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USAGE OF ANTIMICROBIAL ACTIVITY AGAINST E. COLI O157:H7 ISOLATED FROM LOCAL MEAT AND VEGETABLES IN ERBIL CITY

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Abstract

The antimicrobial effect of *Escherichia coli* O157:H7 was evaluated in Erbil City's fresh vegetables and meat. A total of 250 samples were collected in Erbil City. *E. coli* O157:H7 was successfully extracted using the ISO-16654:2001 standard. After that, the samples were isolated for antimicrobial susceptibility for ten different antimicrobials using the disk diffusion method. Only 0.51 percent of the meat and vegetable samples tested had *E. coli* O157: H7. According to kanamycin and ampicillin susceptibility testing results, all of the microorganisms examined were invulnerable. Erbil City's meat and vegetables are contaminated with *Escherichia coli*, according to this research. Despite the disease's low incidence, the fact that it may be found in a raw food product raises questions about public health implications.

Keywords: Antimicrobial activity, E. coli O157:H7, Meat and vegetables.

استعمال النشاط المضاد للأحياء المجهرية تجاه E. coli O157:H7 المعزولة من اللحوم والخضروات المحلية في مدينة اربيل

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الخلاصة

ان البكتريا *Escherichia coli* O157:H7 هي أحد مسببات الأمراض التي تنتقل عن طريق الأغذية. هدفت هذه الدراسة إلى تقدير انتشار ونمط النشاط المضاد للميكروبات ضد *Escherichia coli* O157:H7 في اللحوم والخضروات الطازجة من مدينة أربيل. تم جمع ما مجموعه 250 عينة من اللحوم والخضروات من مدينة أربيل. تم عزل وتحديد *E. coli* O157: H7 باتباع معيار ISO–16654: 2001. تم إجراء مزيد من الاختبارات على العزلات لمعرفة مدى التأثر بمضادات الميكروبات لعشرة مضادات جرثومية باستخدام طريقة الانتشار القرصي. من بين 250 عينة من اللحوم والخضروات التي تم فحصها، حملت اثنتان 0.51٪ *Escherichia coli* O157:H7 أظهر نمط الحساسية لمضادات الميكروبات للسلالات مقاومة للأمبيسيلين والكاناميسين بنسبة 100٪. تظهر نتائج هذه الدراسة وجود *Escherichia coli* O157:H7 المقاومة للأدوية في اللحوم والخضروات في مدينة أربيل. على الرغم من انتشاره المنخفض، فإن وجوده في منتج يؤكل نيئًا يسلط الضوء على المخاطر المحتملة على الصحة العامة في المنطقة المرتبطة بهذا العامل الممرض.

كلمات مفتاحية: نشاط مضادات الميكروبات، Escherichia coli O157:H7، اللحوم والخضروات.

Introduction

Foodborne disease is a significant source of sickness and death and a substantial impediment to global economic progress. Worldwide, food-borne illnesses seem to be increasing in prevalence. An estimated one-third of people in developed nations become ill every year because of something they ate. Food-borne illnesses are more widespread in the underdeveloped countries because of sanitation issues (9).

Escherichia coli is a bacterium that may be found in the intestines of both humans and animals. More than 99% of all strains of *E. coli* are thought to be safe members of the normal intestinal flora. Some Strains of *E. coli*, like as O157: H7, may release toxins that are toxic to humans (24). All the way up to thrombotic-thrombic purpura, hemorrhagic necrotizing fasciitis (HCF), hemolytic uremic syndrome (HUS), even death are possible symptoms of infection in humans with *E. coli* O157:H7. Someone

with suppressed immune system and children below the age of five are the most susceptible to the sickness (18).

Escherichia coli O157:H7-infected food has been linked to various outbreaks. Several outbreaks of *E. coli* O157:H7 have been linked to meat and vegetables. Outbreaks of *E. coli* O157:H7 in the US have been connected to uncooked meat (14). *E. coli* O157:H7 outbreaks have often been linked to the consumption of raw or undercooked meat or vegetables. From the farm to the store, dangerous bacteria might infiltrate the supply chain (3). The contamination of food by cow dung has been linked to the closeness of vegetable farms and water supplies to cattle ranches (12). Antibiotic resistance is affecting an overwhelming number of individuals throughout the world (13). Cattle and other ruminants that contain *E. coli* O157:H7 and are exposed to antimicrobial medications may propagate antimicrobial-resistant bacteria throughout the livestock production system (17). This type of fast food is becoming more widespread in Erbil City eateries, raising concerns about the health and safety of this type of cuisine. Antimicrobial activity and O157:H7 infection are unknown in Erbil. For the present study, the researchers focused on Erbil vegetable and meat and retail shops, the incidence of O157:H7 serotype, and antibiotic sensitivity patterns for O157:H7 serotypes and the results agreement with (10).

Materials and Methods

Sample Collection: Across-sectional investigation was performed. Between September 1, 2021 and March 1, 2022, samples were collected and screened. Samples were collected by sampling methods. Samples consisting of two different types of fresh beef meat abattoir and retail shops with three types of vegetables, which consist of cabbage, lettuce, and salad, were collected randomly from different locations in Erbil city. For each meat and vegetable type, fifty samples were taken; 100 samples for both meat types and 150 for the three vegetable types; a total of 250 samples were screened. After scanning, samples for E. coli placed in labeled plastic containers and transported to the laboratory for further analysis, in which all positive isolates were further screened for O157:H7.

Preparation and Enrichment the Sample: stomacher bags containing 25 grams of samples (meat or vegetables) were filled with 225 milliliters of Novobiocin-fortified Tryptone Soya Broth and mixed for two minutes at high speed in a homogenizer were used to thoroughly blend the ingredients. Mixing continued for one minute until agglutination emerged in the enrichment mixture at 41.5°C, which was incubated for 24 hours. During the latex test, samples and controls were tested. Despite the samples (*E. coli* O157) being positive for agglutination, the control test came out negative. For 18–24 hours, the isolate was incubated at 37 degrees Celsius before being plated onto blood agar. The H7 test latex required between 18 and 24 hours to disseminate the development on the blood-agar plate. The agglutination response colonies tested positive for O157:H7.

Isolation: The ISO-16654:2001 standard was used to detect *E. coli* O157:H7 (15). All enriched broths were plated onto cefixime tellurite sorbitol MacConkey agar (CT-SMAC) (Oxoid, England), supplemented with 0.05 mg/L cefixime and potassium 2.5

mg/L tellurite (Oxoid, England), and incubated at 37 C for 24 hours. Following the incubation period, the CT-SMAC agar plates were checked for the presence of non-sorbitol fermenter colorless colonies and subcultured on Rainbow agar O157. The plates were then incubated at 37°C for 20-4 hours to look for typical black or gray coloration on Rainbow agar O157, which shows pure colonies (4).

Biochemical Confirmation: Five representative colonies from each Colorful agar O157 plate were grown on nutritional agar to validate indole production, plates were then incubated Celsius for 18 to 24 hours. Single cell from the culture broth on nutrient agar was injected into tryptone/tryptophan medium and incubated for 24 hours at 37°C. Afterwards, the tube was incubated for 5 - 10 min with 1 cc of Kovacs before analysis. The color red is known to elicit positive emotions in people (15).

Detection of O157 and H7 Antigens by Serology: The presence of *E. coli* O157:H7 was confirmed using kits from Oxoid, England (4). A test reagent, a control reagent, positive and negative controls are included in the latex kit. An antibody against *E. coli* serotype O157 or H7 was used to coat each reagent's particles, and the control was rabbit globulin. Formalin-killed *E. coli* O157:H7 cells serve as both positive and negative controls.

RIM Antiserum was checked for indole-positive colonies using an O157:H7 latex test (Oxoid, England). Sorbitol MacConkey agar was used to transfer Indole-positive colonies. A single drop of latex was needed for each isolate on a test plate. On a second slide well, a drop of the *E. coli* control latex was added. The NSFC from sorbitol MacConkey agar was combined with the *E. coli* O157 test latex on the slide and applied to the response region using a plastic stick. A fresh plastic stick was used to emulsify the remaining NSFC in the *E. coli* control latex. This particular slide has rabbit globulin on it. *E. coli* O157:H7 cells that have been formalin-killed serve as both positive and negative controls.

The reaction of indole-positive colonies to antiserum was examined using the RIM O157:H7 latex test, Sulfite MacConkey agar was used to cultivate Indole-positive colonies. A drop of latex was placed in each test slide well of each isolate before further testing. One drop of E. coli control latex was placed in each well on the test slide. After being emulsified with *E. coli* O157 test latex, an NSFC was extracted from sorbitol MacConkey agar. The NSFC was then put on a slide. The new material is put to good use. Homogenization of the remaining NSFC in *E. coli* control was repeated. Agglutination developed when the plate was whirled in circles for a minute or more. To test for the presence of *E. coli* O157, the isolate was streaked onto blood agar mixture was incubated at 37°C for 18–24 hours for flocculation with the test latex and negative results with the control latex. Using H7 test latex, the growth of blood agar plates was emulsified after 18–24 hours. O157:H7-positive colonies were identified by agglutination tests (25).

Susceptibility to Antimicrobial: The conventional agar disk diffusion technique and commercial antimicrobial disks were used for the antimicrobial susceptibility testing (6), as described Table 1.

Each bacterium where isolated colony was incubated for six hours at 37 °C in TSB. To obtain a turbidity of 0.5 McFarland standards, either sterile saline solution or more

colonies were used to alter the cultural broth's turbidity (approximately 3×10^8 CFU per mL). All Mueller-Hinton agar plates were produced in accordance with the manufacturer's instructions, as specified by the manufacturer. It was necessary to remove the surplus fluid from the Mueller-Hinton plates by dipping a cotton swab into the suspension, rotating it against the tube's wall, and then wiping it over the surface in three directions. After drying, sterile forceps were used to insert antibiotic disks on the inoculated plates. After being carefully pushed into the agar surface to achieve firm contact. According to CLSI recommendations, the clinical interpretive criteria breakpoints indicated for the isolates were classified as, intermediate, resistant and sensitive1 (14).

Table 1 Antibiotic disks to measure the concentration of antibiotics against *Escherichia coli* O157: H7.

Antibiotic disks	Symbol	Potency (µg)	Resistant (≤)	Intermediate	Susceptible (≥)
Ampicillin	AMP	10	13	14–16	17
Amoxycillin-clavulanic acid	AMC	20/ 10	13	14–17	18
Amikacin	AK	30	14	15–16	17
Ciprofloxacin	CIP	5	21	22–25	26
Ceftriaxone	CRO	30	19	20–22	23
Gentamicin	GM	10	12	13–14	15
Cefepime	CPM	50	14	19–24	17
Kanamycin	KM	30	18	14–17	25
Tetracycline	TE	10	11	12–14	15
Streptomycin	**STR**	**10**	**11**	**12–14**	**15**

CLSI (6)

Data Analysis: Analysis of the data was performed by SPSS version 25 (22).

Results and Discussion

Prevalence: More than two hundred and fifty samples, comprising two kinds of fresh meat abattoirs and three kinds of retail lettuces, cabbages, and salads, were tested for the presence of *E. coli* and *E. coli* O157:H7. Results for the two types of meat, each with 100 samples, are shown in Table 2. Only 4% of the samples tested positive for *E. coli* O157:H7, while 50% of the samples tested positive for *E. coli* from fresh beef. The prevalence of bacteria was increased by 6% and 60%, respectively, at retail locations.

E. coli H7 and *E. coli* were found in 150 samples of vegetables from three different types. Food infected with H7 and *Escherichia* had a contamination level of 2 percent and a contamination level of 44 percent, respectively, in the study. Microorganisms were found in lettuce at rates of 8 percent and 40 percent, respectively. Cabbage is free of O157: H7 and *E. coli.*

Table 2 Prevalence rates in meat.

Meat	No. of Sample Analysis	No. (%) with *E. coli* O157:H7	No. (%) with *E. coli*
Fresh meat (Abattoir)	50	2 (4)	25 (50)
Fresh meat (Retail shops)	50	3 (6)	30 (60)
Total	100	5 (5)	55 (55)

% = percentages based on No. of samples analysis.

SPSS version 25 (22).

Table 3 Prevalence rates in vegetables.

Vegetable	No of samples Analysis	No. (%*) of *E. coli* O157:H7	No. (%) of *E. coli*
Lettuce	50	4 (8)	20 (40)
Cabbage	50	0 (0)	0 (0)
Salad	50	1 (2)	22 (44)
Total	150	5 (3.33)	42 (28)

SPSS version 25 (22).

(21) isolated and identified 39 (19.5%) of *E. coli* from 200 samples of raw vegetables in which highest percentage (90%) was isolated from parsley, while lowest percentage (10%) was isolated from tomato. No *E. coli* O157 detected among all isolated *E. coli*. Eighteen isolates (32.7%) of *E. coli* were isolated from salads served in restaurants and cafeteria in which 80% from salads served in cafeteria and 22.22% from salads served in restaurants. No *E. coli* O157 was detected among all isolated *E. coli* from salads served in both restaurants and cafeteria. (10) were collected 390 retail lettuces samples from vegetable retailer outlets in Addis Ababa, 39 in each of the 10 subcities. Out of 390 lettuce samples examined, two were positive to *E. coli* O157:H7. However, the prevalence of *E. coli* O157:H7 was not statistically significant ($\chi 2 = 8.041$; $p > 0.05$) among the subcities of vegetable retail outlets.

The results of (16) study revealed that out of 50 samples, 20 samples were found to be positive for *E. coli*. Isolates were characterized as bright pink color on MacConkey agar plates and showed blue-greenish metallic sheen on EMB agar plate. Upon Gram's staining of the isolates under 100× using light microscope, pink-colored, small rod-shaped organisms arranged in single, pairs or short-chain were identified.

Escherichia coli were isolated by (8) from different samples and it suspended in Tryptone Soya Broth. Samples were vortexed and incubated overnight at 37°C. After selective enrichment, 50 µl of product was streaked onto MacConkey agar for primary isolation of *E. coli* and incubated aerobically at 37°C for 24 hours. The plates were observed for the growth of *E. coli* (pink colony; lactose fermenter). A single, isolated colony was picked and subcultured on Eosin Methylene Blue (EMB) agar for formation of metallic sheen.

This study of (23) has focused on cooked doner kebabs. Out of 80 samples, thirty-seven (46.25%) samples gave positive results for *E. coli* O157:H7 with the O157 antiserum. All of these O157 antiserum positive results, however, could not be confirmed by the biochemical tests carried out. Only twenty-one (26.25%) samples positive for *E. coli* O157 using O157 antisera were compiled well with the biochemical tests, whereas, seven (8.75%) isolates found positive for *E. coli* O157 with O157 antiserum test could not be confirmed by the biochemical tests.

The biochemical characteristics of *E. coli* isolate showed positive for catalase, Methyl red and indole test but negative for Vogues-Proskauer, urease, and citrate. In addition, reactions in TSI agar slant revealed yellow but with gas and production of hydrogen sulfide was observed. Almost all the isolates of *E. coli* fermented lactose, sucrose and glucose with the production of both acid and gas (16). Pure colonies were identified according to their Gram staining and other microscopically characteristic. The

bacterial isolates were gram-negative rods in shape, non-spore forming, with peritrichous flagella (19).

Antimicrobial Susceptibility Pattern of O157:H7: Ten isolates of *Escherichia coli* O157:H7 were screened for their resistance to ten widely used antibiotics including (Ampicillin, amoxicillin-clavulanic acid, Amikacin, Ciprofloxacin, Ceftriaxone, Gentamicin, Cefepime, Kanamycin, Tetracycline, and Streptomycin).

All isolates showed significantly different resistance patterns to different antimicrobials (Table 4). Ninety-nine percent of the isolates showed resistance to OX (n=99), followed by P (97%), E (53%), AZM (47%), VA (38%), CD (30%), TE (23%), NIT (21%), TOB (18%), NOR (18%), CIP (11%), and LEV (9%), while the lowest resistant percent (1%) was recorded against each of AK, G, NET, and SXT.

Ampicillin and kanamycin resistance was found in ten out of 10 isolates, as shown in table 4. The medications Ciprofloxacin, Gentamicin, and Tetracycline were all effective against ten different strains. Three medicines, Amikacin, Cefepime, and Streptomycin, were shown to be effective against all five isolates.

Table 4 *Escherichia coli* O157:H7 isolates' antimicrobial susceptibility patterns, n=10.

Antimicrobial agents	Symbol	Susceptibility and resistance pattern of all *E. coli* O157:H7isolates		
		E. coli O157:H7		
		S *n.* (%)	I *n.* (%)	R *n.* (%)
Ampicillin	AMP	0(0)	0(0)	10(100)
Amoxicillin-clavulanic acid	AMC	0(0)	10(100)	0(0)
Amikacin	AK	5(50)	5(50)	0(0)
Ciprofloxacin	CIP	10(100)	0(0)	0(0)
Ceftriaxone	CRO	5(50)	5(50)	0(0)
Gentamicin	GM	10(100)	0(0)	0(0)
Cefepime	CPM	5(50)	5(50)	0(0)
Kanamycin	KM	0(0)	0(0)	10(100)
Tetracycline	TE	10(100)	0(0)	0(0)
Streptomycin	STR	5(50)	5(50)	0(0)

S: Susceptible, I: intermediate, R: resistant.

SPSS 25 (22).

MDR was defined as an isolate showing resistance to three or more antibiotic classes (20). In the study of (5), 66 (61.68%) isolates were MDR and among these isolates, 31 (28.97%) were ESBL-producing, 5 (4.67%) were carbapenemase-producing and 30 (28.04%) were non-ESBL. In addition, 16 (14.95%) isolates were resistant to less than three antibiotic classes and 25 (23.36%) were fully sensitive. The majority of the isolates was susceptible to Fosfomycin (n = 105; 98.13%). Furthermore, the resistance rate to aminoglycosides (n = 50; 46.72%), fluoroquinolones (n = 56; 52.33%), trimethoprim (n = 52; 48.59%), and trimethoprim-sulfamethoxazole (n = 48; 44.85%) was high, compared to the resistance rates to piperacillin/Tazobactam and nitrofurantoin which were 13.08 and 3.73%, respectively. Observed antibiotic resistance profiles including MDR could be linked to the genetic background of *E. coli* isolates. (2) was recorded the highest sensitivity of Ciprofloxacillin and Norfloxacillin were recorded in 98.2% of the isolates. Norfloxacillin and tetracycline

for all isolates did not show intermediate resistance while another tested drug was recorded as intermediate resistance in one or more of the tested isolates. The highest resistance isolates were recorded for neomycin (76.8%) followed by amoxicillin (48.2%). Resistance to chloramphenicol and Norfloxacillin were observed in 1.8% of the isolates.

The Clinical and Laboratory Standards Institute's Kirby Bauer disc diffusion method on Mueller Hinton agar was used to determine the antimicrobial susceptibility pattern of *E. coli* O157:H7 isolates (11). The isolates of *E. coli* O157:H7 were evaluated by (7) for sensitivity to the most commonly used antimicrobials, including ampicillin (AMP/10 µg), cefoxitin (FOX/30 µg), streptomycin (S/10 µg), tetracycline (TE/30 µg), ciprofloxacin (CIP/5 µg), gentamicin (GEN/10 µg), sulfamethoxazole (RL/100 µg), trimethoprim (TR/25 µg) and doxycycline (DO/30 µg). Furthermore, isolates that showed resistance to two or more antimicrobials were labeled as multidrug-resistant. The findings of (7) were done to antimicrobial sensitivity of *E. coli* O157:H7 recovered from different sample types revealed that, of the total 27, all isolates were resistant to ampicillin and cefoxitin. On the other hand, 70.37% (n = 19), 59.26% (n = 16), 37.04% (n = 10), 25.93% (n = 7), 22.22% (n = 6) and 14.81% (n = 4) were found to be resistant to streptomycin, tetracycline, doxycycline, gentamicin, sulfamethoxazole and trimethoprim, respectively. None of the isolates was resistant to ciprofloxacin. In addition, multidrug resistance analysis showed that 27/27 (100%) of tested *E. coli* O157:H7 isolates were resistant to different combinations of two or more antimicrobials. A multidrug-resistance pattern consisting of seven drugs was seen in 3/27 (11.11%) isolates.

Antibiotic susceptibility testing results of (1) showed that all the 16 isolates were resistant to ampicillin, Ceftazidime and cefuroxime. Further, 14 (87.5%) of the isolates were resistant to Augmentin, 8 (50%) were resistant to nitrofurantoin and 4 (25%) were resistant to ciprofloxacin and gentamicin. None of the isolates were resistant to Ofloxacin. All isolates were resistant to at least three antibiotics.

Conclusions: *Escherichia coli* O157:H7 prevalence in fresh meat abattoirs was determined to be under 4% in two samples, according to our investigation. The *Escherichia coli* O157:H7 determined to be nil in cabbage and salad contains just 2%. Ten of the isolates tested negative for ampicillin and kanamycin, indicating widespread antimicrobial resistance

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