considered a significant food-borne microorganism that causes food poisoning infections in humans every year. *E. coli* O157:H7 has various virulence factors such as Shiga-toxin encoding (*Stx1* and *Stx2*). Meat and its products are considered the best meals that consumers eat every day worldwide, but meat and its products are exposed to contamination through unhygienic processing, handling, and storage. The aim of the study was the isolation of *E. coli* O157:H7 and detection of the *uidA*, *Stx1*, and *Stx2* genes. 504 samples of meat and butchers' shops were gathered from diverse areas in Mosul city. Classical and molecular biology techniques were used to isolate and identify *E. coli* O157:H7. The results appeared to indicate the total number of *E. coli* isolates in this study was 138 and the spread rate of *E. coli* O157:H7 isolated was 9.4% (13/138). The spread rate of *E. coli* O157:H7 was high in workers hands 4 (20%), while we did not detect *E. coli* O157:H7 in Machines. Additionally, all *E. coli* O157:H7 have the *uidA*, and *Stx2* genes at 100%, while 92.3% of *E. coli* O157:H7 possess the *Stx1* gene. The study concludes *E. coli* O157:H7 occurrences in meats and butchers' shops and that all equipment and tools used were capable of transmitting *E. coli* O157:H7 to meats. Meats and butchers' shops are a risk to humans who consume the uncooked meats.

Keywords: *E. coli* O157:H7, Meats and butchers' shops, PCR, *Stx1*, and *Stx2* genes, *uidA*.

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INTRODUCTION

E. coli is the most substantial food-borne microorganism that brings about the different types of diseases that lead to the death of humans (Tarr ***et al.*, 2005**). *E. coli* possesses various types of virulence factors encoding genes such as a Shiga toxin one (*Stx1*) and Shiga toxin two (*Stx2*) genes (Kruger and Lucchesi, 2015). *E. coli* O157:H7 is enabled to produce the Shiga toxin (stx1 and stx2) that causes the food poisoning outbreak in humans **(Vallance and Finlay, 2000).** Shiga-toxigenic *E. coli* O157:H7 is a significant serotype of *E. coli* capable of causing a variety of diseases in humans and animals **(Yamasaki *et al.*, 2015)**. Cattle are a major source of disease transmission by infecting humans with *E. coli* O157:H7 bacteria through contaminated animal food or direct contact with infected animals and the environment **(Howie *et al.*, 2003).** In recent decades, *E. coli* O157:H7 has caused food-poisoning infections worldwide. *E. coli* O157:H7 is responsible for the outbreaks of bloody diarrhea in humans in 1982 **(Zhang *et al.*, 2006).** 280 thousand food poisoning cases were reported as a result of eating food contaminated with E. coli O157: H7, with an annual spread rate of 43.1 cases per 100,000 people **(Majowicz *et al.*, 2014).** in the United States, the illness statistics of gastroenteritis caused by *E. coli* O157:H7 yearly were 62000 **(Mead *et al.*, 1999).**

Beef meat and its products are the major route to transferring the *E. coli* O157:H7 to consumers **(Llorente *et al.*, 2014).** The hide of cattle will be contaminated with *E. coli* O157:H7 by direct contact through shedding feces of infected cattle which leads to the contamination of meat during the slaughter process **(Beutin, 2006).** The tools and equipment used in the slaughterhouse such as knives, machines, non-potable water, workers hands, hooks, tables, and carriages contribute to the transmission of *E. coli* O157:H7 to blocks and minced meats **(Marta Hernández *et al.*, 2009)**. The high contamination average of *E. coli* O157:H7 in meats indicates that the meats were produced under unhygienic condition and under bad hygienic practice **(Diercke *et al.*, 2014).** There are two

methods used to reveal *E. coli* O157:H7 including the conventional methods and molecular biology techniques. The conventional methods are based on selective media and biochemical tests to isolate and identify *E. coli* O157:H7 **(Kristen *et al.*, 2016).** The molecular biological techniques are based on detecting the target sequence of the specific-species gene and using the molecular biological techniques to confirm the results of the classical tests. Molecular biology techniques characterize by simpler, cheaper, faster, and more accurate results.

The present study was carried out because there is limited information about the prevalence rate of *E. coli* O157:H7 in veal meats and butchers' shops in Iraq. The objectives of the current research are (i) to isolate and identify *E. coli* O157:H7 from meat and butchers' shops, (ii) to determine the most important equipment that plays a role in spreading *E. coli* O157:H7 in meats and inside butchers' shops, and (iii) to reveal the *uidA*, *Stx1*, and *Stx2* genes in *E. coli* O157:H7 isolates using the PCR technique.

MATERIALS AND METHODS

Sampling

The total number of samples of meat and different parts of a butchers' shops gathered from different areas of the right and left side of Mosul city was 504 samples (84 samples from each Knife, Hook, Table, Machine, Workers' hands, and Meat). The collection period of samples began from September 2021 to January 2022. All the samples were taken using antiseptic containers, while other collected samples were gathered using swabs which were put in antiseptic containers and then transported to the Researchers Center in the department of Veterinary Public Health, College of Veterinary medicine, University of Mosul for identification of zoonotic pathogenic bacteria.

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

Selective media and biochemical tests were utilized to isolate and recognize *E. coli* O157:H7 isolates according to the morphology, shape, and color of *E. coli* O157:H7 colonies. All collected specimens were injected into the nutrient broth (LAB, United Kingdom) and then incubated overnight at 37°C. Based on the conventional culture method, one loop of nutrient broth was transported on the Eosin Methylene Blue Agar (EMB), and MacConkey agar (LAB, United Kingdom) and then incubated overnight at 37°C. In addition, Brilliance *E. coli*/coliform Agar (Oxoid, United Kingdom) is a chromogenic agar which was used in this study to differentiate between *E. coli* and coliform. The suspect *E. coli* was streaked on the HiCrome *E. coli* O157:H7 agar (Himedia Laboratories, India) to allow the differentiation between non-*E. coli* O157:H7 isolates and *E. coli* O157:H7 isolates. All *E. coli* isolates were confirmed by using biochemical tests such as Gram stain, Indole test, Methyl Red test, Citrate Utilization test, Voges-Proskauer test, Catalase, Oxidase, and Triple Sugar Iron agar **(Momtaz*, et al.*, 2013).** All the *E. coli* were maintained in Nutrition broth with 15% glycerol at -80ºC until further use.

DNA Isolation and Template Production

The suspected *E. coli* O157:H7 were proliferation on HiCrome *E. coli* O157:H7 agar for 24 h at 37°C. The Deoxyribonucleic acid of *E. coli* O157:H7 was isolated based on the instructions of the DNeasy Blood and Tissue kit (Geneaid, Korea). The quantity of Deoxyribonucleic acid of *E. coli* O157:H7 was valued by applying the Bio-drop instrumentation and then stored at minus twenty Celsius for further analysis.

Amplification of the *uidA*, *Stx1*, and *Stx2* genes

The PCR assay was based on the sequence of the *uidA*, *Stx1*, and *Stx2* genes of *E. coli* O157:H7 was amplified (Table 1). 25 µL (total volume of PCR reaction) (12.5 µL of 2×Go Taq Green Mix Master (Promega Corporation, USA), including 2 µL of Primer-F and Primer-R, 6.5 µL of DNeasy-free water (Promega Corporation, USA), and (v) 4 µL DNA template of *E. coli* O157:H7. The whole mixture was placed in the Eppendorf tube of 200 µL (Biozym, Oldenhorf, Germany). Finally, by using gel electrophoresis, DNA ladder 100 bp, and 2% agarose gel (Peqlab, Erlangen, Germany), the amplicons of the specific sequence were determined.

Table 1: PCR programs and primers for detecting *E. coli* O157:H7 *uidA*, *Stx1*, and *Stx2* genes

Gene	Primer	Sequence (5- 3)	Amplicon Size [bp]	PCR Programme*	Reference
uidA	uidA-1	5-CCAAAAGCCAGACAGAGT-3	623	I	(Moyo *et al.*, 2007)
	uidA-2	5-GCACAGCACZTCAAAGAG-3			
Stx1	Stx1-1	5-AGTTAATGTGGTGGCGAAGG-3	347	II	(Fujioka *et al.*, 2013)
	Stx1-2	5-CACCAGACAATGTAACCGC-3			
Stx2	Stx2-1	5- TTCGGTATCCTATTCCCGG-3	592	II	(Fujioka *et al.*, 2013)
	Stx2-2	5- CGTCATCGTATACACAGGAG-3			

*PCR program: I: 35 times (94°C – 30s, 57°C – 30s, 72°C – 30s), II: 35 times (94°C – 30s, 55°C – 30s, 72°C – 30s).

RESULTS

Based on the results of the selective media, biochemical tests, and the PCR technique, the gross issue of *E. coli* isolated in the current study was 138. Among of *E. coli* isolates, 13 were *E. coli* O157:H7. The spread rate of *E. coli* O157:H7 isolates were higher in the workers hands 4 (20%) and meat 4 (11.4%). While, the prevalence rate of *E. coli* O157:H7 isolated from knives, hooks, and tables was 2 (7.7%), 1 (7.1%), and 2 (7.7%), respectively as shown in the table 2.

Table 2: Number and percentage of positive *E. coli* O157:H7 isolated from meats and butchers' shops.

Sample	No. of Samples	*E. coli* No.	*E. coli* O157:H7 No.(%)
Knives	84	26	2 (7.7%)
Hooks	84	14	1 (7.1%)
Tables	84	26	2 (7.7%)
Machines	84	17	-
Workers' hands	84	20	4 (20%)
Meat	84	35	4 (11.4%)
Total	504	138	13 (9.4%)

According to our findings, 100% of positive *E. coli* O157:H7 have the *uidA*, and *Stx2* genes, while 92.3% have the *Stx1* gene (Table 3 and Figs. 1,2, 3).

Table 3: The variation rate of the *uidA*, *Stx1*, and *Stx2* genes in *E. coli* O157:H7 isolates.

Gene	No. of positive *E. coli*	Percent of positive *E. coli*
uidA	13/13	100%
Stx1	12/13	92.3%
Stx2	13/13	100%

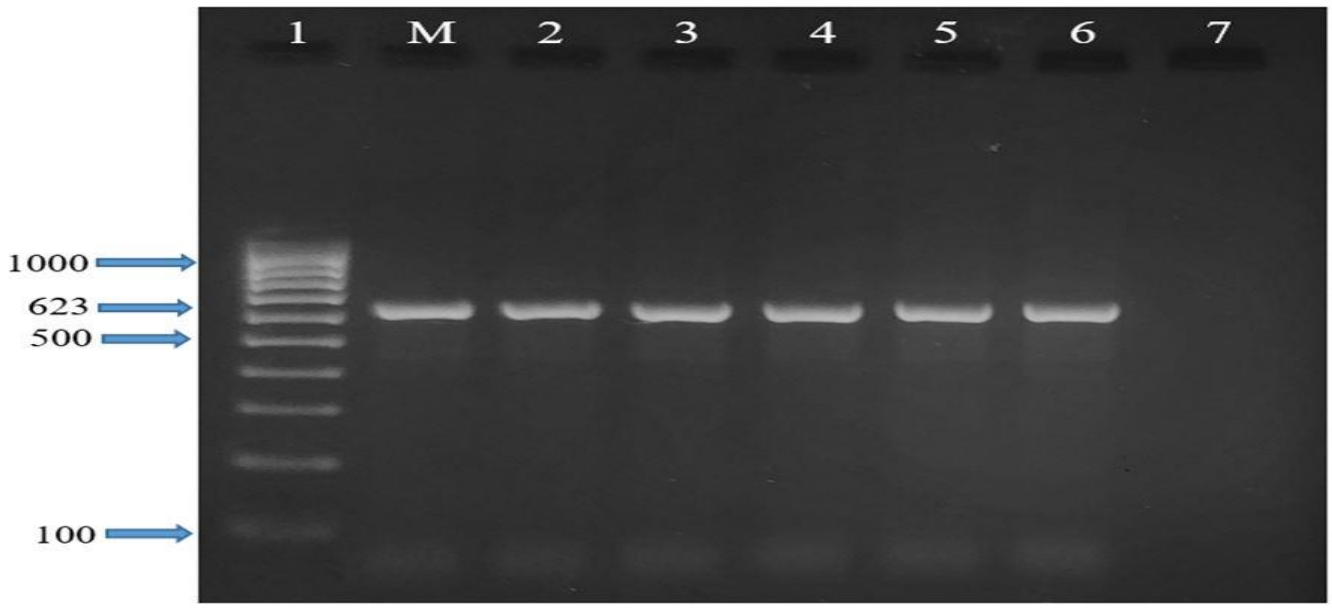

Fig.1: *UidA* gene product in *E. coli* O157:H7isolates (623 bp).

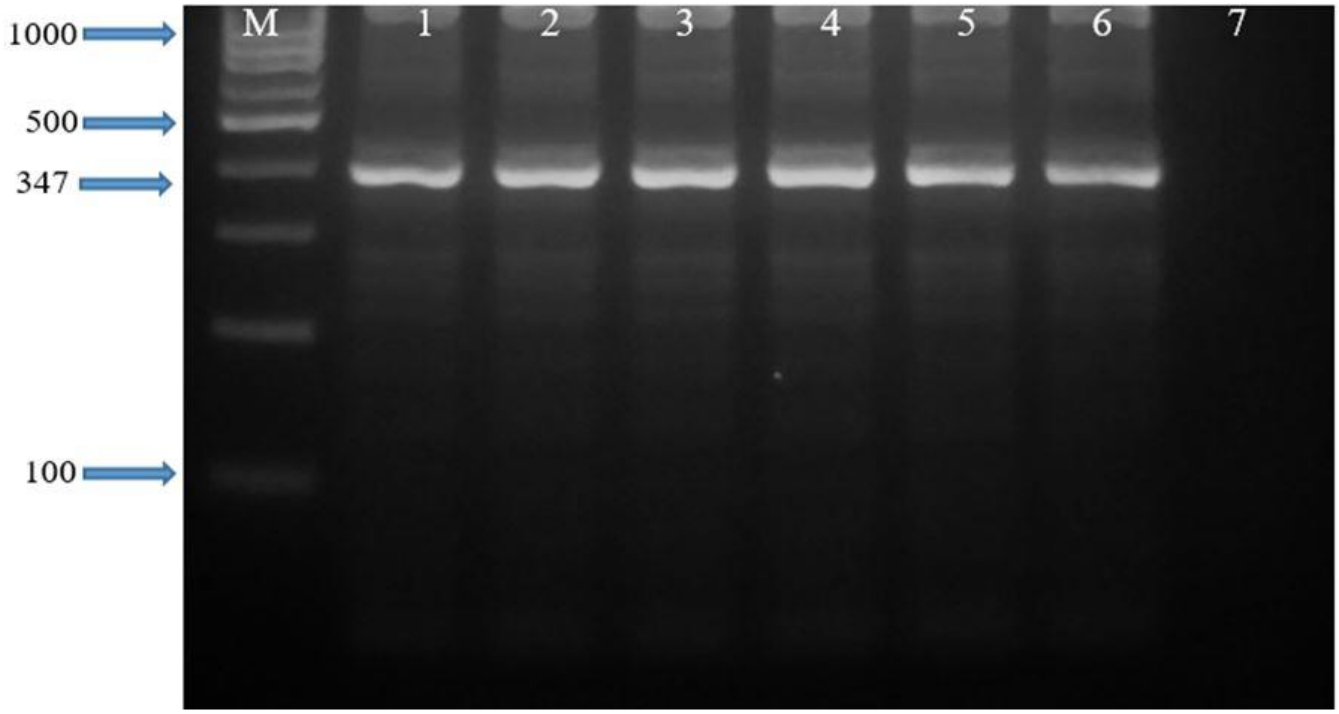

Fig.2: *Stx1* gene product in *E. coli* O157:H7 isolates (347 bp).

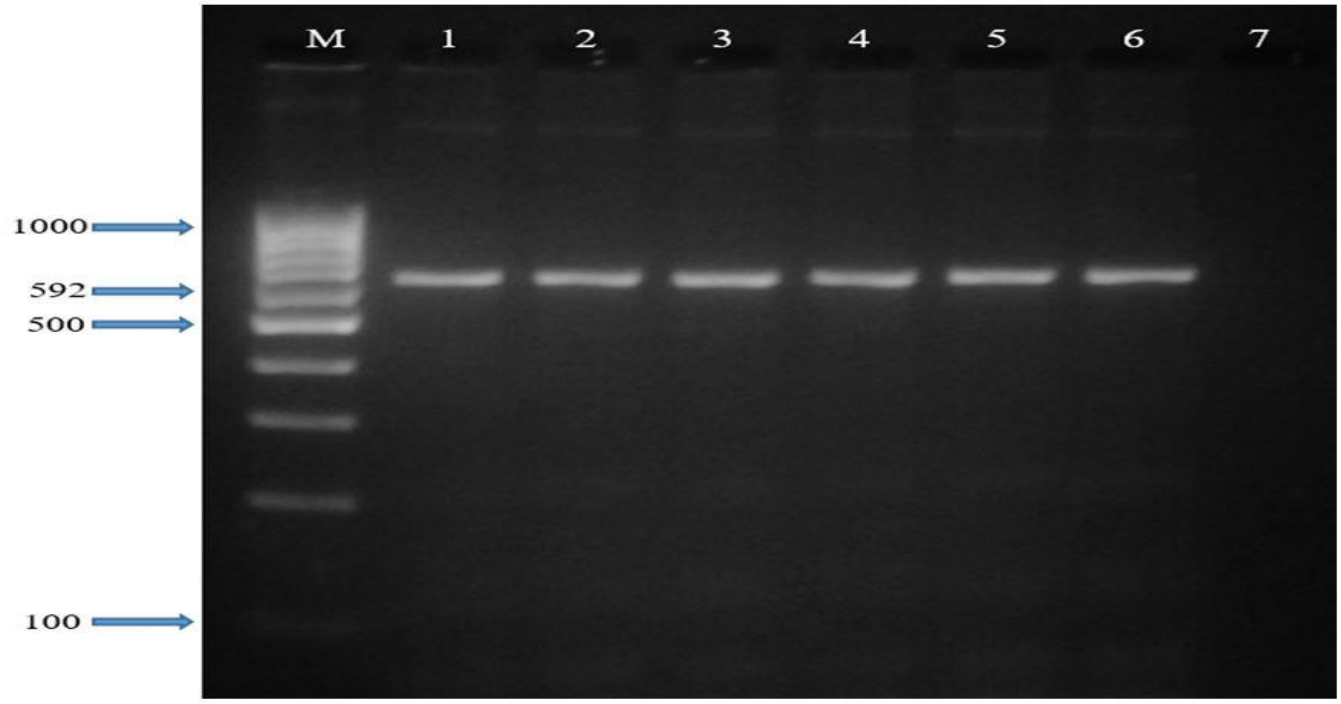

Fig.3: *Stx2* gene product in *E. coli* 157:H7 isolates (592 bp).

DISCUSSION

Animal-derived foods are regarded as a major source of E. coli O157:H7 infection in humans **(Jo *et al.*, 2004).** Ruminants such as cattle, sheep, and goats play an important role in saving and spreading *E. coli* O157:H7 that causes human disease **(Griffin and Tauxe, 1991).** Meat and its products exposed to *E. coli* O157:H7 contamination are a major causative agent of food poisoning in humans **(Hussein, 2007).** The results of the current study found that the spread rate of *E. coli* O157:H7 in meats was 11.4% (4/35). In the current study, the spread rate of *E. coli* O157:H7 was elevated compared to the previous studies which reported 1% positive for *E. coli* O157:H7 in the UK **(Chapman *et al.*, 2000),** 2.8% in the USA **(Padhye and Doyle, 1991),** 3.8% in Argentina **(Chinen *et al.*, 2001),** and

5% in China **(Zhou *et al.*, 2002).** While, the spread rate of *E. coli* O157:H7 is lower than in other studies, which found 16.8% positive for *E. coli* O157:H7 in the USA **(Samadpour *et al.*, 2002),** 25.5% in Argentina **(Brusa *et al.*, 2012),** and 29% in Canada **(Sekla *et al.*, 1990).** The *E. coli* O157:H7 isolate from the feces and carcasses of animals is similar to those that have been contaminated during the slaughter process **(Paiba *et al.*, 2003).** There are many factors were played an important role to contaminate meat and its products by *E. coli* O157:H7 during the slaughter process such as equipment, knives, cutting tables, and machines **(Harhoura *et al.*, 2012).**

In addition, the workers' hands contributed to transmitting the *E. coli* O157:H7 to meats through cutting or handling **(Yilmaz *et al.*, 2006).** Furthermore, rodents, airborne, insects, and other animals contribute to transmitting *E. coli* O157:H7 to meat and its products **(Laury *et al.*, 2009).** Humans would be infected due to consumption of the contaminated meat and its products by *E. coli* O157:H7 which causes several diseases to humans **(Frye and Jackson, 2013).** However, the results of various studies show that it is difficult to compare the results between them because they differ in geographic distribution, sampling collection, and isolation and identification methods of *E. coli* O157:H7.

Additionally, the spread rate of *E. coli* O157:H7 in butchers' shops is 9.4% (13/138). The results of the current study declared that the spread rate of *E. coli* O157:H7 is higher than the prior studies which showed that 1.1% was positive for *E. coli* O157:H7 in the UK (Chapman *et al.*, 2000), 2.3% in Switzerland **(Fantelli and Stephan, 2001),** and 7% in Ethiopia **(Atnafie *et al.*, 2017).** Several studies mentioned the carcasses being exposed to contamination by *E. coli* O157:H7 in abattoirs during the killing or evisceration of animals before transporting the carcasses to the supermarket and butcher shops **(McCluskey *et al.*, 1999); De Boer and Heuvelink, (2000); Dutta *et al.*, (2000).** The higher spread rate of *E. coli* O157: H7 in the developing countries is due to processing the meats under unhygienic conditions, did not apply the hygienic practices, poor meat transport, low level of education, and quality control **(Nicolas *et al.*, 2007).**

According to the PCR method, the results declared that all positive *E. coli* O157:H7 possess the specific-species *uidA* gene. *E. coli* O157:H7 has the *Stx1* gene (92.3%) and *Stx2* (100%). The previous studies had detected the *Stx1* and *Stx2* genes in the difference ratio. The *E. coli* O157:H7 isolates possessed the *Stx1* (3.4%) and *Stx2* (2.5%) **(Aslan *et al.*, 2018).** In Nigeria, the *Stx2* detected in the *E. coli* O157:H7 isolates was 87.5% (7/8) **(Oloyede *et al.*, 2016).** The majority of *E. coli* O157:H7 isolates possess the *Stx2* gene (Majowicz *et al.*, 2014). While some *E. coli* O157:H7 strains have both the *Stx1* and *Stx2* genes, some strains only have the *Stx1* gene **(Zhang *et al.*, 2000).** There are several methods used to isolate and identify *E. coli* O157:H7 and detect all the genes (*Stx1* and *Stx2*), such as conventional methods, immunosorbent assay (ELISA) methods, and PCR methods. The PCR assay used for detecting the *Stx1* and *Stx2* target genes of *E. coli* O157:H7 is suitable and effective from feces or fecal enrichment broth cultures **(Staples *et al.*, 2017).**

CONCLUSION

E. coli O157:H7 is the main food-borne microorganism that causes food poisoning in humans. *E. coli* O157:H7 occurrences in butcher's shops and meats. The equipment used in the butcher's shops (knives, hooks, tables, machines, and worker hands) plays an important role in the spread and transmission of *E. coli* O157:H7 to meats. Most *E. coli* O157:H7 possesses the *Stx1* and *Stx2* genes, and it can produce the Shiga-toxins which cause food poisoning. The PCR methods are helpful in detecting all genes encoding virulence factors of *E. coli* O157:H7.

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Conflict of interest

The authors declaire that there is no conflict of interest regarding the research idea and tools, actual, potential and financial, directly or indirectly.

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