Indian J. Fish., 69(2): 93-99, 2022
DOI: 10.21077/ijf.2022.69.2.88404-11

Molecular characterisation and antibiotic resistance profile of *Escherichia coli* isolated from seafood

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ABSTRACT

The present study made an attempt to investigate the occurrence of pathogenic *Escherichia coli* in seafood, its antimicrobial susceptibility patterns and the biofilm forming capacity. Total of 43 seafood samples collected from ten commercial markets of Kochi was screened for the presence of pathogenic *E. coli*. The PCR results revealed that out of 21 isolates screened, only one isolate from fish sample showed positive for *rfb E* gene of Enterohaemorrhagic *E. coli* (EHEC). Antibiotic susceptibility pattern of the isolates revealed that 90% of them were multidrug resistant to more than 5 antibiotics of four structurally different classes. The MAR index of all the isolates was found between 0.2 and 0.5. Out of the 15 moderate biofilm forming *E. coli* obtained, 2 of them exhibited maximum multidrug resistance to 8 antibiotics of six different classes whereas remaining 13 isolates showed multidrug resistance to 5 antibiotics. Hence, this study revealed the emergence of antibiotic resistance in biofilm forming *E. coli* in seafood from Kochi markets, which may pose future threat to develop control strategies in the fish processing industry and also pose significant treatment challenge to physicians.

Keywords: Biofilm, Multidrug resistance, Pathogenic *E. coli*, Seafood

Introduction

Seafood is an essential part of a balanced diet which provides excellent sources of protein and contributes to a good nutritional status worldwide. Seafood being highly perishable food commodity and also a source of food-borne infections, there is need of meticulous control of its bacteriological quality (Croci and Suffredini, 2003). Considering the seafood borne infection outbreaks till date, majority of seafood types involved were molluscan shellfish (45%), followed by fin fish (39%) and crustaceans (16%) (Iwamoto *et al*., 2010). In a developing country like India with dense population, the faecal contamination of natural water bodies has emerged as a main confront (Taneja and Sharma, 2019). Water body contaminated with pathogenic *E. coli* can cause a variety of diarrhoeal diseases in hosts due to the presence of specific colonisation factors, virulence factors and pathogenicity associated genes (Kaper *et al*., 2004). Pathogenic *E. coli* is renowned as one of the most significant food borne human pathogens (Cray and Moon, 1995). *E. coli* that cause diarrhoeal diseases are of six pathotypes namely, Enterohaemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC) and Diffusely adherent *E. coli* (DAEC) (Nataro and Kaper, 1998). Food-borne outbreaks related with pathogenic *E. coli* have been reported worldwide. The incidence of EHEC, mainly Shigatoxigenic *E. coli* (STEC) in seafood has already been reported from India (Kumar *et al.,* 2001; Prakasan *et al*., 2018). The extent of human health risk due to antibiotic resistant bacteria has been reported by Duran and Marshall (2005) in which ready-to-eat shrimps from 4 countries (India, Thailand, Oman and United States) were found to harbour multi-drug resistant *E. coli, Enterococcus* sp., *Salmonella* sp., *Shigella flexneri*, *Staphylococcus* sp. and *Vibrio* sp. Kumar and Schweizer (2005) and Van *et al*. (2007) reported the presence of multiple antibiotic resistant strains of *E. coli* in seafood sold in India and Vietnam. Ryu *et al*. (2012) reported the presence of antibiotic resistant *E. coli* harbouring class I and class II integrons in commercial fish and seafood marketed in Korea. In order to prevent the seafood being a means of transport for antibiotic resistant bacteria, it is important to thoroughly examine the incidence of antibiotic resistant pathogenic bacteria which will help to prevent their human transmission. The resistant microbes may function as a potential source in the transportation

of antimicrobial resistance to human beings. Antibiotics once effective at controlling *E. coli* infections are now ineffective due to the acquired resistance to these compounds (Van den Bogaard *et al*., 2001; Schroeder *et al*., 2002).

There is little information about the biofilm-forming abilities of pathogenic *E. coli* isolated from food. The incidence of pathogenic, biofilm forming antibiotic resistant *E. coli* on food processing apparatus and other food contact surfaces can act as important source of contamination threatening the microbiological quality and safety of seafood (Abebe, 2020). Hence, the present study was to investigate the antibiotic resistance and biofilm-producing abilities of potentially human pathogenic *E. coli* from fresh seafood.

Materials and methods

Sample collection

Forty three fresh/chilled seafood samples (wild caught) [fin fish (n=20), clams (n=10) and shrimps (n=13)] were collected during a period of 6 months from January 2017 to June 2017, from major ten commercial markets in Kochi, Kerala, south India. The samples were aseptically transferred to polythene bags and transported in ice box immediately to the laboratory and checked for the presence of pathogenic *E. coli*.

Isolation and identification of pathogenic E. coli

Isolation and identification of pathogenic *E. coli* was conducted by following Feng *et al.* (2020). Seafood samples (25 g each) were weighed aseptically and homogenised with 225 ml of sterile brain heart infusion (BHI) broth and incubated for 10 min at room temperature with periodic shaking. The samples were then allowed to settle for 10 min and the medium was decanted carefully into a sterile container. In order to revive the metabolically injured cells, the medium was incubated for 3 h at 35°C. After incubation, the contents were transferred to 225 ml of sterile double strength tryptone phosphate broth and incubated for 20 h at 44°C. After incubation, a loopful of the inoculum was streaked on to L-Eosin Methylene Blue agar (L-EMB) and MacConkey agar and incubated at 35°C for 24 h. To check the presence of *E. coli* O157 strains (EHEC), the enriched samples were serially diluted in Butterfield's phosphate buffer solution (10^{-7}) and then plated onto Sorbitol MacConkey agar with potassium tellurite and cefixime as supplement (TC-SMAC) and was incubated at 35°C for 24 h (Feng *et al.,* 2020). The isolates were biochemically analysed by IMViC test, catalase test, oxidase test, urease test, carbohydrate fermentation test, ONPG test and triple sugar iron (TSI) agar test.

Molecular characterisation of E. coli

Polymerase chain reaction (PCR) was done to amplify the virulent genes of four pathotypes *viz.* EHEC, EPEC, ETEC and EIEC of *E. coli* isolated from seafood. The details of the primer sequences used for the identification of pathotypes of *E. coli* are listed in Table 1.

Extraction of DNA

For DNA extraction, 1.5 ml overnight culture in Tryptone soya broth (TSB) was used (contains approximately 10^9 cells ml^{-1}). The DNA extraction of the *E. coli* isolates was done using the DNeasy® Tissue Kit (Qiagen, Germany) as per the instructions given by the manufacturers. The extracted DNA was stored at -20ºC for further use.

PCR amplification

To detect the presence of different pathotypes of *E. coli* in the isolates under study, uniplex PCR was performed in a final reaction volume of 25 µl containing 10X PCR buffer, 10 mM solution of four deoxynucleoside triphosphates (dNTPs) (pH 8.0), 5U Taq DNA polymerase and 1 µl of each forward and reverse primer (10 pM) of respective virulent genes targeted for each pathotype. PCR reaction of 30 cycles were run in a thermal cycler with initial denaturation at 95^0C for 2.5 min, denaturation at 95^0C for 30 s, annealing time for 1 min, extension 72^0C

Table 1. Primer sequences used for detection of virulent genes of different pathotypes of *E. coli.*

Pathotype	Primer	Target gene	Nucleotide Sequence (5'-3')	Product length (bp)	Reference
EHEC	rfbE	*rfbE*	F: GGATGACAAATATCTGCGCTGC R: GGTGATTCCTTAATTCCTCTCTTCC	213	Gordillo *et al*. (2011)
EPEC	bfpA	*bfpA*	F: TTCTTGGTGCTTGCGTGTCTTTT R: TTTTGTTTGTTGTATCTTTGTA	367	Nguyen *et al*. (2005)
ETEC	LT	*eltB*	F: TCTCTATGTGCATACGGAGC R: CCATACTGATTGCCGCAAT	322	
ETEC	ST	*estA*	F: GCTAAACCAGTAGGGTCTTCAAAA R:CCCGGTACGGGCAGGATTACCAACA	147	
EIEC	SHIG	*ial*	F: CTGGTAGGTATGGTGAGG R: CCAGGCCAACAATTATTTCC	367	

for 1 min and final extension at 72^{0}C for 5 min. Annealing temperature for each primers used were: 58^{0}C for *rfbE*, 55^{0}C for *bfpA*, 60^{0}C for *eltB*, 55^{0}C for *estA* and 60^{0}C for *ial* (Nguyen *et al*., 2005; Gordillo *et al*., 2011). The amplicons were analysed by electrophoresis in 1.5% agarose gel in 1X TAE buffer (pH 8.0) with Ethidium bromide stain (0.5 µg ml^{-1}) at 85 V in a horizontal gel electrophoresis system.

Antibiogram analysis

Antibiotic susceptibility patterns for the *E. coli* isolates were determined using commercial antibiotic discs in accordance with the Clinical and Laboratory Standards Institute (CLSI, 2017) guidelines by Kirby-Bauer disc diffusion method (Bauer *et al*., 1966). A total of 16 antibiotics from 8 structurally different classes *viz*. Doxycycline (DO 30 µg ml^{-1}), Nalidixic acid (NA 30 µg ml^{-1}), Trimethoprim (TMP 5 µg ml^{-1}), Azithromycin (AZM 30 µg ml^{-1}), Sulphamethoxazole (SMX 25 µg ml^{-1}), Amoxyclav (AMC 30 µg disc^{-1}), Nitrofurantoin (NIT 300 µg disc^{-1}), Gentamicin (GEN 10 µg disc^{-1}), Norfloxacin (NX 10 µg disc^{-1}), Amikacin (AK 30 µg disc^{-1}), Chloramphenicol (C 30 µg disc^{-1}), Ciprofloxacin (CIP 5 µg disc^{-1}), Erythromycin (E 15 µg disc^{-1}), Polymyxin-B (PB 30 units disc^{-1}), Streptomycin (S 10 µg disc^{-1}) and Tetracycline (TE 30 µg disc^{-1}) (HiMedia, Mumbai, Indiaµ) were used for the present study. The antibiotic discs were placed onto Muller Hinton agar plates swabbed with *E. coli* isolates (adjusted to 0.5 McFarland turbidity standards). After the incubation period of 24 h at 35ºC, antibiotic susceptibility patterns were observed (CLSI, 2017) by measuring the zones of growth inhibition using *E. coli* ATCC 25922 as standard strain.

Multiple antibiotic resistance (*MAR*)

Multiple antibiotic resistance (MAR) index was determined for those isolates which showed resistance to more than three antibiotics (Riaz *et al*., 2011).

$$\text{MAR index} = \frac{a}{b}$$

where, a is the number of antibiotics to which the isolate shows resistance and b is the number of antibiotics to which the isolate was exposed.

Determination of Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the selected antibiotics to which the maximum isolates were showing resistance was determined by the standard agar diffusion test (Bauer *et al*., 1966) on Muller-Hinton agar and were incubated for 24 h at 35ºC. Ezy MIC™ strips (HiMedia) of antibiotics Amikacin (AK 0.016-256 µg ml^{-1}), Polymixin B (PB 0.016-256 µg ml^{-1}), Chloramphenicol (C 0.016-256 µg ml^{-1}), Sulphamethaxazole (SMX 0.002-32 µg ml^{-1}), Ciprofloxacin (CIP 0.002-32 µg ml^{-1}), Gentamicin (GEN 0.064-1024 µg ml^{-1}), Tetracycline (TE 0.016-256 µg ml^{-1}) and Trimethoprim (TMP 0.002-32 µg ml^{-1}) were used.

Detection of biofilm formation

Biofilm forming capacity of all the 21 isolates of *E. coli* was determined by tube method (Christensen *et al*., 1982). A loopful of the isolates were inoculated in 10 ml trypticase soy broth (TSB) with 1% glucose in the test tubes and incubated at 37ºC for 24 h. Tubes were washed with phosphate buffered saline (pH 7.3) and dried after the incubation period and then was stained with crystal violet (0.1%) and excess stain was washed with deionised water. The amount of biofilm formed was measured according to the area of coverage of the stain and scored as 1 - weak/none, 2 - moderate and 3 - high/strong in comparison with the control strain (*Staphylococcus aureus* ATCC 27217) (Fig. 2).

Results and discussion

Isolation and identification of E. coli

Out of the 43 samples analysed for the presence of pathogenic *E. coli*, more than 2 colonies showing dark centered, flat, with or without green metallic sheen on EMB agar and dark pink coloured colonies on MacConkey agar for each samples analysed were selected (FAO, 1992) and biochemical analysis was performed for further confirmation. Considering the results of biochemical analysis performed, 21 isolates were identified as *E. coli*. Out of the 21 *E. coli* isolates obtained from various seafood samples, 9 were from fish (45%); seven and five isolates were from clams (70%) and shrimps (38%), respectively. This seems to be normal, as the shellfish are reared in the estuarine environment where faecal contamination occurs frequently and has been reported by several studies in the past (Kumar *et al*., 2001; Sehgal *et al*., 2008; Mieszkin *et al*., 2013; Prabhakar *et al*., 2017). On TC-SMAC, one of the isolates (ECF5) formed colourless colonies, which is presumptive for EHEC strain *E. coli* O157. There are previous reports suggesting shellfish from coastal environments acting as vehicle for STEC transmission (Kumar *et al*., 2001; Gourmelon *et al*., 2006). However, the presence of *E. coli* detected in finfish in the present study might represent post-harvest contamination from the landing centre and fish market from the use of unpotable water and contaminated ice (Prabhakar *et al*., 2017).

Molecular characterisation

The primer sequences used for the amplification of virulent genes of EHEC, EIEC, EPEC and ETEC showed 100% similarity with the sequence submitted in the GenBank and EMBL database libraries. The results of

PCR detection revealed that out of 21 isolates screened for the presence of EIEC, EPEC, ETEC and EHEC, only one isolate (ECF5) from fish sample showed positive result for *rfbE* gene of EHEC (*E. coli* O157) with specific band of 213 bp (Fig. 1). From 1996 to 2005, among 190 *E. coli* isolates from seafood, 16 (8.4%) showed positive for *E. coli* O157 strain in India (Sehgal *et al*., 2008). Surendraraj *et al*. (2010) isolated *E. coli* O157:H7 from fish and shellfish marketed in Kochi harbouring intimin protein (*eaeA*), enterohemolysin (*hlyA*) and shiga toxin (*stx*) genes. Kumar *et al*. (2001) reported the presence of *stx2*, *hlyA* and *eaeA* genes in STEC isolates from fish marketed in Mangalore, India.

Antibiogram analysis

All the 21 isolates were subjected to antibiotic susceptibility test by disc diffusion method in which, 19 isolates (90%) showed resistance to antibiotics of 5 classes (13 antibiotics) under study, whereas all the isolates showed sensitivity to Doxycycline, Naladixic acid and Azithromycin (100%). Among the isolates, 52% showed resistance to Polymyxin-B while 38% were resistant to Ciprofloxacin and Chloramphenicol. Resistance to Nitrofurantoin and Erythromycin was observed in 28% of the isolates. Similarly, 24% of the isolates were resistant to Streptomycin and Norfloxacin, while 19% of the strains were resistant to Amoxyclav (Table 2). The emergence of resistance in *E. coli* against these antibiotics may be due to their frequent usage in diarrhoea treatment. Antibiotics such as Trimethoprim and Sulfamethoxazole that were previously effective for diarrhoea, are now found ineffective. Azithromycin with Fluoroquinolones (Ciprofloxacin and Levofloxacin) are now preferred as first-line antibiotics for the effective treatment of diarrhoea (Tribble, 2017). As reported by Van den Bogaard *et al*. (2001) and Schroeder *et al*. (2002)), the present study also shows that the indiscriminate use of antibiotics has resulted in *E. coli* acquiring resistance against antibiotics that were effective previously.

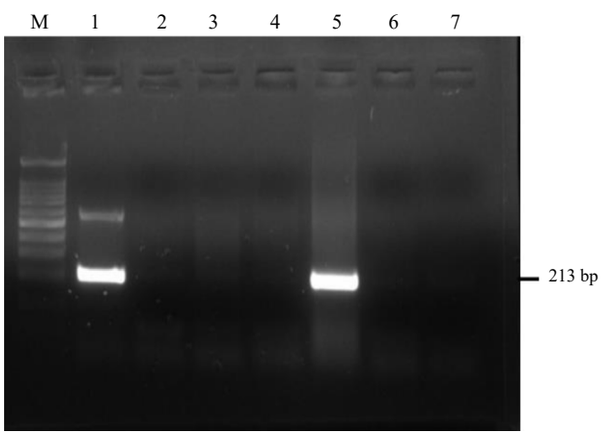

Fig. 1. PCR amplification of *rfbE* gene of *E. coli* O157 isolated from seafood. Lane M: 100 bp molecular weight marker; Lane 1: Positive control (A multiplex PCR amplified product of E. coli O157 for both *rfbE* and fliCh7); Lane 2: Negative control; Lane 5: Positive for *E. coli* O157 (ECF5); Lane 3, 4, 6, 7: Negative for *E. coli* O157

Multiple Antibiotic Resistance (MAR) index and Multi Drug Resistance (MDR) profile

Among the 21 isolates, 17 isolates showed MAR index values greater than 0.2, which indicates that those seafood samples were from highly polluted sources as the hospital and domestic sewage is disposed to the major water resources, whereas only 4 isolates were found to have originated from least contaminated sources as their MAR index values were 0.2 (Table 2). Among the 21 isolates, MDR profile of 2 *E. coli* isolates, ECF4 and ECC3 showed 50% resistance to the total antibiotics tested in this study. MDR profile of more than 15 isolates was above 30%, whereas only one isolate (ECF5) showed resistance below 20%. High prevalence of multidrug resistance indicates that little attention has been paid to the use of antibiotics in both human and animal health sectors, which needs serious attention for antibiotics surveillance program. *E. coli* CE21 isolated from fishes caught from sea/estuary and also from seafood processing plants were reported as Chloramphenicol and Ampicillin resistant strains by Kumaran *et al*. (2010). Due to random utilisation of antimicrobial agents, such high frequency of multidrug resistance might have occurred, which may eventually replace the drug susceptibility of microorganisms (Van den Bogaard *et al*., 2001).

Minimum Inhibitory Concentration (MIC)

Those antibiotics to which most of the isolates showed maximum resistance in antibiotic susceptibility test were selected for MIC test. MIC of 8 different antibiotics was determined by using Ezy MIC™ strips. Highest MIC value was obtained for Gentamicin and Polymixin ranging between 98-128 mcg ml^{-1} whereas, lowest MIC value was shown for Ciprofloxacin ranging between 0.016-0.094 µg ml^{-1} (Table 3). Boss *et al*. (2016) had studied antibiotic resistance of 44 samples of *E. coli* from Salmon, Pangasius, Shrimps and Oysters against Colistin, Cefotaxime, Meropenem, Ceftazidime and Tigecycline and found that the MIC of Azithromycin was ≤8 mg l^{-1}. Jiang *et al.* (2012) also showed that the *E. coli* isolated from seafood possessed plasmid-mediated quinolone resistance (PMQR) genes and extended-spectrum β-lactamases (ESBLs) with reduced susceptibility to Ampicillin (MIC≥8 mg l^{-1}) and Ciprofloxacin (MIC≥0.06 mg l^{-1}).

Table 2. Antibiotic resistance profile, MAR index, Multi drug resistance profile and biofilm formation of *E. coli* isolated from fresh seafood

Isolates code	Antibiotics (µg ml^{-1})																MAR Index	MDR (%)	Biofilm score
	CIP (5)	AK (30)	AMC (30)	S (10)	C (30)	NIT (30)	E (15)	GEN (10)	PB (300)	TE (30)	NX (10)	AZM (15)	DO (30)	SMX (25)	TMP (5)	NA (30)			
ECF1	-	R	-	-	R	-	-	-	-	-	-	-	-	R	R	-	0.2	25	1
ECF2	R	-	-	-	-	-	-	-	R	R	R	-	-	-	-	-	0.2	25	1
ECF4	R	-	-	R	R	-	-	-	R	R	R	-	-	R	R	-	0.5	50	2
ECF5	-	-	-	-	-	-	-	-	R	-	-	-	-	R	R	-	0.2	19	2
ECF6	-	-	-	-	-	-	R	-	R	-	-	-	-	R	R	-	0.2	25	2
ECF7	-	R	-	-	R	-	-	R	-	R	-	-	-	R	R	-	0.3	38	2
ECF8	-	-	-	-	-	-	R	R	R	-	-	-	-	R	R	-	0.3	31	2
ECF9	-	-	R	-	R	-	-	-	-	R	-	-	-	R	R	-	0.3	31	2
ECF10	-	-	-	-	R	R	-	R	R	-	R	-	-	-	-	-	0.3	31	2
ECS3	-	-	R	R	R	-	-	-	-	R	-	-	-	-	R	-	0.3	31	2
ECS4	R	-	-	-	-	-	-	-	R	R	R	-	-	R	R	-	0.3	38	2
ECS5	-	-	-	R	-	-	R	-	-	R	-	-	-	R	R	-	0.3	31	2
ECS6	-	-	R	R	-	-	R	-	-	R	-	-	-	-	R	-	0.3	31	2
ECS7	R	-	-	R	-	-	R	R	R	-	-	-	-	R	R	-	0.4	44	2
ECC1	-	-	R	-	-	R	R	R	-	R	-	-	-	R	R	-	0.4	44	2
ECC2	R	R	-	-	-	-	-	R	R	R	-	-	-	R	R	-	0.4	44	1
ECC3	R	R	-	-	-	R	-	R	R	R	-	-	-	R	R	-	0.5	50	2
ECC4	-	-	-	-	R	-	-	R	-	R	-	-	-	R	R	-	0.3	31	1
ECC5	-	R	-	-	-	R	-	R	-	R	-	-	-	-	R	-	0.3	31	1
ECC6	R	R	-	-	R	R	-	-	-	R	R	-	-	-	R	-	0.4	44	2
ECC7	R	R	-	-	-	R	-	R	R	R	-	-	-	-	R	-	0.4	44	1

*R = Resistant; - = Sensitive/intermediate; CIP - Ciprofloxacin, AK - Amikacin, AMC - Amoxyclav, S - Streptomycin, C - Chloramphenicol, NIT - Nitrofurantoin, E - Erythromycin, GEN - Gentamicin, PB - Polymyxin B, TE - Tetracycline, NX - Norfloxacin, AZM - Azithromycin, DO - Doxycycline, SMX - Sulphamethoxazole, TMP - Trimethoprim, NA - Nalidixic acid.

Table 3. MIC of *E. coli* isolates against the antibiotics tested

Isolate code	Ezy MIC™ Strips (µg ml^{-1})							
	CHL (0.016-256)	TMP (0.002-32)	TET (0.016-256)	SMX (0.002-32)	GEN (0.064-1024)	CIP (0.002-32)	PB (0.016-256)	AMK (0.016-256)
ECF1	-	-	12	-	64	0.50	96	1.5
ECF2	64	6	2	0.047	128	0.023	64	1.5
ECF4	-	-	-	-	48	0.094	128	1
ECF5	48	-	12	-	32	0.023	96	1.5
ECF6	96	-	24	-	32	0.064	64	1.5
ECF7	-	-	-	-	64	0.50	64	1.5
ECF8	32	-	24	-	128	0.064	32	1.5
ECF9	-	-	-	-	48	0.064	48	1
ECF10	32	8	2	0.047	32	0.023	-	1.5
ECS3	-	-	8	0.094	32	0.016	-	2
ECS4	64	-	-	-	48	0.064	64	1
ECS5	64	-	8	-	128	0.016	128	1.5
ECS6	32	-	-	0.064	32	0.064	64	1
ECS7	48	-	12	-	32	0.023	96	1.5
ECC1	32	-	12	-	32	0.023	48	1.5
ECC2	96	-	24	-	128	0.064	32	1.5
ECC3	96	-	-	-	128	0.094	48	1
ECC4	-	-	-	-	48	0.064	64	1
ECC5	96	-	8	0.094	3	0.016	128	2
ECC6	-	-	8	0.094	3	0.016	64	2
ECC7	64	-	-	-	1	0.094	32	1

Detection of biofilm formation

By analysing the biofilm forming capacity of different isolates by conventional tube method, out of 21 isolates, 15 isolates (71%) showed moderate biofilm formation (score 2) and 6 isolates (29%) were weak or are non-biofilm formers (score 1) (Table 2). While comparing the ability to produce biofilm and antibiotic resistance of *E. coli* isolates under study, it was found that 2 isolates with biofilm forming capacity (ECF4 and ECC3) had showed resistance against more than 7 antibiotics of 6 structurally different classes used in this study. With this observation, we can assume that biofilm forming bacteria has the capacity to acquire antimicrobial resistance, as the biofilm provides protection against several environmental stresses allowing them to survive and grow in a hostile environment. Even though most of the isolated strains from seafood were biofilm formers, it was tough to distinguish between moderate, weak and non-biofilm producers due to the unpredictability in the results obtained by conventional methods. Hence, quantitative analysis of biofilm production and screening of genes involved in biofilm development need to be done in future to get more insight into the development of biofilm and its mechanism against antibiotic susceptibility. Procedures such as transposon mutagenesis and genome-wide screening may lead to the identification of genes involved for the production of biofilm matrix components and the regulatory principles governing biofilm development.

Current study revealed the presence of *E. coli* O157 (ECF5) in fresh seafood marketed in Kochi which clearly indicates that seafood can act as a vehicle for transmission of enterohaemorrhagic *E. coli* into human. In addition to that we found a connection between the emergence of antibiotic resistance and the presence of biofilm in *E. coli*, which may pose future threat to develop control strategies in the fish processing industry which may lead to a long lasting effect on the human health. Intensive efforts are needed from all stakeholders to produce fish and shellfish that are free from pathogens and antibiotics which adversely affect human health.

Acknowledgements

The authors are grateful to the Kerala University of Fisheries and Ocean Studies (KUFOS), for the financial support to carry out the work. We acknowledge the Dean, Faculty of Fisheries Science, KUFOS and Centre of Excellence in Food Processing Technology (CEFPT) for all the necessary support during the study.

References

Abebe, G. M. 2020. The role of bacterial biofilm in antibiotic resistance and food contamination. *Int. J. Microbiol*., https://doi.org/10.1155/2020/1705814.

Bauer, A. W., Kirby, W. M., Sherris, J. C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardised single disk method. *Am. J. Clin. Pathol*., 45(4): 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493.

Boss, R., Overesch, G. and Baumgartner, A. 2016. Antimicrobial resistance of *Escherichia coli, Enterococci, Pseudomonas aeruginosa* and *Staphylococcus aureus* from raw fish and seafood imported into Switzerland. *J Food Prot*., 79(7): 1240-1246. doi: 10.4315/0362-028X.JFP-15-463.

Christensen, G. D., Simpson, W. A., Bisno, A. L. and Beachey, E. H. 1982. Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. *Infect. Immun*., 37(1): 318-326. doi: 10.1128/iai.37.1.318-326.1982.

CLSI 2017. *Performance standards for antimicrobial susceptibility testing*. 27th edn. CLSI supplement M100, Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA.

Cray, W. C., Jr. and Moon, H. W. 1995. Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. *Appl. Environ. Microbiol*., 61(4): 1586-1590. doi: 10.1128/aem.61.4.1586-1590.1995.

Croci, L. and Suffredini, E. 2003. Microbiological risk associated with seafood consumption. *Ann. Ist Super Sanita*, 39(1): 35-45.

Duran, G. M. and Marshall, D. L. 2005. Ready-to-eat shrimp as an international vehicle of antibiotic-resistant bacteria. *J. Food Prot*., 68(11): 2395-2401. doi: 10.4315/0362-028x-68.11.2395.

Feng, P., Weagant, S. D., Grant, M. A. and Burkhardt, W. 2020. Enumeration of *Escherichia coli* and the coliform bacteria. In: *Bacteriological analytical manual (BAM)* . United States Food and Drug Administration (USFDA) Administration Center for Food Safety,Washington, DC, USA.

FAO 1992. Microbiological analysis. In: *Manual of food quality control 4, Rev. 1*. Food and Agricultural Organisation of the United Nations, Rome, Italy, p. 13-26.

Gordillo, R., Cordoba, J. J., Andrade, M J., Luque, M. I. and Rodríguez, M. 2011. Development of PCR assays for detection of *Escherichia coli* O157:H7 in meat products. *Meat Sci.,* 88(4): 767-773. doi: 10.1016/j.meatsci.2011.03.011.

Gourmelon, M., Montet, M. P., Lozach, S., Le Mennec, C., Pommepuy, M., Beutin, L. and Vernozy-Rozand, C. 2006. First isolation of Shiga toxin 1d producing *Escherichia coli* variant strains in shellfish from coastal areas in France. *J. Appl. Microbiol*., 100(1): 85-97. doi: 10.1111/j.1365-2672.2005.02753.x.

Iwamoto, M., Bjorklund, T., Lundberg, C., Kirik, D. and Wandless, T. J. 2010. A general chemical method to regulate protein stability in the mammalian central nervous system. *Chem. Biol.,* 17(9): 981-988. doi: 10.1016/j.chembiol.2010.07.009.

Jiang, H. X., Tang, D., Liu, Y. H., Zhang, X. H., Zeng, Z. L., Xu, L. and Hawkey, P. M. 2012. Prevalence and characteristics of lactamase and plasmid-mediated quinolone resistance genes in *Escherichia coli* isolated from farmed fish in China. *J. Antimicrob. Chemother.,* 67(10): 2350-2353.

Kaper, J. B., Nataro, J. P. and Mobley, H. L. 2004. Pathogenic *Escherichia coli*. *Nat. Rev. Microbiol*., 2(2): 123-140. doi: 10.1038/nrmicro818.

Kumar, S. H., Otta, S. K., Karunasagar, I. and Karunasagar, I. 2001. Detection of Shiga-toxigenic *Escherichia coli* (STEC) in fresh seafood and meat marketed in Mangalore, India by PCR. *Lett Appl Microbiol*., 33(5): 334-338.

Kumar, A. and Schweizer, H. P. 2005. Bacterial resistance to antibiotics: Active efflux and reduced uptake. *Adv. Drug Deliv. Rev*., 57(10): 1486-1513. doi: 10.1016/j.addr.2005.04.004.

Kumaran, S., Deivasigamani, B., Alagappan, K., Sakthivel, M. and Karthikeyan, R. 2010. Antibiotic resistant *Esherichia coli* strains from seafood and its susceptibility to seaweed extracts. *Asian Pacific Journal of Tropical Medicine*, 3(12): 977-981. DOI:10.1016/S1995-7645(11)60013-8.

Mieszkin, S., Caprais, M. P., Le Mennec, C., Le Goff, M., Edge, T. A. and Gourmelon, M. 2013. Identification of the origin of faecal contamination in estuarine oysters using Bacteroidales and F-specific RNA bacteriophage markers. *J. Appl. Microbiol*., 115(3): 897-907. doi: 10.1111/jam.12260.

Nataro, J. P. and Kaper, J. B. 1998. Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev*., 11(1): 142-201. doi: 10.1128/CMR.11.1.142.

Nguyen, T. V., Le Van, P., Le Huy, C., Gia, K. N. and Weintraub, A. 2005. Detection and characterization of diarrheagenic *Escherichia coli* from young children in Hanoi, Vietnam. *J. Clin. Microbiol*., 43(2): 755-760. doi: 10.1128/JCM.43.2.755-760.2005.

Prabhakar, P., Lekshmi, M., Nayak, B. B. and Kumar, S. 2017. Incidence of potentially pathogenic *Escherichia coli* in fresh seafood in mumbai. *Poll Res*., 36 (3): 541-546.

Prakasan, S., Prabhakar, P., Lekshmi, M., Nayak, B. B. and Kumar, S. 2018. Isolation of Shiga toxin-producing *Escherichia coli* harboring variant Shiga toxin genes from seafood. *Veterinary World*, 11(3): 379-385. doi: 10.14202/vetworld.2018.379-385.

Riaz, S., Faisal, M. and Shahida, H. 2011. Antibiotic susceptibility pattern and multiple antibiotic resistances (MAR) calculation of extended spectrum β lactamase (ESBL) producing *Escherichia coli* and *Klebsiella* species in Pakistan. *Afr. J. Biotechnol*., 10(33): 6325-6331.

Ryu, S. H., Park, S. G., Choi, S. M., Hwang, Y. O., Ham, H. J., Kim, S. U. and Chae, Y. Z. 2012. Antimicrobial resistance and resistance genes in *Escherichia coli* strains isolated from commercial fish and seafood. *Int. J. Food Microbiol*., 152(1-2): 14-18. doi: 10.1016/j.ijfoodmicro.2011.10.003.

Sehgal, R., Kumar, Y. and Kumar, S. 2008. Prevalence and geographical distribution of *Escherichia coli* O157 in India: a 10-year survey. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 102: 380-383.

Schroeder, C. M., Zhao, C., DebRoy, C., Torcolini, J., Zhao, S., White, D. G. and Meng, J. 2002. Antimicrobial resistance of *Escherichia coli* O157 isolated from humans, cattle, swine and food. *Appl. Environ. Microbiol*., 68(2): 576-581. doi: 10.1128/AEM.68.2.576-581.2002.

Surendraraj, A., Thampuran, N. and Joseph, T. C. 2010. Molecular screening, isolation, and characterization of enterohemorrhagic *Escherichia coli* O157:H7 from retail shrimp. *J. Food Prot*., 73(1): 97-103. doi: 10.4315/0362-028x-73.1.97.

Taneja, N. and Sharma, M. 2019. Antimicrobial resistance in the environment: The Indian scenario. *Indian J. Med. Res.*, 149(2): 119-128.

Tribble, D. R. 2017. Antibiotic therapy for acute watery diarrhea and dysentery. *Military Medicine*, 182(9/10): 17-25.

Van den Bogaard, A. E., London, N., Driessen, C. and Stobberingh, E. E. 2001. Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. *J. Antimicrob. Chemother*., 47(6): 763-771. doi: 10.1093/jac/47.6.763.

Van, T. T. H., Moutafis, G., Tran, L. T. and Coloe, P. J. 2007. Antibiotic resistance in food-borne bacterial contaminants in Vietnam. *Appl. Environ. Microbiol*., 73(24): 7906-7911. doi: 10.1128/AEM.00973-07.

Date of Receipt : 01.04.2019
Date of Acceptance : 27.02.2022