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Isolation and Detection of *Escherichia coli* Bacteria from Fish in Al-Shirqat City

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ABSTRACT
Escherichia coli is considered one of the most bacterial contaminants, serving as an indicator for meat spoilage. The study aimed to isolate and detect *Escherichia coli* bacteria in some parts of fish, workers hands and some tools used for cutting fish in the local markets of Al-Shirqat City. In this study, a total of 80 samples were tested, of which 55 tested positive for *Escherichia coli*, resulting in an overall diffusion rate of 68%. *Escherichia coli* isolates appeared on MacConkey agar as bright pink in color, on eosin methylene blue was greenish metallic sheen, and as blue colonies on chromogenic agar. The results of Vitek II system for isolates of *Escherichia coli* showed positive identification with 97% accuracy. In conclusion, the results recorded unsatisfactory levels for safety and quality of fish due to contamination by *Escherichia coli*. A higher contamination in fish has occurred due to poor hygiene practices during marketing. These bacteria can be transmitted to humans and cause diseases. The results highlight the need to control and prevent *Escherichia coli* contamination in aquaculture systems, production and transport to market to reduce microbiological risks on consumer.

INTRODUCTION

Fish meat is considered an important component of our diets that is in increasing demand, since it is considered as a food source that is balanced due to their low cost and higher nutritional values. Its nutritional value lies in being a good source of proteins, lipids, vitamins and minerals **(Ali *et al*., 2022)**. **Luo *et al*. (2022)** reported that fish meat is important for the growth and health of humans, since it promotes the growth of the body and prevents mental diseases and rickets of children. Freezing food usually preserves it from contamination compared to being refrigrated which results in shorter-shelf life **(Ježek & Buchtová, 2007; Dakheel & Jasim, 2021)**. Freezing time (short or fast freezing) has a great effect on the quality of fish meat. Slow freezing causes tissue damage and denaturation of protein **(Hassoun *et al*., 2020; Jumaa *et al*., 2024)**.

The fish is spoilt during storage due to different damage mechanisms, such as lipid oxidation, autolytic degradation, and microbiological spoilage **(Ghaly *et al*., 2010; Bashar *et al*., 2025)**. The bacterial activity increases after days of freezing. These bacteria are pathogenic and harmful, leading to the decomposition of fish meat that become not suitable for consumption **(Wambui *et al*., 2018; Ajena, 2021)**. *Escherichia coli* is considered one of the most bacterial contaminants, serving as an indicator for meat spoilage **(Ahmed *et al*., 2019; Ayat & Shakir, 2021; Jumma, 2024)**. Pathogenic *Escherichia coli* consists of many strains which differ according to their pathological effects or virulence factors. The intestinal pathogenic *Escherichia coli* groups include diffusely adherent, enteroinvasive, enteropathogenic, enteroaggregative, enterotoxigenic, and verocytotoxigenic *Escherichia coli* **(O'Sullivan *et al*., 2007; Sabah *et al*., 2024)**. Extra-intestinal pathogenic *Escherichia coli* include sepsis-causing, *E. coli* associated with neonatal meningitis, and *E. coli* uropathogenic **(Köhler & Dobrindt, 2011)**. Globally, the rates of mortalities and morbidities asociated with *Escherichia coli* are high. Estimates of the year 2010 showed that food-borne *Escherichia coli* caused 16.1% of global diseases. In addition, *Escherichia coli* was responsible for 196,617 deaths due to food poisioning, representing 0.02% of global mortality from food-borne diseases **(Kirk *et al*., 2015)**. This study aimed to isolate and detect *Escherichia coli* bacteria in some parts of fish, workers' hands and some tools used for cutting fish in the local markets of Al-Shirqat City.

MATERIALS AND METHODS

Samples

25g of fish meat were prepared and cut into small pieces, then placed into a labeled vial containing peptone water and buffered peptone water. The small fragments were standardized, and then the peptone water was streaked using a sterile loop onto nutrient agar for bacterial growth. A total 80 swabs were collected from different sources of fish including the meat, body cavity, gills, in addition to workers' hands, knives and tables from the local market in Al-Shirqat City/Iraq.

Isolation and detection *Escherichia coli* bacteria

The initial samples were incubated in buffered peptone water in 37°C for 24h. Then, a sterile loopful subculture on nutrient medium was used for incubation of sample before being transferred into solid media. Selective agar medium was used for the isolation of *Escherichia coli*, such as MacConkey agar broth, that was incubated in 37°C for 24h, followed by sub culturing on Eosin Methylene Blue (EMB) agar for 24h of incubation at 37°C. Subsequently, Gram staining and group of biochemical tests were performed for identification **(Quinn *et al*., 2002; Othman *et al*., 2024)**. Additionally, chromogenic media agar of Tryptone Bile X-Glucuronide (TBX) was used to demonstrate *Escherichia coli* **(Evans *et al*., 2008; Hirvonen *et al*., 2012)**. The VITEK II

Compact System was used for the confirmation of isolation and detection of *Escherichia coli* bacteria from the samples, using its card system which contained 64 biochemical tests (Fig. 4). This technique is characterized by being very accurate and quick in detecting all types of pathogenic bacterial.

RESULTS AND DISCUSSION

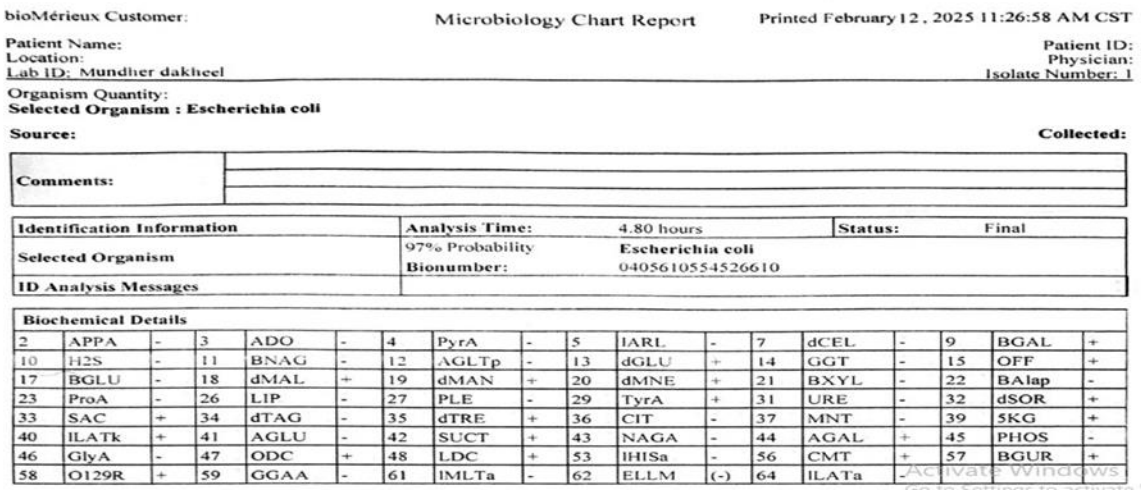
The result of present study for 80 samples were collected from different sources including the body cavity of fish, gills, workers' hands, knives and tables from the local market in Al-Shirqat City to determine the rate of diffusion of *Escherichia coli*. A total of 80 samples were tested, of which 55 tested positive for *Escherichia coli,* resulting in an overall diffusion rate of 68% (Table 1).

Table 1. Occurrence of *Escherichia coli* isolates from fish meat body cavity, gills, workers hands and knives samples

Sources of Samples	Number of Samples test	*Escherichia coli* positive (%)
Fish meat	**20**	**13 (16%)**
Body cavity	**20**	**12 (15%)**
Gills	**20**	**10 (12%)**
Workers hands	**8**	**8 (10%)**
Knives	**7**	**7 (8%)**
Tables	**5**	**5 (6%)**
Total	**80**	**55 (68%)**

Escherichia coli colonies growth on MacConkey agar showed bright pink color plates (Fig. 1) and on EMB agar plate showed greenish metallic sheen (Fig. 2). *Escherichia coli* colonies appear as blue colonies on chromogenic agar (Fig. 3). All isolates of *Escherichia coli* gave positive results for Vitek II system and rapid agglutination reaction (Fig. 4).

Fig. 1. *Escherichia coli* colonies appear as (A) Pink color on MacConkey agar, (B). Greenish metallic sheen color on EMB, (C). Chromogenic TBX Agar



bioMérieux Customer: Microbiology Chart Report Printed February 12 , 2025 11:26:58 AM CST

Patient Name: Patient ID:
Location: Physician:
Lab ID: Mundher dakheel Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	4.80 hours	**Status:**	Final
Selected Organism	97% Probability **Bionumber:**	**Escherichia coli** 0405610554526610		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	+
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	(-)	64	ILATa	-			

Fig. 4. Result of Vitek II system for detection of *Escherichia coli*

This study aimed to evaluate the sanitary quality of fish meat in local markets of Al-Shirqat City. In this study, the results of the isolation and detection of *Escherichia coli* bacteria from some parts of fish, workers hands and some tools used for cutting fish were significantly high with 68% diffusion rate (68%). The *Escherichia coli* colonies exhibited different shapes and colors in selective agars. In MacConkey agar, the colonies were smooth, round, pink morphology, on Eosin Methylene Blue (EMB) agar colonies were green metallic sheen and the colonies of *Escherichia coli* demonstrated the typical blue-green color. This result agrees with results reported by **Quinn *et al*. (2002)**, **Evans *et al*. (2008)**, **Hirvonen *et al*. (2012)** and **Othman *et al*. (2024)**. Additionally, these results coincide with those of **Alttai *et al*. (2023)**, who reported increased rates of *Escherichia coli* isolated from fish in local markets of Nineveh Governorate. In this context, **Neshtiman *et al*. (2025)** reported higher isolation rates of *Escherichia coli* in fish meat and gills. These results are similar to the present outcomes. The poor hygiene during fish handling and preparation is associated with higher microbial burdens **(Obe *et al*., 2021)**. Additionally, **Jahan *et al*. (2020)** reported that fish treated in non-sterile market circumstances had major total viable counts. The higher contamination rates were in fish meat (16%), body cavity (15%), gills (12%), tables (6%), knives (8%), and workers' hands (10%). These results indicate significantly hygiene challenge during post-harvest transport, storage, and processing. This result agrees with **Yohans *et al*. (2022)**, who reported the inappropriate infrastructure and handling were causes of higher contamination levels. Contamination was higher on post-harvest surfaces such as workers' hands, tables and knives. This shows that inappropriate hygiene contributes to spread of antimicrobial resistance **(Yohans *et al*., 2022; Sharef *et al*., 2025)**. Moreover, **Essa *et al*. (2025)** reported that *Escherichia coli* and *Aeromonas hydrophila* were the most frequently bacterial species isolated from the examined fish samples. The existence of *E. coli* in meat may pose risk to consumers, which is consistent with findings of

Abuelhassan *et al*. (2014), who explained that the presence of pathogenic *Escherichia coli* bacteria is a potential risk to human health due to their ability to survive in meat.

CONCLUSION

The results of the study recorded that contamination by *Escherichia coli* was at levels unsatisfactory for safety and quality of fish. Higher contamination occurred due to poor hygiene practices during marketing. These bacteria can be transmitted to humans and cause diseases. The results highlight the need to control and prevent *Escherichia coli* contamination in aquaculture systems, production and transport to market to reduce microbiological risks on consumer.

REFERENCES

Abuelhassan, N.N.; Abdul, M. S.; Muin, N.M.; Tibin, E.; Doni, F. and Yusoff, W. (2014). Antibiotic resistance pattern among *Escherichia coli* O157\:H7 isolated from retail imported frozen meat. Asian J. Microbiol. Biotechnol. Environ. Sci., 16(3), 739-744. https://www.researchgate.net/publication/265979302

Ahmed, N. A.; Mahmood, S. S. and Abbas, A. H. A. (2019). comparative study of some virulence factors and phylogenetic characterization of *Escherichia coli* isolates causing urinary tract infection and the commensal gut. The Iraqi Journal of Agricultural Science, 50(3), 1193-1198. https://doi.org/10.36103/ijas.v50i4.763

Ajena, S. J. M. (2001). The effect of frozen storage periods and packaging treatment on the suitability of veal for human consumption using chemical, sensory and bacterial evidence, Master's thesis, College of Agriculture/University of Baghdad.

Ali, A.; Wei, S.; Ali, A.; Khan, I.; Sun, Q.; Xia, Q.; Wang, Z.; Han, Z.; Liu, Y. and Liu, S. (2022). Research Progress on Nutritional Value, Preservation and Processing of Fish-A Review. Foods (Basel, Switzerland), 11(22), 3669. https://doi.org/10.3390/foods11223669

Alttai, N. A.; Alsanjary, R. A. and Sheet, O.H. (2023). Isolation and molecular identification of *Escherichia coli* strain from fish available in farms and local markets in Nineveh governorate, Iraq. Iraqi Journal of Veterinary Sciences, 37(2), 431-435. https://doi.org/10.33899/ijvs.2022.135441.2478

Ayat, A. A. and Shakir, K. A. (2021). Functional properties of catfish skin collagen hydrolysates. Iraqi Journal of Agricultural Sciences, 52(6), 1528-1540. https://doi.org/10.36103/ijas.v52i6.1494

Bashar, S. N.; Qusai, S. J.; Muthanna, S.; Raghad, Z. S.; Khalid, A. H.; Ali, A. A. (2025). Preventive and Ameliorative Role of Lactoferrin in Improving Blood, Lipid and Liver Profiles Toward Immune Homeostasis in Lipopolysaccharides (LPS) Induced Dysregulation of Bio-Vital Parameters in the Common Carp (*Cyprinus carpio*). Egyptian Journal of Aquatic Biology and Fisheries, 29(2), 1173-1180. https://doi.org/10.21608/ejabf.2025.419336

Dakheel, M. and Jasim, H. (2021). Evaluation the microbial and chemical properties of refrigerated chicken meat coated by bionanocomposite of gelatin mix with zinc oxide nanoparticles. Biochem. Cell. Arch., 21(2), 3375-3384. https://connectjournals.com/03896.2021.21.3375

Essa, M.; kadori, A.; Suleiman, J.; Jumma, Q. and Aziz, A. (2025). Evaluation of the Quality of Fish Storage, Isolation and Identification of the Bacteria *Escherichia coli* and *Aeromonas hydrophila*. Egyptian Journal of Aquatic Biology and Fisheries, 29(5), 455-468. https://doi.org/10.21608/ejabf.2025.419776.6506

Evans, J.; Knight, H. I.; Smith, A. W.; Pearce, M. C.; Hall, M.; Foster, G.; Low, J. C. and Gunn, G. J. (2008). Cefixime-tellurite rhamnose MacConkey agar for isolation of Vero cytotoxin-producing *Escherichia coli* serogroup O26 from Scottish cattle and sheep faeces. Letters in applied microbiology, 47(3), 148–152. https://doi.org/10.1111/j.1472-765X.2008.02402.x

Hassoun A.; Shumilina E.; Di Donato F.; Foschi M.; Simal-Gandara J. and Biancolillo A. (2020). Emerging Techniques for Differentiation of Fresh and Frozen-Thawed Seafoods: Highlighting the Potential of Spectroscopic Techniques. Molecules, (25), 4472. https://doi.org/10.3390/molecules25194472

Hirvonen, J. J.; Siitonen, A. and Kaukoranta, S. S. (2012). Usability and performance of CHROMagar STEC medium in detection of Shiga toxin-producing *Escherichia coli* strains. Journal of clinical microbiology, 50(11), 3586–3590. https://doi.org/10.1128/JCM.01754-12

Jahan, N.; Hussain, M. A. and Rahman, M. (2020). Comparative assessment of microbial load in fish samples from market and lab-processed sources. Food Sci. Technol. 40(2), 345-350.

Ježek, F. and Buchtová, H. (2007). Physical and Chemical Changes in Fresh Chilled Muscle Tissue of Common Carp (Cyprinus Carpio L.) Packed in a Modified Atmosphere. Acta Vet. Brno. (76), S83-S92 https://doi.org/10.2754/avb200776S8S083

Jumaa, Q.S.; Al-Bayati, H.H.K.; Abdullah, S.A.; Shihab, T.J. and Sultan, M. (2024). Immunogenicity of culture filtrated proteins and whole-cell killed formalin of Listeria monocytogenes to induced cellular immune response in vivo. Open Vet J.14 (12):3581-3598. https://doi.org/10.5455/OVJ.2024.v14.i12.40

Jumma, Q. (2024). Detection of ESBL *E.coli* That Carried STX1 and STX2 Form Common Carp (*Cyprinus carpio*) in Salhaldeen Province. Egyptian J. Vet. Sci, 55(4), 1165-1170. https://doi.org/10.21608/ejvs.2024.255648.1728

Kirk, M. D.; Pires, S.M.; Black, R. E.; Caipo, M.; Crump, J. A. and Devleesschauwer, B. (2015). World Health Organization estimates of the global and regional disease burden of 22 food-borne bacterial, protozoal, and viral

diseases, 2010: a data synthesis. PLoS Medicine, 12(12), e1001921. https://doi.org/10.1371/journal.pmed.100192

Köhler, C. D. and Dobrindt, U. (2011). What defines extraintestinal pathogenic Escherichia coli? International Journal of Medical Microbiology, 301:642-647. https://doi.org/10.1016/j.ijmm.2011.09.006

Luo, J.; Amenyogbe, E.; Fu, W.; Huang, J. and Chen, G. (2022). Hepatic transcriptome profiles reveal the hepatoprotective effects of dietary quercetin and sodium quercetin-5′-sulfonates supplementation in hybrid grouper (*Epinephelus fuscoguttatus*♀× *Epinephelus polyphekadion*♂) Aquaculture. 738483. https://doi.org/10.1016/j.aquaculture.2022.738483

Lupindu, A. M. (2017). Isolation and Characterization of lt;igt;Escherichia colilt;/igt; from Animals, Humans, and Environment. InTech. https://doi.org/10.5772/67390

Neshtiman, A.; Sharef, Z. and Eman, D. (2025). Evaluating the Hygienic Quality of Fish Meat and Environmental Contamination with *Escherichia coli*. Egyptian J. of Aquat. Biol. & Fisheries, 29(4), 149-162. https://doi.org/10.21608/ejabf.2025.437645

O'Sullivan, J.; Bolton, D. J.; Duffy, G.; Baylis, C.; Tozzoli, R. and Wasteson, Y. (2007). editors. Methods for Detection and Molecular Characterisation of Pathogenic *Escherichia coli*. Dublin: Pathogenic *Escherichia coli* Network (PEN), 2007. 34 p.

Obe, T.; Berrang, M. E.; Cox, N. A.; House, S. L. and Shariat, N. W. (2021). Comparison of selective enrichment and plating media for *Salmonella* isolation from broiler carcasses. J. Food Saf.; 41(6): 1-14. https://doi.org/10.1111/jfs.12928

Othman, C. O.; Khidhir, Z. K. and Arif, E. D. (2024). Prevalence of the copA Gene in *Escherichia coli* Isolated from Common Carp in Sulaymaniyah Province. Pak. Vet. J.; 44(1):190-194. http://dx.doi.org/10.29261/pakvetj/2023.125

Quinn, P. J.; Markey, B. K.; Carter, M. E.; Donnelly, W. J. and Leonard, F.C. Veterinary Microbiology and Microbial Disease. 1st ed. Blackwell Science Ltd; London. 163-167(2002). https://vetbooks.ir/veterinary-microbiology-and-microbial-disease-2nd-edition/

Sabah, M. A.; Qusai, J. and Ali, A. (2024). Oxidative Stress and Blood Parameters Affected by Carbaryl Insecticide in *Cyprinus carpio*. Egyptian J. Aquatic Biology and Fisheries, 28(6), 2195-2201. https://doi.org/10.21608/ejabf.2024.400585

Sharef, N. A.; Zaid, K. K. and Eman, D. A. (2025). Evaluating the Hygienic Quality of Fish Meat and Environmental Contamination with Escherichia coli. Egyptian Journal of Aquatic Biology and Fisheries, 29(4), 149-162. https://doi.org/10.21608/ejabf.2025.437645

Wambui, J.; Lamuka, P.; Karuri, E.; Matofari, J. and Njage, P. K. (2018). Microbial contamination level profiles attributed to contamination of beef carcasses,

personnel, and equipment: case of small and medium enterprise slaughterhouses. J Food Prot. (81), 684–91. https://doi.org/10.4315/0362-028X.JFP-17-402

Yohans, H.; Mitiku, B. A. and Tassew, H. (2022). Levels of *Escherichia coli* as Bio-Indicator of Contamination of Fish Food and Antibiotic Resistance Pattern Along the Value Chain in Northwest Ethiopia. Veterinary medicine (Auckland, N.Z.), (13), 299-311. https://doi.org/10.2147/VMRR.S373738