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Quinolone and Colistin Resistance Genes in Extended-Spectrum Beta-Lactamase (ESBL)-Producing *Escherichia coli* of Diverse Phylogenetic Groups Isolated from Seafood in Mumbai, India

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Abstract: Contamination of coastal-marine environment with multidrug-resistant *Escherichia coli* has resulted in such bacteria increasingly being detected in the seafood chain. This study aimed to determine the quinolone and colistin resistance genes in extended spectrum-β-lactamase (ESBL)-producing *E. coli* from seafood. ESBL-producing *E. coli* isolates (n = 269) were tested for quinolones and colistin resistance phenotypes by disk diffusion and broth microdilution methods, respectively. The isolates were further PCR screened for the plasmid-mediated quinolone resistance (PMQR) genes *qnrA*, qnrB, and *qnrS*, genomic mutations in *gyrA* and *parC* genes, and the colistin resistance genes *mcr-1* and *mcr-2*. Phylogroup was determined by PCR using the Clermont *E. coli* phylotyping method. Of 269 isolates tested, 73.60% of *E. coli* isolates were resistant to moxifloxacin and 8.55% to ofloxacin, the least of all the quinolones tested. Further, 150 (55.76%) *E. coli* isolates carried at least one of the three PMQR genes tested, where *qnrS* was the most prevalent gene (53.90%). The colistin resistance gene (*mcr-2*) was detected in 38 (14.12%) isolates. Twenty-one of these isolates (55.26%) had a colistin minimum inhibitory concentration (MIC) of 16 µg/mL. Based on the Clermont *E. coli* phylotyping of the isolates harboring at least one of the *qnr* genes, 66 (44%) belonged to the phylogroup B1, followed by 23 (15.33%) to phylogroup A. Among 38 *E. coli* isolates carrying colistin resistance gene *mcr-2*, 27 (71.05%) isolates belonged to phylogroup B1, followed by 4 (10.52%) isolates to phylogroup A. The results suggest that *E. coli* phylogroups B1 and A harboring plasmid-mediated quinolone and colistin resistance genes are predominant in the seafood supply chain.

Keywords: ESBL; *qnr*; *mcr*; seafood; quinolone resistance; colistin; *Escherichia coli*

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1. Introduction

The extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* has become a severe challenge to chemotherapy [1,2]. The ESBLs are classified into several groups, the most prominent of them being TEM, SHV, and CTX-M types [3,4]. Pathogenic *E. coli* are distinct from their commensal counterparts due to the virulence genes they possess, based on which they are classified into at least five pathovars, namely enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enteroaggregative/adherent *E. coli* (EAEC), and enteroinvasive *E. coli* (EIEC). *E. coli* causes diverse infections ranging from wound infection to meningitis and is one of the major

ESBL-producing Gram-negative bacteria. ESBLs confer resistance to a broad spectrum of cephalosporins, and together with AmpC lactamases, bacteria producing them can resist a majority of cephalosporins and their inhibitor combinations [5–7]. The emergence and spread of carbapenemases have further confounded the problem, as carbapenems have been the preferred antibiotics to control ESBL-producing *Enterobacteriaceae*. The broad-spectrum antibiotics of the fluoroquinolone group are also widely used to treat gastrointestinal and urinary infections caused by *E. coli*. However, quinolone resistance has dramatically increased in recent years, particularly with the emergence of certain specific lineages of bacteria such as ST131-H30 [8,9]. Quinolone resistance occurs due to amino acid changes in topoisomerase subunits *GyrA* and *ParC*, which become recalcitrant to binding by the antibiotic due to structural changes [10]. The plasmid-mediated quinolone resistance (PMQR) genes, collectively called *qnr* genes and first reported in a *Klebsiella pneumoniae* isolate, encode pentapeptide proteins that bind with DNA gyrase and topoisomerase IV enzymes, protecting them from the inhibitory effects of quinolones [10–12]. The genes *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, and *qnrVC*, with over 100 variants, are widely distributed in Gram-negative bacteria [13]. Plasmids harboring *qnr* may also carry extended-spectrum β-lactamases (ESBLs) [14]. With ESBL- and carbapenemase-resistant *Enterobacteriaceae* becoming challenging to treat, polymyxins are increasingly being considered as therapeutic options despite their potential nephrotoxicity. Colistin (polymyxin E) is a cationic, multi-component, lipopeptide antibacterial that has been used in veterinary medicine for decades to treat Gram-negative intestinal tract infections. Colistin was initially restricted to ophthalmic and topical use for treating human infections due to its systemic toxicity [15]. The growing evolution of multidrug-resistant (MDR) Gram-negative bacterial infections with limited available therapeutic options has compelled its clinical use as a primary treatment option. The most commonly found mechanism of colistin resistance is associated with the chromosomal mutation in the genes, which leads to modification of the lipid A of LPS, the primary target of colistin, as an adaptative strategy. However, plasmid-mediated polymyxin resistance is attributed to the *mcr* genes, with at least ten variants, *mcr-1* to *mcr-10* [16,17]. The horizontal transfer of the plasmid-carried *mcr-1* gene encoding PEtN has become an important cause of the spread of colistin resistance among various Gram-negative bacteria [17,18]. The emergence of *mcr* genes has focused global attention on colistin resistance, one of the last means of treatment. The *mcr*-gene-containing bacteria (MGCB) are disseminated by horizontal/lateral transfer into diverse ecosystems, including aquatic, soil, botanical, wildlife, animal, and environment ecosystems, and public places.

The anthropogenic contamination of the coastal-marine environment can introduce multidrug-resistant (MDR) *E. coli*, eventually leading to their presence in seafood. *E. coli* from seafood may show different levels of quinolone and colistin resistance due to mutations in QRDR genes and the acquisition of plasmids containing quinolone and colistin resistance genes by horizontal gene transfer. The plasmid-mediated quinolone and colistin resistance genes can be mobilized into other *Enterobacterales* members, leading to wider dissemination of antibiotic resistance phenotypes and genotypes. Phylogenetic evaluation of *E. coli* can help understand the distribution and origin of MDR *E. coli* in seafood and the aquatic environment. The present study aimed to understand the quinolone and colistin resistance in ESBL-producing *E. coli* from seafood in Mumbai, India and their phylogenetic diversity.

2. Materials and Methods

2.1. Detection of ESBL Phenotype in Escherichia coli Isolates

Escherichia coli isolates used in this study were from our previous study [19], in which we reported the prevalence of ESBL-producing *E. coli* isolates from seafood samples from

markets of Mumbai, India. Briefly, for the phenotypic detection of ESBL production, the double-disk synergy test was followed. The isolate was grown to 0.5 McFarland units in Mueller–Hinton (MH) broth and 100 µL of the culture was spread plated evenly on a Mueller–Hinton (MH) agar plate. An amoxicillin/clavulanic acid (30/10 µg) disk was placed at the center, and cefpodoxime (10 µg), cefotaxime (30 µg), and ceftazidime (30 µg) disks were positioned at 20–30 mm away from the central disk. An extension in the zone of inhibition around the peripheral disk towards the centrally placed disk by at least 5 mm was considered positive for ESBL production. This was further confirmed by using the Triple ESBL detection Ezy MIC™ Strip (Hi-Media, Mumbai, India) according to the manufacturer's instructions. The test strain was spread plated evenly on a Mueller–Hinton (MH) agar plate and then a Triple ESBL detection Ezy MIC™ Strip was placed and minimum inhibitory concentrations (MICs) were recorded. A total of 269 isolates of ESBL-positive *E. coli* were included in this study.

2.2. Quinolone Susceptibility Testing Using Disk Diffusion Assay

The isolates were retrieved from −80 °C glycerol stock on Mueller–Hinton agar (HiMedia, Mumbai, India) by streak plating. A single colony from the plate was inoculated into Mueller–Hinton (MH) broth and was grown for 12 h at 37 °C. The broth culture was evenly spread on the MH agar using a sterile swab and allowed to dry for 5 min before placing antibiotic disks. The isolates were screened for quinolone resistance by the standard disk diffusion method using the following quinolone antibiotics: ciprofloxacin (CIP)—5 µg, nalidixic acid (NA)—30 µg, levofloxacin (LE)—5 µg, ofloxacin (OF)—5 µg, norfloxacin (NX)—10 µg, and moxifloxacin (MO)—5 µg. The zones of inhibition were measured and results were interpreted according to the Clinical Laboratory Standards Institute guidelines [20].

2.3. Screening of Colistin-Resistant Isolates Using Chromogenic Agar Medium

A fresh single pure colony was streaked on HiCrome™ colistin-resistant agar (Hi-Media, India) and incubated for 18–24 h at 37 °C. Luxuriant purple colonies indicate the presence of presumptive colistin-resistant *E. coli*.

2.4. PCR Detection of Quinolone and Polymyxin Resistance Genes

Bacterial DNA was extracted using the Wizard DNA kit (Promega, Madison, WI, USA) according to the instructions therein. The quinolone resistance genes *qnrA*, *qnrB*, and *qnrS*, and the colistin resistance gene *mcr-1* and *mcr-2* were tested by PCR using previously described oligonucleotide primers and protocols [21–24]. Briefly, each PCR reaction mixture of 30 µL consisted of 15 µL of 2X EmeraldAmp® GT master mix (TaKaRa Bio Inc., Tokyo, Japan), 10 µM of each of forward and reverse primers, and 3 µL of template DNA. The genomic mutations in *gyrA* (Ser83 and Asp87) and *parC* (Ser80 and Glu84) genes were tested by multiplex allele-specific PCR [25]. All the PCRs were performed in a SimpliAmp thermocycler (Thermo Fisher Scientific, Agawam, MA 01001, USA). The PCR products were separated on 1.5% agarose gel, stained with 0.5 µg/mL ethidium bromide, and photographed using the Gel Doc XR+ gel documentation system (Bio-Rad, Hercules, CA 94547, USA).

2.5. Determination of Colistin Minimum Inhibitory Concentration (MIC) by Broth-Microdilution

The colistin minimum inhibitory concentrations (MICs) of the isolates were determined by the broth microdilution method according to CLSI protocol using cation-adjusted Mueller–Hinton II broth (CA-MHBII) [26]. From a stock solution of 64 µg/mL, colistin was serially diluted in CA-MHBII up to a concentration of 0.125 µg/mL. The test isolate was grown to 0.5 McFarland units in CA-MHBII and inoculated into microtiter plates to

obtain 5 × 104 CFU/well. The plates were incubated at 35 ± 2 °C for 16 to 20 h. The lowest concentration inhibiting the visible growth of test isolates was recorded, and the streak plating method confirmed the absence of growth. According to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), isolates with a MIC of ≥2 µg/mL were classified as resistant to colistin [27].

2.6. Phylotyping of E. coli Isolates

The primer sequences and their target genes for *E. coli* phylotyping and the interpretations of the results were conducted according to the Clermont method [28]. *E. coli* isolates of this study were subjected to phylogroup PCRs targeting 7 housekeeping genes. A quadruplex genotype corresponding to the presence/absence of the four genes (*arpA*, *chuA*, *yjaA*, and *TspE4.C2*) was determined for each strain. Then, based on the quadruplex genotype obtained, an isolate was either immediately assigned to a phylogroup or subsequently based on the results of the C and E group PCRs. Some strains of *E. coli* belonging to a group intermediate between the F and B2 phylogroups were classified as phylogroup G. Hence, B2 group and F group isolates were screened for phylogroup G/F PCRs targeting three housekeeping genes *trpA*, *cfaB*, and *ybgD* [29].

3. Results

3.1. Quinolone and Colistin Resistance in ESBL-Producing E. coli and Their Genetic Determinants

Of 269 isolates tested, 198 isolates (73.60%) were resistant to moxifloxacin, 156 isolates (57.99%) were resistant to ciprofloxacin, 132 isolates (49.07%) were resistant to nalidixic acid, 47 isolates (17.47%) were resistant to norfloxacin, 39 isolates (14.49%) were resistant to levofloxacin, and 23 isolates (8.55%) were resistant to ofloxacin (Table 1).

Table 1. Antibiotic susceptibility profiles of *E. coli* (n = 269) isolates based on the disk diffusion test.

Antibiotic	No. (%)		
	Resistant	Intermediate	Sensitive
Nalidixic Acid (NA)	132 (49.07%)	75 (27.88%)	62 (23.04%)
Ciprofloxacin (CIP)	156 (57.99%)	84 (31.22%)	29 (10.78%)
Ofloxacin (OF)	23 (8.55%)	6 (2.23%)	240 (89.21%)
Levofloxacin (LE)	39 (14.49%)	110 (40.89%)	120 (44.60%)
Norfloxacin (NX)	47 (17.47%)	91 (33.82%)	131 (48.67%)
Moxifloxacin (MO)	198 (73.60%)	-	71 (26.39%)

The occurrence of plasmid-mediated quinolone resistance (PMQR) genes in ESBL *E. coli* isolates was studied using PCR for *qnrA*, *qnrB*, and *qnrS*. Of 269 isolates tested, 150 (55.76%) isolates were found positive for at least one of these genes conferring resistance to quinolones. The *qnrS* gene was found in the maximum number of isolates (145 isolates, 53.90%), followed by *qnrB* (20 isolates, 7.43%). The *qnrA* gene was not found in any of the isolates tested (Table 2).

Table 2. Distribution of quinolone and colistin resistance genes in *E. coli*.

Gene	No. (%) Positive
qnrS	145 (53.9%)
qnrB	20 (7.43%)
qnrA	0
mcr-2	38 (14.12%)
mcr-1	0

Total number of *E. coli* isolates tested, n = 269.

Overall, 235 isolates resistant to at least one of the quinolones tested were screened for the occurrence of genomic mutations in *gyrA* and *parC* genes, of which *gyrA83* mutation was found in 162 (68.93%) isolates, *gyrA87* mutation in 166 (70.63%) isolates, *parC80* mutation in 162 (68.93%) isolates, and *parC84* mutation in 186 (79.14%) isolates (Table 3). At least one tested mutation was found in 193 (82.12%) isolates of *E. coli.*

Table 3. Molecular detection of QRDR mutations in *E. coli*.

QRDR Mutation	No. (%) Positive
gyrA83	162 (68.93%)
gyrA87	166 (70.63%)
parC80	16 (68.93%)
parC84	186 (79.14%)

Total number of *E. coli* isolates tested, n = 235.

3.2. Incidence of Colistin Resistance Gene mcr-1 and mcr-2 in E. coli

The presumptive colistin-resistant isolates from HiCrome™ colistin-resistant agar were further screened for *mcr-1* and *mcr-2* using PCR. The n = 38 (14.12%) isolates were found to be harboring the *mcr-2* gene, and the *mcr-1* gene was not detected in any isolates (Table 2). All (n = 38) *mcr-2* positive isolates were tested for colistin minimum inhibitory concentration (MIC) determination by the broth micro dilution method. For 21 isolates (55.26%), the minimum inhibitory concentration (MIC) was 16 µg/mL; it was 8 µg/mL for 10 (26.31%) isolates and 4 µg/mL for 7 (18.42%) isolates.

3.3. Phylogroup Evaluation of E. coli

All (n = 269) *E. coli* isolates of this study were subjected to phylogroup PCRs targeting seven housekeeping genes. The most prevalent phylogroup was found to be B1 (46.46%), followed by type UN (23.04%), type A (11.52%), type D (7.80%), type C (6.31%), type B2 (2.23%), type F (1.11%), and type E (three isolates; 1.11%). Six B2 group and four F group isolates were screened for phylogroup G/F PCR targeting three housekeeping genes *trpA*, *cfaB*, and *ybgD*. One isolate belonging to phylogroup G was identified (Table 4, Figure 1).

Table 4. Phylogroup of *E. coli* isolates tested.

Phylogroup	No. of Isolates (%)	No. of Isolates Harboring One or More qnr Genes (%)	No. of Isolates Harboring mcr-2 Gene (%)
B1	125 (46.46%)	66 (44%)	27 (71.05%)
UN	62 (23.04%)	23 (15.33%)	2 (5.26%)
A	31 (11.52%)	23 (15.33%)	4 (10.52%)
D	21 (7.80%)	18 (12%)	2 (5.26%)
C	17 (6.31%)	13 (8.66%)	1 (2.63%)
B2	6 (2.23%)	3 (2%)	1 (2.63%)
F	3 (1.11%)	2 (1.33%)	0
E	3 (1.11%)	1 (0.66%)	1 (2.63%)
G	1(0.37%)	1 (0.66%)	0

Total number of *E. coli* isolates tested, n = 269; total number of *E. coli* isolates harboring one or more *qnr* genes, n = 150; total number of *E. coli* isolates harboring *mcr-2* gene, n = 38.

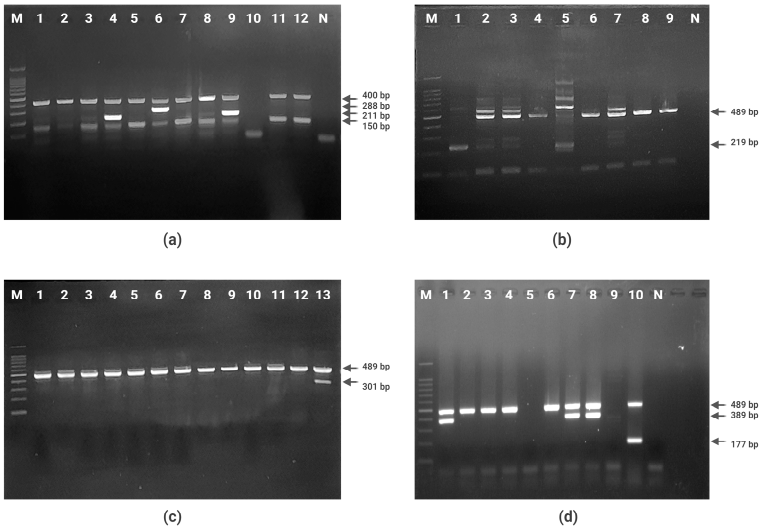

Figure 1. Agarose gel electrophoresis of PCR products. (**a**) Quadruplex PCR for *arpA*, *chuA*, *yjaA*, and *TspE4.C2* genes (Lane 1–12: strains of *E. coli*; lane M: 100 bp molecular size marker; lane N: negative control). (**b**) PCR for phylogroup C identification (Lane 1–9: *E. coli* strains of A or C phylogroup; lane M: 100 bp molecular size marker; lane N: negative control). (**c**) PCR for phylogroup E identification (Lane 1–13: *E. coli* strains of D or E phylogroup; lane M: 100 bp molecular size marker). (**d**) PCR for phylogroup G identification (Lane 1–10: *E. coli* strains of F or B2 phylogroup; lane M: 100 bp molecular size marker; lane N: negative control).

In the present study, of 150 quinolone-resistant *E. coli* isolates harboring one or more *qnr* genes, 44% belonged to phylogroup B1, followed by 15.33% to phylogroup A, 15.33% to phylogroup UN, 12% to phylogroup D, 8.66% to phylogroup C, 2% to phylogroup B2, 1.33% to phylogroup F, and 0.66% each to phylogroups E and G. Of the 38 *E. coli* isolates carrying colistin resistance gene *mcr-2*, 71.05% of the isolates belonged to phylogroup B1, followed by 10.52% to phylogroup A and 5.26% each to phylogroups D and UN, while one isolate each belonged to phylogroups B2, C, and E (Table 4).

4. Discussion

Fluroquinolone resistance is often caused by specific point mutations in the quinolone resistance-determining regions (QRDRs) of either the topoisomerase IV (*parC* and *parE*) or DNA gyrase (*gyrA* and *gyrB*) genes. The most common mutations identified in *E. coli* are at Ser83 and/or Asp87 in *gyrA* and Ser80 and/or Glu84 in *parC* [25]. In the present study, of the 235 isolates resistant to at least one of the quinolones tested, *gyrA* and *parC* mutations were observed in the majority of the isolates. Further, 42 (17.87%) strains were negative for the all four mutations tested. Novel mutations in QRDRs involved in resistance to nalidixic acid, ciprofloxacin, and ofloxacin in *E. coli* isolated from imported shrimp in USA were at positions 68, 83, and 87 in *gyrA* and at positions 80 and 84 in *parC* genes [30]. The *qnrA* gene was the first plasmid-mediated quinolone resistance gene discovered, followed by *qnrB* and *qnrS*, which share 40% and 59% amino acid identity, respectively, with *qnrA* [23]. Among the plasmid-mediated quinolone-resistant genes tested in this study, 55.76% of isolates were found to be positive for at least one of these genes conferring resistance to quinolones, whereas the *qnrS* gene was found in the maximum number of isolates (53.90%). In a study from the German food chain, among the *qnr*-positive *E. coli* isolates, the most common gene was *qnrS* (92.2%) followed by *qnrB* (5.8%), while *qnrA* and *qnrVC* were detected in only one isolate each and none had *qnrC* or *qnrD* [31]. *E. coli* isolated from food producing animals predominantly carried the *qnrS1* gene (63.2%), followed by *qnrB4* (36.7%) [32]. The *qnr* genes have been detected in several members of the *Enterobacteriaceae* family, mainly in *K.*

pneumoniae, *E. coli*, *Enterobacter spp.*, *Citrobacter freundii*, and *Providencia stuartii* in different countries [14]. Very few studies have reported the incidence of quinolone resistance in seafood-borne *E. coli*. Plasmid-mediated *qnrA*, *qnrB*, and *qnrS* have been reported in 73.8% of ESBL-positive *E. coli* from farmed fish in China, with *qnrB* being the most dominant gene, followed by *qnrS* [33]. The relative abundance of different plasmid-mediated quinolone resistance genes can vary in clinical, environmental, and food isolates of *E. coli* according to different studies from India [34,35]. The present study corroborates the general finding that *qnrB* and *qnrS* are the predominant PMQR genes in *E. coli*, while *qnrA* is relatively less prevalent. Co-occurrence of β-lactamase genes such as ESBL and MBL with the quinolone resistance genes in bacteria leaves little scope for chemotherapeutic control of such bacteria.

In the present study, the mobile colistin resistance gene *mcr-2* was detected in 14.12% of the isolates, whereas the *mcr-1* gene was not detected. In Belgium, an *E. coli* strain carrying the mcr-2 gene was isolated from pigs and cattle [36]. More than 18% of ESBL/AmpC-producing *E. coli* isolates from poultry in Lebanon carried the *mcr-1* gene [37]. The occurrence of *mcr-1* and *mcr-5* colistin resistance genes in *E. coli* and *Salmonella*, respectively, has been reported in the broiler meat supply chain from Indonesia [38]. A study from Barcelona, Spain suggests the occurrence of *mcr-1* harboring multidrug-resistant *E. coli* in sewage from waste water plants [39]. These colistin-resistant *E. coli* were the commonly circulating pulsotypes in the community, suggesting that environmental contamination with colistin-resistant bacteria was occurring due to the community. Several studies from India have reported a high prevalence of colistin resistance among clinical isolates [40,41]. A study conducted in Chennai, India analyzed raw food materials including poultry meat, mutton meat, fish, fruits, and vegetables collected from food outlets and found that 46.4% of the samples, including seafood, were contaminated with colistin-resistant bacteria [42]. Studies have demonstrated co-transfer of bla_{NDM} and *mcr-1* genes through conjugation [40]. These studies emphasize the need to monitor the presence and spread of multidrug-resistant *Enterobacteriaceae* carrying colistin resistance genotypes in the community and the environment.

An *E. coli* strain belonging to ST48 and phylogroup A with quinolone resistance harboring the *mcr-1* gene on the IncHI2, IncN, and IncX3 plasmids was isolated from Scampi shipped to Norway from Bangladesh [43], indicating that the seafood trade may be a possible means of intercontinental transmission. In the present study, of 150 quinolone-resistant *E. coli* isolates harboring one or more *qnr* genes, 44% belonged to phylogroup B1, followed by 15.33% to phylogroup A, 15.33% to phylogroup UN, 12% to phylogroup D, 8.66% to phylogroup C, 2% to phylogroup B2, 1.33% to phylogroup F, and 0.66% each to phylogroups E and G. Among the 38 *E. coli* isolates carrying the colistin resistance gene *mcr-2*, 71.05% of the isolates belonged to phylogroup B1, followed by 10.52% to phylogroup A, and 5.26% each to phylogroups D and UN, while one isolate each belonged to phylogroups B2, C, and E. In a study on *Escherichia coli* in chicken meats, phylogroups A, C, D, and F were found among colistin-resistant *E. coli*, suggesting that colistin-resistant *E. coli* strains are genetically more diverse [44]. A study on colistin-resistant *E. coli* isolated from diseased pigs in France showed that isolates belonged to groups A (63%), D (17.3%), B1 (11.1%), E (5%), B2, C, and F (1.2% each) using the Clermont *E. coli* phylotyping method [45]. Our results showed that *E. coli* phylogroups B1 and A harboring plasmid-mediated quinolone and colistin resistance genes are predominant in the seafood supply chain. It has been reported that isolates belonging to the B1 and A clade carry diverse virulence and antibiotic resistance genes with no linkage between phylogeny and antibiotic resistance gene presence [46]. A study by on the *mcr-1* resistance gene and plasmidome in *E. coli* pathogenic strains showed variable phylogroups A, B1, B2, C, D, E, and G with no correlation for particular genotypes with pathotypes [47]. *E. coli* ST131 from the phylogroup B2 was found in a study of seafood to be bla_{NDM}-positive [19].

5. Conclusions

In conclusion, the present study indicates the predominance of plasmid-mediated quinolone and colistin resistance genes in seafood isolates of *E. coli*. We did not characterize the plasmids carrying quinolone and colistin resistance genes or study their mobility, which is a limitation of this study. Nevertheless, the occurrence of such ESBL-producing *E. coli* in seafood and the coastal environment conferring multi-drug resistance is a concern since the genetic traits of antibiotic resistance can easily be disseminated among other members of *Enterobacterales*. Quinolone and colistin antibiotics are "critically important" in human medicine, and the rapid emergence of resistance to these can severely compromise their clinical efficacy. Phylogenetic typing is a valuable tool for understanding the diversity and sources of ESBL-producing *E. coli* and the emergence of any new resistant lineage in the environment. The present study emphasizes the need to develop strategies to mitigate the contamination of coastal-marine waters with antibiotic-resistant *E. coli* from human and animal sources. This study also draws attention to developing reliable and faster detection methods to ascertain the risk of ESBL-producing *E. coli* in seafood harvest and post-harvest environments. This is necessary to ensure the safety of fish and shellfish for human consumption and control the spread of antibiotic-resistant *E. coli* in the community.

Author Contributions: S.H.K. conceptualized the study. S.H.K., B.B.N., S.K.G. and M.L. designed the experiments. C.K.D. performed the benchwork and collected the data. C.K.D., S.H.K., S.K.G. and M.L. performed data analysis. B.B.N. contributed to data analysis and interpretation of results. C.K.D. wrote the original draft of the manuscript and created the tables and figures. All authors have read and agreed to the published version of the manuscript.

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