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PREVALENCE OF MULTIPLE ANTIBIOTIC RESISTANT AND EXTENDED SPECTRUM BETA-LACTAMASE (ESBL) PRODUCING *ESCHERICHIA COLI* IN A TRADITIONAL FISHING HARBOUR AND SURROUNDING WATER BODIES IN THE SOUTH-WEST COAST OF INDIA

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Abstract: In the present study, a total of 106 isolates of *Escherichia coli* were obtained from 390 samples collected from Ponnani harbour and surrounding water bodies like Ponnani estuary, Puthuponnani backwaters and Canoli canal over a period of two years. Antibiotic sensitivity studies were carried out by employing 12 antibiotics to determine the multiple antibiotic resistance (MAR) and the resistance profile of different isolates. Extended-Spectrum Beta-Lactamase(ESBL) production and virulence characteristics like haemolysis, cell surface hydrophobicity, and serum resistance of the strains were also investigated in the current study. Results revealed that 58.4% of isolates were resistant to ampicillin. Resistance to other antibiotics was comparatively lower (<20%), and all the isolates were sensitive to imipenem.Altogether 16(15%) isolates demonstrated multiple antibiotic resistance in the present investigation. MAR index of *E. coli* isolates from Ponnani harbour, and surrounding water bodies ranged from 0.25 to 0.41.But the MAR Index of all the sampling locations were less than 0.25. ESBL production was observed in 6.6% of *E. coli* isolates, and the study also revealed a significant association between multiple antibiotic resistance and ESBL production. Twenty-six (24.5%) isolates were found to be haemolytic twelve (11.3%) were hydrophobic, and twenty (18.8%) isolates exhibited serum resistance.

Key words: Antibiotic resistance, ESBL production, MAR index, Serum resistance

INTRODUCTION

Escherichia coli is a common bacteria found in the intestine of warm-blooded organisms and its presence in food and water indicates faecal contamination. However, horizontal gene transfer has resulted in the emergence of pathogenic forms among them and many life-threatening infections caused by such strains have been reported from around the world. Strains producing both intestinal and extra-intestinal infections have been isolated and characterized by different workers (Kaper *et al.*, 2004: Platell *et al.*, 2011). Shiga toxigenic serotype like O157:H7 has been associated with various food-borne infections occurring in both developed and developing countries (Nataro and Kaper, 1998) It is found that extra-intestinal strains of *E. coli* are the prime cause of urinary tract infections reported annually from different countries around the world (Russo and Johnson, 2000). Non-pathogenic strains acquire virulent properties by gene transfer and become pathogenic. It is challenging to differentiate such strains from commensal ones in diagnostic laboratories.

Fish often get contaminated during post-harvest handling in fishing vessels, harbours, and landing centres. Unhygienic practices such as washing with nearshore water are the leading cause of contamination of fish and fish contact surfaces with pathogenic bacteria in landing centres. Due to the dumping of municipal waste and sewage, water

bodies are polluted with pathogenic bacteria which reach near-shore water regularly as there is no natural or anthropogenic mechanism to prevent it. Due to the contamination, fish may consist of pathogenic and non-pathogenic strains of *E. coli* and consumption of such fish without adequate precaution may lead to intestinal infection and the transmission of ExPEC strains. The ability of *E. coli* to cause disease is determined by the presence of virulence factors as these virulence factors help the bacteria to survive and multiply in host cells and withstand adverse conditions (Johnson, 1991: Nataro and Kaper, 1998; Oelschlaeger *et al*., 2002). Production of hemolysin, serum sensitivity studies, cell surface hydrophobicity, etc. is usually carried out to assess the virulence potential of *E. coli*.

Antibiotic-resistant forms have emerged among both gram-positive and gram-negative bacteria, and in gram-negative pathogens, β-lactamase production remains an essential factor in antibioticresistance. The β- lactamases can hydrolyse the β-lactam ring of the penicillin group of antibiotics. When new medicines were introduced, strains with novel β-lactamases emerged due to selection pressure exerted by those antibiotics. One such group of new antibiotics were oxyimino-cephalosporins employed to treat bacterial infection caused by gram-negative bacteria. But resistance to these expanded-spectrum-lactam antibiotics emerged quickly. Because of their increased spectrum of activity, especially against the oxyimino-cephalosporins, these enzymes were designated as extended-spectrum β- lactamases (ESBLs). Today, over 150 different ESBLs have been reported from different genera of *Enterobacteriaceae* and is divided into three groupsTEM, SHV, and CTX-M(Bradford, 2001) ESBL producing strains of *E. coli* are resistant to third-generation cephalosporins likecefotaxime, ceftriaxone, ceftazidime but are sensitive to carbapenems like imipenem, meropenem, and ertapenem. The CTX-M β-lactamases, now exceeding 50 different types, can be divided into five groups based on their amino acid identities. This CTX- M β-Lactamases are often produced by *E. coli* and seem to be the actual community ESBL pathogen (Pitout and Laupland, 2008). It may be noted that recently a novel β-lactamase, New Delhi Metallo β-lactamase (NDM-1) has been identified capable of mediating resistance to different β-lactam agents including carbapenems.Resistance to carbapenems by these novel strains limits the treatment option and represents a significant challenge in the field of infectious disease.(Walsh *et al*., 2001).

Therefore, a study has been conducted to make out the incidence of antibiotic resistance among *E. coli* isolated from Ponnani fishing harbour and the surrounding water bodies and to identify the ESBL producing strains if present among the isolates. Ponnani fishing harbour, being an emerging destination of the seafood landing in the south-west coast of India and source of raw material for the international market, needs particular attention to assure the quality of seafood. Precautionary measures should be taken if antibiotic-resistant strains of *E. coli* are present in the vicinity of the harbour to prevent contamination of seafood originating from the harbour and the dissemination of resistant strains

MATERIALS AND METHODS

Samples were collected from Ponnani harbour, and the surrounding water bodies such as Ponnani estuary, Puthuponnani backwaters and Canoli canal. From Ponnani harbour Samples were collected from fish water, ice, utensils, net, floor and worker's hand. Water, fish and sediment samples were collected from different stations in water bodiesdescribed above (Fig. 1). A total of 390 samples comprising 237 samples from the harbour and 153 samples from surrounding water bodies were collected seasonally over a period of two years. Samples were brought to the Microbiology laboratory of the Postgraduate department and research centre in Aquaculture and Fishery Microbiology of MES PonnaniCollegefollowing standard procedure (ICMSF, 1986).

Isolation of *Escherichia coli*

Samples were enriched in Lauryl tryptose broth for isolation of *E.coli*. From Tubes showing colour change and gas production, one loopful was transferred to EC broth for confirmation. For isolation of *E. coli*, inocula from EC broth was transferred to Eosin Methylene Blue Agar and incubated at 37^0C for 24 hours. Colonies with blue metallic sheen were presumptively identified as *E. coli* and later confirmed by IMViC tests. (Downs and Ito, 2001).

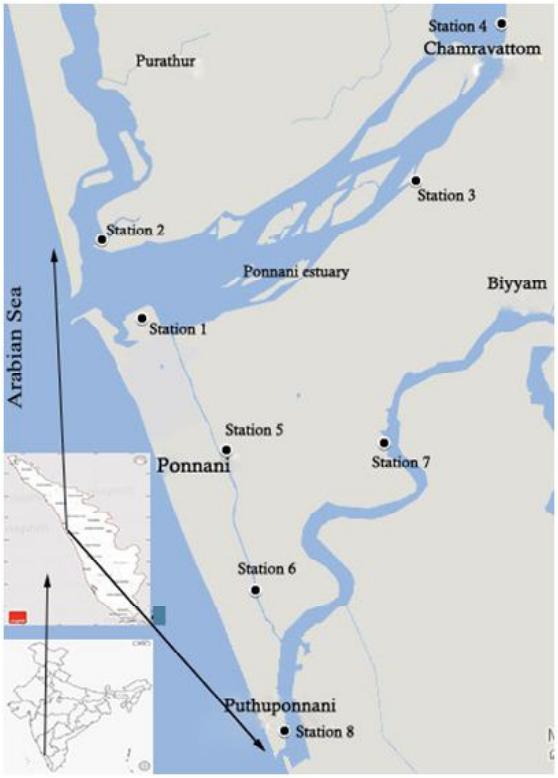

Fig. 1. Map showing Ponnani estuary and sampling stations

Statation-1 Ponnaniharbor, Station-2, Thirur River, Station-3, Ponnani Estuary, Station-4, Bharathapuzha (River), Station-5, 6, Canoli Canal, Station -7, 8, Puthuponnani Backwaters

Antibiotic sensitivity studies

Antibiotic sensitivity studies were carried out by disc diffusion method (Bauer *et al.*, 1966) following the guidelines of Clinical and Laboratory Standards Institute (2013) Antibiotics employed for the sensitivity studies included amikacin (30µg), ampicillin (10µg), cefotaxime (30µg), ceftazidime (30µg), ceftriaxone (30µg), ciprofloxacin (5µg), imipenem (10µg), nitrofurantoin (300µg), gentamycin (10µg), cotrimoxazole, (25µg) tobramycin (5µg) and nalidixic acid (30µg)

Multiple antibiotic resistanceand multiple antibiotic resistant (MAR) indexe

Multiple antibiotic resistance is defined as non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos *et al*., 2011). MAR index for a single isolate is defined as **a/b** where a= Number of antibiotics to which an antibiotic is resistant and b =Total number of antibiotics employed. For MAR indexing of samples, the formula **a/ (bc)** was employed where a= aggregate antibiotic resistance score of all isolates from the sample, b= number of antibiotics employed, c= number of isolates obtained from that sample (Krumperman, 1983; Titilawo *et al*., 2014).

ESBL production

Some strains of *E. coli* are resistant to third-generation cephalosporins like cefotaxime, ceftazidime, and ceftriaxone. These strains are capable of producing extended-spectrum beta-lactamase and are usually found in Pathogenic strains. Strains resistant to third-generation cephalosporin are considered potential ESBL producers. An inhibition zone of <22 for ceftazidime and <27 for cefotaxime indicate ESBL production. Cefotaxime (30ìg) vsCefotaxime/clavulanic acid (30/10ìg) and ceftazidimevsceftazidime/clavulanic acid were employed for the confirmatory test. An increase of >5m in the zone diameter of combination discs with clavulanic acid confirms the ESBL production (CLSI, 2013)

Detection of virulence factors

Serum resistance

An inoculum of *E. coli* diluted with the help of 0.9% salt solution was added to 0.05ml fresh serum and incubated for 180 minutes at 37°C. The viable count was obtained at the beginning and the end of incubation. Serum resistance was measured as the percentage of bacteria survived in serum when compared to initial count after incubating for 180 minutes. If 90% of bacteria survived after incubation for 180 minutes, such strains are called serum resistant (Siegfried *et al*., 1994; Sharma *et al*., 2007)

Hemolytic activity

Haemolytic activity was determined by streaking on prepared sheep blood agar plates supplied by Himedia. Plates were incubated for 24 hours at 37°C. Formation of a clear zone around the colonies is a positive reaction and is considered as a virulent property (Rijavec *et al*., 2008)

Cell surface hydrophobicity

Cell surface hydrophobicity was determined by salt aggregation test. The bacterial sample prepared in Phosphate buffer was mixed with Ammonium

sulphate solution of different molarities (0.002 to 4.00) on a glass slide and examined for clumping of cells. The highest dilution of samples showing clumping was considered as salt aggregation test (SAT) value. *E. coli* strains with less than 1.25 SAT is regarded as highly hydrophobic (Siegfried *et al*., 1994; Raksha *et al*., 2003).

Statistical analysis

Chi-square tests were employed to find out the association between various categorical variables such as multi-antibiotic Resistance, and ESBL production. Statistical analysis was carried out by SPSS version 2

RESULTS AND DISCUSSION

Antibiotic resistance studies

In the present study, a total of 106 isolates of *E.coli* were obtained from 390 samples collectedfrom Ponnani harbour and surrounding water bodies like Ponnani estuary, Puthuponnani backwaters and Canoli canal over a period of two years. Of the 106 isolates, 58.4% were resistant to ampicillin, whereas all the isolates were sensitive to imipenem.Apart from ampicillin *E.coli* showed resistance to cotrimoxazole (16.9.%) gentamycin(15.9%) nalidixic acid (16.9%) tobramycin (13.2%) ciprofloxacin (10.3%), ceftazidime (11.3%), nitrofurantoin (10.3%), andceftriaxone (7.5%) (Fig-2)

Multiple antibiotic resistance and multiple antibiotic resistance index of *E.coli*isolated from Ponnani harbour and surrounding water bodies

Some of the isolates were multi-antibiotic resistant, showing resistance to at least one antibiotic in three or more antimicrobial categories. Major categories of antibiotics employed in the present investigation include aminoglycosides, penicillins, cephalosporins, fluoroquinolones, quinolones, carbapenem, and sulphonamides. Table-1 shows the multiple antibiotic resistance patterns of *E.coli* isolates obtained in the present investigation from Ponnani harbour and surrounding water bodies. Majority of the multiple antibiotic-resistant strains were resistant to ampicillin. The Table-2 shows the MAR index of samples. It may be noted that MAR index of the Ponnani estuary is comparatively higher than that of other sources. Altogether 16(15%) isolates demonstrated multiple antibiotic resistance in the present investigation. Isolates from Puthuponnanibackwaters did not exhibit multiple antibiotic resistances in the present investigation.

Resistance to commonly employed antibiotics like ampicillin, gentamycin, cotrimoxazole, nalidixic acid, and cefotaxime was found in the current investigation. It may be noted that Divya *et al*. (2015) reported the incidence of resistance of *E.coli* isolated from Cochin estuary to the above antibiotics. Multiple-antibiotic resistance was found only in15% of isolates in the present study. The result obtained is found to be quite contrary to the results of an earlier investigation in Cochin estuary, where 95% of isolates were multi-resistant (Chandran *et al*., 2008). In another study in Matang estuary in Malaysia, 34% of 128 isolates of *E. coli* were resistant to three or more antibiotics (Ghaderpour *et al*., 2015). The emergence of multiple antibiotic resistance might be due to the indiscriminate use of antibiotics in hospitals as well as for nonclinical purposes such as in aquaculture and animal husbandry (McManus and Stockwell, 2001)

The isolates with MAR index above 0.25 are considered to be originated from high-risk sources (Krumperman, 1982; Chitanand, 2010). In the current investigation MAR index of *E.coli* isolates from Ponnani harbour and surrounding water bodies ranged between 0.25 to 0.41 indicating contaminations from high-risk sources such as the faecal matter of human origin. But the MAR Index of all the sampling locations were less than 0.25, as shown in table -5. So there is a little ambiguity in considering these locations as high-risk contamination sites. In a similar investigation in Cochin estuary, the MAR index of *E.coli* isolates ranged from 0.33 to 1 and probably originated from high-risk sources. The MAR indexes of all sampling station were also higher than 0.25 establishing the fact that these stations were polluted with faecal bacteria from high-risk sources (Chandran *et al*., 2008

ESBL production

In the current investigation, after initial screening, 14 out of 106 isolates were presumptively identified as ESBL producers, but only 7(6.6%) isolates were confirmed as ESBL producers after double-disc synergy test. Of the seven ESBL isolates four isolates

were from Canoli canal, and three were from Ponnani harbour, and the six of the isolates originated from water and the remaining one from fish. Another essential feature is that all the ESBL producers showed resistance to ampicillin. All the seven ESBL producing *E.coli* was also exhibited multiple antibiotic resistances.Extended-spectrum beta-lactamase (ESBL) positive strains are resistant to third-generation cephalosporins (e.g., cefotaxime, ceftazidime) and monobactams (e.g., aztreonam), but cannot hydrolyzecephamycins (cefoxitin) or carbapenems (imipenem) and are inhibited by beta-lactamase inhibitors such as the clavulanic acid (Philippon *et al.*, 1989). The incidence of ESBL positive strains in clinical samples has become a public health problem as cephalosporins cannot be employed against such strains during infection. ESBL producing strains have regularly been reported in clinical samples from almost all part of the world. But recently, such strains have been isolated from food, vegetables and environmental samples (Calbo *et al.*, 2011; Doi *et al.*, 2010; Shaheen *et al.*, 2011).In Cambodia Nadimpali *et al.* (2019) detected ESBL-producing *E. coli* in 93 (62%) of 150 food samples, including 32 (53%) of 60 fish, 45 (75%) of 60 pork, and 16 (53%) of 30 chicken samples. In the current investigation after initial screening, 14 isolates were presumptively identified as ESBL producers, but only 7(6.6%) isolates were confirmed as ESBL producers after double-disc synergy test. Of the seven ESBL isolates one originated from fish while the rest were form water In a similar study in China 29(11.2%), ESBL producing strains were obtained from a total of 252 samples comprising chicken faeces and River water (Gao *et al.*, 2014). The antibiotic susceptibility test detected ESBL producing *E. coli* in raw chicken meat, chevon, milk, and also in human clinical samples in a recent study conducted at Chhattisgarh, India. Altogether 10% of isolates (21/191) showed ESBL production by the phenotypic method in that investigation (Bhoomika *et al.*, 2016). In another study conducted at Veraval, Gujrat 14% (4/18) of *E. coli* isolates from fish samples were ESBL producers (Sivaraman *et al.*, 2017). The incidence of ESBL producing *E. coli* is comparatively higher in clinical samples, and 61.4% of 200 clinical isolates were ESBL producers in a clinical investigation carried out in various hospitals in Karnataka (Rao *et al.*, 2014). The current research reveals the spread and the proliferation of clinically evolved ESBL producing strains into nonclinical sources such as estuaries and coastal areas. Such strains are introduced into water bodies through the effluents from clinical sources or by indiscriminate sewage disposal. Selection pressure imposed by careless and unwanted uses of antibiotics will increase the percentage of such strains in natural environments like rivers and estuaries. The aquatic environment acts as a reservoir for the aggregation of bacteria of different origins leading to the exchange and proliferation of resistant genes among various strains (Baquero *et al.*, 2008)

Association between multiple antibiotic resistance and ESBL production

Statistically, a significant association was found between multiple antibiotic-resistant and ESBL positive strains ($P<0.05$). In the present study, all seven ESBL positive isolates were multiple antibiotics resistant. This may be because ESBL is encoded by genes located on large plasmids and these also carry genes for resistance to other antimicrobial agents such as aminoglycosides, trimethoprim, sulphonamides, tetracycline and chloramphenicol (Paterson, 2000). Due to this, ESBL producing organisms often show co-resistance to multiple classes of antibiotics (Coque *et al.*, 2008). The widespread incidence of multiple antibiotic resistance was found among ESBL producing strains of *E. coli* isolated from food, poultry and fish samples collected from various markets in Taiwan recently. More than 50% of samples were multi-antibiotic resistant in that study (Le *et al.*, 2015). Multiple antibiotic-resistant and ESBL producing *E. coli* have also been reported from fresh seafood sold in the retail market of Western Mumbai India (Singh *et al.*, 2017). Similarly ESBL-producing atypical enteropathogenic *Escherichia coli* isolated from diarrheal patients, animals, and raw meat in China displayed co-resistance to aminoglycosides, tetracycline, nalidixic acid, trimethoprim-sulfamethoxazole, and nitrofurantoin (Xu *et al.*, 2018) ESBL strains with multidrug resistance have become a worldwide problem since they demonstrate resistance to other classes of antibiotics leaving few or no treatment option. The

incidence of these strains in Ponnani harbour and water bodies point to the chances of community-acquired infections by such strains.

Virulence factors

Various virulence factors like serum resistance, cell surface hydrophobicity, and haemolytic activity were observed in some of the strains isolated during the study. Twenty-six (24.5%) isolates were found to be haemolytic, and twelve (11.3%) were hydrophobic, and twenty (18.8%) isolates exhibited serum resistance. Onlyfour (3.7%) isolates showed all the three virulent factors and 11(10.3%) isolates displayed two virulence factors. (Table-3)

Haemolytic ability by the production of hemolysin is considered as an essential virulence property and is commonly found in ExPEC strains (Caprioli *et al*., 1989). Around 25% of isolates were found to be positive for haemolysis during the current investigation. Maximum incidence (42%) of hemolysin production was noted among isolates from water which may be an indication of ExPEC strains in water. About 27% of *E. coli* from fish samples also showed a hemolytic property. More or less similar to the present outcome, all *E. coli* isolates from fish and ready to eat fish products in Ludhiana showed haemolytic ability (Gupta *et al*., 2013). Hemolysis has also reported from *E. coli* isolated from water samples collected from Tigris River in Baghdad, Iraq (Israa *et al*., 2014). Hemolytic *E. coli*, especially Uropathogenic strains, are frequently reported from hospitals and intensive care units (Raksha *et al*., 2003: Sharma *et al*., 2007).

Bacteria may be destroyed by the action of complement substances present in serum. The complement system consists of a group of proteins which cooperate to attack the 188 extracellular pathogens entering the body. The balance is activated spontaneously or by antigen-antibody reactions. The complement either facilitates phagocytosis of pathogens or kills specific pathogen directly (Ananthanarayan and Panikar, 2005). But some strains have acquired resistance to complements and are called serum-resistant strains. Surface proteins and polysaccharides protect serum resistant bacteria from complement destruction (Tayler, 1983). Altogether 18.8% of Isolates in the present investigation exhibited such property. Such strains thus are capable of invading and surviving in blood during infection. Incidences of serum- resistant strains are frequently reported from clinical specimens like urine and pus. In a clinical investigation, 37% of *E. coli* isolated from urine showed serum-resistance (Raksha *et al*., 2003). Serum-resistance has also been reported in *E. coli* isolates from water and wastewater samples in Cape Town South Africa. Of the 47 isolates, 60% showed serum resistance. (Doughari *et al*., 2011)

Bacterial cell surface hydrophobicity is a newly determined virulence mechanism which helps bacteria to adhere to mucosal cell surfaces and is due to the presence of crystalline surface layers on cell wall (Sleyter *et al*., 1983). Around 11% of samples in the present study showed cell surface hydrophobicity. A comparatively higher incidence is usually encountered in clinical specimens (Shetty *et al*., 2014; Sharma *et al*., 2007)

Out of the seven ESBL producing strains obtained from various sources, two strains exhibited all the three virulence factors, and the three ESBL producing strains shown two virulence factors. More than 200 ESBL producing *E. coli* were isolated from river water, vegetables, fish, poultry meat animal faeces, healthy individuals, and primary care patients in Switzerland and 82 % of isolates tested positive for one or more virulent factors of uropathogenic *E. coli* (Müller *et al*., 2016). But in clinical isolates, multiple virulent factors were observed more in ESBL negative strains than in ESBL positive strains (Sharma *et al*., 2007). Isolation of ESBL strains with virulent factors from Ponnani harbour and surrounding water bodies reflect the fact that these strains are no longer confined to hospital premises but have become widespread among various niches such as estuaries, fishing harbours and coastal areas. Apart from hospital effluents and sewage, waste from poultry farms and retail chicken shops might also contribute to the dissemination of these virulent strains. There are many reports of isolation of such strains from retail chicken meat (Johnson *et al*., 2005; Vincent *et al*., 2010; Jakobsen *et al*., 2010). Stringent measures should be imposed to prevent the disposal of sewage and chicken waste into estuaries and other water bodies, whereby spread of such virulent strains can be minimized to some extent.

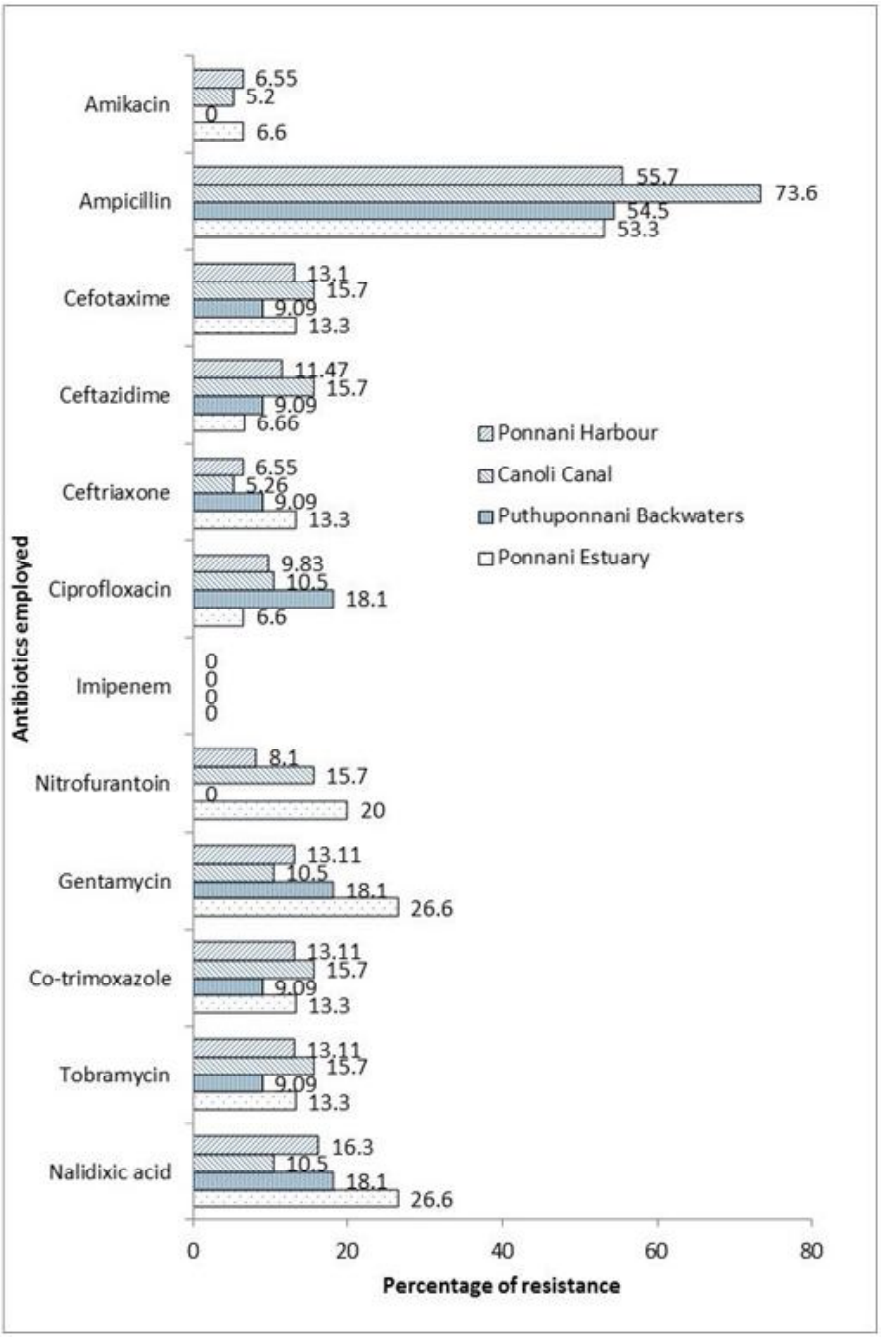

Fig. 2. Antibiotic resistance status of *E. coli* isolated from Ponnani harbour and surrounding water bodies

Table 1. MAR pattern and MAR index of *E.coli* isolates from Ponnaniharbor and surrounding water bodies

Station	Multiple antibiotic resistant pattern	MAR index
Ponnani harbor	1, AMP, NA, AK, CAZ	0.33
	2, AMP, NA, CTX, COT	0.33
	3, AMP, CTX, NIT	0.25
	4, AMP. CTR. NIT	0.25
	5, AMP, CTR.GEN	0.25
Ponnani estuary	1, AK, AMP, CTX, CIP, NIT	0.41
	2, AK, AMP, CAZ, CIP, GEN	0.41
	3, AMP, CTR,NIT, GEN, NA	0.41
	4, CTR, NIT, GEN, NA	0.33
	5, AMP, CTX, TOB	0.33
Canoli canal	1, AMP, CIP, COT, TOB, NA	0.41
	2, AK. AMP, CAZ, COT	0.33
	3, AMP, CTR, TOB, NA	0.33
	4, AMP, CAZ, COT	0.25
	5, AMP.COT, NA	0.25
	6, AMP, TOB, NA	0.25

AK-Amikacin, AMP-Ampicillin, CTX-cefotaxime, CAZ-Ceftazidime, CTR-ceftriaxone, CIP-Ciprofloxacin, NIT-Nitrofurantoin, GEN-Gentamycin, COT-Cotrimox, TR-Trimethoprim, NA-Nalidixic acid

Table 2. Multiple antibiotic resistance index of *E.coli* from different station

Stations	No of *E. coli*	% of MAR strains	MAR index of station
Ponnani harbour	61	4.71%	0.0915
Ponnani estuary	15	4.71%	0.2388
Canoli Canal	19	5.66%	0.2236

Table 3. Virulence factors of *E. coli* isolated from Poannai fishing harbour and surrounding water bodies

Sampling location	No of samples	No of *E.coli* isolated	virulence factors		
			Serumr-esistance	Hydro-phobicity	Haemolysis
Ponnani harbor	237	61	8	4	12
Ponnani estuary	61	15	4	1	6
Puthuponnanibackwater	53	11	2	1	2
Canoli canal	39	19	6	6	8
Total	390	106	20	12	26

CONCLUSION

The incidence of Pathogenic*E.coli* in Ponnani harbour and surrounding area is a severe public health problem and is a threat to the local population as they make use of the estuarine water for various purposes. Isolation of strains with virulent factors is an indication of the occurrence of uropathogenic forms within the estuary and nearby areas. Such forms might have originated from human, animal or avian sources. The incidence of multiple antibiotic-resistant and ESBL producing strains within the harbour and surrounding areas warrants continuous monitoring of these bacteria to prevent the dissemination of such strains among the population.

The fish from Ponnaniharbour reaches various processing centres located far away to produce ready to eat as well as frozen products to cater to the needs of overseas consumers. Negligence and failure to implement the quality management system like HACCP in Ponnani harbourcan result in the contamination of seafoodwithin theharbour during post-harvest operations.Isolation of ESBL strainsfrom the harbour and surrounding waterbodies points to the possibility of the global spread ofsuch strains through the sea-food originating from the harbour. Thereforeproper measures should be taken to upgrade the harbour to global standards to ensure the quality and safety of seafood exported to different countries.

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