Original Research Article

Prevalence and Antibiotic Resistance Profiling of Escherichia coli from Seafoods and Water from Malokun Sea, Ilaje, Ondo State, Nigeria

Abstract

Background

This study investigates the prevalence, and antibiotic resistance profiles of *Escherichia coli* isolated from aquatic samples including fish, shrimps, crab, and water collected from the Malokun Sea, Ilaje, Ondo State, Nigeria. The aim was to assess contamination levels and antibiotic resistance patterns to inform microbial risk assessments and public health interventions.

Methodology

Standard microbiological and molecular techniques were employed for the isolation and identification of *E. coli* using Most Probable Number (MPN) assays and Polymerase Chain Reaction (PCR). Antibiotic susceptibility testing was conducted with eight commonly used antibiotics using the disc diffusion method (Kirby-Bauer), and results were interpreted according to standard CLSI guidelines.

Results

A total of 37 *E. coli* isolates were recovered: 8 from fish, 6 from shrimps, 5 from crab, and 18 from water samples. Comparative analysis revealed higher *E. coli* loads for MPN-PCR (3.00 ± 0.89 log CFU/mL) indicating enhanced detection of viable but non-culturable cells. In contrast, MPN/EMB detected significantly higher bacterial loads in shrimps (4.46 ± 0.22 log CFU/g) and fish (3.36 ± 0.31 log CFU/g) compared to PCR. Crab samples showed relatively low *E. coli* levels, with minimal difference between methods. All isolates exhibited 100% resistance to penicillin, ceftazidime, and doripenem, while remaining fully susceptible to amikacin and norfloxacin. Multiple Antibiotic Resistance (MAR) indices ranged from 0.375 to 0.625. The resistance pattern CAZ-DOR-P was consistently found across all sample types, indicating widespread multidrug resistance.

Conclusion

The presence of multidrug-resistant *E. coli* in seafood and water from the Malokun Sea presents a significant public health concern. While MPN/EMB detected more viable cells in seafood, PCR demonstrates superior sensitivity in water, detecting a broader spectrum of bacterial states. These findings underscore the need for integrated culture-based and molecular surveillance and improved seafood hygiene practices, to mitigate the transmission of resistant pathogens through the aquatic food chain.

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Keywords: *Escherichia coli,* MPN-PCR, antibiotic-resistance, seafoods, water, Nigeria

1.0 Introduction

Fisheries play a pivotal role in global food security, livelihoods, and economic development, particularly in developing nations like Nigeria, where fish production has shown significant growth. Small-scale fisheries alone contribute approximately 40% of the global catch and 44% of the economic value, supporting the livelihoods of over 500 million people, predominantly in low- to middle-income countries (Franz et al., 2023; Bazurto *et al*., 2025). In Nigeria, fish contributes significantly to animal protein intake, with aquaculture production experiencing substantial growth, increasing at an annual average rate of 11.2% from 2000 to 2020, while capture fisheries production also grew by an annual average of 2.6% over the same period (Akegbejo-Samsons, 2022; Fakoya *et al*., 2022). Despite being a vital source of high-quality animal protein and income, fish and other aquatic products can serve as vehicles for foodborne infections and intoxications, especially when harvested and processed under unhygienic conditions (Bedane *et al*., 2022; Samarajeewa, 2023). Common foodborne pathogens associated with seafood include *Bacillus cereus, Campylobacter jejuni, Hepatitis A virus, Listeria monocytogenes, Norwalk virus, Salmonella,* and *Vibrio parahaemolyticus*, often linked to inadequate cooking, cross-contamination, or poor hygiene practices (Roy *et al.*, 2022; Salama and Chennaoui, 2024; Akinsemolu and Onyeaka, 2024). Aquatic environments are frequently exposed to various disease-causing organisms, including bacterial pathogens, many of which are zoonotic and can be transmitted to humans through direct contact, ingestion of contaminated water or food, or contact

with infected animals (Jenkins *et al*., 2021; Stec *et al*., 2022). Examples include *Aeromonas spp*., *Salmonella spp*., and *Escherichia coli* (Akinsemolu and Onyeaka, 2024).

Escherichia coli (*E. coli*), a well-studied bacterial species, is commonly found in the intestinal tracts of healthy hosts and endothermic animals (Yu *et al*., 2021). Its presence is traditionally recognized as a crucial indicator of fecal contamination in water and fish, reflecting unhygienic conditions at landing sites, storage facilities, and markets, which pose significant public health risks (Bedane *et al.,* 2022). The World Health Organization (WHO) considers the presence of *E. coli* in water as definitive evidence of recent fecal contamination and potential co-occurrence of other harmful pathogens (Oon *et al*., 2023). The problem is exacerbated in developing countries due to fecal contamination of natural water bodies from various waste discharges, including untreated municipal wastewater and agricultural runoff, particularly in densely populated urban areas (Singh *et al*., 2022).

The management of bacterial infections in aquaculture has led to a considerable reliance on antibiotics, which are used for growth promotion, feed efficiency, prophylaxis, and treatment (Okeke *et al* ., 2022). This extensive use, often unregulated, contributes to the emergence and dissemination of antimicrobial-resistant bacteria, a global health threat. The overuse and misuse of antibiotics in animal agriculture, including aquaculture, lead to the selection and proliferation of resistant bacteria, which can then spread through the food chain and environment (Manyi-Loh, *et al*., 2018; Singh, *et al*., 2024. *E. coli* is considered a candidate vehicle for the transfer of antibiotic resistance genes, accumulating resistance genes primarily through horizontal gene transfer mechanisms such as plasmids, transposons, and integrons, rendering previously effective antibiotics ineffective against its infections (Singh *et* al., 2022). The acquisition of multiple antibiotic resistances among enteric bacterial species presents a serious challenge due to the

potential for resistance transfer to humans via the food chain, leading to therapeutic failures and making treatment difficult (Manyi-Loh, *et al*., 2018; Singh, *et al*., 2024).

Given the increasing pollution of aquatic environments and the potential for antibiotic-resistant pathogens to undermine effective health outcomes and prolong hospitalization, it is critical to document the prevalence and antibiotic susceptibility profiles of *E. coli* in seafood and water sources (Mumbo *et* al., 2023). Aquatic ecosystems are increasingly impacted by anthropogenic activities, including sewage discharges and agricultural runoff, which contribute to the persistence, emergence, and spread of antimicrobial resistance (Singh *et al*.,~~ 2024~~, 2024). Monitoring *E. coli* in these environments serves as an important indicator for both fecal contamination and the presence of antimicrobial resistance (Singh *et al*., 2024). This study aims to ~~determined~~determine the prevalence and antibiotic susceptibility profiles of *E. coli* isolated from Fish, Shrimps, Crab, and water samples in Malokun Sea. This evaluates the collective potential public health risk associated with consuming Malokun Sea aquatic products.

2.0 Methodology

The study utilized samples collected from the Malokun Sea in Awoye, Ilaje community, Ondo State, Nigeria (Longitude 4°54'32" E and Latitude 5°58'34" N). This location is notable as a fishing source and for its cultural significance.

2.1 Sample Collection: Fish samples, including Sole fish (*Cynoglossus*), Africa fish (*Clarias gariepinus*), were collected from fishermen. Shrimps, Crab, and water samples were also collected from the sea. All seafood samples were placed in sterile stomacher bags while water samples were collected in 1 ~~litre~~liter sterile plastic bottles and transported on ice to the microbiology laboratory for immediate analysis. Precautions included not opening sample containers until collection,

avoiding finger contact with container interiors, and ensuring analysis within 24 hours of collection.

2.2 Sample Preparation Isolation and Identification of Presumptive E. coli using Most Probable Number (MPN) Assay

The enumeration of *E. coli* spp. was conducted using the 3-tube by 5-dilution end-point MPN-PCR and MPN-EMB methods as described by Copin *et al*., 2012. Ten grams of homogenized crab, crayfish, and fish samples were each transferred into separate sterile conical flasks containing 90 mL of sterile Tryptic Soy Broth. From each dilution, 1 mL aliquots were inoculated into tubes containing 9 mL of sterile APW in triplicate and incubated at 37 °C for 24 hours.

Water samples were processed similarly, with the initial dilution prepared in double-strength Tryptic Soy Broth. Following incubation, tubes exhibiting turbidity were recorded, and the corresponding MPN values were extrapolated using the FDA-BAM MPN Excel spreadsheet. These values were considered as the estimated bacterial density. DNA was subsequently extracted from 1 mL of each turbid culture and subjected to PCR analysis for confirmatory detection.

2.3 Sample Enrichment for Target Isolation

To enhance the isolation of target *E. coli* spp., an additional 10 g of homogenized seafood (crab, crayfish, and fish) and water samples were inoculated into 90 mL of sterile Tryptic Soy Broth. The enrichment cultures were gently agitated and incubated at 37 °C for 24 hours. Post-incubation, a loopful from the surface pellicle was aseptically streaked onto fresh sterile Eosin Methylene Blue agar plates for selective isolation. For water samples, a volume of 100 ml was also filtered through 0.45 µm pore-size membrane filters, which were then placed on EMB agar plates and incubated at

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37°C overnight. Green metallic sheen colonies, characteristic of presumptive *E. coli*, were carefully picked and purified on fresh sterile EMB agar, followed by nutrient agar plates. Purified isolates were stored in cryovials for further analysis

2.4 Determination of *E. coli* Density Total *E. coli* density in fish, Shrimps, and crab samples was determined using the 3-tube, 5-dilution Most Probable Number (MPN)-PCR and MPN-EMB method. Turbid tubes were recorded, and MPN values were extrapolated using the BAM-MPN excel spreadsheet (US Food and Drug Administration, 2024). For water samples, presumptive *E. coli* colony counts on EMB agar plates were expressed as colony forming units per 100 mL (CFU/100 mL), a standard approach for quantifying bacterial load in water samples (APHA, 2017).

2.5 Morphological and Biochemical Characterization

Bacterial isolates were subjected to Gram staining and standard biochemical tests to determine their morphological and physiological characteristics. Gram staining was performed following the method described by Olutiola (2018). Briefly, thin smears of 18–24-hour-old cultures were heat-fixed, stained with crystal violet, treated with iodine, decolorized using ethanol, and counterstained with safranin. Gram-negative bacteria appeared pink to red under microscopic observation.

Subsequently, biochemical tests were conducted for the identification of *E. coli* isolates. These included the indole, methyl red (MR), Voges-Proskauer (VP), citrate utilization, and oxidase tests.

2.5 Molecular Identification Molecular identification of *E. coli* isolates was confirmed by PCR amplification of the 16S rRNA gene, using specific primers (ECO-1 and ECO-2) as shown in Table 1, that yield a 585 bp amplicon. *E. coli* strain ATCC 25922 served as the positive control (Adefisoye and Okoh, 2016)

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Table 1: Sequence of the *E.coli* primer

Type	Primer Designation	Primers (5 to 3)	Target gene	Amplicon size (bp)
ECO	ECO-1	GACCTCGGTTTAGTTCACAGA	*16SrRNA*	585
	ECO-2	CACACGCTGACGCTGACCA		

2.6 Antibiotic Susceptibility Testing Susceptibility of E. coli isolates to antibiotics was performed using the disc diffusion method (CLSI, 2019). Fresh 18–22-hour old cultures were standardized to 0.5 McFarland standards and spread uniformly on Mueller Hinton agar. Antibiotic discs were placed on the agar surface and incubated at 35 ± 2°C for 18-24 hours. Zones of inhibition were measured and interpreted as susceptible, intermediate, or resistant according to CLSI guidelines (2019).

The antibiotics used in these studies included: Tetracycline (30 µg), Trimethoprim (2.5 µg), Ampicillin (10 µg), Ceftazidime (30 µg), Meropenem (10 µg), Gentamicin (10 µg), Sulfamethoxazole (23.75 µg), Amoxycillin (25 µg), Cephalothin (30 µg), and Ciprofloxacin (5 µg), Kanamycin, Amikacin, Ceftazidime, Norfloxacin, Doxycycline, Penicillin, Streptomycin, and Cefepime.

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2.7 Multiple Antibiotic Resistance (MAR) Indexing The MAR index was calculated for isolates showing resistance to more than two antibiotics, expressed as the ratio of the number of antibiotics an isolate was resistant to ('a') divided by the total number of antibiotics tested against ('b'). A MAR index greater than 0.2 indicates high-risk exposure to antibiotics (Krumperman, 1983).

3.0 Results

3.1 *E. coli* identification and confirmation

A total of 8 samples of Sole fish (*Cynolossus*) and Africa fish (*Clarissa gariepinus*), 30 fresh giant shrimp (Penaeus monodon), 6 Crab *(CALLINECTES SAPIDUS)* and water samples were collected from Malokun sea and densities of *E. coli* were determined by MPN-EMB and MPN-PCR method. The Biochemical results reveal that the isolates were indole-positive, MR-positive, VP-negative, citrate-negative, and oxidase-negative, consistent with the classical biochemical profile of *E. coli*. One hundred and fifty isolates were recovered from all samples. Fifteen (15) presumptive *E. coli* isolates each were randomly selected from Fish, Crab, Shrimps, and 25 from water samples after biochemical analysis and subjected to PCR confirmation. Overall, 54% (37/70) comprising 8, 5, 6 and 18 isolates from Fish, Crab, Shrimps, and water. were confirmed positive as *E. coli*. The gel representative of the molecular confirmation is as shown on Plate 1.

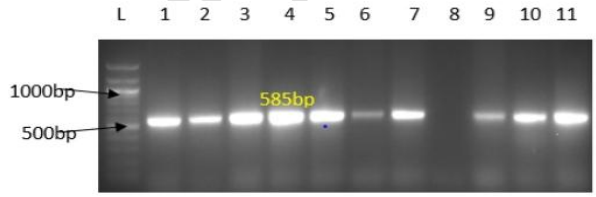

Plate 1: Amplification of 16S rRNA of *Escherichia coli.* L: 100bp ladder; Lane 2: positive control, Lane 8 negative control, Lane 1-7,9-11 confirmed *E. coli strains*

3.2 Density of *E. coli* in selected aquatic samples.

The comparative analysis of *E. coli* densities using MPN/EMB and MPN/PCR methods across water, Shrimps, crab, and fish samples reveals distinct patterns of contamination and detection

sensitivity as shown on table 2. In water samples, PCR yielded a significantly higher mean log CFU/ml (3.00 ± 0.89) compared to the MPN/EMB method (1.20 ± 0.22), indicating that PCR is more sensitive in detecting both viable and non-culturable *E. coli* in aquatic environments. Conversely, in Shrimps, the MPN/EMB method recorded a markedly higher bacterial load (4.46 ± 0.22 log CFU/g) than PCR (2.17 ± 0.22), suggesting that Shrimps harbor a substantial population of viable *E. coli*, with possible PCR underestimation due to DNA extraction challenges or matrix inhibition. Similarly, in fish samples, the MPN method (3.36 ± 0.31 log CFU/g) detected more *E. coli* than PCR (2.35 ± 0.31), supporting the same trend. In crab samples, both methods revealed relatively low *E. coli* levels, with MPN at 1.87 ± 0.30 and PCR at 1.63 ± 0.33, reflecting minimal contamination and closer agreement between methods. Overall, the results suggest that while MPN/EMB is more effective for quantifying viable *E. coli*, particularly in biotic matrices like Shrimps and fish, PCR is more sensitive for detecting a broader range of bacterial states, especially in water. These differences underscore the importance of integrating both culture-based and molecular methods for comprehensive microbial risk assessment in aquatic environments.

Table 2: MPN and PCR assay of *E. coli* from fish, shrimps , water and crab

	MPN/EMB	**MPN/PCR**
Water (log CFU/ml)	1.20±0.22	3.00±0.89
Shrimps (log CFU/g)	4.46 ± 0.22	2.17 ± 0.22
Crab(log CFU/g)	1.87 ± 0.30	1.63 ± 0.33
Fish (log CFU/g)	3.36 ±0.31	2.35±0..31

3.3: The antibiotic susceptibility profile of *E. coli* isolates from seafoods and water samples

The antibiotic susceptibility profile of *E. coli* isolates from crab, water, Shrimps, and fish samples shows significant variations in resistance (R), intermediate (I), and susceptibility (S) across eight

antibiotics tested as detailed in table 3. High levels of resistance were observed against β-lactam antibiotics, with 100% resistance to ceftazidime and penicillin in crab, Shrimps, and fish samples, and over 60% resistance in water samples, indicating the widespread presence of extended-spectrum β-lactamase (ESBL)-producing strains. Notably, doripenem, a carbapenem often reserved as a last-line treatment, exhibited 100% resistance across all sample types, highlighting the potential circulation of carbapenem-resistant *E. coli* in the aquatic environment. In contrast, amikacin and norfloxacin maintained high efficacy, with 100% susceptibility in crab, Shrimps, and fish isolates, and over 90% susceptibility in water samples, suggesting these antibiotics remain potent treatment options. Kanamycin showed high susceptibility in water and fish samples but reduced effectiveness in crustaceans, particularly Shrimps, where 85.3% of isolates showed intermediate susceptibility. Streptomycin and cefepime exhibited variable responses, with moderate resistance observed in crab and Shrimps samples, suggesting emerging resistance trends of multidrug resistance across aquatic sources.

Table 3: Percentage antibiotic susceptibility profile of *E. coli* isolated from Crab, water, Shrimp and Fish

ANTIBIOTIC CODES	**CRAB**			**WATER**			**SHRIMPS**			**FISH**		
	R%	I%	S%	R%	I%	S%	R%	I%	S%	R%	I%	S%
Kanamycin (K)	20	0	80	0	0	100	16.6	85.3	0	0	0	100
Amikacin (AK)	0	0	100	0	0	93	0	0	100	0	0	100
Ceftazidime (CAZ)	100	0	0	62	0	25	100	0	0	100	0	0
Norfloxacin (NOR)	0	0	100	0	0	100	0	16.6	83.3	0	0	100
Doripenem (DOR)	100	0	0	100	0	0	100	0	0	100	0	0
Penicillin (P)	100	0	0	92	2	6	100	0	0	100	0	0
Streptomycin (S)	0	20	80	0	6	93	33	0	66	37.5	0	62.5
Cefepime (FEP)	60	20	20	33	66	0	33	0	66	0	62.5	37.5

3.4 The Multiple Antibiotics Resistance index

As shown on Table 4, *E. coli* isolates exhibited resistance to various combinations of antibiotics, with the most frequent patterns including penicillin (P), doripenem (DOR), cefepime (FEP), and ceftazidime (CAZ). Some isolates also showed resistance to amikacin (AK), kanamycin (K), and streptomycin (S), indicating resistance across multiple antibiotic classes. Among the water isolates, common resistance patterns were P-DOR-FEP, P-DOR-FEP-CAZ, and DOR-FEP-CAZ, while Shrimps isolates frequently exhibited CAZ-DOR-P, CAZ-DOR-P-S, and CAZ-DOR-P-FEP combinations. The calculated MAR index values ranged from 0.375 to 0.625, with most isolates having values ≥ 0.5, which is significantly higher than the 0.2 threshold commonly used to indicate high-risk contamination sources.

Table 4: Multiple antibiotic resistance pattern and index of *E. coli* spp from fish.

Isolate code & frequency	Antibiotic pattern	Multiple Antibiotics Resistance index (MARI)
ISOLATES FROM FISH		
MFE2 (3/8)	**CAZ-P-DOR**	**0.375**
MFE6 (3/8)	**CAZ-P-DOR**	**0.375**
MFE8 (3/8)	**CAZ-P-DOR**	**0.375**
MFE10 (3/8)	**CAZ-P-DOR**	**0.375**
MFE11 (4/8)	**CAZ-S-P-DOR**	**0.5**
MFE12 (3/8)	**CAZ-P-DOR**	**0.375**
MFE13 (4/8)	**CAZ-S-P-DOR**	**0.5**
MFE15 (4/8)	**CAZ-S-P-DOR**	**0.5**

ISOLATES FROM CRAB		
CB 13 (3/8)	**CAZ-DOR-P**	**0.375**
CB 15 (5/8)	**K-CAZ-DOR-P-FEP**	**0.625**
CB 2 (4/8)	**CAZ-DOR-P-FEP**	**0.5**
CB 7 (3/8)	**CAZ-DOR-P**	**0.375**
CB 8 (4/8)	**CAZ-DOR-P-FEP**	**0.5**
ISOLATES FROM WATER		
MW 1(3/8)	**P- DOR- FEP-AMK**	**0.5**
MW 2(4/8)	**P- DOR- FEP- CAZ**	**0.5**
MW 3(3/8)	**P- DOR- FEP**	**0.375**
MW 4(3/8)	**P- DOR- FEP**	**0.375**
MW 5(3/8)	**P- DOR-FEP**	**0.375**
MW 6(3/8)	**DOR- FEP- CAZ**	**0.375**
MW 7(4/8)	**P- DOR- FEP- CAZ**	**0.5**
MW 8(3/8)	**P- DOR- FEP**	**0.375**
MW 9(3/8)	**P- DOR-FEP**	**0.375**
MW 10(3/8)	**P- DOR-FEP**	**0.375**
MW 11(4/8)	**P- DOR- FEP- CAZ**	**0.5**
MW 12(4/8)	**P- DOR- FEP- CAZ**	**0.5**
MW 13(4/8)	**P- DOR- FEP- CAZ**	**0.5**
MW 14(4/8)	**P- DOR- FEP- CAZ**	**0.5**
MW 15(4/8)	**P- DOR- FEP- CAZ**	**0.5**
MW19 (3/8)	**CAZ-DOR-P**	**0.375**

MW 20 (3/8)	**CAZ-DOR-P**	**0.375**
MW 29 (3/8)	**CAZ-DOR-P**	**0.375**
ISOLTATES FROM SHRIMPS		
MCF 1 (3/8)	**CAZ-DOR-P**	**0.375**
MCF 2 (3/8)	**CAZ-DOR-P**	**0.375**
MCF 3 (4/8)	**CAZ-DOR-P-S**	**0.5**
MCF 5 (4/8)	**CAZ-DOR-P-FEP**	**0.5**
MCF 6 (5/8)	**K-CAZ-DOR-P-S**	**0.625**
MCF 7 (4/8)	**CAZ-DOR-P-S**	**0.5**

Penicillin (P), Amokacin (Ak), Norfloxacin (Nor), Kanomycin (K), Dornipenem (Dor), Cefepime (Fep), Streptomycin (S) And Ceftazidime (Caz).

3.5 Common resistance patterns across all seafoods and water samples

Resistance to three antibiotics, CAZ-DOR-P, is the most widespread and recorded across the *E. coli* isolates recovered from seafoods and water samples, making it the most common resistance pattern. Other combinations like CAZ-DOR-P-FEP which shows extended resistance patterns to four antibiotics occur in isolates from three out of four samples i.e Crab, Shrimps and water but are not recorded from fish sample isolates. Resistance patterns involving Streptomycin (S) or Kanamycin (K) were not consistently seen in *E. coli* isolates across all samples as shown in Fig 1.

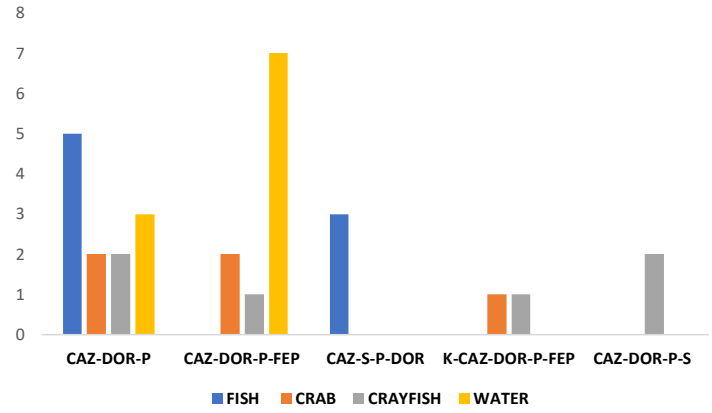

Fig 1: Common Resistance Patterns Across All Four Samples (Fish, Crab, Shrimps, and Water)

Discussion

Escherichia coli is a significant foodborne pathogen globally and a key indicator of fecal contamination (Khan and Gupta, 2020). This study confirms the widespread occurrence and high prevalence of *E. coli* in fish, Shrimps, crab, and water samples from Malokun Sea, Ilaje, Ondo State. The prevalence rate of 53.3% in fish samples suggesting significant contamination of the aquatic environment. The high *E. coli* densities observed in Shrimps (4.46 ± 0.22 Log10 MPN/g) and crab (1.87 ± 0.30 Log10 MPN/g), along with colony counts in water samples, further corroborate the widespread contamination. This high occurrence is likely attributable to the discharge of manure effluents and sewage wastewater into Malokun Sea, enabling *E. coli* to persist in the aquatic environment for extended periods (Korajkic and Gupta, 2019; Chaturvedi, *et al*., 2021).

A critical finding of this study is the alarming antibiotic resistance profiles demonstrated by *E. coli* isolates. Consistent 100% resistance to Penicillin, Ceftazidime, and Doripenem across all fish, Shrimps, crab, and water samples is a major public health concern. This indicates these antibiotics are entirely ineffective against *E. coli* strains prevalent in Malokun Sea. Such widespread resistance suggests significant exposure of these bacterial populations to these antimicrobial agents, likely due to their extensive use in aquaculture and human/animal waste discharged into the environment (Manyi-Loh, *et al*., 2018; Singh, *et al*., 2024). Researchers have highlighted that the excessive and misuse of antimicrobials contributes to increased antimicrobial resistance, posing a severe public health threat (Serwecinska, 2020;, Ahmed *et al*., 2024).

Conversely, the consistent 100% susceptibility to Amikacin and Norfloxacin (with some intermediate resistance for Norfloxacin in certain samples) identifies these as highly effective antibiotics against the isolated *E. coli* strains from Malokun Sea. While Streptomycin showed some susceptibility, its recorded resistance (37.5% in fish and 14.2-33% in other samples) suggests it

may not be a reliable treatment option. Similarly, Cefepime exhibited varying degrees of resistance and intermediate susceptibility, making its efficacy questionable.

The consistently high Multiple Antibiotic Resistance (MAR) indices (ranging from 0.375 to 0.625) across all samples reinforce the severity of the antibiotic resistance problem in Malokun Sea. An MAR index greater than 0.2 is indicative of high-risk exposure to antibiotics and potential contamination from sources where antibiotics are heavily used (Titilawo *et al*., 2015; Al-Badaii and Abdul Halim, 2021). The presence of *E. coli* with multiple antibiotic resistant phenotypes, including co-resistance to four or more unrelated families of antibiotics, is indeed a serious health concern. These multi-drug resistant *E. coli* strains are not limited to cultured fish but are also prevalent in raw food items and food distribution systems, capable of carrying resistance and virulence genes (Sivaraman, *et al*., 2020; Mumbo *et al*., 2023; Ramirez-Castillo *et al*., 2023).

The findings suggest that consuming fish, Shrimps, and crab from Malokun Sea, particularly if raw or undercooked, poses a direct health risk to humans due to potential exposure to antibiotic-resistant *E. coli* and other associated infections (Salama and Chennaoui, 2024; Omeje *et al*., 2024). The presence of *E. coli* in these aquatic products also serves as an indicator for other enteric pathogens that may be present due to fecal contamination (Dissasa *et al*., 2022).

Conclusion

This study highlights the widespread presence of *Escherichia coli,* including multidrug-resistant strains, in seafood and water samples from the Malokun Sea, Ilaje, Ondo State, Nigeria. The complete resistance of all isolates to penicillin, ceftazidime, and doripenem, and elevated Multiple Antibiotic Resistance (MAR) indices, indicates significant exposure to antimicrobial agents. The resistance pattern CAZ-DOR-P consistently found across all sample types, emphasized the

prevalent nature of multidrug resistance in the study area. These findings underscore the urgent need for integrated environmental monitoring programs, strict regulation of antibiotic use, and improved seafood handling practices to mitigate the risks associated with antibiotic-resistant pathogens in the aquatic environment and food chain.

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