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Isolation and molecular identification of *Escherichia coli* strain from fish available in farms and local markets in Nineveh governorate, Iraq

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Abstract

This research work was conducted as well as the determination of the resistance to antibiotics of these isolated species of *E. coli* in Nineveh governorate to assess the incidence of *Escherichia coli* (*E. coli*) contamination in different fish farms and local fish markets. The total number of fish samples used in the present study was 153, including 75 samples from various fish farms and 78 samples from different local markets in Mosul. The current study showed that the percentage of *E. coli* isolated from fish farms was 24% (18/75) and 35.9% (28/78) from local markets. While it showed a positive result for *E. coli* with serotype (O157:H7) with a percentage was 9.3 and 14.1% from both farmed fish and market fish samples, respectively. Additionally, all *E. coli* positive isolates possess the specific *uidA* gene, which was detected using the PCR technique. The highest sensitivity of *E. coli* bacteria to the antibiotic's ciprofloxacin, trimethoprim, and gentamicin was 96, 94, and 86%, respectively. At the same time, the highest percentage of resistance of *E. coli* to the antibiotics cephalothin, tetracycline, erythromycin, and amoxicillin was 100, 64, 64, and 62%, respectively. To reduce health risks to consumers, these results provide useful basic information for the proper management of these environments in order to prevent fecal contamination in fish farms and the fish sold in local markets.

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Introduction

The safety of aquatic life, especially fish, is one of the most critical public health issues directly related to agriculture and healthy food production, as is the case with other types of food (1), therefore fish plays a significant role in providing nutrients foods to many animals as well as humans. Sixty percent of fish contribute to the global protein supply, and the developing countries derive more than 30% of animal protein from fish (2), whereas the provision of healthy fish products is necessary from a food safety point of view in order to maintain the health of the consumer because the health of the consumer is part of the safety of the food consumed (3). Besides being a food source, fish also protects humans from diseases prevalent worldwide through daily fish consumption, preventing human heart disease (4). Aquatic organisms, especially fish, contain the many pathogens they transmit to humans. These diseases associated with consuming fish and sea foods, significantly contaminated with microbial pathogens, have increased when they are produced under poor sanitary conditions. Therefore, the contamination of fish causes many risks to consumers' health (5). The pollution of the aquatic environment of fish with sewage water from homes and toxic waste from factories leads to fish contamination with industrial metals resulting from factory waste thrown into running water (6-8). Moreover, bacterial contamination resulting from surface contact with food, a significant source of many foodborne diseases, affects public health through the transmission of microbes from the equipment surfaces to processed foods while dealing with fish during fishing, cleaning, and removing their guts that contaminate fish meat. Additionally, during storage, especially the use of crushed ice in preserving fish until they reach the market, which

contains large numbers of disease-causing bacteria that poses a potential threat to public health through the consumption of contaminated fish products and seafood which leads to the food poisoning to consumers (9,10). One of the most important bacterial contaminants is *E. coli*, which is generally used as an indicator of fish contamination and spoilage. Researchers have conducted several studies about *E. coli* bacteria in fresh fish and their ready-to-eat products in sales markets (11). *E. coli* are characterized by their production of toxins, especially Shiga toxin, which leads to food poisoning in the consumer due to eating fish contaminated with bacteria or their toxins (12).

The study aimed to detect the presence and spread of *E. coli* in fish available on farms and local markets and determine the resistance to antibiotics of these isolated species of *E. coli*.

Materials and methods

Ethical approve OR data collection permit

University of Mosul, College of Veterinary Medicine, the approval issue number and date are 1650 at 21/11/2021.

Sampling

One hundred and fifty-three fish samples (*Cyprinus carpio*) were collected in the current study, including: 75 fish samples collected from various fish farms (Hawi Church area, Wana sub-district, and Hamdaniya district) and 78 fish samples collected from different local markets in the Mosul city (Al-Midan area, Albaladiat, and Nabi Yunus markets) at the period from November 2021 to January 30, 2022.

Isolation of bacteria

The isolation of these bacteria was done in the laboratory of the Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, Iraq. Based on the conventional methods, the swabs were taken from the fish samples' skin using sterile swabs, placed in tubes containing peptone water, and incubated for18 - 24 h at 37°C as pre-enrichment. Then 1 ml of peptone water was taken and transferred to test tubes containing 9 ml of MacConkey broth and incubated at 37°C for 24 h. From these selective enrichment medium tubes, 1-2 Bacteriological loops were taken and cultured on Eosin-methylene blue agar (EMB), MacConkey agar, Brilliance *E. coli*/coliform agar, and lastly cultured on Chrome agar *E. coli* O157:H7 to show the brilliant metallic sheen, pink colonies as lactose -the fermenting characteristic for *E. coli*, purple colonies and to isolate *E. coli* O157:H7, and all cultured Petri dishes are incubated at 37°C for 18-24 h (13). To identify these isolates of *E. coli*, standard biochemical reaction tests (IMVC) are used, including Indole production, citrate utilization test, methyl red, Voges-Proskauer test, oxidase and catalase test, urease and typical reactions on the triglyceride sugar iron agar (14).

Confirmation of *E. coli* isolates by conventional PCR

The phenotypic-identified *E. coli* was confirmed by PCR assay to amplify the *uidA* gene by using PCR that encodes the B-glucuronidase enzyme, which is common in all *E. coli* species. According to Moyo (15), a 25 µl PCR mixture consisted of 12.5 µl of hot start premix,1 µl of each (10 pmol) of primer F: 5-CCAAAAGCCAGACAGAGT-3 and R:5-GCACAGCACTTCAAAGAG-3, 4 µl of sample DNA, and the remained was filled with nuclease-free water. PCR amplification was carried out in PCR system 9700 GeneAmp with pre-PCR heating at 95°C for 5 min, subsequently exposed to 35 cycles (1 min at 94°C, 1 min at 58°C, 1 min at 72°C), and a final cycle for 5 min at 72°C. The amplified product was run at 85 Volt for 40 min. The amplified PCR products 623 bp in 2% agarose gel prepared in 1x TAE buffer and stained by red safe DNA staining solution were verified.

Antibiotic sensitivity test

The sensitivity of bacteria to antibiotics was tested based on the method (16), and this is done by transferring about 5-6 pure bacterial colonies from the selective media by the bacteriological loop and cultivated in tubes containing 5 ml of peptone water and then incubated for 5 h at a temperature of 37°C (17). Sensitivity test was conducted using 12 types (tetracycline, gentamicin, cephalothin, erythromycin, ciprofloxacin, trimethoprim, ceftriaxone, amoxicillin, streptomycin, nitrofurantoin, cefixime, chloramphenicol) of antibiotics belonging to different groups of antibiotics which obtained as ready-made discs from Bioanalyse Company. A sensitivity test of these isolates on Muller Hinton agar was carried out using the disc diffusion method (18).

Results

The results of the current study showed that the number of isolates of *E. coli* bacteria isolated from fish samples, which numbered 75 fish samples from fish farms was 18 positive samples, at a rate of 24%, and 78 fish samples from local markets, 28 positive samples with a percentage of 35.9% as shown in table 1. This study showed that all *E. coli* bacteria isolated from fish samples, whether farmed or local market fish, possessed the *uidA* gene with a molecular weight of 623 bp (Figure 1).

The number of samples that showed a positive result for the serotype *E. coli* bacteria (O157:H7) was 7 (3.9%) of the farmed fish samples, while its percentage in the local market fish samples was 11 (14.1%) as present in table 2.

The different results of the resistance of *E. coli* to the antibiotics used in our study showed in (Table 3 and Figure 2). The highest sensitivity of *E. coli* bacteria to the antibiotic ciprofloxin, trimethoprim, and gentamicin was 96, 94, and 86%, respectively. In comparison, the highest percentage of resistance of *E. coli* to the antibiotics cephalothin, tetracycline, erythromycin, and amoxicillin was 100, 64, 64, and 62%, respectively.

Table 1: Number and percentages of isolates of *E. coli* from fish samples of local market and fish farm

Location	Number of samples	Positive samples	%	Negative samples	%
Farm fish					
Hawi Church area	25	4	16	21	84
Wana sub-district	25	6	24	19	76
Hamdaniya district	25	8	32	17	68
Total count	75	18	24	57	76
Local market fish					
Al medan market	28	17	60.7	11	39.3
Albaladiat market	25	6	24	19	76
Al nabi younis market	25	5	20	20	80
Total count	78	28	35.9	50	64.1

Table 2: Number of positive *E. coli* O157:H7 isolated from farmed fish and local marked fish

Location	No. of examine sample	Positive sample	No. (%) of *E. coli* O157:H7
Farm fish			
Hawi Church area	25	4	1 (4%)
Wana sub-district	25	6	2 (8%)
Hamdaniya district	25	8	4 (16%)
Total count	75	18	7 (9.3%)
Local market fish			
Al medan market	28	17	7 (25%)
Albaladiat market	25	6	3 (12%)
Al nabi younis market	25	5	1 (4%)
Total count	78	28	11 (14.1%)

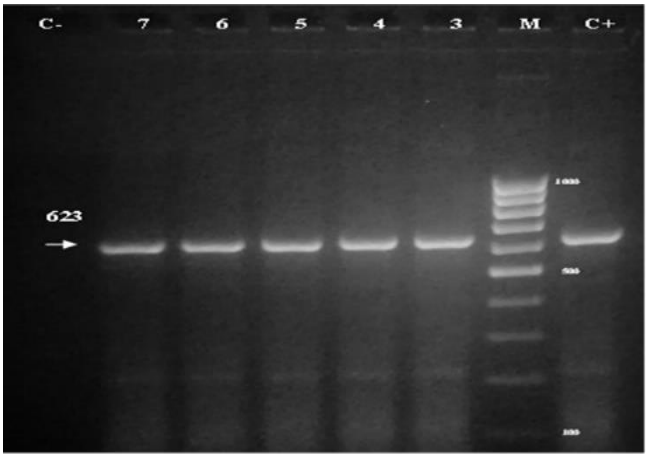

Figure 1: Electrophoresis of PCR products for *E. coli* isolates, where lane C+ represents the positive control, lane M represents Ladder DNA 100bp, lanes 3, 4, 5, 6, 7 represent the positive samples of *E. coli* uidA gene with a molecular weight of 623 bp and lane C- represents the negative control.

Table 3: Number of resistance and sensitivity of isolates *E. coli* to the antibiotics used in this study

Antibiotics	Sensitive %	Intermediate %	Resistant %
Tetracycline	16	20	64
Gentamycin	86	14	0
Cephalothin	0	0	100
Erythromycin	16	20	64
Ciprofloxacin	96	4	0
Trimethoprim	94	2	4
Ceftriaxone	72	22	6
Amoxicillin	26	12	62
Streptomycin	62	8	30
Nitrofurantoin	30	42	28
Cefixime	72	16	12
Chloramphenicol	72	4	24

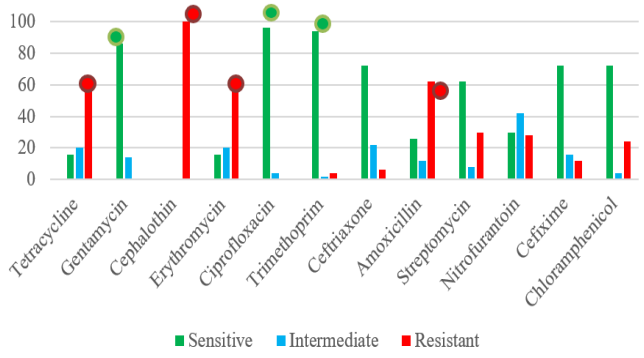

Figure 2: Resistance and sensitivity of isolated *E. coli* to the antibiotics collected from local markets and farmed fish.

Discussion

In the past decades, fish farming has been directed to fill the shortage in the provision of animal protein (19). Safe handling of food is an important issue due to the transmission of microorganisms from fish to humans (20). One of these

microorganisms is the family of *Enterobacteriaceae*, usually found in fish's skin and digestive system due to the pollution that occurs in the aquatic environment in which these fish live (21,22).

The current study showed that the percentage of *E. coli* bacteria isolated from 75 fish farms and 78 samples of local fish markets was 24 and 35.9%, respectively. Aynadis and Engdawork (23) in Southern Ethiopia revealed that the isolation of *E. coli* done by taking swab samples from the skin of *Cyprinus carpio* was 32.5%. Our study's results also agreed with Taha and Yassin's findings (24) when they studied *Cyprinus carpio* fish in Dohuk province, Iraq, where they obtained 39.1%.

The current study showed a positive result for *E. coli* with serotype (O157:H7) with 16% and 25.6% for both farmed and market fish samples, respectively. Although various methods were developed to detect this particular serotype, standard bacteriological methods remain the gold standard (25). Also, all *E. coli* positive isolates showed that they had their specific *uidA* gene, which was detected by using the PCR technique because these isolates of *E. coli* contained in their DNA. The molecular method was used to detect the species-specific *uidA* gene in *E. coli* isolates and to confirm the results of the classical methods (26,27). In order to prevent fecal contamination in fish farms and the names sold in local markets, the results of this study provide useful introductory information that must be used to reduce health risks to consumers.

Antibiotics have been widely used in aquaculture to prevent infection and economic loss. However, the indiscriminate use of antibiotics has led to the emergence of resistant strains, a hazardous situation for consumers due to the transmission of bacterial resistance to humans (28-30). The highest sensitivity of *E. coli* bacteria to the antibiotic ciprofloxin, trimethoprim, and gentamicin was 96%, 94%, and 86%, respectively. In contrast, the highest percentage of resistance of *E. coli* to the antibiotic cephalofen, tetracycline, erythromycin, and amoxicillin was 100, 64, 64, and 62%, respectively. In India, the study of Chakravarty and coworkers (31), his results agreed somewhat with these results, where the antibiotic resistance of *E. coli* was 100% to Penicillin-G, Tetracycline, and Ampicillin, while 100% sensitive to chloramphenicol, nalidixic acid and ciprofloxacin. On the other hand, Ryu *et al.* (32) confirmed that commercial fish in retail markets in Seoul, Korea, may constitute reservoirs of multi-antibiotic-resistant bacteria, high resistance to tetracycline 30.7%, cephalotin 11.7%, ampicillin 6.7% and low resistance to ticarcillin 6.1% in strains of *E. coli* isolated from fish.

Conclusion

In conclusion, we isolated several *E. coli* associated with intestinal bacterial infection from fish collected from fish farms and local markets in Mosul city. The presence of the O157:H7 serotype of *E. coli* in the isolates indicated that these fish were contaminated, and these contaminants may be transmitted to consumers. The results of this study provide useful introductory information that must be used in the proper management of these environments in order to prevent fecal contamination in fish farms and the names sold in local markets, thus reducing health risks to consumers.

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Conflict of interest

The authors declare that there is no conflict of interest.

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التشخيص الجزيئي لسلالات الايشيريكيا القولونية المعزولة من اسماك المزارع والأسواق المحلية في محافظة نينوى، العراق

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الخلاصة

تم إجراء هذا البحث لتقييم معدل حدوث التلوث بجرثومة الإيشريكيا القولون في مزارع الأسماك وأسواق الأسماك المحلية المختلفة، بالإضافة إلى تحديد مقاومة المضادات الحيوية لهذه الأنواع المعزولة من الإيشريكيا القولونية في محافظة نينوى. بلغ العدد الكلي لعينات الأسماك المستخدمة في الدراسة الحالية ١٥٣ عينة شملت ٧٥ عينة من مزارع الأسماك و ٧٨ عينة من الأسواق المحلية المختلفة في مدينة الموصل. أظهرت نتائج الدراسة الحالية أن النسبة المئوية للإشريكية القولونية المعزولة من المزارع السمكية كانت ٢٤٪ (٧٥/١٨) و ٣٥,٩٪ (٧٨/٢٨) من الأسواق المحلية. بينما أظهرت نتيجة إيجابية للإشريكية القولونية ذات النمط المصلي و١٧٥ هـ٧ بنسبة ٩,٣٪ و ١٤,١٪ لكل من عينات أسماك المزارع وعينات الأسواق المحلية على التوالي. كما أظهرت جميع عزلات الإيشريكيا القولونية بأنها تمتلك جين *uidA* والذي تم الكشف عنه باستخدام تقنية تفاعل البلمرة المتسلسل. أعلى حساسية لبكتريا الإيشريكيا القولونية للمضادات الحيوية السبروفلوكساسين والتراي مثبريم و الجنتامايسين كانت ٩٦ و ٩٤ و ٨٦٪ على التوالي. بينما كانت أعلى نسبة مقاومة للإشريكية القولونية للمضادات الحيوية سيفالوثين وتتراسيكلين وإريثروميسين وأموكسيسيلين ١٠٠ و ٦٤ و ٦٤ و ٦٢٪ على التوالي. لتقليل المخاطر الصحية على المستهلكين، توفر هذه النتائج معلومات أساسية مفيدة للإدارة السليمة لهذه البيئات من أجل منع التلوث البرازي في المزارع السمكية وكذلك الأسماك التي تباع في الأسواق المحلية.