Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Detection of some virulence gene *Stx1*, *Stx2* and *rfb* of *Escherichia coli* isolated from fish in Nineveh governorate, Iraq

N.A. Alttai, R.A. Alsanjary and O.H. Sheet

Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Article information

Article history:
Received September 30, 2022
Accepted January 22, 2023
Available online February 28, 2023

Keywords:
Fish farms
Local fish markets
E. coli
Stx1 and Stx2 genes

Correspondence:
R.A. Alsanjary
ralsanjary61@uomosul.edu.iq

Abstract

The aim of the current study is to detect the presence of some virulence genes of *Escherichia* (*E.*) *coli* in fish. A total of 46 strains of *E. coli* were previously isolated from 153 fish samples, including 28 and 18 isolate of *E. coli* from local markets and fish farms, respectively, in Nineveh Governorate from November 2021 to January 2022. The results of the study show that all isolates of *E. coli* possessed the *uidA* gene with a molecular weight of 623 bp. In addition, they show that 88.9% (16/18) isolates from farmed fish samples possessed the *Stx1* gene with a molecular weight of 347 bp, while 72.2% (13/18) of them carried the *Stx2* gene with a molecular weight of 589 bp. Also, the study unveils that 89.3 % (25/28) isolated from the market fish samples possessed the *Stx1* gene with a molecular weight of 347 bp and 85.7% (24/28) isolates carried the *Stx2* gene with a molecular weight of 589 bp. The *rfb* gene is not detected in this study, neither in farm fish nor in the samples from the local fish markets using the PCR technique. Likewise, it shows that *E. coli* isolated from fish possessed the *Stx1* and *Stx2* genes, which are major causative agents of food poisoning for consumers. Finally, the study gives important information about the application of health conditions on fish farms and fish markets to prevent contamination and reduce infections by foodborne pathogens that cause food poisoning for humans.

DOI: 10.33899/ijvs.2022.136232.2571,

Introduction

Fish is one of the important foods for humans, because it is rich in easily digestible proteins and has a high nutritional value. Yet, it is characterized by being a perishable food product, which leads to a decrease in its quality, especially during treatment and storage, a thing that limits its life span (1). Several researchers recently indicated that these bacteria were isolated from mollusks (oysters) found in polluted coastal waters (2). In addition, *E. coli* were isolated from fish found in coasts contaminated with sewage water (3). Also, *E. coli* was isolated from different animals such as cows and their environment (4) and from meat and the butchers' shop (5). Freshwater fish may contaminate with various types of pathogenic microorganisms from the various sources, which are usually either primary sources through the presence of these fishes in the aquatic environment or secondary sources during the harvesting of these fishes, as well as from transportation, marketing and storage, which lead to the pollution of these fishes and make them unsuitable for human consumption or to be carriers of some pathogens to humans (6). Therefore, the low count of bacteria and decreased contamination with pathogens bacteria in fresh fish indicate that fish is suitable for human consumption and fish is of high quality (7). The offal of fish such as intestines, scales, fins, gills and the bad handling of them play an important role in the spread of harmful bacteria, which leads to cause hygienic problems. Although the presence of some types of microorganisms such as *E. coli* bacteria in fish is uncommon (8), these pathogenic microorganisms were linked to food-borne diseases through food routes from eating fish or fish products (9). *Enterobacteriaceae* are

widespread in all parts of fish due to the imbalance between the bacteria and the host environment (10). This family of bacteria is a major contaminant and pathogen in farmed fish (11), but in most cases, these microorganisms are considered part of the normal microorganisms of fish. They are opportunistic bacteria that can cause some diseases when colonizing human body (12) such as urinary tract infections as well as causing hemorrhagic colitis, acute renal failure, irritable bowel syndrome or death (13,14). There are different strains of *E. coli* such as EPEC, ETEC, EHEC, STEC, which give an indication of wastewater contamination, but the Shiga toxin-producing *E. coli* (STEC), in addition to the enteric pathogen (EPEC) are considered important pathogens due to their zoonotic origin that transport diseases to humans, especially in developing countries through consuming the contaminated fish (15,16).

Selling fresh fish in traditional fish markets or retailers, or even farm-raised fish, which do not apply hygienic conditions during the breeding of fish may contaminate fish with pathogenic bacteria that were originally in water (17). Therefore, fish traders in the markets paid attention to how to deal with these foods in terms of transportation, storage, and used clean water to wash fish fielded in traditional fish markets (18). Henceforth, this study aims at detecting the presence of some virulence genes of *E. coli* in fish in fish farms and local markets in Nineveh Governorate.

Materials and methods

A total of 46 *E. coli* isolates were previously isolated from 153 fish samples, which included 28 isolates of *E. coli* from local markets and 18 isolates from local markets and fish farms from various regions in Nineveh Governorate from November 2021 to January 2022. The samples were directly transferred to the laboratories of the Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, Iraq, for processing, isolation and the molecular detection of *E. coli* bacteria (19,20).

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.

DNA Extraction and Amplification

The selected colonies were placed to 200 µl of sterile distilled water in a 1.5 mL Eppendorf tube (21). Then, they were mixed with a vortex mixing device. Cells were lysed for at least 15 seconds and DNA extracted using a laboratory kit prepared by (Jena) Bioscience (22). The conventional species-specific polymerase chain reaction (PCR) technology was used to confirm *E. coli* isolates using *uidA* universal primers and the virulence factors (*stx1, stx2,* and *rfb*) (Table 1).

Table 1: Primers Sequence of Virulence Gene (*uidA*, *Stx1, Stx2*, and *rfb*) of *E. coli*

Gene	Primer	Sequence (5- 3)	Amplicon Size [bp]	Reference
uidA	uidA-1	5-CCAAAAGCCAGACAGAGT-3	623	Moyo *et al.* (20)
	uidA-2	5-GCACAGCACATCAAAGAG-3		
Stx1	Stx1-1	5-AGTTAATGTGGTGGCGAAGG-3	347	Fujioka *et al.* (23)
	Stx1-2	5-CACCAGACAATGTAACCGC-3		
Stx2	Stx2-1	5- TTCGGTATCCTATTCCCGG-3	592	Fujioka *et al.* (23)
	Stx2-2	5- CGTCATCGTATACACAGGAG-3		
Rfb	*rfb*-1	5-CGGACATCCATGTGATATGG-3	259	Paton *et al.* (24)
	rfb-2	5-TTGCCTATGTACAGCTAATCC-3		

The master mix for PCR reaction was prepared using the Gen Net Bio kit by calculating the required volumes of reaction components for each sample. The additives were mixed well and dispensed in a volume of 20 µl (12.5 µl master mix, 1 µl for each primer and 5.5 µl of Nuclease-Free Water) put into 0.2 ml PCR tubes for amplification. Then, 5 µl of DNA extracted from each sample were added separately to each tube to reach the total volume of 25 µl. The PCR reaction was carried out in a T100TM thermocycler (Bio-Rad, USA), using the following parameters for all genes used in this study at 95°C for 5 min as initial denaturation, then for all, the final elongation was 72°C for 5 min, but the rest of program was, for *uidA*: (94°C - 1 min, 57°C - 1 min, and 72°C - 1 min) and for *stx1*: (94°C - 30s, 57°C - 30s, 72°C - 30s), for *stx2*: (94°C - 30s, 55°C - 30s, 72°C - 30s), and for *rfb*: (94°C - 30s, 52°C - 30s, 72°C - 30s) for 35 cycles for each. The tubes were removed from the apparatus and placed in a refrigerator at 4-8°C until Gel electrophoresis was performed to confirm the size of amplified products for the genes used in this study.

Results

The results of the current study declared that all isolates of *E. coli* isolated from fish samples, from both farmed fish and local market fish were positive for the *uidA* gene with a molecular weight of bp 623 (Table 2 and Figure 1).

Detection the *Stx1, Stx2* and *rfb* gene

The results of the current study showed that the number of *E. coli* isolated was 18 isolates from farmed fish samples. In addition, this study found that *E. coli* isolated from fish

farm having the *stx1* gene was 88.9% (16/18) with a molecular weight of 347 bp, while *E. coli* isolated from fish market samples having the *stx1* gene was 89.3% (25/28). The results of the current study found that *E. coli* isolated from farmed fish samples having the *Stx2* gene was 72.2% (13/18) with a molecular weight of 589 bp. Moreover, *E. coli* isolated from the local fish market samples having the *Stx2* gene was 85.7% (24/28) with a molecular weight of 589 bp, while we didn't detect the *rfb* gene in *E. coli* isolated from fish farm and local fish markets by using polymerase chain reaction and its primers (Table 3 and Figure 2).

Table 2: Number of positive samples of *E. coli* isolate and *uidA* gene in all study areas

Location	Number of the samples		
	Examine	+ve *E. coli*	+ve *uidA* gene
Hawi church	25	4	4
Wana	25	6	6
Hamdaniya	25	8	8
Total	75	18 (24%)	18 (24%)
Al-Medan	28	17	17
Albaladiat	25	6	6
Nabi younis	25	5	5
Total	78	28 (35.9%)	28 (35.9%)

Table 3: Detection the *Stx1*, *Stx2* and *rfb* gene in *E. coli* isolates

Location	Number of the samples				
	Examine	+ve to *E. coli*	+ve to *Stx1* gene	+ve to *Stx2* gene	+ve to *rfb* gene
Hawi church	25	4	3	2	-
Wana	25	6	5	4	-
Hamdaniya	25	8	8	7	--
Total	75	18 (24%)	16 (88.9)	13 (72.2%)	0
Al-Maidan	28	17	15	13	-
Al-Baladiyat	25	6	6	6	-
Nabi younis	25	5	4	5	-
Total	78	28 (35.9)	25 (89.3%)	24 (85.7)	0

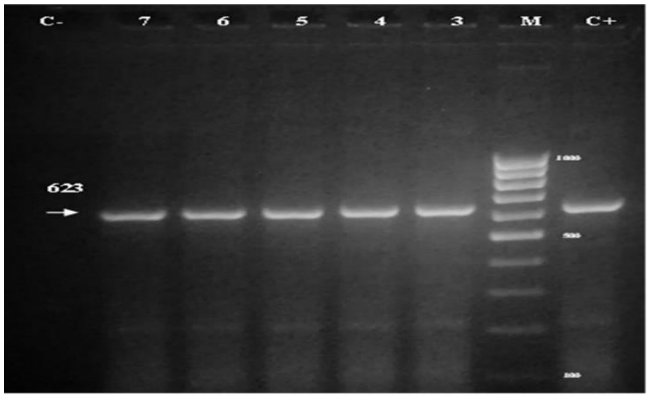

Figure 1: Shows electrophoresis of PCR products for *E. coli* isolates, where lane C+ represents the positive control, lane M represents Ladder DNA 100bp, lanes 3, 4, 5, 6, 7 represent the positive samples of *E. coli* uidA gene with a molecular weight of 623 bp and lane C- represents the negative control.

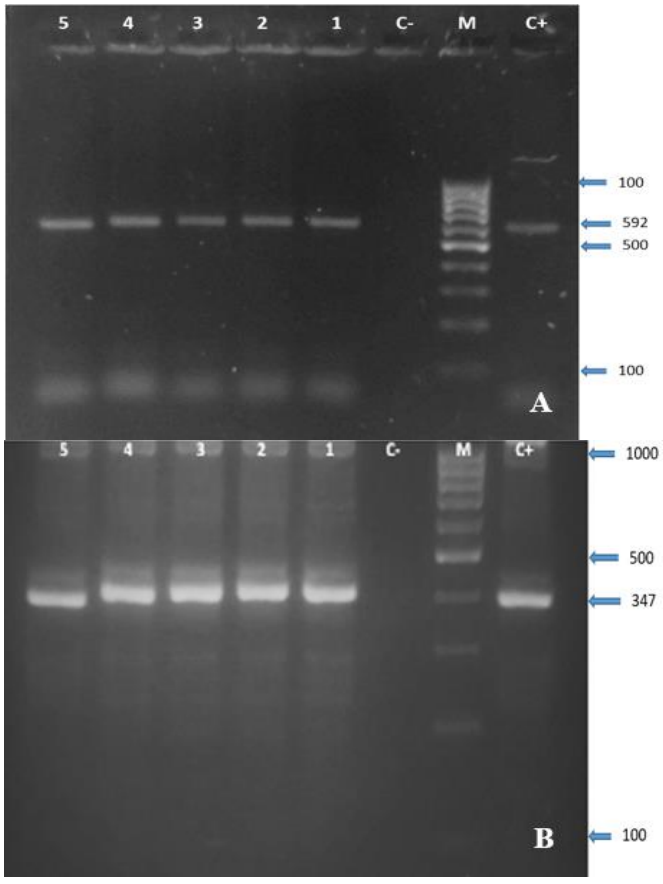

Figure 2: A and B: the virulence factor *Stx1* with a molecular weight of 347 bp and *Stx2* virulence factor with a molecular weight of 592 bp, where lane 1 represents the positive control, lane 2 represents the Ladder DNA 100 bp, lane 3 represents the negative control, and lanes 1, 2, 3, 4 and 5 represent positive samples.

Discussion

In recent decades, the consumption of fish and its products increased because fish has vitamins, minerals, and fat that are important for humans. Modern techniques are used for detecting the virulence factors encoding genes, which are responsible for producing several types of toxins that cause food poisoning in humans. The previous studies revealed that *E. coli* can produce the *Stx1* and *Stx2* genes that cause problems such as high morbidity, high mortality rate of humans, high cost of therapeutics. In addition, they unveiled that *E. coli* has the ability to resist different types of antibiotics via *E. coli* possession of the specific gene, which prevents the activity of antibiotics on bacteria (22,25). The results showed that the number of samples that gave positive results for the *Stx1* gene from farmed fish was 18 samples with a rate of 88.9%, while the number of samples *Stx2* was 15 samples with a percentage of 72.2%. As for the number of samples that gave positive results for the *Stx1* gene from the local fish market, it was 25 samples at a rate of 89.3%. As for the *Stx2* gene, the number of samples was 24 samples at a rate of 85.7%, while these results of *Stx1* and of *Stx2* were not detected and do not identify with another study conducted in Duhok Province/ Iraq (26), as well, the results of this study were higher than the results of Siddhnath *et al.* (27), when they revealed the percentage 27% of the positive *Stx* genes detected by direct PCR. In his research, which he conducted on 18 fish samples, where 8 samples contained *Sxt1* by 44% and 14 samples contained *Stx2* by 77%, Ribeiro *et al.* (28) revealed that out of the 115 isolates, 2 (1.74%) were positive only for the *Stx1* gene and six isolates 5.21% for *Stx2* gene. The isolates positives for *Stx2* were found in water and fish gastrointestinal tract samples, while there are no virulence genes detected in skin. In our study, in which the encoding of the O antigen was used for the detection of O157 serotype, we did not detect the *rfb* genes. This result is in harmony with that yielded in another study, where no detection is made (29).

Effluents from slaughterhouses and municipal wastewater treatment, as well as sewage, leakage of sewage systems, uncontrolled discharge of feces and excreta from wildlife and runoff of manure and fecal residues deposited in fields and pastures, may pollute the water and thus contaminate the fish that live in this water (30,31).

Conclusion

Fish contaminated with *E. coli* is deemed a major cause in food poisoning for humans. The fish market applying poor methods to clean and store fish help to grow and multiply bacteria, which leads to cause food poisoning. Importantly, *E. coli* has the *Stx1* and *Stx2* genes that can synthesize the stx1 and stx2 toxins, which may be transmitted to humans while consuming contaminated food with *E. coli* that causes health problems. *E. coli* isolates used in this study did not contain the *rfb* gene encoding *E. coli* O157:H7. Finally, the PCR technique is regarded as a modern method to detect *E. coli* based on the target sequence of the gene.

Acknowledgments

The authors express their gratitude for the efforts of College of Veterinary Medicine, University of Mosul, in granting them all possible facilitations.

Conflict of interest

The researchers confirmed that there is no conflict of interest.

References

1. Norman RA, Crumlish M, Stetkiewicz S. The importance of fisheries and aquaculture production for nutrition and food security. Rev Sci Tech. 2019;38(2):395-407. DOI: 10.20506/rst.38.2.2994
2. Sharp JH, Clements K, Diggens M, McDonald JE, Malham SK, Jones DL. *E. coli* is a poor end-product criterion for assessing the general microbial risk posed from consuming norovirus contaminated shellfish. Front Microbiol. 2021;12:150. DOI: 10.3389/fmicb.2021.608888
3. Kamala K, Rajeshkumar S, Sivaperumal P. The predominance of Shiga toxin-producing *E. coli* in the southeast coast of India. Mar Pollut Bull. 2022;174:113188. DOI: 10.1016/j.marpolbul.2021.113188
4. Alsanjary LH, Sheet OH. Molecular detection of uidA gene in *Escherichia coli* isolated from the dairy farms in Nineveh governorate, Iraq. Iraqi J Vet Sci. 2022;36(3):599-603. DOI: 10.33899/ijvs.2021.131046.1913
5. Othman SM, Sheet OH, Alsanjary LH. Isolation and identification of *Escherichia coli* O157:H7 isolated from veal meats and butchers' shops in Mosul city, Iraq. J Appl Vet Sci. 2022;7(4):55-61. DOI: 10.21608/javs. 2022.152213.1164
6. Petronillah R, Robert K, John V, Nyoni S. Isolation and identification of pathogenic bacteria in edible fish: A case study of Fletcher dam in Gweru, Zimbabwe. Int J Sci Res. 2013;2:269-73. DOI: 10.1.1.680.9981
7. Costa RA. *Escherichia coli* in seafood: A brief Overview. Adv Biosci Biotechnol. 2013;4(3):450-454. DOI: 10.4236/abb.2013.43A060
8. Gordon D, Cowling A. The distribution and genetic structure of *Escherichia coli* in Australian vertebrates: Host and geographic effects. Microbiol. 2003;149(12):3575-3586. DOI: 10.1099/mic.0.26486-0
9. Aleksender N, Margarita T, Inga E, Olga V, Aivars B. Major foodborne pathogens in fish and fish products: A review. Ann Microbiol. 2016;66:1-15. DOI: 10.1007/s13213-015-1102-5
10. Newaj A, Mutani A, Ramsubhag A, Adesiyun A. Prevalence of bacterial pathogens and their anti-microbial resistance in tilapia and their pond water in Trinidad. Zoonoses Public Health. 2008;55(4):206-213. DOI: 10.1111/j.1863-2378.2007.01098.x
11. Noor El-Deen AE, Atta NS, Abd El-Aziz MA. Oral vaccination of Nile tilapia (*Orechromis niloticus*) against motile Aeromonas septicemia. Nat Sci. 2010;8(2):21-25. [available at]
12. Sanyal S, Banerjee S. Bacterial population of fish and their environment in ponds utilized for natural purification of wastewater. Appl Ecol Environ Sci. 2021;9(7):649-655. DOI: 10.12691/aees-9-7-4
13. Nagamatsu K, Hannan TJ, Guest RL. Dysregulation of *Escherichia coli* α-hemolysin expression alters the course of acute and persistent urinary tract infection. Proc Natl Acad Sci USA. 2015;112(8):871-880. DOI: 10.1073/pnas.1500374112
14. Spinale JM, Ruebner RL, Lawrence C. Long-term outcomes of Shiga toxin hemolytic uremic syndrome. Pediatr Nephrol. 2013;28:2097-2105. [available at]

15. Galal HM, Hakim AS, Sohad M. Dorgham. Phenotypic and virulence genes screening of *Escherichia coli* strains isolated from different sources in delta Egypt. J Life Sci. 2013;10(2):352-361. [available at]
16. Wogu MD, Maduakol M. Evaluation of microbial spoilage of some aqua cultured fresh fish in Benin city, Nigeria. Ethiop J Environ Stud Manag. 2010;3(3):18-22. DOI: 10.4314/ejesm.v3i3.63960
17. Fouz B, Toranzo AE, Milan M, Amaro C. Evidence that water transmits the disease caused by the fish pathogen *Photobacterium damselae subsp. damselae*. J Appl Microbiol. 2000;88:531-535. DOI: 10.1046/j.1365-2672.2000.00992.x
18. Lokollo E, Mailoa MN. Handling techniques and microbial contamination of fresh flying fish in traditional markets in Ambon city. J Pengolah Has Perikan Indones. 2022;23(1):103-11. [available at]
19. Brown AE, Smith HR. Benson's microbiological applications, laboratory manual in general microbiology. 14th ed. New York: McGraw Hill Higher Education; 2017: 438 p.
20. Moyo SJ, Matee MI, Largeland N, Mylvaganam H. Identification of diarrheagenic *Escherichia coli* isolated from infant and children in Dares Sallaam, Tanzania. BMC Infect Dis. 2007;7:92. DOI: 10.1186/1471-2334-7-92
21. Dashti AA, Mehrez MJ, Abdulsamad MA, Hussein MD. Heat treatment of bacteria: A simple method of DNA extraction for molecular techniques. J Kuwait Med Assoc. 2009;41(2):117-122. [available at]
22. Cardozo MV. Pathogenic *Escherichia coli* in wild fish and intensive fish farming for human consumption. J Microb Biochem Technol. 2013;5(4):79. DOI: 10.4172/1948-5948.S1.010
23. Fujioka M, Otomo Y. Ahsan CR.A novel single-step multiplex polymerase chain reaction assay for the detection of diarrheagenic Escherichia coli. J Microbial Methods.2013; 92(3): 289-92. Dol: 10.1016/j-mimet.2012.12.010.
24. Paton, J. C. ; Paton, A. W. (1998). Pathogenesis and diagnosis of shiga toxin-producing Escherichia coli infections. Clin. Microbiol. Rev. 11, 450-479. https://doi.org/10.1128/CMR.11.3.450
25. Ashraf AA, El-Hofy F, El-Gamal AM, Ibrahim HO. Bacteriological and molecular studies on bacteria transmitted from fishes to human. Int J Adv Res. 2018;6(1):1047-1059. DOI: 10.21474/IJAR01/6319
26. Taha ZM, Yassin NA. Prevalence of diarrheagenic *Escherichia coli* in animal products in Duhok province, Iraq. Iran J Vet Res Shiraz Univ. 2019;20(4):255-262. [available at]
27. Siddhnath K, Majumdar RK, Parhi J, Sharma S, Mehta NK, Laishram M. Detection and characterization of Shiga toxin-producing *Escherichia coli* from carps from integrated aquaculture system. Aquac. 2018;487:97-101. DOI: 10.1016/j.aquaculture.2018.01.008
28. Ribeiro LF, Barbosa MC, de Rezende Pinto F., Guariz CL, Maluta RP, Rossi JR, Rossi GM, Lemos MF, do Amaral LA. Shiga toxigenic and enteropathogenic *Escherichia coli* in water and fish from pay-to-fish ponds. Appl Microbiol. 2015;62:216-220. DOI: 10.1111/lam.12536
29. Kumar HS, Otta SK, Karunasagar I. Detection of Shiga-toxigenic *Escherichia coli* (STEC) in fresh seafood and meat marketed in Mangalore, India by PCR. Lett Appl Microbiol. 2001;33(5):334-338. DOI: 10.1046/j.1472-765X.2001.01007.x
30. Ritter L, Solomon K, Sibley P, Hall K, Keen P, Mattu G, Linton B. Sources, pathways, and relative risks of contaminants in surface water and groundwater: A perspective prepared for the Walkerton inquiry. J Toxicol Environ Health. 2002;65:1-142. DOI: 10.1080/152873902753338572
31. Simpson JM, Santo-Domingo JW, Reasoner DJ. Microbial source tracking: State of the science. Environ Sci Technol. 2002;36:5279-5288. [available at]

الكشف عن بعض جينات الفوعة *stx1* و *stx2* و *rfb* لجراثيم الاشيريكيا القولونية المعزولة من الأسماك في محافظة نينوى، العراق

نور عبد الجبار الطائي و رعد عبد الغني السنجري و عمر هاشم شيت

فرع الصحة العامة البيطرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

الهدف من الدراسة الحالية هو الكشف عن وجود بعض جينات الفوعة للإيشريكية القولونية في الأسماك. إذ عُزل ما مجموعه ٤٦ سلالة من جراثيم الإيشريكيا القولونية من ١٥٣ عينة من الأسماك، بما في ذلك ٢٨ و ١٨ عزلة من جراثيم الإيشريكيا القولونية من الأسواق المحلية ومزارع الأسماك، على التوالي، في محافظة نينوى خلال الفترة من تشرين الثاني ٢٠٢١ إلى كانون الثاني ٢٠٢٢. تُظهر نتائج الدراسة أن جميع عزلات جراثيم الإيشريكيا القولونية تمتلك جين *uidA* بوزن جزيئي يبلغ ٦٢٣ نقطة أساس. بالإضافة إلى ذلك، تُظهر النتائج أن ٨٨,٩٪ (١٨/١٦) من العزلات المستخلصة من عينات الأسماك تحمل الجين الخاص بسموم الشيكا الأول بوزن جزيئي ٣٤٧ نقطة أساس، بينما تحمل نسبة ٧٢,٢٪ (١٨/١٣) منها الجين الخاص بسموم الشيكا الثاني بوزن جزيئي قدره ٥٨٩ نقطة أساس. كما تكشف الدراسة أن نسبة ٨٩,٣٪ (٢٨/٢٥) من العزلات المأخوذة من عينات أسماك السوق تحمل الجين بسموم الشيكا الأول بوزن جزيئي ٣٤٧ نقطة أساس ونسبة ٨٥,٧٪ (٢٨/٢٤) من العزلات تحمل الجين بسموم الشيكا الثاني بوزن جزيئي ٥٨٩ نقطة أساس. كذلك، لم تكشف الدراسة عن وجود جين *rfb* لا في المزارع السمكية ولا في عينات من أسواق الأسماك المحلية باستخدام تقنية تفاعل البلمرة المتسلسل، على حد سواء. أيضا، تُظهر الدراسة الحالية أن جراثيم الإيشريكيا القولونية المعزولة من الأسماك تحمل جينات سموم الشيكا الأول والثاني والتي تعتبر من العوامل الرئيسية المسببة للتسمم الغذائي للمستهلكين. أخيرا، أعطت الدراسة معلومات مهمة حول تطبيق الشروط الصحية على مزارع الأسماك وأسواق الأسماك لمنع التلوث وتقليل العدوى بمسببات الأمراض المنقولة بالغذاء، التي تسبب التسمم الغذائي للإنسان.