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Phenotypic and genotypic characterizations of *Escherichia coli* Isolated from veal meats and butchers' shops in Mosul city, Iraq

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Abstract

Foodborne pathogens bacteria can cause various diseases and death worldwide. *Escherichia coli* is the most crucial microorganism transmitted through meat and its products. Pathogenic *E. coli* is one of the major groups that can produce the *Stx1* and *Stx2* toxins. The present study aims to isolate and identify the *E. coli* bacteria using the classical methods, and to detect the specific-species *uidA* gene, and *Stx1* and *Stx2* genes using the PCR assay. Five hundred four samples were collected randomly from meats and different parts of a butcher shops from various regions of the right and the left sides of Mosul city. The results found that the prevalence rate of *E. coli* in this study was 27.4% (138/504). Additionally, the prevalence rate of *E. coli* was higher in meat, 41.7% (35/84). At the same time, the prevalence rate of *E. coli* was lower in hook 16.7% (14/84). Additionally, the prevalence rate of *E. coli* in meats and butcher shops on the right and left sides of Mosul city was 31.9% (65/204) and 24.3% (73/300), respectively. Furthermore, all *E. coli* isolates possessed the specific species *uidA* gene. 30/138 (21.7%) of *E. coli* isolates possess the *Stx1* gene, while 17/138 (12.3%) of *E. coli* isolates have the *Stx2* gene. Finally, most *E. coli* isolates possessed the *Stx1* and *Stx2* genes 91/138 (66%).

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Introduction

Many Zoonotic foodborne microorganisms are transmitted from animals and their products to consumers while consuming the contaminated foods (1). The Zoonotic foodborne microorganisms have been able to contaminate meat while processing of carcasses in the abattoir (2). The most critical foodborne microorganisms isolated from meat and its products were *Escherichia coli* (*E. coli*), *Salmonella* spp., *Listeria monocytogenes*, and *Campylobacte*r spp., which isolated from several cases of outbreaks of human illness (3-5). The most important source of proteins for human beings is meat which may be exposed to contamination by *E. coli* during the slaughter of the animals under unhygienic conditions and slaughter practices, pollutants, insects, and rodents (6,7). *E. coli* is a Gram-negative, rod-shaped, facultatively anaerobic, which lives in the intestines of animals and humans (8). Humans may be infected with pathogenic Shiga toxin-producing *E. coli* (STEC) which causes various types of disease and sometimes death (9,10). STEC can cause outbreaks and food poisoning worldwide due to consuming contaminated food like meat (meat products) and milk (dairy products) (11,12), water, fruits, and vegetables (13). In addition, equipment such as knives, tables, saws, hooks, and other utensils may play a significant role in spreading the STEC during processing carcass and meats cuts (14). STEC can produce the Shiga toxins (15). The Shiga toxins (*Stx1* and *Stx2*) are the primary virulence genes (16). *Stx1* and *Stx2* are responsible for causing diseases in consumers and animals (17). The methods used to identify the STEC are based on the classical methods including the selective media,

chromogenic media, and biochemical tests, also on the Molecular biology techniques such as polymerase chain reaction (PCR) and RT-PCR used to detect the target sequence gene (18). In the last decades, few studies have been performed to identify the STEC and its toxins isolated from meat. Therefore, the project was carried out to determine the prevalence of STEC in meats and butcher shops in Mosul city, Iraq.

The study aimed to identify the phenotypic characterization of *E. coli* isolated from meats and butcher shops by using selective media, chromogenic media, and biochemical tests, as well as to identify the genotypic characterization of *E. coli* by detecting the *uidA*, *Stx1,* and *Stx2* gene by using the PCR assay.

Materials and methods

Ethical approve

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Sampling

In the current study, 504 samples were collected randomly from meats and different parts of a butcher's shops (84 samples from each knife, hooks, tables, machines, worker's hands, and veal meat) of the right and the left part of Mosul city. The study period started in September 2021 and ended in January 2022. Meats were collected using sterile containers, while other collected samples were gathered using swabs which were put in containers (sterile) and then transported to the Researchers Center for diagnosis of zoonotic pathogenic bacteria.

E. coli isolation and identification

Meat samples and butcher shops parts swabs were analyzed to isolate and identify of pathogenic *E. coli*. All samples and swabs were inoculated in the nutrient broth (LAB, United Kingdom) and then incubated overnight at 37°C. For the classical culture method, one loop of nutrient broth was plated 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) was used in this study to differentiate between generic *E. coli* and coliform. The suspected *E. coli* isolates were confirmed using biochemical tests such as Gram stain, Indole test, Methyl Red test, Citrate Utilization test, and Voges-Proskauer test, Catalase, Oxidase, and Triple Sugar Iron agar (19). All the *E. coli* isolates were frosted in Nutrition broth (15% glycerol) at -80ºC until further use.

DNA isolation and template production

The suspected *E. coli* were cultured on the Brilliance *E. coli/coliform* agar overnight at 37°C. Deoxyribonucleic acid of *E. coli* was isolated according to the instructions of the DNeasy Blood and tissue kit (Geneaid, Korea). The concentration of Deoxyribonucleic acid of *E. coli* was estimated using the Bio-drop device then and stored at -20°C for further analysis.

UidA, Stx1, and *Stx2* genes amplification

The PCR assay, amplified the sequence of the *uidA, Stx1*, and *Stx2* of *E. coli* bacteria isolated (Table 1). Twenty-five µL (total volume of PCR reaction) (12.5 µL of 2×Go Taq Green Mix Master including (Promega Corporation, USA), 1 µL of primer-F, 1 µL primer-R, 6.5 µL of nuclease-free water (Promega Corporation, USA), and (v) 4 µL DNA template of *E. coli*. The whole mixture was put in the Eppendorf tube at 200 µL (Biozym, Oldenhorf, Germany). Finally, the amplicons of the target sequence were determined by using gel electrophoresis, DNA marker 100 bp (ladder), and 2% agarose gel (Peqlab, Erlangen, Germany).

Table 1: PCR program and Primers for detection of *uidA, Stx1,* and *Stx2* in *E. coli*

Gene	Primer	Sequence (5- 3)	Amplicon Size [bp]	PCR Program*	Reference
uidA	uidA-1	5-CCAAAAGCCAGACAGAGT-3	623	I	(20)
	uidA-2	5-GCACAGCACZTCAAAGAG -3			
Stx1	Stx1-1	5-AGTTAATGTGGTGGCGAAGG-3	347	II	(21)
	Stx1-2	5-CACCAGACAATGTAACCGC-3			
Stx2	Stx2	5- TTCGGTATCCTATTCCCGG-3	592	II	(21)
	Stx2	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

According to the morphology of colonies, the positive *E. coli* isolates appeared color with a metallic green sheen on the EMB agar, dark pink color on the MacConkey agar, and purple color on the Brilliance *E. coli* / coliform Agar. In addition, all isolates of *E. coli* were positive for the special biochemical tests used for identifying the isolates of *E. coli*. Our findings showed that the prevalence of *E. coli* in meat and butcher shops was 27.4% (138/504). Furthermore, the percentage of *E. coli* was higher in meat, 41.7% (35/84). In contrast, the percentage of *E. coli* was lower in hooks in

butcher shops, 16.7% (14/84). Additionally, the percentage of *E. coli* isolated from knives, tables, worker hands, and machines was 31% (26/84), 31% (26/84), 23.8% (20/84), and 20.2% (17/84), respectively (Table 2).

In addition, our result showed that the percentage of *E. coli* isolated from meats and butchers' shops on the right side of Mosul city was 31.9% (65/204) which is more than on the left side 24.3% (73/300). Furthermore, the prevalence rate of the *E. coli* isolated from knives, tables, machines, workers hands on the right side was 41.2%, 35.3%, 29.4%, and 29.4% that were higher contaminated from the knives, tables, machines, workers' hands on the left side of the Mosul city (Table 3).

According to table 4, our findings showed that all *E. coli* isolates are possessed the specific species *uidA* gene. The results of the culture method were confirmed using the PCR technique. The results of the culture method and the results of the PCR assay were concorded (Figure 1). In addition, the *Stx1* gene was detected in *E. coli* isolates 30/138 (21.7%) (Figure 2), while the *Stx2* was detected in *E. coli* isolates 17/138 (12.3%) (Figure 3). Our study also revealed that most *E. coli* isolates possessed the *Stx1* and *Stx2* genes 91/138 (66%).

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

Sample	No. of Sample	Positive No. (%)
Knives	84	26 (31)
Hooks	84	14 (16.7)
Tables	84	26 (31)
Machines	84	17 (20.2)
Hands	84	20 (23.8)
Meat	84	35 (41.7)
Total	504	138 (27.4)

Table 3: The percentage of positive *E. coli* isolated from meats and butchers' shops on the right and left banks of Mosul city

Sample	Right side Left side			
	No. Sample	Positive No. (%)	No. Sample	Positive No. (%)
Knives	34	14 (41.2%)	50	12 (24%)
Hooks	34	5 (14.7%)	50	9 (18%)
Tables	34	12 (35.3%)	50	14 (28%)
Machines	34	10 (29.4%)	50	7 (14%)
Worker Hands	34	10 (29.4%)	50	10 (20%)
Meat	34	14 (41.2%)	50	21 (42%)
Total	204	65 (31.9%)	300	73 (24.3%)

Table 4: The variation rate of the *uidA*, *Stx1*, and *Stx2* genes in *E. coli* isolates

Gene	No. +ve *E. coli*	% +ve *E. coli*
uidA	138/138	100%
Stx1	30/138	21.7%
Stx2	17/138	12.3%
Stx1, Stx2	91/138	66%

Discussion

In recent decades, *E. coli* has been considered the prime etiology of food poisoning worldwide. The food animal originated significantly transmitted *E. coli* to humans via the consuming contaminated foods with *E. coli* (22). our results demonstrate that the percentage of *E. coli* isolated from meat and butcher shops was 27.4% (138/504). In addition, this study showed the percentage of *E. coli* was rising in meat at 41.7% (35/84), which agreed with the previous surveys that found the percentage of *E. coli* in meat was 43.1% in India (23), 43.4 % in the United States (24). However, our results are lower than the previous surveys that appeared the percentage of *E. coli* from meat in Egypt was 54% (27/50) (25), 74.5% in South Africa (26), and 100% in Burkina Faso (27). In addition, our results were higher than other studies which found the prevalence rate of *E. coli* in meat was 1.5% in Iran (28), 17.8% in Australia (29), and 21.1% (49/232) in the United States (30). Various contamination rates of *E. coli* in meats may be due to variations in the national or geographic features of animal feeding systems, differences in meat processing, and differences in the methods used in the microbiological tests (31). Many studies found that raw and processed meat was more contaminated with *E. coli* (32). Meat and its products are exposed to contamination with *E. coli* from entering the animals to the slaughterhouse until consuming meat. Many factors contribute to transmitting *E. coli* to meat and its products such as the skin of animals, cutting tools and machines, an unhygienic environment, loss of workers' hygiene (33). In developing countries, most people want to buy cheap meats from the informal markets which do not apply hygiene instructions and safety standards when cutting meat (34).

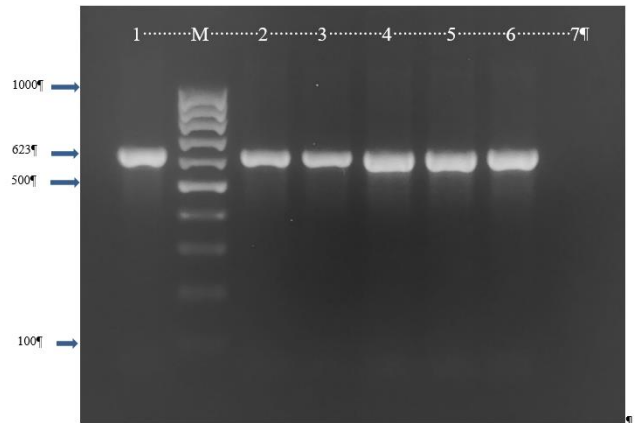

Figure 1: *UidA* gene product in *E. coli* isolates (623 bp).

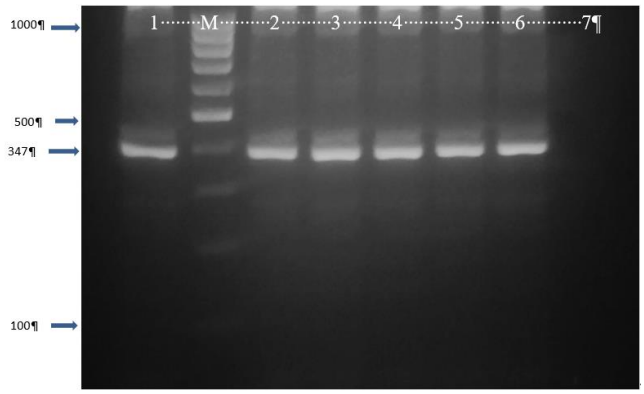

Figure 2: *Stx1* gene product in *E. coli* isolates (347 bp).

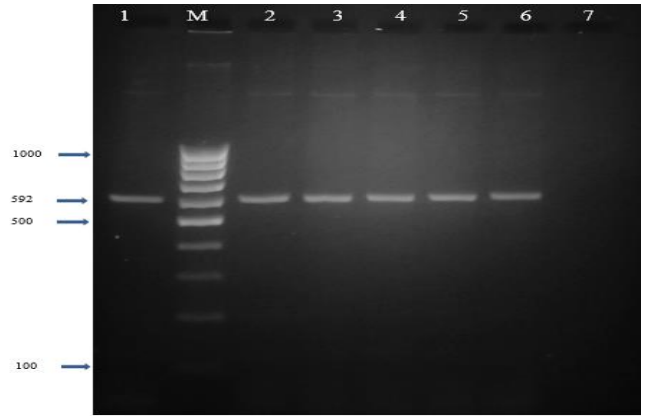

Figure 3: *Stx2* gene product in *E. coli* isolates (592 bp).

Additionally, the current study showed that *E. coli* possesses the *Stx1* gene in 30/138 (21.7%), while *E. coli* has the *Stx2* gene in 17/138 (12.3%). Also, our study revealed that most *E. coli* isolates possessed the *Stx1* and *Stx2* genes in 91/138 (66%). The *Stx1* gene is more predominantly detected than *Stx2* in *E. coli* isolates. Our results were similar to previous contributions, which showed that the *Stx1* was more frequently found in *E. coli* (35,36). Many studies showed a difference between the frequent found of the *Stx1* and *Stx2* genes in *E. coli* isolates. The previous study showed that 29% of isolates possess the *Stx1* gene, 51% isolates carry the *Stx2* gene, and 20% possess both *Stx1* and *Stx2* (37). The previous studies showed that 5.3% of isolates carried *Stx1*, 86% of isolates had *Stx2*, and 8.8% of isolates possessed *Stx1* and *Stx2* (38), another study declared that 40.68% of *E. coli* isolates contained *Stx1* and 5.88% of *E. coli* isolated have *Stx2* (36). At the same time the *Stx1* and *Stx2* genes did not detect *E. coli* isolated from meat and fish in Vietnam (39).

Conclusion

Escherichia coli is significant foodborne microorganism in humans due to consuming meats and meat products. Our findings revealed that *E. coli* was isolated from different samples (Knives, Hooks, Tables, Machines, and Worker Hands) which have a role in spreading the *E. coli* isolates in meats and meat products. The meat was the most contaminated with *E. coli* due to passing meat in different contamination stages starting from slaughterhouse until sale meats in the supermarket and meat shops. In addition, the molecular characteristics of *E. coli* isolates have appeared that some isolates possess only the *Stx1* gene, while anther *E. coli* possess only the *Stx2* gene, and most *E. coli* possess the *Stx1* and *Stx2* genes together.

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Conflict of interest

There are no conflicts of interest.

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التوصيفات المظهرية والجينية لجرثومة الإشريكية القولونية المعزولة من لحوم العجول ومحلات الجزارة في مدينة الموصل، العراق

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الخلاصة

تمتلك البكتيريا المسببة للأمراض المنقولة عن طريق الغذاء القدرة على التسبب في حدوث أمراض مختلفة والوفاة في جميع أنحاء العالم. تعتبر جراثيم الإشريكية القولونية من أهم أنواع الجراثيم التي تنتقل عن طريق اللحوم ومنتجاته. تعتبر جراثيم الإشريكية القولونية التي تنتج سموم الشيجا كواحدة من اهم المجاميع الرئيسية التي لها القدرة على إنتاج نوعين من السموم *Stx1* و *Stx2* .أهداف هذه الدراسة هي عزل وتشخيص جراثيم الاشريكيا القولونية باستخدام الطرق التقليدية، وكذلك الكشف عن جين محدد *uidA*، وكذلك جينات *Stx1* و *Stx2* باستخدام طريقة تفاعل البلمرة المتسلسل. جمعت ٥٠٤ عينة عشوائيا من اللحوم وأجزاء مختلفة من محلات الجزارين من مناطق مختلفة من جانبي الأيمن والأيسر من مدينة الموصل. أظهرت نتائج دراستنا أن نسبة انتشار جراثيم الاشريكيا القولونية المعزولة من محلات اللحوم والجزارة كانت ٢٧,٤٪ (١٣٨/٥٠٤). بالإضافة إلى ذلك، كان معدل انتشار الإشريكية القولونية بنسبة عالية في اللحوم ٤١,٧٪ (٣٥/٨٤). بينما كان معدل انتشار بكتيريا جراثيم الاشريكيا أقل في الخطاف ١٦,٧٪ (١٤/٨٤). بالإضافة إلى ذلك، بلغ معدل انتشار الإشريكية القولونية من محلات اللحوم والجزارين في الجانب الأيمن والجانب الأيسر من مدينة الموصل ٣١,٩٪ (٦٥/٢٠٤) و ٢٤,٣٪ (٧٣/٣٠٠). بالإضافة إلى ذلك، فإن جميع عزلات الإشريكية القولونية تمتلك نوعا معينا من جين*uidA* . وجد جين *Stx1* في عزلات جراثيم الاشريكيا القولونية وبنسبة ٢١,٧%(٣٠/١٣٨)، بينما وجد *Stx2* في عزلات جراثيم الاشريكيا القولونية ١٢,٣%(١٧/١٣٨). أخيرا، امتلكت معظم عزلات الإشريكية القولونية الجينات *Stx1* و *Stx2* ٦٦%(٩١/١٣٨).