International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 17s, 2025
https://theaspd.com/index.php

Molecular Identification Of Virulence Genes In E. Coli From Fishes In Local Markets In Erbil City

Ibrahim Ramadhan Ibrahim[1], Blnd Ibrahim Mohammed[2], Khalid Esmahel Aziz[3]
[1]Department of Fish Recourses and Aquatic Animals, College of Agriculture Salahaddin University-Erbil, Kurdistan Region, Iraq
[2]Department of Biology, College of Science, Salahaddin University-Erbil, Erbil, Iraq
[3]Department of Food technology, College of Agriculture Engineering Sciences, Salahaddin University-Erbil, Erbil, Iraq

Abstract
Fish serves as a critical source of protein and nutrients in Erbil, Iraq, yet its potential role in transmitting pathogenic bacteria poses significant public health risks. This study investigated the prevalence of Escherichia coli (E. coli) and its virulence-associated genes in fresh and frozen fish sold in Erbil's local markets. A total of 80 fish samples (40 fresh, 40 frozen) were analyzed using culture-based methods and PCR to detect virulence genes (stx1, stx2, eaeA, hlyA, lt). Results revealed a higher contamination rate in fresh fish (65%) compared to frozen samples (38%). Virulence genes were identified in 18% of fresh fish isolates versus 12% of frozen isolates, though none exhibited high-risk gene combinations (e.g., stx2 + eaeA) linked to severe disease. The predominant genes detected were stx1 and eaeA. These findings underscore the elevated microbial risk associated with fresh fish, likely due to unhygienic handling and inadequate temperature control during supply chain processes. The study emphasizes the need for stringent hygiene protocols in local markets to mitigate foodborne pathogen transmission and protect consumer health.
Keywords: *Escherichia coli; Virulence genes; Food safety; Erbil city; PCR; Fish contamination*

INTRODUCTION

Fish is a great source of high-quality protein and vital elements for human consumption. Local fish markets in many areas, including Erbil, are essential to supplying the population's daily nutritional needs (Paruğ et al., 2024). Nonetheless, fish may also act as a conduit for the spread of harmful germs, such as Escherichia coli (E. coli), a bacterium frequently present in both human and animal systems (Chatreman et al., 2020). Some pathogenic strains of E. coli include virulence genes that can cause severe gastrointestinal and extraintestinal infections; however, the majority of strains are safe (Sarowska et al., 2019).

The detection of virulence-associated genes in E. coli is particularly significant in food safety, as it provides direct insight into the pathogenic potential of contaminating strains (Ramatla et al., 2023). In regions like Erbil, where fish consumption is integral to daily nutrition, understanding the genetic profile of E. coli isolates becomes essential for mitigating health risks associated with foodborne pathogens (Paixao et al., 2016; Mohamed and Habib, 2023). Furthermore, the rise of antimicrobial resistance (AMR) in bacterial populations amplifies concerns, as virulent E. coli strains carrying resistance genes could lead to challenging clinical infections (Marano et al., 2023). By employing PCR-based methods, this study not only identifies hazardous strains but also contributes to broader surveillance efforts, aiding in the development of region-specific hygiene protocols and preventive strategies to safeguard public health (Loayza et al., 2020).

Fish can become contaminated with pathogenic E. coli at any stage of the supply chain, such as when they are harvested, handled, transported, or displayed in unhygienic market settings (Diaz-Decaro, 2018). The existence of genes linked to virulence suggests that these strains have the capacity to infect consumers with illness (Grema et al., 2020). Molecular techniques, especially those based on the polymerase chain reaction (PCR), offer a sensitive and targeted way to identify these genes and evaluate the possible harm to public health (Madani et al., 2022).

This study aimed to identify the presence of virulence genes in Escherichia coli isolates obtained from fish samples collected in local markets within Erbil city using molecular techniques.

MATERIALS AND METHODS

Sample Collection

A total of eighty fresh fish samples were randomly collected from various local markets across Erbil city, Iraq, in 2024. Samples included both freshwater and breeding once commonly sold for human consumption. Each sample was aseptically placed in sterile bags, transported in ice cooled containers to the laboratory, and processed within 4 hours of collection.

Isolation and Identification of E. coli

Fish samples were dissected, and portions of gill, intestine, and muscle tissues were homogenized in sterile buffered peptone water and incubated at 37°C for 18–24 hours. Aliquots were streaked on MacConkey agar and incubated at 37°C for 24 hours. Lactose-fermenting colonies with characteristic morphology were sub-cultured on Eosin Methylene Blue (EMB) agar for further confirmation. Colonies showing a metallic green sheen on EMB were presumptively identified as
E. coli and confirmed by standard biochemical tests (indole, methyl red, Voges–Proskauer, and citrate utilization) (Hariri, 2022).

DNA Extraction

Genomic DNA was extracted from confirmed E. coli isolates using a boiling method (Rashid, 2023). Briefly, 1 mL of overnight culture was centrifuged at 10,000 rpm for 5 minutes, and the pellet was suspended in 100 µL of sterile distilled water. The suspension was boiled at 95°C for 10 minutes, then immediately chilled on ice and centrifuged at 12,000 rpm for 5 minutes. The supernatant was collected and used as a DNA template for PCR.

Detection of Virulence Genes by PCR

The presence of virulence genes was determined by conventional PCR using specific primers targeting the following genes: stx1, stx2 (Shiga toxins), eaeA (intimin), hlyA (hemolysin), and lt (heat-labile enterotoxin). PCR reactions were carried out in a 25 µL total volume containing 12.5 µL of Master Mix (Promega, USA), 1 µL each of forward and reverse primers (10 pmol), 2 µL of DNA template, and 8.5 µL of nuclease-free water. Primers sourced from previous studies (Dimitrakopoulou et al., 2020; Moeinizadeh and Shaheli, 2021). PCR cycling conditions were as follows: initial denaturation at 94°C for 5 minutes; followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58–60°C (depending on primer) for 30 seconds, extension at 72°C for 1 minute; and a final extension at 72°C for 10 minutes.

Statistical analysis

Data were collected in Microsoft Excel and analyzed using SPSS version 25. Results were compared across patient variables with a significance level of ≤ 0.05 and presented as rates, ratios, frequencies, and percentages in tables and figures. Statistical analysis included t-tests, Chi-square, or Fisher's exact tests, as appropriate.

RESULTS

In this study, a total of 80 fish samples were examined, comprising both fresh (n=40) and frozen specimens (n=40) collected from various local markets in Erbil city. Escherichia coli was successfully isolated from a greater proportion of fresh fish samples compared to frozen ones. Specifically, 65% of the fresh samples were positive for E. coli, while a lower incidence was observed in frozen samples, with only 38% testing positive. This indicates that fresh fish may be more susceptible to bacterial contamination, likely due to factors such as inadequate hygiene during handling, storage at non-optimal temperatures, and prolonged exposure to open market conditions.

Further analysis using PCR confirmed the presence of virulence-associated genes in several E. coli isolates. These included stx1, stx2, eaeA, hlyA, and lt. Importantly, the distribution of these genes was not uniform between sample types. Virulence genes were detected more frequently in E. coli isolates from fresh samples than those from frozen ones, suggesting that fresher products may present a higher microbial risk to consumers. For example,

18% of the fresh E. coli isolates carried at least one virulence gene, while only 12% of the frozen isolates tested positive for these genes as shown in Table (1), and Figure (1).

Despite the detection of some virulence markers, highly virulent gene profiles typically associated with severe human illness were not recorded in any of the isolates from either fresh or frozen samples. None of the samples showed the simultaneous presence of multiple key virulence genes, such as both stx2 and eaeA, which are commonly linked to enterohemorrhagic E. coli (EHEC) strains. This suggests that while some isolates possessed potential pathogenic traits, their overall virulence level may be considered very low.

These findings highlight the relatively higher risk of contamination and presence of virulence genes in fresh fish compared to frozen fish available in local markets. It emphasizes the importance of proper handling, storage, and temperature control to reduce microbial risks in fish intended for human consumption.

Table 1: Comparison of E. coli Contamination and Virulence Gene Detection in Fresh and Frozen Fish Samples

Parameter	Fresh Fish (n=40)	Frozen Fish (n=40)
E. coli positive samples (%)	26 (65%)	15 (38%)
Samples negative for E. coli (%)	14 (35%)	25 (62%)
E. coli isolates with virulence genes (%)	7 (18% of 40)	5 (12% of 40)
High-risk virulence profiles detected	0	0

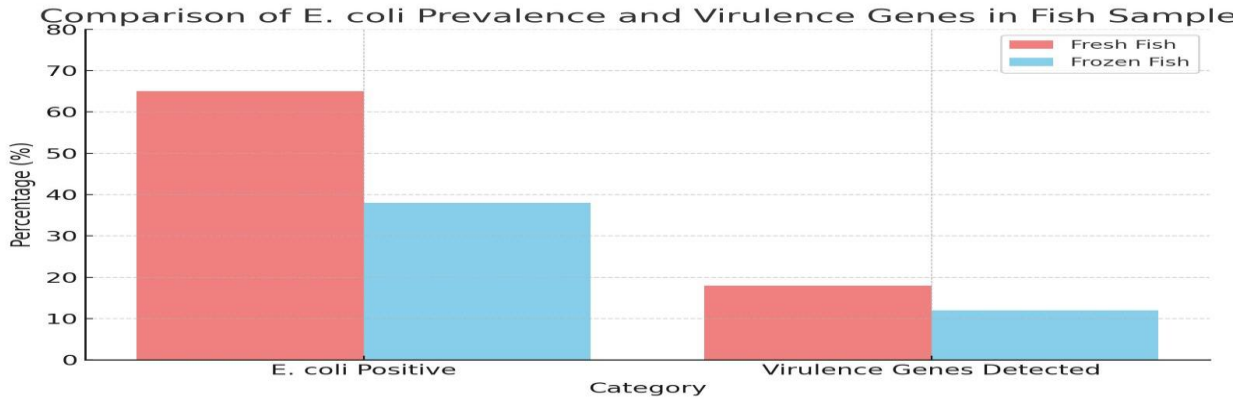

Figure 1: E coli prevalence and virulence gens

DISCUSSION

The detection of E. coli in fish samples from Erbil's markets highlights critical gaps in food safety practices, particularly in fresh fish handling. The significantly higher contamination rate in fresh fish (65%) compared to frozen samples (38%) aligns with studies from other regions, where improper storage, prolonged exposure to ambient temperatures, and cross-contamination during market display have been identified as key contributors to bacterial proliferation (Chatreman et al., 2020; Paruğ et al., 2024). Freezing, while not eliminating E. coli, likely reduces bacterial viability and gene expression, explaining the lower contamination and virulence gene prevalence in frozen fish (Achyar et al., 2021; Malik et al., 2021).

The identification of virulence genes (stx1, stx2, eaeA, hlyA, lt) in isolates from fresh fish raises concerns about consumer exposure to potentially pathogenic strains. Although no isolates carried high-risk gene combinations (e.g., stx2 + eaeA), the presence of stx1 and eaeA associated with Shiga toxin production and intestinal colonization suggests these strains could still cause mild to moderate gastrointestinal illness (Stella et al., 2022; Haque et al., 2022). The absence of highly virulent profiles may reflect the environmental origin of the E. coli strains, possibly introduced via contaminated water or unhygienic handling rather than direct fecal contamination from human or animal hosts.

Notably, the higher frequency of virulence genes in fresh fish isolates underscores the role of supply chain

practices in microbial risk amplification. Fresh fish in Erbil's markets are often stored at ambient temperatures for extended periods, creating ideal conditions for bacterial growth contrast, freezing disrupts cellular integrity and inhibits virulence gene expression, offering partial protection against pathogen transmission (Erikson et al., 2021; Zhang et al., 2023). These findings corroborate global data advocating for cold chain management as a critical intervention in reducing foodborne hazards (Ruxton, 2011). Improved refrigeration, regular infection testing, and vendor hygiene training are urgently needed at Erbil's fish markets. Inform customers about safe cooking and thawing techniques. Check for the dangers of antimicrobial resistance (AMR) in E. coli.

Limitations and Future Directions

Study limited by focused virulence gene analysis and sample size. Future work should integrate AMR profiling, larger samples across seasons, and trace contamination sources (water, equipment).

CONCLUSION

According to this study, fresh fish from Erbil local markets are more likely to be contaminated with E. coli of fresh samples (65%) tested positive compared to (38%) of frozen ones. Fresh fish isolates were more likely to have virulence genes like stx1 and eaeA, but no high-risk combinations (such stx2 + eaeA) were discovered. The results highlight the need for improved cleanliness, cold chain procedures, and vendor training in addition to ongoing surveillance and public education to safeguard consumer health, even while the lack of highly virulent pathogens lessens immediate worry.

Acknowledgements

This work was financially supported from funds of the Salahaddin University-Erbil, Erbil, Kurdistan Region, Iraq.

REFERENCES

1. Achyar, A., Putri, A.I., Putri, D.H. and Ahda, Y., 2021. Primer design, in silico PCR and optimum annealing temperature for Escherichia coli detection in refillable drinking water samples. Tropical Genetics, 1(2), pp.52-60.
2. Chatreman, N., Seecharran, D. and Ansari, A.A., 2020. Prevalence and distribution of pathogenic bacteria found in fish and fishery products: A review. Journal of Fisheries and Life Sciences, 5(2), pp.53-65.
3. Diaz-Decaro, J.D., 2018. Combatting Circulating Infectious Diseases in California: Evaluating New Approaches to Surveillance and the Costs of Outbreaks on Public Health Agencies. University of California, Los Angeles.
4. Dimitrakopoulou, M.E., Stavrou, V., Kotsalou, C. and Vantarakis, A., 2020. Boiling extraction method vs commercial kits for bacterial DNA isolation from food samples. Journal of Food Science and Nutrition Research, 3(4), pp.311-319.
5. Erikson, U., Uglem, S. and Greiff, K., 2021. Freeze-Chilling of whitefish: effects of capture, on-board processing, freezing, frozen storage, thawing, and subsequent chilled storage—a review. Foods, 10(11), p.2661.
6. Grema, H.A., Kwaga, J.K.P., Bello, M. and Umaru, O.H., 2020. Understanding fish production and marketing systems in North-western Nigeria and identification of potential food safety risks using value chain framework. Preventive Veterinary Medicine, 181, p.105038.
7. Haque, M., Bosilevac, J.M. and Chaves, B.D., 2022. A review of Shiga-toxin producing Escherichia coli (STEC) contamination in the raw pork production chain. International Journal of Food Microbiology, 377, p.109832.
8. Hariri, S., 2022. Detection of Escherichia coli in food samples using culture and polymerase chain reaction methods. Cureus, 14(12).
9. Loayza, F., Graham, J.P. and Trueba, G., 2020. Factors obscuring the role of E. coli from domestic animals in the global antimicrobial resistance crisis: an evidence-based review. International journal of environmental research and public health, 17(9), p.3061.
10. Madani, A., Esfandiari, Z., Shoaei, P. and Ataei, B., 2022. Evaluation of virulence factors, antibiotic resistance, and biofilm formation of Escherichia coli isolated from milk and dairy products in Isfahan, Iran. Foods, 11(7), p.960.
11. Malik, I.A., Elgasim, E.A., Adiamo, O.Q., Ali, A.A. and Mohamed Ahmed, I.A., 2021. Effect of frozen storage on the biochemical composition of five commercial freshwater fish species from River Nile, Sudan. Food Science & Nutrition, 9(7), pp.3758-3767.
12. Marano, G., Mazza, M., Lisci, F.M., Ciliberto, M., Traversi, G., Kotzalidis, G.D., De Berardis, D., Laterza, L., Sani, G., Gasbarrini, A. and Gaetani, E., 2023. The microbiota–gut–brain axis: psychoneuroimmunological insights. Nutrients, 15(6), p.1496.
13. Moeinizadeh, H. and Shaheli, M., 2021. Frequency of hlyA, hlyB, hlyC and hlyD genes in uropathogenic Escherichia coli isolated from UTI patients in Shiraz. GMS Hygiene and Infection Control, 16, p.Doc25.
14. Mohamed, M.Y.I. and Habib, I., 2023. Pathogenic E. coli in the food chain across the Arab countries: a descriptive review. Foods, 12(20), p.3726.
15. Paixao, A.C., Ferreira, A.C., Fontes, M., Themudo, P., Albuquerque, T., Soares, M.C., Fevereiro, M., Martins, L. and De Sá, M.C., 2016. Detection of virulence-associated genes in pathogenic and commensal avian Escherichia coli isolates. Poultry science, 95(7), pp.1646-1652.

16. Paruğ, Ş., Jabbr, A. and Lazrag, F.A., 2024. Enhancing sustainable fisheries and aquaculture in North Africa: Challenges, opportunities, and future directions. Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi, 10(1), pp.90-105.
17. Ramatla, T., Ramaili, T., Lekota, K.E., Ndou, R., Mphuti, N., Bezuidenhout, C. and Thekisoe, O., 2023. A systematic review and meta-analysis on prevalence and antimicrobial resistance profile of Escherichia coli isolated from water in africa (2000–2021). Heliyon, 9(6).
18. Rashid, R., 2023. EPIDEMIOLOGY AND MOLECULAR CHARACTERIZATION OF MULTIDRUG RESISTANT ESCHERICHIA COLI ISOLATED FROM FISH (Doctoral dissertation, Chattogram Veterinary & Animal Sciences University, Khulshi, Chattogram).
19. Ruxton, C.H., 2011. The benefits of fish consumption. Nutrition Bulletin, 36(1), pp.6-19.
20. Sarowska, J., Futoma-Koloch, B., Jama-Kmiecik, A., Frej-Madrzak, M., Ksiazczyk, M., Bugla-Ploskonska, G. and Choroszy-Krol, I., 2019. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic Escherichia coli isolated from different sources: recent reports. Gut pathogens, 11(1), p.10.
21. Stella, R., Mastrorilli, E., Pretto, T., Tata, A., Piro, R., Arcangeli, G. and Biancotto, G., 2022. New strategies for the differentiation of fresh and frozen/thawed fish: Non-targeted metabolomics by LC-HRMS (part B). Food Control, 132, p.108461.
22. Zhang, H., Yang, Z., Wang, Z., Li, H., Mo, H., Ma, S., Hu, L. and Zhou, X., 2023. Freezing enhances the killing of S. aureus by thymol via a unique bactericidal mechanism in cold chain food. Food Control, 153, p.109936.