Prakasan et al | Article 10694
J Pure Appl Microbiol. 2025;19(4):2931-2943. doi: 10.22207/JPAM.19.4.32
Received: 17 June 2025 | Accepted: 11 October 2025
Published Online: 27 November 2025

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Multidrug-resistance in Diverse *Escherichia coli* Pathogroups Isolated from Fish and Shellfish

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Abstract

Escherichia coli **are serious pathogens of concern responsible for intestinal and extraintestinal disorders. The presence of antibiotic-resistant pathogenic *E. coli* in seafood is a growing concern for food safety. This study investigated the antibiotic resistance profile of *E. coli* (n = 33) representing different pathogroups isolated from seafood. Pathogenic *E. coli* isolates from fresh seafood samples collected in Western and Southern Mumbai, India, were used for antibiotic susceptibility testing. The Kirby-Bauer disc diffusion method was used for analysing the susceptibility patterns, and the results were interpreted according to the CLSI (Clinical & Laboratory Standards Institute) guidelines. The multiple antibiotic resistance (MAR) index was determined to understand the level of antibiotic resistance. The highest resistance was observed against the third-generation cephalosporins cefotaxime (97%) and cefpodoxime (87.8%), while the least resistance was against chloramphenicol (12.1%) and Co-trimoxazole (18.2%). More than 50% of the isolates were resistant to third-generation cephalosporins, nalidixic acid, ciprofloxacin, aminoglycosides such as gentamicin and amikacin, imipenem, meropenem, piperacillin-tazobactam, Amoxycillin-clavulanic acid, and colistin. The highest (0.95) and the lowest (0.09) MAR indices were recorded for isolates belonging to enterohaemorrhagic *E. coli* (EHEC) and enteroaggregative *E. coli* (EAEC) pathogroups, respectively. The high resistance to multiple drugs in various pathogroups of *E. coli* from seafood emphasizes the need to trace and contain the sources of resistant bacteria to ensure the safety of seafood for consumption and prevent dissemination of such strains in the seafood consumer community.**

Keywords: *Escherichia coli*, Antibiotic Resistance, Seafood, MDR, Pathogroup, Safety

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Citation: Prakasan S, Stephen J, Lekshmi M, Nayak BB, Kumar SH. Multidrug-resistance in Diverse *Escherichia coli* Pathogroups Isolated from Fish and Shellfish. *J Pure Appl Microbiol.* 2025;19(4):2931-2943. doi: 10.22207/JPAM.19.4.32

INTRODUCTION

The growth of antimicrobial drug resistance in bacteria is a one-health issue that poses significant health challenges to the public, impacts food security, and undermines sustainable development globally.[1] The surge in the occurrence of resistant superbugs has become a global concern and has threatened the future of antimicrobial therapy.[2] Recurrent misuse and overuse of antibacterial agents like antibiotics in humans and animal health has a direct influence on the emergence of drug resistance in bacterial pathogens of human health significance.[3] Humans can acquire resistant enteric pathogens through various sources, such as contaminated food and water. The coastal-marine environment is readily prone to faecal contamination from human and animal wastes introduced through land runoff, sewage discharge, and various other anthropogenic activities.[4] Consequently, fish and shellfish harvested from faecally contaminated waters harbour enteric pathogens. Among others, *E. coli* is an important bacterium from a human health perspective, found associated with fish and shellfish exposed to faecal contamination.[5] Although *E. coli* strains are well-known common commensals residing in the digestive tract of humans and the endotherms, distinct clonal types have acquired virulence traits, making them highly pathogenic, capable of triggering various intestinal and extraintestinal infections.

The traditional indicator status of *E. coli* changed with the identification of pathogroups that can cause diverse infections across all age groups. *E. coli* indicates the existence of other enteric bacteria, viruses, and parasites, which are introduced via faecal contamination, and is also a pathogen itself capable of causing diverse infections. Based on the serovar distribution, presence of virulence genes, and the interactions with the cultured cells, pathogenic *E. coli* are broadly classified into five pathogroups, namely enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic (Shiga toxin-producing) *E. coli* (EHEC/STEC), enteroaggregative *E. coli* (EAEC), and enteroinvasive *E. coli* (EIEC).[6]

Resistance to antibiotics is increasingly being reported in food-associated *E. coli*. The imprudent use of antibiotics in healthcare services and agriculture is one of the key determinants contributing to the rising resistance against antibiotics in pathogenic *E. coli*. Food as a vehicle for resistant pathogens can have serious implications for the health of the consumer community, as well as the dissemination and evolution of resistant clones.[7]

Anthropogenic contamination of coastal-marine waters contributes to the incidence of enteric bacterial and viral pathogens. The level of faecal contamination, the incidence of *E. coli*, and their different pathogroups in fresh and processed seafood have been reported from India.[8,9] However, the problem is more confounding when multidrug-resistant strains are encountered in seafood, like the extended-spectrum β-lactamase (ESBL) or the carbapenemase-producing strains.[10,11] The incidence of bla_{NDM}-harboring *E. coli* in wild-caught seafood from India has emphasized the need to focus on the consequences for public well-being due to seafood-originated antibiotic-resistant bacteria. The ability of *E. coli* to persist continuously in seawater over an extended period can contribute to its wider dissemination and exposure to horizontal gene transfer events, leading to the acquisition of Antimicrobial resistance (AMR) genes from the environment. *E. coli* contamination of seafood is a significant challenge for food safety in developing economies with strained sanitation infrastructure, owing to the large population, particularly in urban areas.[12] Recently, we reported the isolation of *E. coli* belonging to all pathogroups (EHEC/STEC, EPEC, ETEC, EAEC, and EIEC) from fresh finfish and shellfish samples marketed in Mumbai, Maharashtra, India.[9] In this study, we investigated the pattern of resistance of pathogenic *E. coli* isolates from seafood representing distinct pathogroups towards important antibiotics. This will further help us understand the implications of such bacteria on consumer health.

MATERIALS AND METHODS

Isolates of *Escherichia coli*

Confirmed isolates of *E. coli* (n = 33) used in this study were previously recovered from fresh seafood samples collected from fish landing centres, retail fish markets, and a retail supermarket, all located in Mumbai, India

Table 1. *Escherichia coli* isolates used in this study, their pathogroup affiliations, serogroups and the source of isolation

No.	Isolate	Pathogroup	Serogroup	Source
1	PSE64	EHEC	O120	*Parapenaeopsis stylifera*
2	PMH55	EHEC	O157	*Penaeus monodon*
3	MPH7	EHEC	026	*Polydactylus heptadactylus*
4	LSHM6	EHEC	083	*Harpadon nehereus*
5	4SBD12	EAEC	O18	*Harpadon nehereus*
6	TIS71	EHEC	O120	*Parapenaeopsis stylifera*
7	TIE83	ETEC	07	*Parapenaeopsis stylifera*
8	CRS10	EIEC	07	*Metapenaeus affinis*
9	HNE10	EHEC	083	*Harpadon nehereus*
10	NET87	EHEC	O149	*Odontamblyopus roseus*
11	4MSH40	EHEC	O134	*Parapenaeopsis stylifera*
12	DHS39	ETEC	07	*Johnius macrorhynus*
13	TMOT1	EHEC	O20	*Opisthopterus tardoore*
14	TEC19	ETEC	O83	*Meretrix casta*
15	TMSA2	EHEC	O157	*Opisthopterus tardoore*
16	SC2	EHEC	083	*Harpadon nehereus*
17	PSM65	EHEC	O120	*Parapenaeopsis stylifera*
18	BDS6	ETEC	O7	*Harpadon nehereus*
19	BD651	EHEC	O83	*Harpadon nehereus*
20	TMA7	ETEC	O134	*Fenneropenaeus indicus*
21	MLV17	EHEC	O135	*Metapenaeopsis stridulans*
22	LSBD21	EHEC	O120	*Harpadon nehereus*
23	MAM8	EHEC	O135	*Megalaspis cordyla*
24	BDE6	EHEC	O120	*Harpadon nehereus*
25	4MSH38	EHEC	O135	*Parapenaeopsis stylifera*
26	MLV18	EHEC	O134	*Metapenaeopsis stridulans*
27	4SSH61	EHEC	O135	*Parapenaeopsis stylifera*
28	TIM651	EPEC	O120	*Parapenaeopsis stylifera*
29	TSOT13	EHEC	O134	*Opisthopterus tardoore*
30	TECL3	EHEC	O7	*Meretrix casta*
31	LEBD13	EAEC	O134	*Harpadon nehereus*
32	PMM31	EHEC	O157	*Penaeus monodon*
33	PMH14	EPEC	O135	*Penaeus monodon*

(Table 1).[9] Among 33 *E. coli* isolates, 16 were isolated from finfish and 17 were from shellfish (Table 1). Of these, 23 isolates belonged to EHEC/STEC, five to ETEC, two to each of EPEC and EAEC, and one to EIEC. The EHEC isolates consisted of serotypes O120, O157, O26, O83, O149, O134, O20, O135 and O7. ETEC isolates belonged to O7, O83, and O134; EPEC isolates to O120 and O135; EAEC to O18 and O134; and EIEC to O7. The isolates were stored in glycerol broth at -80 °C till further analysis.

Antibiotic susceptibility testing

The susceptibility of *E. coli* isolates to 21 antibiotics was studied using the disc diffusion method. The following antibiotics were tested; Cefotaxime (CTX; 30 µg), Ceftazidime (CAZ; 30 µg), Cefoxitin (CX; 30 µg), Cefpodoxime (CPD; 10 µg), Ceftriaxone (CTR; 30 µg), Cephalothin (CEP; 30 µg), Chloramphenicol (C; 30 µg), Ciprofloxacin (CIP; 5 µg), Co-Trimoxazole (COT; 25 µg), Gentamicin (GEN; 10 µg), Imipenem (IPM; 10 µg), Meropenem (MRP; 10 µg), Nalidixic acid (NA; 30 µg), Ertapenem (ETP; 10 µg), Piperacillin/Tazobactam (PIT; 100/10 µg), Aztreonam (AT; 30 µg), Amoxycillin-clavulanic acid (AMC; 30 µg), Colistin (CL; 10 µg), Amikacin (AK; 30 µg), Tetracycline (TE; 30 µg) and Trimethoprim (TR; 5 µg).

The Kirby-Bauer method was used to determine the antibiotic sensitivity of *E. coli*.

Table 2. Antibiotic susceptibility patterns of *E. coli* isolates

Antibiotics used	No. (%) resistant	No. (%) intermediate resistant	No. (%) sensitive
Cefotaxime (CTX)	32 (97)	1 (3)	0
Ceftazidime (CAZ)	26 (78.8)	4 (12.1)	3 (9.1)
Cefoxitin (CX)	19 (57.6)	7 (21.2)	7 (21.2)
Cefpodoxime (CPD)	29 (87.8)	2 (6.1)	2 (6.1)
Ceftriaxone (CTR)	22 (66.7)	3 (9.1)	8 (24.2)
Cephalothin (CEP)	21 (63.6)	8 (24.2)	4 (12.1)
Chloramphenicol (C)	4 (12.1)	8 (24.2)	21 (63.6)
Ciprofloxacin (CIP)	22 (66.7)	4 (12.1)	7 (21.2)
Co-Trimoxazole (COT)	6 (18.2)	17 (51.5)	10 (30.3)
Gentamicin (GEN)	26 (78.8)	3 (9.1)	4 (12.1)
Imipenem (IPM)	22 (66.7)	7 (21.2)	4 (12.1)
Meropenem (MRP)	25 (75.8)	3 (9.1)	5 (15.1)
Nalidixic Acid (NA)	24 (72.7)	5 (15.2)	4 (12.1)
Ertapenem (ETP)	16 (48.5)	10 (30.3)	7 (21.2)
Piperacillin/Tazobactam (PIT)	28 (84.8)	3 (9.1)	2 (6.1)
Aztreonam (AT)	28 (84.9)	1 (3.0)	4 (12.1)
Amoxycillin-clavulanate (AMC)	19 (57.6)	10 (30.3)	4 (12.1)
Colistin (CL)	31 (93.9)	2 (6.1)	0
Amikacin (AK)	29 (87.9)	3 (9.1)	1 (3.0)
Tetracycline (TE)	10 (30.3)	5 (15.2)	18 (54.5)
Trimethoprim (TR)	9 (27.3)	15 (45.4)	9 (27.3)

Bacteria were grown in Mueller-Hinton (MH) medium (Hi-Media, Mumbai, India) to 0.5 McFarland turbidity unit. The broth culture was inoculated onto a Mueller-Hinton agar plate by spreading it uniformly on the agar with a sterile swab. After drying the plates for 5 minutes, the antibiotic discs were placed on the agar surface using sterile forceps. After incubation at 37 °C for 18 hours, the diameter of the zones of inhibition was measured. Interpretations as susceptible, intermediate and resistant were made as per the guidelines of Clinical and Laboratory Standards Institute (CLSI).[13]

Multiple Antibiotic Resistance (MAR) index

The level of resistance against antibiotics was calculated employing the formula,

MAR index = a/b,

where

a is the number of antibiotics to which the bacterium is resistant, and b is the total number of antibiotics tested.[14]

RESULTS

Susceptibility patterns of isolates of pathogenic *E. coli* against antibiotics

Table 1 presents the details of *E. coli* isolates used in this study, including their pathogroup affiliations, serogroups, and the source of isolation. The majority of the isolates screened belonged to the EHEC pathogroup, followed by ETEC, EPEC, EAEC, and EIEC. The serogroup O120 was the most prevalent serogroup among EHEC isolates, and O7 was the most prevalent among ETEC isolates. The susceptibility patterns of pathogenic *E. coli* isolates against selected antibiotics are shown in Table 2 and Figure 1. Third-generation cephalosporin resistance was found to be common in the tested isolates, with 32 out of 33 (97%) isolates being resistant to one or more cephalosporins. The highest resistance was against cefotaxime (97%), followed by cefpodoxime (87.8%), ceftazidime (78.8%), ceftriaxone (66.7%), cephalothin (63.6%), and cefoxitin (57.6%).

Further, 28 (84.9%) isolates were resistant to aztreonam, 25 (75.8%) to meropenem, 22 (66.7%) to imipenem, and 16 (48.5%) were resistant to ertapenem. A high level of resistance was noted against quinolone antibiotics, with 24 (72.7%) isolates being resistant to nalidixic acid and 22 (66.7%) isolates being resistant to ciprofloxacin. The aminoglycoside resistance was also significant. Twenty-nine (87.9%) and 26 (78.8%) isolates were resistant to amikacin and gentamicin, respectively. Thirty-one (93.9%) isolates were resistant to colistin.

Among other antibiotics, 28 (84.8%) exhibited resistance to piperacillin/tazobactam, and 19 (57.6%) were resistant to the amoxycillin-clavulanate antibiotic-inhibitor combination. The isolates were relatively more susceptible to co-trimoxazole, tetracycline, trimethoprim, and chloramphenicol antibiotics, with 6 (18.2%), 10 (30.3%), 9 (27.3%), and 4 (12.1%) isolates,

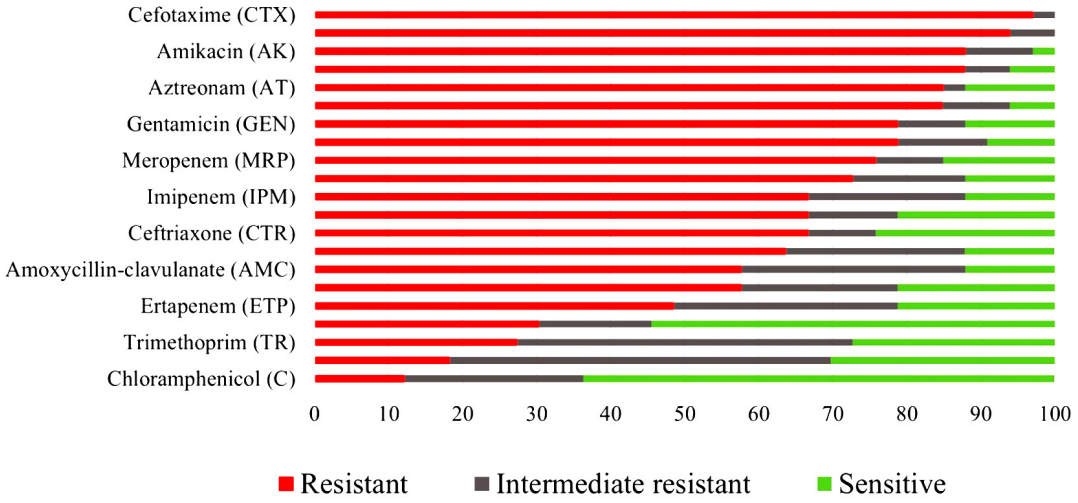

Figure 1. Antibiotic susceptibility patterns of pathogenic *E. coli*

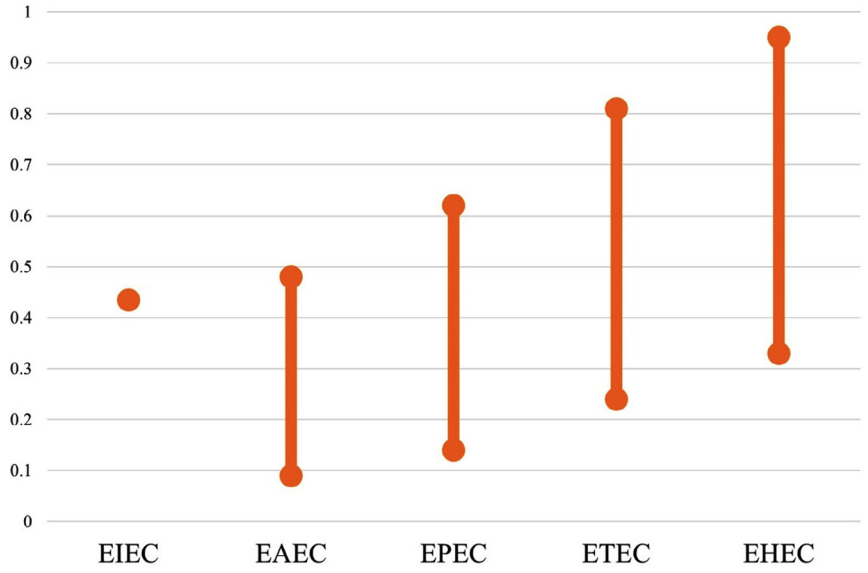

Figure 2. The multiple antibiotic resistance (MAR) index ranges of *E. coli* patho groups

Table 3. Multiple antibiotic resistance (MAR) indices of the isolates

Isolate	Patho-group	Sero-group	Number of antibiotics to which resistant	MAR index
PSE64	EHEC	O120	20	0.95
TSOT13	EHEC	O134	20	0.95
TIS71	EHEC	O120	19	0.9
MLV17	EHEC	O135	19	0.9
LSBD21	EHEC	O120	19	0.9
BDE6	EHEC	O120	19	0.9
PMH55	EHEC	O157	18	0.86
4MSH38	EHEC	O135	18	0.86
TIE83	ETEC	O7	17	0.81
4MSH40	EHEC	O134	17	0.81
TMSA2	EHEC	O157	17	0.81
TECL3	EHEC	O7	17	0.81
MPH7	EHEC	O26	16	0.76
SC2	EHEC	O83	16	0.76
PSM65	EHEC	O120	16	0.76
TMA7	ETEC	O134	15	0.71
MLV18	EHEC	O134	15	0.71
LSHM6	EHEC	O83	13	0.62
BD651	EHEC	O83	13	0.62
4SSH61	EHEC	O135	13	0.62
TIM651	EPEC	O120	13	0.62
HNE10	EHEC	O83	12	0.57
NET87	EHEC	O149	12	0.57
BDS6	ETEC	O7	12	0.57
4SBD12	EAEC	O18	10	0.48
CRS10	EIEC	O7	9	0.43
DHS39	ETEC	O7	9	0.43
TMOT1	EHEC	O20	9	0.43
MAM8	EHEC	O135	8	0.38
PMM31	EHEC	O157	7	0.33
TEC19	ETEC	O83	5	0.24
PMH14	EPEC	O135	3	0.14
LEBD13	EAEC	O134	2	0.09

respectively exhibiting resistance to these antibiotics.

None of the isolates were sensitive to cefotaxime and colistin. A very few isolates showed sensitivity towards amikacin (1, 3.0%), cefpodoxime (2, 6.1%), and piperacillin/Tazobactam (2, 6.1%), indicating a high level of resistance of the tested isolates towards these antibiotics. A relatively high level of sensitivity was noted against tetracycline (18, 54.5%) and chloramphenicol (21, 63.6%), where the number of sensitive isolates exceeded that of resistant and intermediate-resistant isolates. However, in the case of co-trimoxazole and trimethoprim, the number of intermediate-resistant isolates exceeded that of resistant and sensitive isolates, at 17 (51.5%) and 15 (45.4%), respectively.

Multiple drug resistance profiles of *E. coli* isolates and the MAR index

Most of the tested isolates displayed multiple drug resistance (MDR) phenotypes. The MAR index of the tested *E. coli* ranged from 0.09 to 0.95 (Table 3 and Figure 2). Two isolates, PSE64 and TSOT13, belonging to the EHEC pathogroup, had a MAR index of 0.95. On the contrary, isolate LEBD13, which belonged to EAEC, showed the least resistance (two antibiotics), with a MAR index of 0.09. Two other isolates, PMH14 (EPEC) and TEC19 (ETEC), exhibited resistance to three and five antibiotics, respectively, with minimum MAR indices of 0.14 and 0.24 (Table 3). TIS71, MLV17, LSBD21, and BDE6, belonging to the EHEC pathogroup, exhibited a high level of resistance, with each having an MAR index of 0.9. All isolates of EHEC O120 serogroup, the most prevalent among others, had a comparatively high MAR index, ranging from 0.76 to 0.95 (Table 3).

The one isolate representing the EIEC pathogroup (CRS10) showed resistance to 9 out of 21 antibiotics, with an MAR index of 0.43 (Table 3 and Figure 2). The two EAEC isolates (4SBD12 and LEBD13) exhibited a difference of 0.39 in value. The range of MAR index obtained for the five ETEC and two EPEC isolates tested varied from low to high, with values of 0.24 to 0.81 and 0.14 to 0.62, respectively. Fifteen out of 23 (65.2%) EHEC isolates had a MAR index above the average MAR index of 0.65. Overall, the MAR index range of isolates belonging to the EHEC pathogroup ranged between 0.33 and 0.95, and the least MAR index was noted for the isolate PMM31 (0.33). All three EHEC O157 isolates, PMH55, TMSA2, and PMM31, tested in this study showed a varied MAR index of 0.86, 0.81, and 0.33, respectively. These were isolated from *shrimp* (*Penaeus monodon*) and fish (*Opisthopterus tardoore*) samples.

Of 23 EHEC isolates tested in this study, two were resistant to 20 antibiotics, 4 to 19, 2 to 18, 3 to 17, 3 to 16, 1 to 15, 3 to 13, 2 to 12, and one each to 9, 8, and 7 antibiotics (Table 3). Multidrug-resistance patterns of EHEC isolates are shown in Figure 3. All the EHEC isolates tested

showed resistance to colistin, and 21 isolates each exhibited resistance to aminoglycosides, monobactams, and amoxycillin-clavulanate. Five isolates were resistant to phenicol (Figure 3). Five ETEC isolates were tested, with one isolate each being resistant to 17, 15, 12, 9, and 5 antibiotics. Five isolates, three of which belonged to the O7 serotype and one each to O83 and O134, exhibited varying drug resistance patterns, with their MAR indices ranging from 0.24 to 0.81 (Figure 3). All the isolates were resistant to third-generation cephalosporins (Figure 3).

Two EPEC isolates tested were resistant to 13 and three antibiotics, respectively, while two isolates of EAEC were resistant to 10 and two antibiotics. Two EPEC isolates differed significantly in terms of antimicrobial resistance, with isolate TIM651 exhibiting resistance to 13 antibiotics, including cephalosporins, carbapenems, aminoglycosides, β-lactam/inhibitor combinations, aztreonam, and colistin. In contrast, isolate PMH14 was resistant to only three antibiotics: cefotaxime, aztreonam, and colistin (Table 4). A similar trend was observed in EAEC isolates also.

The least resistance was observed in an isolate of EAEC (LEBD13), which was resistant to only two antibiotics, colistin and amikacin (Table 4). This isolate was recovered from a sample

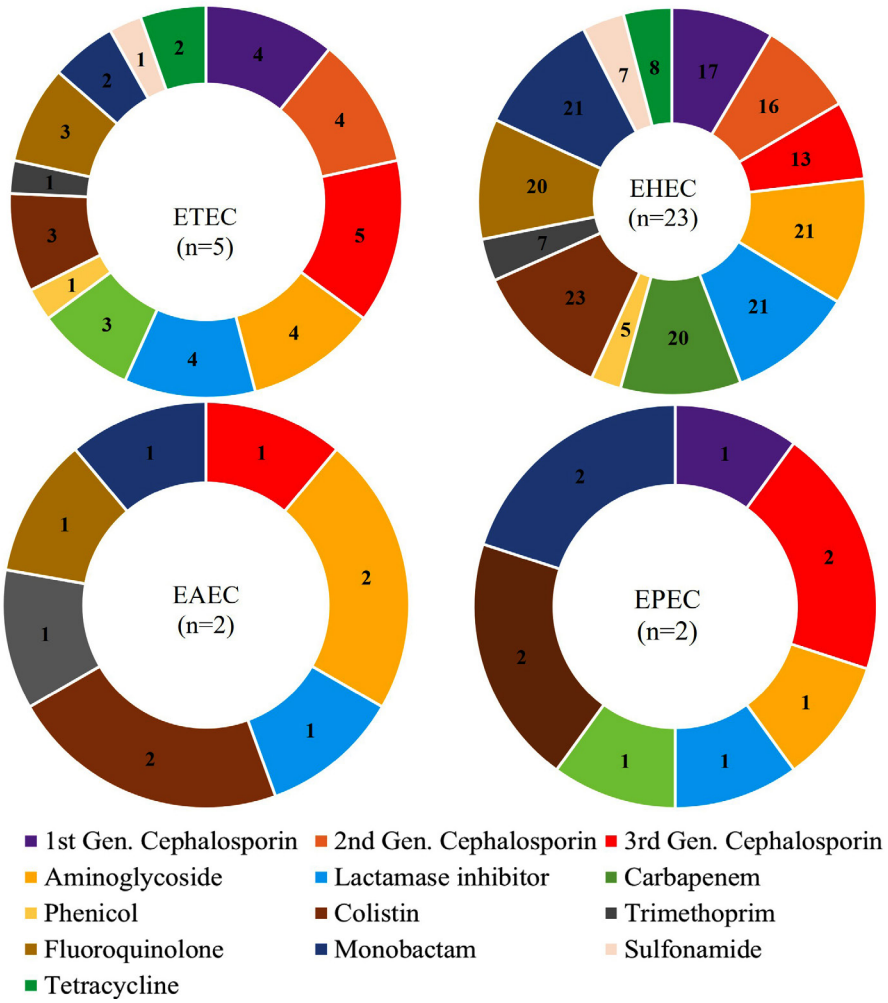

Figure 3. Multidrug-resistance patterns of *E. coli* pathogroups

Table 4. Antibiotic resistance profiles of *E. coli* isolates exhibiting multidrug-resistance (MDR) phenotypes

Isolate	No. of antibiotics to which resistant	Resistance profile
PSE64	20	CAZ, CTR, CTX, CX, CPD, CEP, GEN, CIP, COT, NA, IPM, MRP, ETP, PIT, AT, AMC, CL, AK, TE, TR
TSOT13	20	CAZ, CTR CTX, CX, CEP, CPD, CIP, GEN, COT, IPM, MRP, ETP, NA, PIT, AT, AMC, CL, AK, TE, TR
MLV17	19	CAZ, CTX, CX, CTR, CPD, CEP, GEN, COT, MRP, CIP, NA, PIT, ETP, AT, AMC, C, CL, AK, TE
TIS71	19	CTX, CTR, CAZ, CX, CPD, CEP, CIP, GEN, COT, IPM, MRP, ETP, PIT, AT, NA, AMC, CL, AK, TR
LSBD21	19	CAZ, CTR, CTX, CX, CPD, CEP, CIP, COT, GEN, MRP, NA, ETP, PIT, AMC, C, CL, AK, TE, TR
BDE6	19	CAZ, CTR, CX, CTX, CPD, GEN, CEP, CIP, IPM, MRP, NA, ETP, PIT, AT, AMC, C, CL, AK, TE
PMH55	18	CAZ, CTX, CPD, CX, CEP, GEN, MRP, IPM, CIP, NA, PIT, AT, ETP, C, CL, AK, TE, TR
4MSH38	18	CAZ, CTX, CX, CTR, CPD, GEN, CEP, CIP, IPM, MRP, NA, ETP, PIT, AT, AMC, CL, AK, TE
TIE83	17	CTX, CTR, CAZ, CX, CEP, GEN, CIP, IPM, COT, PIT, AT, NA AMC, CL, AK, TE, TR
4MSH40	17	CTX, CTR, CAZ, CX, CPD, CEP, GEN, CIP, IPM, MRP, NA, ETP, PIT, AT, AMC, CL, AK
TMSA2	17	CAZ, CTX, CX, CTR, CPD, CEP, GEN, CIP, IPM, MRP, NA, ETP, PIT, AT, AMC, CL, AK
TECL3	17	CAZ, CTX, CX, CTR, CPD, CEP, CIP, IPM, MRP, GEN, NA, ETP, PIT, AT, AMC, CL, AK
MPH7	16	CTX, CX, CAZ, CTR, CPD, CEP, IPM, MRP, GEN, CIP, NA, PIT, AT, AMC, CL, AK
SC2	16	CAZ, CTX, CX, CTR, CPD, CEP, GEN, CIP, IPM, MRP, NA, ETP, PIT, AT, CL, AK
PSM65	16	CAZ, CTX, CX, CTR, CPD, GEN, CEP, CIP, IPM, MRP, ETP, PIT, AT, AMC, CL, AK
TMA7	15	CAZ, CTX, CX, CTR, CPD, GEN, CEP, CIP, IPM, MRP, ETP, PIT, AT, CL, AK
MLV18	15	CTX, CAZ, CX, CPD, CTR, CEP, GEN, IPM, MRP, NA, PIT, AT, AMC, CL, TR
LSHM6	13	CTX, CPD, CAZ, CIP, GEN, COT, MRP, PIT, NA, AT, AMC, CL, AK
BD651	13	CAZ, CTX, CTR, CX, CEP, CIP, CPD, NA, GEN, AT, AMC, C, CL
4SSH61	13	CTX, CPD, CAZ, CTR, GEN, IPM, NA, MRP, PIT, ETP, AT, CL, AK
TIM651	13	CAZ, CTX, CEP, GEN, CPD, MRP, IPM, PIT, ETP, AT, AMC, CL, AK
HNE10	12	CTX, CPD, CIP, GEN, IPM, MRP, NA, PIT, AT, AMC, CL, AK
NET87	12	CAZ, CTX, CIP, CPD, GEN, PIT, MRP, NA, AT, AMC, CL, AK
BDS6	12	CTX, CAZ, CX, CPD, CEP, MRP, NA, ETP, PIT, AMC, C, CL
4SBD12	10	CTX, CPD, CIP, GEN, NA, PIT, AT, CL, AK, TR
CRS10	9	CTX, CAZ, CPD, CIP, GEN, MRP, NA, CL, AK
DHS39	9	CTX, CAZ, CPD, CTR, CEP, NA, PIT, AK, TE
TMOT1	9	CTX, CAZ, CPD, CTR, CEP, NA, PIT, AT, CL
MAM8	8	CTX, CPD, CTR, MRP, PIT, AT, CL, AK
PMM31	7	CTX, COT, GEN, CL, AK, TE, TR
TEC19	5	CTX, CX, CPD, MRP, AK
PMH14	3	CTX, AT, CL
LEBD13	2	CL, AK

of Bombay duck fish (*Harpadon nehereus*) and belonged to the serotype O134. The second EAEC (4SBD12) isolate of this study was resistant to 10 antibiotics (Table 4). The isolate was sensitive to carbapenems, some cephalosporins, amoxicillin-clavulanate, tetracycline, chloramphenicol, etc. Both the EAEC isolates were resistant to colistin and the aminoglycoside antibiotic amikacin. In contrast, both the EPEC isolates showed resistance to third-generation cephalosporins in addition to these antibiotics (Figure 3). A single isolate of EIEC (CRS10) from shrimp was susceptible to multiple cephalosporins, as well as some carbapenems, including imipenem and ertapenem, amoxicillin-clavulanate, piperacillin-tazobactam, and tetracycline (Table 4).

DISCUSSION

Addressing antibiotic resistance has become an international focus as it affects humans, animals, and agricultural systems. In this investigation, *E. coli* isolates representing different pathogroups recovered from seafood samples collected from Mumbai were examined for their susceptibility to antimicrobials. Considering the

persistent contamination of coastal waters in this densely populated metropolitan city, we anticipated an increased incidence of antibiotic-resistant *E. coli*.

The results of antibiotic susceptibility testing indicated the occurrence of *E. coli* pathogroups resistant to most clinically relevant antibiotics. The result is alarming, as *E. coli* in general is intrinsically susceptible to nearly all the antimicrobial agents of clinical significance.[15] However, *E. coli* is known for its receptive capacity to accumulate resistant genes, especially through horizontal gene transfer; this might have played an important role in its evolution with respect to antimicrobial resistance and its rapid spread among pathogroups.[5,16] For the last decades, the number of resistance genes in *E. coli* has been steadily increasing, which has made *E. coli* a bacterium with the highest burden of antibiotic resistance.[17,18]

Resistance to third-generation cephalosporins was prevalent (97%) among the pathogenic isolates (Table 2, Figure 1). We observed the highest resistance against cefotaxime (97%) and the lowest resistance against ceftriaxone (66.7%) in our *E. coli* isolates. Singh et al. reported a similar level of resistance in Enterobacterales isolated from seafood in Mumbai, where a majority (>90%) of the tested isolates showed resistance to cefotaxime, cefpodoxime, and ceftazidime, which are third-generation cephalosporins.[11] The percentage of resistance shown by the isolates towards cefotaxime (95%) was high and comparable to our results. High cephalosporin resistance in *E. coli* isolates from frozen shrimp has been reported from Saudi Arabia.[19] However, this study reported high resistance towards first-generation cephalosporins compared to resistance to third-generation cephalosporins observed in our study. Our study also showed high resistance to aminoglycosides, monobactams, carbapenems, quinolones, and fluoroquinolone antibiotics. Ibrahim and Elhadi reported a different susceptibility pattern for penicillin (ampicillin 90.7%, piperacillin 87.1%), quinolones (nalidixic acid 64.2%), sulfonamides (trimethoprim/sulfamethoxazole 50.7%), and tetracycline (41.4%).[19] Contrary to our findings, a study from China reported high resistance of *E. coli* isolates isolated from fish and shellfish towards chloramphenicol (72.1%) and tetracycline (93.7%).[20] *E. coli* isolated from fish samples in Cameroon, Africa, showed high resistance to trimethoprim-sulfamethoxazole, ampicillin, and ticarcillin compared to other antibiotics.[21] Notably, different resistance patterns are usually observed for pathogenic and non-pathogenic strains of *E. coli* owing to the presence of resistant genes on plasmids. In addition to several plasmid-borne antibiotic resistance genes, *E. coli* possesses the *marRAB* locus. This chromosomally encoded intrinsic resistance mechanism confers resistance to various antibiotics, including tetracyclines, chloramphenicol, cephalosporins, nalidixic acid, penicillins, rifampin, and fluoroquinolones.[22] Overall, the isolates screened in this present study were largely resistant towards beta-lactam antibiotics, and relatively more sensitive to non-beta-lactam antibiotics. Resistance towards beta-lactam antibiotics is common among bacteria, and its emergence is on the rise due to their widespread use.[23]

Among 23 EHEC isolates tested, a large proportion of EHEC/STEC isolates were resistant to 7-20 antibiotics with their MAR indices ranging from 0.33-0.95 (Tables 3 and 4). Some of these included the well-known EHEC serogroups O157 and O26 involved in several food-borne outbreaks. EHEC O26 is an important non-O157 serogroup along with O103, O111, and O145 recognized as emerging, virulent non-O157 EHEC capable of causing bloody diarrhoea and haemolytic uraemic syndrome (HUS).[24] Other STEC serogroup such as O7, O20, O149 (Table 3) have been reported to be associated with cattle, which are the major reservoirs of STEC strains.[25,26] Three isolates of EHEC O157, isolated from different seafood samples, were resistant to 7, 17, and 18 antibiotics, respectively (Table 3). The isolation of serogroup O157 resistant to only ciprofloxacin from fish was reported by Onmaz et al. in 2020.[27] Two EHEC/STEC isolates were resistant to each antibiotic tested except chloramphenicol. Overall, the tested EHEC isolates showed high resistance towards colistin, aminoglycosides, and lactamase inhibitor (Figure 3). A recent study characterizing STEC isolated from shellfish in Egypt reported resistance to multiple antibiotics, including β-lactams and β-lactam inhibitors, ciprofloxacin, colistin, tetracycline, and fosfomycin.[28]

Surprisingly, in our study, two EPEC isolates exhibited markedly different resistance profiles. The isolate TIM651 was resistant to 13 antibiotics, while PMH14 was resistant to only three antibiotics (Table 4). Even though many EPEC isolates share similar antibiotic resistance profiles, variations can be expected, as these pathogenic strains are highly diverse in nature.[29] Both the isolates showed resistance towards third-generation cephalosporins, monobactam, and colistin (Figure 3). Studies from India suggest that EPEC clinical strains have gained resistance to multiple antibiotics commonly employed in the treatment of diarrheal diseases.[30,31] A study reported total resistance to cephalothin, cefuroxime, and sulfamethoxazole, as well as very high resistance to tetracycline (76.3%) and streptomycin (84.2%) in clinical EPEC isolates.[29] In our isolates, tetracycline resistance was not found, and also, only one isolate showed resistance to cephalothin.

Varying antibiotic resistance patterns were observed among the ETEC isolates. The isolate TIE83 with a MAR index of 0.81 was sensitive to cefpodoxime, chloramphenicol, ertapenem, and meropenem, and was resistant to all other 17 antibiotics, including third-generation cephalosporins (Table 4 and Figure 3). In contrast, the isolate TEC19 was resistant to five antibiotics, including cefotaxime, cefoxitin, cefpodoxime, meropenem, and amikacin. Two isolates were resistant to ciprofloxacin in addition to cephalosporins and carbapenems. Since the emergence of ciprofloxacin-resistant ETEC in 2001, there has been a trend of increasing resistance patterns of ETEC to fluoroquinolones.[32,33] High prevalence of resistance to trimethoprim-sulfamethoxazole, ampicillin, and tetracycline was seen among the ETEC isolates obtained from ready-to-eat foods in China.[20] This study reports tetracycline resistance as 66.7%, whereas we found 40% resistance against tetracycline. Among 33 isolates screened, 32 isolates showed multidrug-resistance. Studies on the prevalence of seafood-originated drug-resistant *E. coli* from samples collected from Southern India reported the occurrence of strains with multiple drug resistance, implicating seafood as the carrier of MDR bacteria.[34,35] According to a new investigation on the occurrence of ESBL-producing bacteria in seafood, 169 (78.60%) isolates of different Enterobacterales species showed an ESBL-positive phenotype, with *E. coli* representing the major species.[36] Various ESBL-encoding genes were also identified in these isolates. Further, the occurrence of bla_{NDM}-harboring *E. coli* has also been reported in seafood.[10,36] A few other studies have reported the prevalence of antibiotic-resistant *E. coli* in commercial seafood samples in Korea,[37,38] commercial fish captured from Conception Bay, Chile,[39] shellfish from retail markets of Vietnam,[40] shrimps and shrimp farm environments in Thailand,[41] in oysters and mussels in Atlantic Canada,[42] and fish from retail markets of Cambodia.[43] A study from Mizoram, Northeast India, reported high prevalence of multidrug-resistant *E. coli* isolates belonging to EPEC and EIEC pathotypes associated with paediatric diarrhoea.[30] The MDR phenotype was observed in 41.4% of the isolates, which showed high resistance against cephalosporin drugs, aminoglycosides, carbapenem, fluoroquinolone, and sulphonamides. Multidrug-resistance involving β-lactams, third-generation cephalosporins, piperacillin, levofloxacin, and gentamicin has been described in *E. coli* pathogroups isolated from diarrheic children in Bihar, India.[44] Recently, Ghosh et al. reported a high incidence of diarrheagenic *E. coli* resistant to a minimum of six different classes of antimicrobials.[45] The endemicity of different pathogroups of *E. coli* means that they could be found in the environment and consequently in foods, including seafood, when the sanitation infrastructure is inadequately disproportional to the population, particularly in developing nations.[46] All these studies highlight the exposure of seafood to highly antibiotic-resistant *E. coli* from diverse sources, including humans and animals, and the need to identify contaminated sources and contain the spread of MDR pathogens via seafood.

An interesting observation from this study was the increased sensitivity to antibiotics such as chloramphenicol, co-trimoxazole, and tetracycline (Figure 1). The clinical application of these antibiotics has declined significantly over the last three decades due to the development of widespread bacterial resistance.[47-49] The increased susceptibility of diarrheagenic *E. coli* observed in this study warrants further investigation to understand the factors that have

contributed to the susceptibility of *E. coli* to these antibiotics, particularly in light of the drastic rise in resistance to other antibiotics, such as β-lactams, aminoglycosides, and fluoroquinolones.

The MAR indices of 33 isolates ranged from 0.09 to 0.95 (Table 3), suggesting that these strains were from a high-risk environment where they were exposed to higher levels of antibiotics due to extensive use. A similar range of MAR index, extending to 1.0 from 0.09, was also reported from the same location in seafood samples.[11] The antibiotic resistance patterns of seafood isolates of this study are comparable with clinical isolates of pathogenic *E. coli*. The multidrug-resistance traits reported in clinical isolates of diarrheagenic *E. coli* in India suggest that these strains have a human reservoir, and enter the aquatic environment through various routes of contamination.

CONCLUSION

This study reports a high prevalence of antibiotic resistance, as well as multidrug-resistance, among pathogenic *E. coli* isolated from seafood samples. A higher MAR index indicates that these isolates originated from high-risk environments with antibiotic contamination. The presence of extremely resistant pathogenic strains compromises the safety of seafood for consumption. With the rise of extremely drug-resistant clonal strains of *E. coli* that can spread rapidly in the community, causing significant morbidity and mortality, their presence in seafood will further complicate control measures. The aquatic environment is a hotspot for horizontal gene transfer events that can lead to the emergence of extremely antibiotic-resistant strains. In this context, to mitigate the selective pressure from antibiotics, scientific measures under the "One Health concept" are needed to reduce imprudent antibiotic use and to treat wastewater, thereby containing the dissemination of virulent and antimicrobial-resistant *E. coli* through seafood.

ACKNOWLEDGMENTS

The authors would like to thank the Director, ICAR-CIFE, Mumbai, and the Director, ICAR-CIARI, Andaman and Nicobar Islands and ICAR-CIFT, Kochi, for their help and support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

ML and SHK conceptualized the study. ML, SHK and BBN collected resources. SHK applied methodology. BBN and SHK supervised the study. SP performed data collection, investigation and formal analysis. JS and ML performed data analysis. BBN performed data validation. SP wrote the manuscript. JS and ML reviewed the manuscript. JS, ML, BBN and SHK edited the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

This study was supported by ICAR-Central Institute of Fisheries Education, Mumbai, India, vide grant number CIFE-2012/9.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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