



Phenotypic Cefotaxime-Resistant *Escherichia coli* Screening and Detection of Associated Extended-Spectrum β -Lactamase Genes in Online-Marketed Fish from Kochi, India

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Abstract

Online marketplaces are booming, and in cities like Kochi, Kerala, many households rely on them for supplies, including fish. Antimicrobial resistance is a growing trend across the food sector. Therefore, this research investigated the presence of cefotaxime-resistant *Escherichia coli* in fish sold through these online platforms. A total of 100 fish samples obtained from online retailers in Kochi, India, were screened for cefotaxime-resistant *E. coli* using MacConkey agar supplemented with cefotaxime (1 μ g/mL). Confirmed isolates underwent antimicrobial susceptibility testing (17 antibiotics), double-disc synergy testing (DDST), and PCR detection of ESBL-associated genes (*bla*_{CTX-M-1}, *bla*_{CTX-M-2}, *bla*_{CTX-M-9}, *bla*_{TEM}, *bla*_{SHV}, *bla*_{OXA-1-like}). Multiple antibiotic resistance (MAR) index and multidrug resistance (MDR) of the isolates were calculated. Cefotaxime-resistant *E. coli* was detected in 28% of samples, yielding 57 isolates. Among these, 85.9% (49/57) were multidrug resistant (MDR). High resistance was observed to amoxicillin-clavulanic acid (98%), followed by cefpodoxime (84%) and ceftazidime (60%). Phenotypic ESBL production was confirmed in 67% (36/57) of isolates. However, ESBL-associated genes were detected in a smaller proportion: *bla*_{CTX-M-1} (16.6%), *bla*_{TEM} (20.8%), and both genes (4.1%). No isolates carried *bla*_{SHV} or *bla*_{OXA-1-like} genes. The MAR index ranged from 0.059 to 0.824, with 37% of isolates exhibiting MAR > 0.2. While cefotaxime

resistance enabled selection of multidrug-resistant isolates, molecular confirmation demonstrated that only a subset carried ESBL genes, suggesting additional resistance mechanisms were involved. These findings highlight the importance of surveillance of antimicrobial-resistant *E. coli* in emerging food distribution systems such as online seafood markets.

Keywords: *Escherichia coli*, cefotaxime resistance, extended-spectrum β -lactamases (ESBL), antimicrobial resistance, online seafood retail, seafood safety

Introduction

Antibiotics are crucial to modern medicine, essential for treating all types of infections. However, the increasing ability of bacteria to resist these drugs poses a severe threat to public health known as antimicrobial resistance (AMR). This resistance not only diminishes our capacity to fight diseases effectively but also drives up healthcare costs (Salam et al., 2023). This escalating crisis largely contributes to the widespread and often unnecessary use of antibiotics across various sectors, including human healthcare, agriculture, and aquaculture (Okocha, Olatoye, & Adedeji, 2018). An important contributor to this problem is *E. coli*, a common gut bacterium in both humans and animals. *E. coli* has become a significant carrier of genes that confer antibiotic resistance (Ochoa & Gómez-Duarte, 2016). The food supply chain, particularly through animal products, plays a crucial role by allowing these resistant bacteria to spread to consumers (Aarestrup, Wegener, & Collignon, 2008).

Fish and seafood are increasingly recognised as potential carriers of antibiotic-resistant bacteria, including *E. coli* (Kumar, Lekshmi, Parvathi, Nayak,

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& Varela, 2016). Numerous studies from various regions have documented antibiotic-resistant *E. coli* strains in diverse fish species (Ishida et al., 2010; Adzitey, 2015; Silva et al., 2019). This antibiotic resistance in farmed fish is worsened by the widespread use of these agents for prophylactic, metaphylactic, and therapeutic purposes through feed and/or direct application in fish farming (Hossain et al., 2022). When untreated wastewater and agricultural run-off containing these antibiotics are discharged into aquatic environments, they create perfect conditions for resistant bacterial strains to flourish. This proliferation, in turn, makes the fight against AMR even more challenging (Hossain et al., 2022).

A major concern with antibiotic-resistant *E. coli* is the rise of strains that produce ESBLs, which can resist a wide range of β -lactam antibiotics, including third-generation cephalosporins—drugs often used as a last resort for severe bacterial infections (Pitout & Laupland, 2008). The presence of ESBL-producing *E. coli* in our food supply poses a significant public health risk (Sivaraman et al., 2021). Humans can contract these resistant bacteria by consuming or handling contaminated food, potentially leading to infections that are very difficult to treat with standard antibiotics. Specific genes, such as *bla*_{CTX-M}, *bla*_{TEM}, and *bla*_{SHV}, enable *E. coli* to produce ESBL enzymes that hydrolyse third-generation cephalosporins such as cefotaxime (Shaikh, Fatima, Shakil, Rizvi, & Kamal, 2015). Analysing these resistance genes at a molecular level can provide vital insights into the mechanisms driving antibiotic resistance. This knowledge is essential for developing effective strategies to curb the spread of resistant bacteria. Therefore, resistance to cefotaxime is commonly used as a screening criterion for presumptive ESBL-producing isolates. However, cefotaxime resistance may also arise from alternative mechanisms, including AmpC β -lactamase production, porin loss, or efflux pump overexpression.

Hence, the present study aimed to investigate the occurrence of cefotaxime-resistant *E. coli* in fish marketed through online retail platforms in Kochi, India, characterise their AMR profiles, and evaluate the presence of selected ESBL-associated genes. The study does not assume a causal relationship between cefotaxime resistance and ESBL production but examines their association within this selected population.

Materials and Methods

A total of 100 fish samples were collected from 5 online retailers across 11 sampling intervals. Approximately 8–12 samples were collected per sampling schedule. Samples were obtained through convenience sampling from major online retailers delivering within Kochi city. Each sample was processed individually. The sample set included popular marine and freshwater species as well as other locally consumed fish sold through online platforms, as shown in Table 1. The fish were purchased whole, as portioned cuts, or dressed. To maintain anonymity, the samples were delivered to the houses and immediately transported to the laboratory in sterile insulated containers with ice packs and processed within 3 h of receipt at a temperature below 4 °C. All microbiological procedures were performed under aseptic conditions in a biosafety cabinet.

Twenty-five grams of each fish sample was homogenised in 225 mL of lactose broth and incubated for 24 hours at 37 °C for enrichment. After enrichment, a loopful of this culture was streaked onto MacConkey agar plates supplemented with cefotaxime (1 μ g) and incubated for another 24 h at 37 °C (Ahmed & Shimamoto, 2015). The suspected colonies were purified and identified with biochemical tests such as IMViC (indole production, methyl red reaction, Voges-Proskauer test, and citrate utilisation) and Polymerase Chain Reaction (PCR) with the *uidA* gene for the confirmation of *E. coli* (Bej, DiCesare, Haff, & Atlas, 1991; Yu & Thong, 2009) (Table 2). The findings relate specifically to cefotaxime-resistant populations and do not represent overall *E. coli* prevalence in fish. The cefotaxime concentration (1 μ g/mL) was selected based on published protocols for selective screening of presumptive ESBL-producing Enterobacteriaceae (Costa et al., 2004).

The susceptibility of the confirmed *E. coli* isolates to antibiotics was assessed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates, following Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines. A total of 17 antibiotics belonging to different classes were tested, including beta-lactams [amoxicillin-clavulanic acid (20/10 μ g), cefotaxime (30 μ g), ceftazidime (30 μ g), cefpodoxime (10 μ g), aztreonam (30 μ g)], aminoglycosides [gentamicin (10 μ g), tobramycin (10 μ g), amikacin (30 μ g)], quinolones and fluoroquinolones

Table 1. Details of the fishery products sampled from Online retail market

Sampling number	Sample list	Guide to identify the isolate name
1	<i>Penaeus indicus</i> , <i>Etroplus suratensis</i> , <i>Katsuwonus pelamis</i> , <i>Sardinella longiceps</i> , <i>Scomberoides commersonnianus</i> , <i>Epinephelus coioides</i> , <i>Portunus pelagicus</i> , <i>Carangoides malabaricus</i> , <i>Amblygaster sirm</i>	(1/1/1) Sampling number 1/ sample number 1 for prawn/ strain number 1
2	<i>Portunus pelagicus</i> , <i>Arius arius</i> , <i>Katsuwonus pelamis</i> , <i>Aluterus monoceros</i> , <i>Netuma thalassina</i> , <i>Carangoides malabaricus</i> , <i>Istiophorus platypterus</i> , <i>Scoliodon laticaudus</i> , <i>Pastinachus sephen</i> , <i>Carcharhinus limbatus</i>	(2/2/1) Sampling number 2/ sample number 2 for roopchand/ strain number
3	<i>Psettodes erumei</i> , <i>Piaractus brachypomus</i> , <i>Carangoides malabaricus</i> , <i>Coryphaena hippurus</i> , <i>Penaeus indicus</i> , <i>Katsuwonus pelamis</i> , <i>Sardinella longiceps</i> , <i>Penaeus indicus</i> , <i>Leiognathus equulus</i> , <i>Arius arius</i> , <i>Scomberomorus commerson</i>	(3/5/1) Sampling number 3/ sample number 5 for prawn/ strain no
4	<i>Katsuwonus pelamis</i> , <i>Labeo rohita</i> , <i>Piaractus brachypomus</i> , <i>Pangasianodon hypophthalmus</i> , <i>Oreochromis niloticus</i> , <i>Rastrelliger kanagurta</i> , <i>Sardinella longiceps</i> , <i>Parastromateus niger</i> , <i>Penaeus indicus</i> , <i>Macrobrachium rosenbergii</i>	(4/1/1) Sampling number 4, sample no 1, Isolate number – 1
5	<i>Stolephorus indicus</i> , <i>Pangasianodon hypophthalmus</i> , <i>Rastrelliger kanagurta</i> , <i>Labeo rohita</i> , <i>Piaractus brachypomus</i> , <i>Sardinella longiceps</i> , <i>Oreochromis niloticus</i> , <i>Katsuwonus pelamis</i> , <i>Penaeus indicus</i> , <i>Macrobrachium rosenbergii</i> .	(5/1/1) Sampling number 5, sample no 1, Isolate number – 1
6	<i>Katsuwonus pelamis</i> , <i>Uroteuthis duvaucelii</i> , <i>Scomberomorus commerson</i> , <i>Katsuwonus pelamis</i> , <i>Coryphaena hippurus</i> , <i>Lutjanus malabaricus</i> , <i>Penaeus indicus</i> , <i>Uroteuthis duvaucelii</i> , <i>Escualosa thoracata</i> .	(6/1/1) Sampling number 6, sample no 1, Isolate number – 1
7	<i>Comberomorus commerson</i> , <i>Lutjanus malabaricus</i> , <i>Katsuwonus pelamis</i> , <i>Carangoides malabaricus</i> , <i>Coryphaena hippurus</i> , <i>Carangoides malabaricus</i> , <i>Nemipterus japonicus</i> .	(7/1/1) Sampling number 7, sample no 1, Isolate number – 1
8	<i>Mugil cephalus</i> , <i>Stolephorus indicus</i> , <i>Penaeus indicus</i> , <i>Oreochromis niloticus</i> , <i>Pangasianodon hypophthalmus</i> , <i>Gallus gallus domesticus</i> , <i>Sardinella longiceps</i> .	(8/1/1) Sampling number 8, sample no 1, Isolate number – 1
9	<i>Uroteuthis duvaucelii</i> , <i>Penaeus indicus</i> , <i>Meretrix meretrix</i> , <i>Sardinella longiceps</i> , <i>Rastrelliger kanagurta</i> , <i>Mugil cephalus</i> , <i>Leiognathus equulus</i> , <i>Nemipterus japonicus</i> , <i>Oreochromis niloticus</i> , <i>Stolephorus indicus</i> .	(9/1/1) Sampling number 9, sample no 1, Isolate number – 1
10	<i>Rastrelliger kanagurta</i> , <i>Sardinella longiceps</i> , <i>Stolephorus indicus</i> , <i>Penaeus indicus</i> , <i>Johnius dussumieri</i> .	(10/1/1) Sampling number 10, sample no 1, Isolate number – 1
11	<i>Thunnus albacares</i> , <i>Psettodes erumei</i> , <i>Etroplus suratensis</i> , <i>Istiophorus platypterus</i> , <i>Scomberomorus commerson</i> , <i>Lethrinus mahsena</i> , <i>Rastrelliger kanagurta</i> .	(11/1/1) Sampling number 11, sample no 1, Isolate number – 1

[ciprofloxacin (5 µg), levofloxacin (5 µg), gatifloxacin (5 µg), nalidixic acid (30 µg), norfloxacin (10 µg), ofloxacin (5 µg)], folate pathway antagonists (co-trimoxazole) (1.25/23.75 µg), and nitrofurans (nitrofurantoin) (300 µg) were procured from HiMedia Laboratories, Mumbai, India. The double-disc synergy test was performed by placing ceftazidime (30 µg) and ceftazidime-clavulanic acid (30/10 µg) discs 20 mm apart (centre-to-centre) (Fig. 1). An increase in zone diameter ≥ 5 mm in the presence of clavulanic acid was interpreted as phenotypic ESBL-positive according to CLSI (2020) guidelines. *E. coli* ATCC 25922 was used as an ESBL-negative control, and *Klebsiella pneumoniae* ATCC 700603 was used as an ESBL-positive control strains.

PCR was used to confirm the genetic basis of cefotaxime resistance in *E. coli* by identifying the specific ESBL genes responsible for the resistance. *bla*_{CTX-M} (specifically groups 1, 2, and 9), *bla*_{TEM}, *bla*_{SHV}, and *bla*_{OXA-1-like} genes were tested (Pitout et al., 1998; Woodford, Fagan, & Ellington, 2006). PCR amplification of *bla*_{CTX-M} (groups 1, 2, 9), *bla*_{TEM}, *bla*_{SHV}, *bla*_{OXA-1-like} and *uidA* genes was performed using published primers (Dallenne, Da Costa, Decré, Favier, & Arlet, 2010; ICMR, 2019). PCR reaction mixture used was 25 µL. Each PCR reaction contained: 12.5 µL 2× PCR Master Mix (containing Taq DNA polymerase, 1.5 mM MgCl₂, 200 µM dNTPs), 0.5 µL forward primer (10 pmol/µL), 0.5 µL reverse primer (10 pmol/µL), 2 µL template DNA (~50–100 ng), 9.5 µL nuclease-free water. Each run included a negative control (nuclease-free water) and positive control strains. The primer list and reaction conditions are given in Table 2. All reactions were performed in single-plex format. Multidrug resistance (MDR) was defined as acquired non-susceptibility to at least one antimicrobial agent in three or more antimicrobial classes according to the international consensus definition proposed by Magiorakos et al. (2012). Isolates were categorised as MDR based on their resistance profiles across the antimicrobial classes tested. The MAR index was calculated as the ratio of the number of antibiotics to which an isolate was resistant to the total number of antibiotics tested (Krumperman, 1983).

Results and Discussion

In this study, 100 online-marketed fish samples from Kochi city, Kerala, were screened for cefotaxime-resistant *E. coli*. Cefotaxime-resistant *E. coli* was

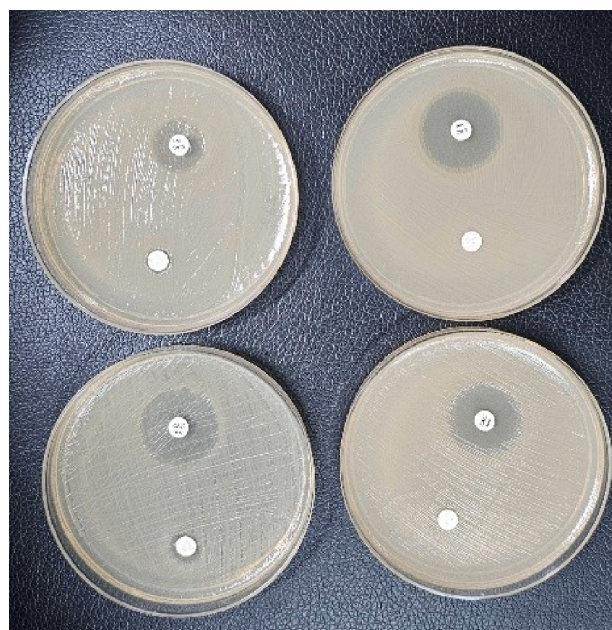


Fig. 1. Double-disc synergy test (DDST) for phenotypic detection of extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli*

found in 28% of the samples, yielding a total of 57 isolates. The occurrence of *E. coli* varied across different fish species and sample types. Specifically, dolphin fish/mahi-mahi had the highest number of cefotaxime-resistant isolates (9), followed by tuna (5), queen fish and mullet (4 isolates each) collected from different retailers. These findings are consistent with previous studies that documented the presence of *E. coli* in various fish species (Prakasan et al., 2022). Research in Korea, Switzerland, Egypt, and Kenya confirms the presence of ESBL-producing *E. coli* in fish and seafood (Ryu et al., 2012; Boss, Overesch, & Baumgartner, 2016; Hussein et al., 2019; Mumbo et al., 2023). In Egypt, ESBL-producing *E. coli* was detected in *Oreochromis niloticus* (32%), *Mugil cephalus* (24%), and sardines (16%) (Saad et al., 2018; Sudaryatma et al., 2025). In Vietnam, screening of chicken meat, pork, beef, and fish/shrimp samples in Ho Chi Minh City demonstrated that animal-based food products represent a major reservoir of ESBL/pAmpC-producing *E. coli* (Nguyen et al., 2016).

The occurrence of cefotaxime-resistant *E. coli* in seafood has been increasingly reported worldwide, highlighting the global dissemination of antimicrobial-resistant bacteria through the aquatic food chain. Resistant *E. coli* has previously been reported in 23.7% of fish samples from China (Jiang et al.,

Table 2. Details of primers used in the study

Sl. No	Name of primer	Primer sequence (5' - 3')	PCR conditions	Product size (bp)	Reference
1	<i>bla</i> _{CTX-M} gp1	TTAGGAARTGTGCCGCTGYA CGATATCGTTGGTGGTRCCAT	Initial denaturation: 95 °C for 5 min	688	Dallenne et al. (2010)
2	<i>bla</i> _{CTX-M} gp2	CGTTAACGGCACGATGAC CGATATCGTTGGTGGTRCCAT	35 cycles of: Denaturation: 95 °C for 30 sec	404	
3	<i>bla</i> _{CTX-M} gp9	TCAAGCCTGCCGATCTGGT TGATTCTCGCCGCTGAAG	Annealing: 60 °C for 30 sec Extension: 72 °C for 60 sec Final extension: 72 °C for 7 min	561	
4	<i>bla</i> _{TEM}	CATTTCCGTGTCGCCCTTATTC CGTTCATCCATAGTTGCCTGAC	Initial denaturation: 95 °C for 5 min	800	Dallenne et al. (2010)
5	<i>bla</i> _{SHV}	AGCCGCTTGAGCAAATTAAAC ATCCCGCAGATAAATCACCAC	35 cycles of: Denaturation: 95 °C for 30 sec	713	
6	<i>bla</i> _{OXA-1}	GGCACCAGATTCAACTTTCAAG GACCCCAAGTTTCCTGTAAGTG	Annealing: 58 °C for 30 sec Extension: 72 °C for 60 sec Final extension: 72 °C for 7 min Hold: 4 °C	564	
7	<i>uidA</i>	CGC CGA TGC AGA TAT TCG GCT GTG ACG CAC AGT TCA TAG	Initial denaturation: 95 °C for 5 min 35 cycles of: Denaturation: 95 °C for 30 sec Annealing: 57 °C for 30 sec Extension: 72 °C for 45 sec Final extension: 72 °C for 7 min	603	ICMR (2019)

2012), and a higher prevalence in commercially important fish species, viz., tuna (87%), pelagic fish (93%), and sardines (70%). Furthermore, frozen shrimp samples have been reported to harbour *E. coli* in 75% of samples (Saif, Mahmoud, Talat, & Ibrahim, 2017). A comparable prevalence of 29.34% in raw fish (Gupta, Ghatak, & Gill, 2013) and a higher prevalence of 90% contamination in fish marketed in Dhaka, Bangladesh, have also been

reported (Akter, Abedin, Mosharaf, Islam, & Hassan, 2022). Variations in prevalence among studies may be attributed to differences in fish species, sampling locations, hygienic handling practices, environmental contamination, aquaculture management, and the selective pressure imposed by antimicrobial use in aquatic environments. Nevertheless, the consistent detection of resistant *E. coli* across geographically diverse regions underscores the need for

continuous surveillance and implementation of prudent antimicrobial stewardship throughout the seafood production and distribution chain.

All isolates were obtained following enrichment with cefotaxime; approximately 82-91% of isolates phenotypically showed ESBL production (Prakasan et al., 2022), indicating that cefotaxime resistance can be a good indicator of ESBL production. Resistance to third-generation cephalosporins was noted in 76–89% of isolates, whereas some isolates showed ESBL phenotypes and were resistant to all tested beta-lactams. Imported freshwater fish showed a 27.2% prevalence of putative ESBL-producing *E. coli* with beta-lactam resistance (Pearce, Conrady, & Guardabassi, 2023). *E. coli* isolates from frozen shrimp showed high resistance to Augmentin (99.4%) and ceftazidime (92.7%), whereas mullet fish isolates were resistant to cefotaxime (81%). This signifies the clinical importance (Alhabib & Elhadi, 2024).

The multidrug-resistant pattern of the cefotaxime-resistant *E. coli* is also alarming in the online seafood samples tested. Eighty-seven percent of isolates qualified as multidrug-resistant, i.e., resistant to ≥ 3 antibiotic classes. The most concerning isolate coded as (7/7/4) showed resistance to 14 of the 17 antibiotics tested. The isolate was obtained from a

threadfin bream species sample collected in June (monsoon). The Multiple Antibiotic Resistance (MAR) index ranged from 0.059 to 0.824, indicating varying degrees of resistance. The isolates with a MAR index >0.20 are considered to originate from high-risk sources of antibiotic contamination (Krumperman, 1983). The percentage of isolates with the MAR index >0.2 is 37.29%. The isolates with a higher MAR index are 5/4/1, 10/9/1, and 10/10/1, isolated from Rohu, Pomfret, and Seer fish samples, respectively. The MAR index suggests exposure to antibiotics in the supply channel or washing in coastal waters with high AMR (Yousuf et al., 2025). Online-marketed samples, with a higher level of AMR among the 57 *E. coli* isolates resistant to more than 3 classes of antibiotics and ESBL production, reflect concern about rising AMR in the food supply chain, particularly in online-sold fish.

Resistance of *E. coli* to ceftazidime, cefpodoxime, ceftriaxone, aztreonam, monobactam, and amoxicillin-clavulanic acid was 60%, 84%, 25%, 52%, 25%, and 98%, respectively. Based on resistance to multiple beta-lactam antibiotics, approximately 45–50 isolates (82–91%) show patterns consistent with ESBL production. Resistance to other antibiotics, such as ciprofloxacin, levofloxacin, and norfloxacin, was found in 58%, 44%, and 9% of the isolates.

Low-level resistance rates were observed for aminoglycosides, including gentamicin, tobramycin, and amikacin (11–22%), compared to other antibiotic classes tested in this study. Previous studies have reported variable susceptibility patterns for aminoglycosides and quinolones in *E. coli* isolated from fish and seafood. Yohans, Mitiku, and Tassew (2022) reported complete susceptibility of isolates to ciprofloxacin and gentamicin, while Saif et al. (2017) observed high sensitivity to amikacin (100%) and ciprofloxacin (97.30%) in mullet-derived isolates. In contrast, Celik et al. (2023) documented resistance rates of 32.35% to aminoglycosides and 29.41% to quinolones in isolates from shrimps and mussels. Similarly, Krahulcová et al. (2023) reported gentamicin resistance in 13% of coliform isolates from fish and seafood. Firdous et al. (2025) reported the highest ceftazidime resistance (95.3%) in seafood samples from Bangladesh. These differences likely reflect variations in geographic location, antimicrobial usage practices, sampling strategies, and methodological approaches. The observed variability highlights the importance of continuous regional surveillance to monitor evolving resistance patterns

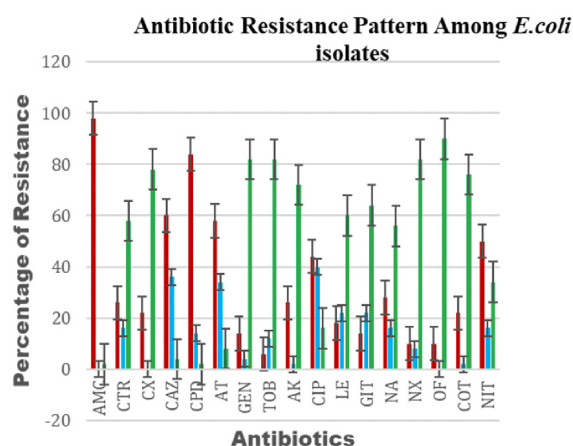


Fig. 2. Antibiotic resistance pattern among *E. coli* isolates (n=57)

AMC- Amoxicillin-Clavulanic Acid; CTR -Ceftriaxone; CTX - Cefotaxime; CAZ - Ceftazidime; CPD - Cefpodoxime; AT - Aztreonam; GEN - Gentamicin; TOB - Tobramycin; AK - Amikacin; CIP - Ciprofloxacin; LE - Levofloxacin; GAT - Gatifloxacin; NA - Nalidixic Acid; NX - Norfloxacin; OF - Ofloxacin; COT - Co-trimoxazole (Trimethoprim-Sulfamethoxazole); NIT - Nitrofurantoin

in seafood-associated bacteria. These findings strongly recommend AMR surveillance in online retail systems and should not be interpreted as clinical treatment recommendations.

To understand the mechanisms behind the observed cefotaxime resistance, molecular characterisation of ESBL-encoding genes in cefotaxime-resistant *E. coli* isolates was carried out. PCR analysis revealed that 16.6% (8/48) of the isolates carried the *bla*_{CTX-M} group 1 gene, a major driver of ESBL production in *E. coli*. Interestingly, no isolates carried *bla*_{CTX-M} group 2 or group 9 genes. Additionally, 20.8% (10/48) of the isolates were positive for the *bla*_{TEM} gene, another common contributor to ESBL production in this bacterium. Two isolates (4.1%) were particularly noteworthy as they harboured both the *bla*_{CTX-M} group 1 and *bla*_{TEM} genes, suggesting a potential for enhanced resistance to beta-lactam antibiotics. Notably, none of the isolates tested positive for the *bla*_{SHV} or *bla*_{OXA-1-like} genes, which are also known to confer ESBL phenotypes in *E. coli*. While ESBL production is a common cause, cefotaxime resistance in *E. coli* can also stem from the hyperproduction of chromosomal AmpC beta-

lactamase (Pitout et al., 1998). The detection of ESBL-encoding genes, particularly *bla*_{CTX-M} and *bla*_{TEM} in *E. coli* isolates from online-sold retail fish sheds light on the specific resistance mechanisms these bacteria use. The spread of ESBL-producing *E. coli* through the food chain represents a serious public health threat, as these resistant strains could lead to increased chances of infections in humans through food (Overdevest et al., 2011).

The double-disk synergy test, using ceftazidime alone and with clavulanic acid, showed that 67% of isolates (36 out of 57) had a zone size difference greater than 5 mm. This finding indicates the known link between cefotaxime resistance in *E. coli* and ESBL production. ESBLs are enzymes that break down and inactivate many beta-lactam antibiotics, including third-generation cephalosporins like cefotaxime. Therefore, resistance to cefotaxime is frequently used as a key indicator for screening ESBL-producing *E. coli*, as these enzymes are particularly effective against such antibiotics. Although 67% of isolates were phenotypically ESBL-positive, only a subset carried the screened ESBL-associated genes. This discordance may be attributed to untested ESBL variants, AmpC β -lactamase production, or other resistance mechanisms. Therefore, cefotaxime resistance alone cannot confirm ESBL production.

Although fewer strains carried the screened ESBL genes, 87% of the isolates were multidrug resistant (MDR) in this study. This indicates that resistance in these isolates extends well beyond β -lactam antibiotics. The high MDR frequency suggests that selection for cefotaxime resistance may have contributed to the co-selection and accumulation of resistance determinants on mobile genetic elements such as plasmids, integrons, and transposons, which frequently carry genes conferring resistance to several unrelated antimicrobial classes. Consequently, isolates lacking the screened ESBL genes may still exhibit extensive resistance due to other β -lactamases and additional non- β -lactam resistance mechanisms. Therefore, MDR represents a broader antimicrobial resistance phenotype, whereas ESBL production reflects only a specific mechanism of β -lactam resistance. The combination of a high prevalence of cefotaxime-resistant isolates (28%), substantial phenotypic ESBL positivity (67%), and an even higher MDR rate (87%) highlights the complexity of antimicrobial resistance in *E. coli* from online seafood. These findings emphasise that

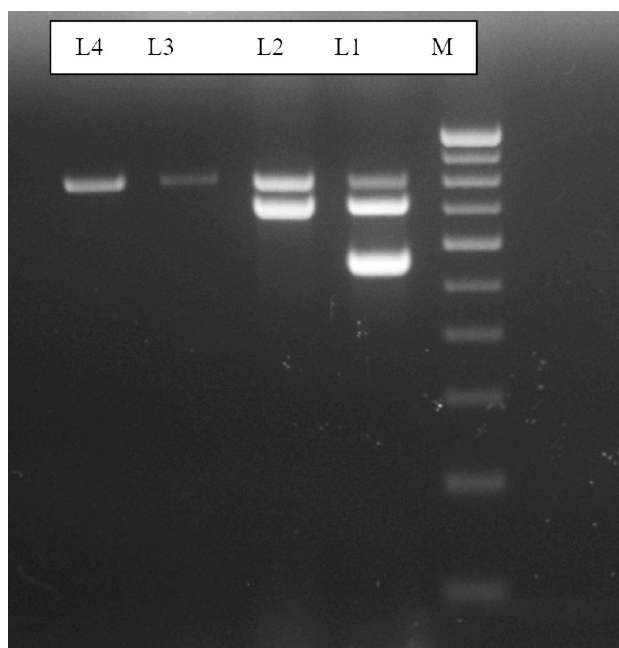


Fig. 3. PCR detection of ESBL-associated genes in cefotaxime-resistant *Escherichia coli* isolates from online-marketed fish in Kochi, India

Lane M: 100 bp DNA ladder; Lane PC: Positive control; Lane S1: Test isolate 1; Lane S2: Test isolate 2; Lane S3: Test isolate 3. Amplicons: 800 bp for *bla*_{TEM}, 713 bp for *bla*_{SHV} and 564 bp for *bla*_{OXA-1-like}

phenotypic screening should be complemented with comprehensive molecular characterisation, including additional ESBL, AmpC, carbapenemase, and whole-genome sequencing approaches, to determine the resistance determinants circulating in the seafood supply chain.

This study documents the occurrence of cefotaxime-resistant *E. coli* in fish (28%) marketed through online retail platforms in Kochi, India. While phenotypic ESBL production was observed in some isolates, molecular screening confirmed ESBL-associated genes in only a subset, suggesting that additional resistance mechanisms may contribute to cefotaxime resistance. The findings apply specifically to cefotaxime-resistant populations rather than overall *E. coli* prevalence. The presence of multidrug-resistant isolates within this food distribution pathway highlights the need for continued AMR surveillance in emerging retail systems.

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