Research Article

Detection and Distribution of *Escherichia coli* O157:H7 Isolated from local Fresh Beef Sold in Baghdad City

Ibrheem E. Ibrhim*, Zina Saab Khudhir

Department of Veterinary Public Health, College of Veterinary Medicine, University of Baghdad, Iraq.

Abstract | *Escherichia coli* (*E. coli*) O157:H7 is a highly pathogenic, food-borne bacterium commonly associated with meat contamination and poses a significant public health risk. This study aimed to investigate the incidence and distribution of coliforms, *E. coli*, and *E. coli* O157:H7 in beef samples collected from butcher shops across Baghdad, Iraq, and to assess the antimicrobial susceptibility of confirmed isolates. A total of 140 beef samples were collected from the Al-Karkh and Al-Rusafa regions of Baghdad. Isolation and identification of coliforms and *E. coli* were performed using conventional culture methods, biochemical testing, and polymerase chain reaction (PCR) analysis. PCR was employed to detect the presence of the *eaeA*, *rfbE*, and *fliCH7* genes in biochemically confirmed isolates to identify *E. coli* O157:H7. The results revealed that 31 (44.29%) and 27 (38.57%) isolates from Al-Karkh and Al-Rusafa, respectively, were identified as *E. coli*. While *E. coli* O157:H7 was not detected in any samples from the Al-Karkh region, 2.86% of the samples from Al-Rusafa tested positive for this pathogenic strain. Antimicrobial susceptibility testing of two confirmed isolates *E. coli* and *E. coli* O157:H7 against ampicillin, cefotaxime, kanamycin, nitrofurantoin, rifampicin, and streptomycin revealed multiple antibiotic resistance (MAR) indices of 0.30 and 0.55, respectively. These findings indicate that a portion of fresh beef sold in the Al-Rusafa region is contaminated with antibiotic-resistant *E. coli* O157:H7, highlighting a significant public health concern and emphasizing the need for improved food safety practices and routine surveillance.

Keywords | Antibiotic resistance, *E. coli* O157:H7, Beef, *eaeA, rfbE, fliCH7*

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***Correspondence** | Ibraheem E. Ibrhim, Department of Veterinary Public Health, College of Veterinary Medicine, University of Baghdad, Iraq; **Email:** ibraheeme.ibraheem@uokufa.edu.iq
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INTRODUCTION

Foods from animal origin, such as chicken, beef, milk, and dairy products, have high protein content, which is essential for the growth and development of the body. However, they transmit various pathogenic and food-spoiling microorganisms which are human health hazards, because they cause death. Therefore, these foods and products from animals represent a public health concern all over the world (Manthoor and Saliem, 2023; saad *et al.*, 2024).

Highly dangerous pathogens such as *E. coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes, Bacillus cereus,* and *Staphylococcus aureus* can be transmitted through eating meat and meat products (Abdulridha and Saliem, 2023; Al-Jaghifi and Khudhir, 2024a). Pathogenic bacteria with resistance to different kinds of antibiotics are gradually increasing, due to frequent and random use of antibiotics for veterinary treatments in attempts to prevent outbreaks of pathogens and to reduce the development of drug resistance among harmful microorganisms (Al-Jaghifi and

Khudhir, 2024b). *E. coli* in animal-based foods is used as a contamination indicator of fecal materials and quality control practices, which include food production, food preparation, processing, handling, and market storage. Hence, the presence of coliform and *E. coli* is an indicator of food contamination by another pathogenic microorganism, such as *Salmonella* spp. and enteropathogenic bacteria, among others, which often threaten the safety of food. This is because these harmful bacteria are a serious public health concern. *E. coli* O157:H7 can be transmitted through the fecal-oral route or unhygienic practices for meat, during dressing livestock for storage and marketing (Dakheel *et al*., 2023). Domestic ruminants, which include cattle, goats, and sheep, are considered to be reservoirs of *E. coli* O157:H7 (Khudhir and Hammad, 2016). Consequently, meat and meat products from these livestock are often contaminated by *E. coli* O157:H7 (Khudhir and Hammad, 2016). Therefore, the objective of this study was to (i) confirm the prevalence and distribution of *E. coli* O157:H7 in beef sold in Baghdad city and (ii) examine the resistance statuses of *E. coli* and *E. coli* O157:H7 isolates from beef samples to some selected antibiotic drugs.

MATERIALS AND METHODS

Study Site and Sampling

This study was done from February 2023 to March 2024 in two regions located in Baghdad city (Al-Karkh and Al-Rusafa) of Iraq. A total of 140 beef samples were collected randomly: seventy (70) samples were obtained from retail butchers' shops in each of the two regions in Baghdad city. Minced meat samples were transported in ice-boxes immediately after collection. They were taken to the post-graduate milk and meat laboratory, Veterinary section of the Public Health Department, University of Baghdad, Iraq. Bacteriological tests were conducted within 2 hours after samples were collected on the same day.

Beef Samples Preparation for Bacteriological Analysis

One (1) kilogram of boneless beef samples was purchased from the local butcher shops in Baghdad city. These shops slaughtered the beef every morning to ensure freshness. The aim was to isolate *E. coli* O157:H7 using laboratory methods. Twenty-five grams of the beef was homogenized after adding 225 ml of modified tryptic soy broth (mTSB, Himedia, India) in the sterile polyethylene bags in the stomacher for 1-3 minutes. The mixture was incubated aerobically at 37 °C for 24 h (Haramain and Yagoub, 2021). After that, the culture was grown on MacConkey agar (MAC, Oxoid, UK) and eosin methylene blue agar (EMB, Oxoid, UK), incubated at 37 °C for 24 hours (Khudhir and Hammad, 2016), to get the visible colonies of bacteria. The isolates were confirmed as *E. coli* O157:H7 by plating onto the cefixime tellurite sorbitol MacConkey agar (CT-SMAC, Oxoid, UK), and HiCrome O157:H7 agar (HEC, Hi-Media, India) following the procedures of Shahid and Yousif, (2022). Isolates were examined using Gram staining, motility test, and biochemical tests (indole, methyl red, Voges-Proskauer, oxidase, and catalase) as shown in Table 1.

Table 1: Cultural and biochemical characterization of *E. coli* O157:H7.

Cultural	**Cultural test**	**Characteristics**	
	MAC agar	Pink colonies	
	EMB agar	Dark colonies with center and a greenish metallic sheen	
	SMAC- CT agar	Colorless colonies	
	Chromogenic agar	Mauve colonies	
Bio-chemical	**Biochemical tests**	**Result**	**Characteristics**
	Gram Stain	Negative	Pink
	Motility Test	Positive	Motile
	Oxidase Test	Negative	light pink
	Indole Test	Positive	Red ring
	Catalase Test	Positive	Bubble formation
	Methyl Red Test	Positive	Red colure
	Voges Proskauer Test	Negative	Yellow colure

DNA Extraction

DNA extraction from bacterial cells was carried out by the G-spin Total DNA Extraction Kit, according to the manufacturer's instructions. One or two mL of each culture was harvested by centrifugation (13000 rpm, 15 min, 65 °C). The sedimented colonies were washed five times, using phosphate buffered saline, and then suspended in 1.0 mL of sterilized water; they were kept at 4°C for the next step (Keeratikunakorn *et al*., 2024).

Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) was performed using genomic DNA extracted from the isolated bacterial strains. A volume of 1 µL of DNA (approximately 200 ng) was added to each PCR reaction mixture. The thermal cycling conditions were as follows: an initial denaturation step at 94°C for 5 minutes; followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 1 minute. A final extension step was carried out at 72°C for 5 minutes to complete the amplification process.

Primers

Primers used in this study were illustrated in Table 2, along with their sequences and concentrations. A total reaction volume was adjusted to 50 µl using deionized water. To prevent evaporation during the reaction, 40 µl of paraffin oil was added.

Table 2: Primers used for detection of *E. coli* O157:H7.

Primer sequence 5'-3'	Genes	size of products	References
F-AAGATTGCGCT-GAAGCCTTTG R-CATTGGCATCGT-GTGGACAG	rfbE	497 bp	(Desmarchelier *et al.*, 1998)
F-GCGCTGTC-GAGTTCTATCGAGC R-CAACGGTGACT-TTATCGCCATTCC	fliCH7	625 bp	Gannon *et al.*, 1997
F-CAGGTCGTCGT-GTCTGCTAAA R-TCAGCGTG-GTTGGATCAACCT	eaeA	1089 bp	Gannon *et al.*, 1993

Agarose Gel Electrophoresis

The amplified PCR products were analyzed by agarose gel electrophoresis, following the procedure described by Sambrook *et al.* (1989). A standard 100 bp DNA ladder was used as a molecular size marker to estimate the fragment sizes of the amplified products.

Antimicrobial Sensitivity Test

Confirmed *Escherichia coli* O157:H7 isolates were subjected to antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (MHA; Merck, Germany), following the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines. A total of 20 commercially available antibiotic discs (Oxoid, UK) were used: Amikacin (30 µg), Amoxicillin/Clavulanic Acid (30 µg), Ampicillin (10 µg), Cefixime (5 µg), Cefotaxime (30 µg), Cefoxitin (30 µg), Chloramphenicol (10 µg), Ciprofloxacin (5 µg), Colistin (25 µg), Doxycycline (30 µg), Gentamicin (10 µg), Imipenem (10 µg), Kanamycin (30 µg), Levofloxacin (5 µg), Nalidixic Acid (30 µg), Nitrofurantoin (300 µg), Ofloxacin (10 µg), Rifampicin (5 µg), Streptomycin (10 µg), and Tetracycline (10 µg).

Bacterial isolates were first cultured on nutrient agar and incubated at 37 °C for 24 hours. Following incubation, colonies were suspended in 5 mL of sterile 0.85% saline to achieve turbidity equivalent to 0.5 McFarland standard. Sterile cotton swabs were dipped into the standardized suspension, excess fluid was removed by pressing the swab against the inner wall of the test tube, and the inoculum was evenly spread over the surface of MHA plates.

Antibiotic discs were then placed on the inoculated plates using sterile forceps, ensuring a minimum spacing of 24 mm between discs and 10 mm from the plate edge. Plates were left at room temperature for 3–5 minutes to allow absorption and then incubated at 37 °C for 24 hours. Post-incubation, the diameters of the zones of inhibition were measured in millimeters using a digital caliper, and results were interpreted as susceptible, intermediate, or resistant according to CLSI standards.

The multiple antibiotic resistance (MAR) index for each isolate was calculated following the method described by Krumperman (1983), using the formula MAR = a/b, where a is the number of antibiotics to which the isolate was resistant, and b is the total number of antibiotics tested.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism version 8.0 (GraphPad Software, Inc., USA). The Chi-square test for equal proportions was employed to assess whether the frequency of quality control bacterial isolates or *E. coli* O157:H7 strains varied significantly across different categorical groups of beef samples. A p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Isolation and Identification of *E. coli* O157:H7 from Raw Beef Samples

The *E. coli* O157:H7 was isolated and identified from beef in Baghdad province using biochemical tests, simple media and selective media. On MacConkey agar, the colony appeared pink and non-mucoid due to the lactose fermentation. On EMB agar, the colonies exhibited a green metallic sheen, which aided in the preliminary identification of organism. These isolates were confirmed as *E. coli* by biochemical tests. The identified *E. coli* colonies were further sub-cultured onto CT-SMAC agar and HiCrome agar, followed by incubation at 37 °C for 24 hours. On CT-SMAC agar, *E. coli* O157:H7 colonies appeared colorless due to their inability to ferment sorbitol, a characteristic that distinguishes them from other *E. coli* serotypes. On HiCrome agar, the *E. coli* O157:H7 colonies exhibited a mauve to pink coloration. These phenotypic traits are consistent with the findings reported by Jabur *et al.* (2016).

Escherichia coli O157:H7 is a highly pathogenic bacterium in humans, and its detection and distribution are best confirmed through molecular characterization using specific virulence genes. In this study, a total of 140 randomly collected beef samples were analyzed to determine the prevalence and regional distribution of *E. coli* O157:H7 in Baghdad. The results showed that in the Al-Karkh region, 31 isolates of *E. coli* were identified, representing 44.29% of the contaminated samples. In the Al-Rusafa region, 27 isolates were detected, accounting for 38.57% of the contaminated samples. These findings highlight the widespread presence of *E. coli* in retail beef and underscore the importance of regional monitoring and molecular diagnostics for food safety surveillance.

The isolation process focused on the Karkh and Rusafa districts of Baghdad. *E. coli* O157:H7 was not detected in any of the samples collected from the Karkh region, as shown in Table 3. In contrast, 2 out of 70 samples (2.86%) from the Al-Rusafa region tested positive for *E. coli* O157:H7, as presented in Table 4. These findings align with previous studies conducted in Baghdad, where *E. coli* O157:H7 was also isolated from raw beef products, such as minced beef and beef burgers (Al-Obaidi and Dawood, 2023; Al-Jaradat et al., 2024b). Overall, the contamination rate of *E. coli* O157:H7 in this study was 2 out of 140 samples, equivalent to 1.42%.

Table 3: Distribution of Coliform, *E.coli* and *E.coli* O157:H7 in beef samples collected from Al-Karkh region.

Districts	No. of samples	Coliform positive isolates (%)	E. coli positive isolates (%)	E. coli O157:H7 positive isolates
Abu Ghraib	10	5 (50%)	5 (50%)	0 (0%)
Al-A`amiriya	10	7 (70%)	3 (30%)	0 (0%)
Al-shu`ala	10	6 (60%)	4 (40%)	0 (0%)
Bayaa	10	3 (30%)	7 (70%)	0 (0%)
Dora	10	3 (30%)	7 (70%)	0 (0%)
Kadhimiya	10	8 (80%)	2 (20%)	0 (0%)
Seaidya	10	7 (70%)	3 (30%)	0 (0%)
Total	70	39 (55.71%)	31 (44.29%)	0 (0%)
Chi-square value		9.61	9.61	--
P-value		0.14 NS	0.14 NS	--

Table 4: Distribution of Coliform, *E.coli* and *E.coli* O157:H7 in beef samples collected from Al-Rusafa region.

Districts	No. of samples	Coliform	E. coli	E. coli O157:H7
		Number of positive isolates (%)	Number of positive isolates (%)	Number of positive isolates(%)
Adhamiyah	10	7 (70%)	3 (30%)	0 (0%)
Bab Al-Moatham	10	8 (80%)	2 (20%)	0 (0%)
Baghdad Al-Jadida	10	5 (50%)	5 (50%)	0 (0%)
Karrada	10	8 (80%)	2 (20%)	0 (0%)
Sadr City	10	3 (30%)	6 (60%)	1 (10%)
Sha`ab	10	5 (50%)	4 (40%)	1 (10%)
Zayonua	10	5 (50%)	5 (50%)	0 (0%)
Total	70	41(58.57%)	27(38.57%)	2 (2.86%)
Chi-square value		8.59	3.15	1.15
P-value		0.16 NS	0.78 NS	0.97 NS

The two *E. coli* O157:H7 isolates were confirmed using conventional polymerase chain reaction (PCR) by detecting the presence of key virulence genes (*eaeA*, *rfbE*, and *fliCH7*), as shown in Figures 1 and 2. These findings are consistent with previous studies conducted both in Iraq and internationally. For instance, a study in Erbil, Iraq, reported that 3 out of 100 meat samples (6%) were positive for *E. coli* O157:H7 (Jalal and Aziz, 2023). Similarly, in Riyadh, Saudi Arabia, Hessain *et al.* (2015) found that 2% of 370 beef samples tested positive for the pathogen.

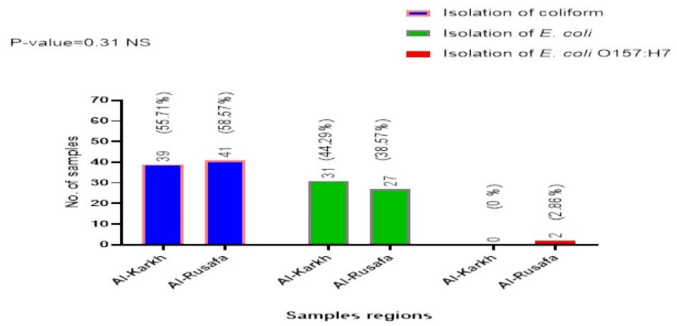

Figure 1: Isolation percentages of Coliform, *E.coli* and *E.coli* O157:H7 from beef meat samples.

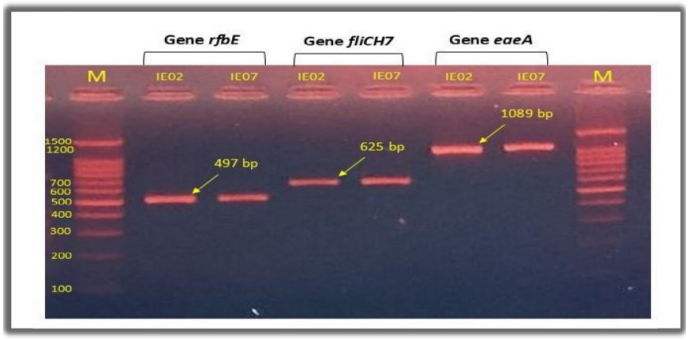

Figure 2: PCR amplification of partial region of three genes responsible for virulence of *E. coli* O157:H7.

Although the overall contamination rate of *E. coli* O157:H7 in the present study was relatively low (1.42%), its presence in fresh beef sold at local butcher shops in Baghdad is concerning. This suggests potential lapses in food hygiene and handling practices that could pose risks to public health. Foods of animal origin, especially raw or undercooked meat, remain the primary sources of enterohemorrhagic *E. coli* (EHEC) infections. Contamination of meat products with this virulent strain underscores the need for improved meat inspection, handling, and consumer education to mitigate the risk of foodborne illness.

Beef carcasses can be significantly affected during the slaughterhouse processes when they come into contact that are contacted with infected workers or equipment under poor hygienic conditions in slaughterhouses. The exposure often leads to a contamination process by high initial bacterial loads, which ultimately have a strong impact on the hygiene characteristics of fresh beef that is sold in the Iraqi local markets. The isolation and distribution rate of

both *E. coli* and *E. coli* O157:H7 between the two regions of Baghdad city may vary due to differences in hygienic practices. Additionally, contamination can occur during the handling of beef in the abattoir, especially if proper cleanliness is not maintained. Ensuring the cleanliness of slaughtering equipment and all subsequent steps leading to consumption is critical to minimizing risks.

In the present study, the *E. coli* O157:H7 isolates demonstrated multidrug-resistant (MDR) properties, as illustrated in Figure 3. The pathogenicity of these isolates was further confirmed by the detection of virulence genes (*eaeA*, *rfbE*, and *fliCH7*), as shown in Table 5. The presence of genes associated with both virulence and antibiotic resistance raises serious public health concerns, particularly as these resistance determinants can be transmitted to consumers through contaminated food and improper handling practices (Rahman *et al*., 2017).

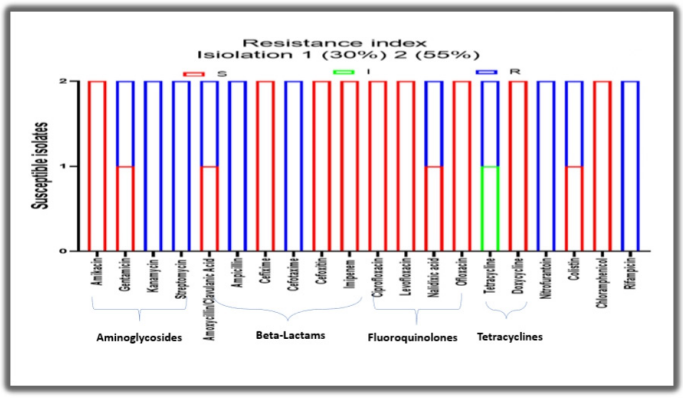

Figure 3: Antibacterial sensitivity profile of the E. coli O157:H7.

Table 5: Number and percentage (%) of *eaeA*, *rfbE*, and *fliCH7* virulence genes detected in *E. coli* O157:H7 isolates.

Source	Positive isolates of E. coli O157:H7	eaeA	rfbE	fliCH7	%
Beef samples	2	2	2	2	100
Total	2	2	2	2	100

The misuse and unregulated application of antibiotics in livestock farming are significant contributing factors to the emergence of MDR pathogens. Commonly used antibiotics in cattle rearing include tetracyclines, penicillins, and sulfonamides. These are frequently administered under poor hygienic conditions, especially in slaughterhouses and during meat processing, increasing the risk of contamination. Khudhir (2020) noted that although various processing methods can significantly reduce the presence of *E. coli* O157:H7 in meat, these reductions may not be sufficient to guarantee food safety. Cross-contamination and inadequate hygiene practices during processing remain critical points of concern (Fayemi *et al*., 2021).

Beta-lactam antibiotics such as penicillin, tetracyclines like tetracycline, and aminoglycosides such as erythromycin, along with trimethoprim/sulfamethoxazole, are widely used in both human and veterinary medicine (Faghihi *et al*., 2017; Mirza, 2015). Interestingly, some *E. coli* O157:H7 strains in this study exhibited susceptibility to certain antimicrobial agents still employed in the veterinary field, a finding consistent with previous studies (Ayenew *et al*., 2021).

Antibiotic-resistant zoonotic *E. coli* pose significant challenges to public health and are associated with increased hospitalization costs. In severe cases, particularly among immunocompromised individuals such as children and the elderly, these infections may be fatal (Onwumere-Idolor *et al*., 2024). The *E. coli* O157:H7 isolates in this study exhibited resistance to multiple antibiotics, including sulfonamides, streptomycin, tetracycline, and ampicillin, consistent with previous findings (Guzmán *et al*., 2019).

These results underscore the urgent need to address the threat posed by antibiotic-resistant *E. coli* O157:H7, especially in the context of widespread antibiotic use for therapeutic and growth-promoting purposes in livestock. The MAR index was used to trace the origin of bacterial contamination (Adzitey, 2015; Davis and Brown, 2016). A MAR index greater than 0.2 suggests that the isolates originated from environments where antibiotics are frequently used or misused (Krumperman, 1983; Mthembu *et al*., 2019).

Consistent with findings by Abebe *et al*. (2023), the *E. coli* O157:H7 isolates in the current study exhibited resistance to more than three antimicrobial agents. Specifically, 56% of the isolates were resistant to 4 antibiotics, while 44% demonstrated resistance to five, highlighting the severity of antibiotic resistance in foodborne pathogens.

CONCLUSIONS AND RECOMMENDATIONS

The results of this study indicated that the prevalence of antibiotic-resistant *E. coli* O157:H7 in local beef samples from Baghdad was relatively low. Nevertheless, the detection of even a small number of contaminated samples is a significant concern and highlights a potential threat to food safety and public health. The low isolation rate observed may be attributed to increased consumer awareness of hygiene and health practices following the COVID-19 pandemic, along with improved adherence to food safety protocols during meat handling. Additionally, stricter health monitoring and regulation of butcher shops likely played a role in minimizing the spread of these resistant bacterial strains.

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NOVELTY STATEMENT

The novelty of this research lies in its focus on determining the distribution and percentage of antibiotic-resistant *Escherichia coli* O157:H7 isolated from locally sold beef in Iraqi markets. This study also evaluates the hygiene standards of local meat by comparing bacterial contamination loads across different regions of Baghdad. Such a regional assessment provides valuable insight into public health risks associated with foodborne pathogens and contributes to the understanding of antimicrobial resistance trends in Iraq.

AUTHOR'S CONTRIBUTIONS

Ibraheem Eibrhim take the responsibility of data collection, bacteriological isolation, preparing the manuscript and data analysis while research design due the supervision of advisor Dr Zina Saab Khudhir. Both the authors approved the final version of the manuscript that submitted to this respect journal.

Conflict of Interest

No potential conflicts of interest relevant to this article are reported.

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