Multiple antibiotic resistance profiles of various *Escherichia coli* serotypes isolated from Cochin Estuary

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Abstract

A total of eighty-one *Escherichia coli* isolates belonging to forty-three different serotypes including several pathogenic strains such as enterotoxigenic *E. coli (ETEC),* enterohaemorrhagic *E. coli (EHEC),* enteropathogenic *E. coli (EPEC)* and uropathogenic *E. coli (UPEC)* isolated from Cochin estuary between November 2001 and October 2002 were tested against twelve antibiotics to determine the prevalence of multiple antibiotic resistance (MAR) and antimicrobial resistance profiles as a measure of high risk source of contamination. The results revealed that more than 95% of the isolates were multiple antibiotic resistant (resistant to more than three antibiotics). The MAR indexing of the isolates showed that all these strains originated from high risk source of contamination. The incidence of multiple antibiotic resistant *E. coli* especially the pathogenic strains in natural waters will pose a serious threat to human population.

Key words: Multiple antibiotic resistance profile, Escherichia coli, MAR index

1 Introduction

Antimicrobial resistance has been recognized as an emerging worldwide problem in human and veterinary medicine (Cohen, 2000) both in developed and developing countries. The effect could be severe in heavily populated developing country such as India where there is no strict monitoring programme of the use of antibiotics in animals and humans. Antibiotics may be used for every mild symptom and discontinued when symptoms disappear but before the pathogen is eliminated. It is also well documented that widespread use of antibiotics in agriculture and medicine is accepted as a major selective force in high incidence of antibiotic resistance among gram-negative bacteria (McKeon et al., 1995). These microorganisms may be shed in feces with subsequent contamination of soil, food, and aquatic environments.The presence and persistence of antibiotic resistant bacteria in soil (Trevors, 1987) sediment (Timoney et al., 1978) sewage (Walter and Vennes, 1985), surface water (Kaspar et al., 1990), municipal drinking water (Moffie and Mouton, 1988), groundwater (McKeon et al., 1995) and sea food (Kumar et al., 2005) is a growing public health concern.

Presence of *Escherichia coli*, the natural intestinal inhabitant of humans and other warm-blooded animals, in food or water is generally considered to indicate direct or indirect fecal contamination of enteric pathogens (Krumperman, 1983). Antimicrobial resistance in *E. coli* is of particular concern because it is the most common Gram-negative pathogen in humans, the most common cause of urinary tract infections, and a common cause of both community- and hospital-acquired bacteraemia (Salvadori et al., 2004). In addition, resistant *E. coli* strains have the ability to transfer antibiotic resistance determinants not only to other strains of *E. coli*, but also to other bacteria within the gastrointestinal tract (Bettelheim, 1997) and to acquire resistance from other organisms (Osterblad et al., 2000). Conjugative and transductional transfer of these factors (R plasmid) among microbial strains in the aquatic environment has already been demonstrated (Hatha et al., 1993). A number of reports have documented the emerging resistance to multiple antimicrobial agents in verocytotoxin-producing *Escherichia coli* including *E. coli* O157:H7 (Schroeder et al., 2002).

Health risk is enhanced when potentially pathogenic bacteria survive for prolonged periods in

aquatic environments, as indicated by the high densities of coliform bacteria in tropical waters in the absence of known human fecal sources (Santiago-Mercado and Hazen, 1987). As commensal bacteria constitute a reservoir of resistance genes for (potentially) pathogenic bacteria their level of resistance is considered to be a good indicator for selection pressure by antibiotic use and for resistance problems to be expected in pathogens (Murray, 1992). Hence, in the present investigation the occurrence of multiple antibiotic resistances profiles of different pathogenic and non pathogenic serotypes of *E. coli* isolated from aquatic environments is examined against commonly used antibiotics and also determined the high risk source of contamination by MAR indexing.

2 Materials and methods

2.1 Description of the study area

The present study was carried out in Cochin Estuary, a part of Vembanad lake, an extensive lake water system in Kerala. It has undergone considerable pollution in the last decade mainly due to the development of satellite townships all along the estuary. Subsequently pollution of the estuary is mainly microbial, since the industrial development in this region has declined. Microbial pollution of Cochin estuary is the result of waste water discharge from many factories of the seafood industry, as the waste water from many factories, which is rich in organic matter, end up in the estuary.

2.2 Sampling site

The water samples were collected from five stations along the Cochin estuary based on proximity to township and waste input for a period of one year from November 2001 to October 2002. Two of the stations such as Chittoor (station 1) and Thevara (station 4) are upstream, two others namely Bolgatty (station 2) and Marine Science Jetty (station 3) in the central part of the estuary, and one at the barmouth (station 5), where the Cochin estuary meets Arabian Sea. The details of the stations are given in Figure 1.

2.3 Collection and Transportation of Samples

One water sample was collected from each station every month between 7.00 - 9.00 A.M. Water samples were collected in sterile plastic bottle (500 ml), one foot below the surface to get a better representation and transported to the laboratory in an icebox and subjected to bacteriological examination within 4 hours of collection.

2.4 Isolation of and identification of Escherichia coli

A three tube most probable number (MPN) method was used for the isolation of *E. coli* using EC broth (Hi-Media) as medium. 10.0 ml, 1.0 ml and 0.1 ml of appropriately diluted samples were inoculated into respective dilution tubes containing inverted Durham's tubes. Inoculated tubes were incubated at 44.5^{o}C for 24 hours and observed for growth and gas production. Tubes showing growth and gas production were recorded as positive. A loopful of culture from each positive tube was streaked on Eosin Methylene Blue (EMB) Agar, and incubated at 37^{o}C for 24 hours. After incubation typical *E. coli* cells with green metallic sheen were picked and restreaked to ensure purity and stored on nutrient agar vials for further biochemical characterization. The presence of *E. coli* was confirmed by Indole, Methyl Red, Voges-Proskauer and Citrate (IMViC) tests. The cultures giving + + - - reaction were confirmed as *E. coli*.

Serotyping: Serotyping of the isolated *E. coli* strains were carried out at National *Salmonella* and *Escherichia* Centre, Kasauli, Himachal Pradesh, India.

E. coli serotypes: A total of eighty-one *E. coli* isolates belongs to forty-three different serotype including several pathogenic strains isolated from different stations from Cochin estuary were used in the study. They are as follows: four O1 (Uropathogenic *E. coli*) serotype, two O39 serotype, one O173 serotype , one O78 serotype

(enterotoxegenic *E. coli*) , three O157 serotype (enterohaemorrhagic *E. coli*), two O8 serotype (enterotoxegenic *E. coli*) , six O101 serotype, one O91 serotype(enteropathogenic *E. coli*), two O165 serotype, one O 106, O104, O107, O14, serotype, two O69 serotype, one O19, two O51 serotype, Seven O25 serotype (enterotoxegenic *E. coli*) , two O139 (enterotoxegenic *E. coli*) serotype, one O86 (enteropathogenic *E. coli*) serotype six O33 serotype, two O3 serotype, three O156 serotype, one O113 (eneterohaemorrhagic *E. coli*) serotype, four O22 (Uropathogenic *E. coli*) serotype, one O102, O117 (eneterohaemorrhagic *E. coli*) ,O2 (uropathoenic *E. coli*), O105, O60, O116 (enterotoxegenic *E. coli*) , O150, O132, O135, O15 (enterotoxegenic *E. coli*), O29 serotype, three O9 serotype, two O88 serotype, one O80, O131, O66, O30, O20 (enteropathogenic *E. coli*) serotype and three UT (untypable) serotypes.

2.5 Antibiotic resistance analysis

Antimicrobial susceptibility testing was performed using a disk diffusion method (Bauer et al., 1966). Disks with the following drugs and concentration were used. Ampicillin (10 mcg); Amikacin (30 mcg); Chloramphenicol (30 mcg); Ciprofloxacin (10 mcg); Gentamycin (10 mcg); Kanamycin (30 mcg); Nalidixic acid (30 mcg); Novobiocin (30 mcg); Oxytetracyline (30 mcg); Streptomycin (30 mcg); Tetracycline (30 mcg) and Vancomycin (30 mcg). All the antimicrobial disks were purchased from Himedia, Bombay.

Pure cultures of different serotypes of *E. coli* were enriched in nutrient broth (Hi-media) at 37°C for 6-8 hrs. The cultures were then streaked over previously prepared Muller Hinton agar plates (Hi-media, Bombay) using a sterile cotton swab. The antibiotic disks were dispensed using a disk dispenser (Hi-media, Bombay) sufficiently separated from each other so as to avoid overlapping of inhibition zones. After 30 minutes, the plates were inverted and incubated at 37°C for 16-18 hours. The inhibition zones were measured and compared with the interpretive chart of performance standards for antimicrobial disks susceptibility tests, supplied by the Hi-media laboratories, Bombay and classified as resistant, intermediate and sensitive.

MAR Index of the individual isolate and the sampling station were done according to Krumperman (1983). MAR index of an individual isolate = the number of antibiotic to which the isolate was resistant ÷ the total number of antibiotics to which the isolate was exposed. The MAR index of a sample site or area = aggregate antibiotic resistance score of all isolate from the sampling station ÷ (number of antibiotics tested × number of isolates from the sample).

3 Results

A total of eighty-one *E. coli* strains belong to forty-three different 'O' serotypes isolated from five stations in Cochin estuary was tested against twelve different antibiotics. The prevalence of *E . coli* was higher at station 1 (38%) followed by station 5 (24%), station 3 (14%), station 2 (12%) and station 4 showed relatively low incidence of E. coli. The results of the antibiotic resistance analysis revealed that more than 95% of *E. coli* was multiple antibiotic resistant. The resistance pattern and the MAR index of the isolates obtained from station 1 (Chitoor) is given in the Table 1. A total of 31 *E. coli* strains were isolated from this station. Among them sixteen isolates belongs to different serotypes including four pathogenic serotypes such as O1, O78, O157 and O8. All the strains showed resistance against more than 4 antibiotics and the MAR index of each isolate ranged between 0.41-0.91. Among the isolates, seven patterns of multiple drug resistance were encountered with the number of antibiotic ranging between 4-11. The predominant resistance patterns observed was ampicillin, amikacin, kanamycin, Novobiocin, oxytetracycline, streptomycin, tetracycline and vancomycin. Emerging pathogenic serotypes O157 (EHEC) O8, O78 (ETEC) O1 (UPEC) were resistant to more than seven antibiotics such as ampicillin, amikacin, ciprofloxacin, gentamycin, kanamycin, novobiocin, oxytetracycline, tetracycline and vancomycin.

The resistance pattern and the MAR index of the isolates obtained from station 2 (Bolgatty) and 3 (Off Marine Science) is presented in Table 2. An interesting observation was that all the strains isolated from station 2 were pathogenic. Enteropathogenic serotype such as O86, O139 and enterotoxigenic serotype O25 were obtained from this station. All these strains showed multiple antibiotic resistance to more than 3 antibiotics. Five patterns of drug resistance were observed and the number of antibiotic ranged from 3-7. The most frequent resistance encountered was towards ampicillin, kanamycin, novobiocin, oxytetracycline, streptomycin tetracycline and vancomycin. None were resistant to amikacin, chloramphenicol, ciprofloxacin, gentamycin, nalidixic acid and the MAR index ranged between 0.25 - 0.58 for each isolate.

A total of 12 *E. coli* serotype was recovered from station 3 (Off Marine Science Jetty) including a pathogenic strain O113 (EHEC). MAR index and resistance patterns of these strains are given in table 2. All the strains from this station were resistant to more than 4 antibiotics and the MAR index ranged between 0.41-0.83. Five different resistance patterns were recorded with the number of antibiotics ranging from 5-10. The pathogenic serotype O113 showed resistance against amikacin, kanamycin, novobiocin, oxytetracycline, tetracycline and vancomycin.

The data presented in Table 3 was obtained from station 4 (Thevara) and 5 (Barmouth). All the *E. coli* serotypes isolated from station 4 showed multiple drug resistance. Uropathogenic *E. coli* serotype such as O2, O22 and enterohaemorrhagic serotype such as O117 was recovered from this station. Three different resistant patterns were obtained with number of antibiotic ranging from 6-10. The most common resistance was recorded against ampicillin, amikacin, chloramphenicol, kanamycin, novobiocin, oxytetracycline, streptomycin tetracycline and vancomycin. None were resistant to gentamycin and the MAR index was between 0.5-0.83. The pathogenic serotypes exhibited variation in their resistance pattern to each of the antibiotic tested and they were resistant to more than six antibiotics.

The resistance profile and MAR index of various serotypes of *E. coli* isolated from station 5 (Barmouth) including four pathogenic strains such as O116, O15, O9 and O20 is given in the Table 5. All the strains obtained from this station were multiple antibiotic resistant. Six different resistance patterns were observed and MAR index ranged between 0.5-0.75. The serotype O80 was resistant to all the twelve antibiotics tested. The predominant resistance was recorded against ampicillin, kanamycin, novobiocin, nalidixic acid, oxytetracycline, streptomycin tetracycline and vancomycin. The pathogenic serotypes O116, O15, O9 and O20 showed resistance against more than six antibiotics such as ampicillin, amikacin, kanamycin, novobiocin, oxytetracycline, streptomycin, tetracycline and vancomycin.

Percentage of isolates resistant to seven or more antibiotic and the MAR index of sampling station from which the samples were taken are given in the Table 4. In station 1 about 90.32% of the isolates were resistant to more than seven antibiotics and the MAR index is 0.68 followed by station 3,4 and 5 (75% resistantance and MAR index 0.64, 75% resistance and MAR index is 0.66, 65% resistance and MAR index 0.64 respectively). In station 2, 40% of the isolates were resistant to more than 7 antibiotics and the MAR index is 0.466.

Number and percentage of *E. coli* isolated from each station resistant to the antibiotic tested is represented in the Table 5. About 70.96 % of the *E. coli* isolated from station 1 was resistant to ampicillin, 64.5 % were resistant to amikacin and more than 90% resistance was obtained for kanamycin, tetracycline and vancomycin. A 100% resistance was recorded against novobiocin and oxytetracycline and more than 85% resistance against streptomycin while all other strains showed relatively low level resistance to the rest of the antibiotics. In station 2 the highest resistance was observed towards novobiocin, oxytetracycline, vancomycin (100%) followed by streptomycin (90%), kanamycin (60%) and ampicillin (40%). None of the

strains were resistant to amikacin, chloramphenicol, ciprofloxacin, gentamycin and nalidixic acid. The E. coli isolates from station 3 recorded 100% resistance to novobiocin, oxytetracycline and vancomycin, 91.66% resistance to kanamycin and tetracycline, and 66.66% resistance to amikacin and streptomycin. Relatively low level of resistance recorded for ampicillin and chloramphenicol. Others showed varying degree of resistance but none of the strains were resistant to gentamycin in station 4. More than 50% resistance was observed against amikacin, kanamycin, nalidixic acid, novobiocin, oxytetrcycline, streptomycin, tetracycline and vancomycin. All others showed considerably low level of resistance.

4 Discussion

The results of the present study revealed that more than 95% of the *E. coli* isolates were multiple antibiotic resistant. The incidence of MAR organisms is higher in the present study (95%) compared to earlier investigations; 45% by Amundsen et al. (1988); 6.1-31.4% by Gaur et al.(1992); Ramteke (1997) and 80% by Parveen et al. (1997) clearly indicating the increasing trend in the spread of drug resistant bacteria. The level of antibiotic resistance observed in this study among all isolates (95%) was greater than that of previous reports for urban and rural waters by Kaspar et al. (1990) who observed that 90% of all isolates were resistant to one or more antibiotics. In another study (Sokari et al., 1988), investigators showed that 80% of strains from municipal waste, river and estuarine water displayed antibiotic resistance. Much lower resistance, ranging from 31 to 75%, has been reported for *E. coli* isolates from various aquatic environments (Jones et al., 1986; Park et al., 2003).

The occurrence of antibiotic resistance among *E. coli* isolates is probably due to widespread use of chemotherapeutic drugs and may reflect the occurrence of plasmid transfer in the alimentary tract of humans and the microbial milieu of sewerage system (Trevors, 1987, Mac Gowan, 1987). Recent studies show that antibiotics can accumulate in the environment, and even persist up to a year (Zuccato et al., 2000). Several studies have shown that plasmid exchange readily occurs between E. coli and other coliform bacteria in stagnant areas of waste water systems (Grabow et al., 1973). It has been also suggested that MAR microorganisms are fit than its nonresistant counterpart and is therefore able to survive under harsh conditions (Bouma and Lenski, 1988).

Although the percentage of resistance to each antibiotic is varied between sample sites, the predominant overall average percentage of resistance observed (Table 5) was as follows: novobiocin (91%), tetracycline (83%), kanamycin (85%), streptomycin (80%), oxytetracycline (84%), ampicillin (62%) and amikacin (50%). Several authors reported varying degrees of resistance to some of these antibiotics. McKeon et al. (1995) observed that the resistance against novobiocin, ampicillin and tetracycline are most common among Gram negative bacteria in rural ground water supplies. A high ampicillin resistance was also noted (Bell et al., 1983; Jones et al., 1986) from rural and urban waters while much lower resistance to ampicillin (4.5-12.5) was reported (Parveen et al., 1997) from point source and non point source respectively. Amundsen et al. (1988) observed the most common resistance was directed towards ampicillin, cephalothin, nitrofurantoin, and tetracycline. Gomathinayagam et al. (1994) reported predominant resistance of *E. coli* to penicillin G, novobiocin and neomycin.

The multiple antibiotic resistance indexing of isolates showed that more than 95% of the isolates originated from high risk source of contamination. According to Krumperman (1983) the choice of MAR index of 0.2 to differentiate between low - and high risks contamination is arbitrary. Indices between 0.2 and 0.25 are in a range of ambiguity, and samples in this range require careful scrutiny. The MAR indexing of the isolates in the present study ranged from 0.33 to 1.00 and is greater than 0.25 and probably originated from high risk source of contamination. Calculation of the FC/FS ratio to

determine the source of contamination showed that it may consider being of human origin. The MAR index of all the sampling stations also exceeded the high risk level (0.25). MAR index of the different stations exceeded the arbitrary level showing that all stations were highly polluted with feacal bacteria originating from high risk source. Gomathinayagam et al. (1994) reported that *E. coli* isolates from Bhavani river originated from high risk sources such as night soil, commercial poultry farms and the MAR index of the sampling location exceeds the high risk level (0.25). The present results substantiates previous observations (Krumpeman, 1983; Kaspar, 1990) that urban sources harbour MAR *E. coli.*

Several pathogenic serotypes of E. coli such as ETEC, EHEC, EPEC, and UPEC were also isolated. The results of the antibiotic resistance analysis revealed that all these strains were multiple antibiotic resistant. Cardonha et al. (2004) reported almost 36% of the *E. coli* strains isolated were resistance to more than one antibiotic including enteroinvasive (O143,O112 and O124) and enteropathogeic (O111 and O125) serotypes. The multiple antibiotic resistance among these human pathogen is worrisome because disease caused by these organisms will be very difficult to treat. The rise in frequency of drug resistant isolates supports the view that widespread use of antibiotics results in the selection of resistant strains carrying plasmid encoding resistance (McGowan, 1987). These resistant strains may spread into different ecological niches, including normal intestinal flora leading to a further increase in the number of drug resistant bacteria.

5 Conclusion

The high diversity of MAR *E. coli* serotypes recorded in the present study indicates its high range of contamination and its presence is a potential human health hazard. Such polluted water is either directly or indirectly a common source of disease in man and animals. The health hazard imposed by the resistant factor (R-factor) is not restricted to drug resistance; they may enhance the infectivity and virulence of some pathogens. The MAR indexing of the isolates revealed that all *E. coli* strains originated from high risk source of contamination and also points to the fact that the water body is subjected to severe contamination and sewage input from in and around Cochin city becoming a 'manmade' reservoir of multiple antibiotic resistant microorganisms. With poor sanitation facilities and inadequate infrastructure to treat and dispose human waste results in high levels of faecal contamination together with high levels of multiple antibiotic resistance amongst the isolated enteric bacteria becoming are a major cause for concern.

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Table 1. MAR index and resistance pattern of *E. coli* serotypes isolated from station 1, Chittoor

E. coli serotype	MAR Index	Resistance pattern
O1#	0.58	Cf KNvOSTVa
O1#	0.75	AkCfKNaNvOSTVa
O1#	0.66	AkCfKNvOSTVa
O1#	0.75	AkCfKNaNvOSTVa
O39	0.41	KNvOSVa
O39	0.66	CfKNaNvOSTVa
O173	0.66	AAkKNvOSTVa
O78*	0.83	AAkCfGKNvOSTVa
O157**	0.58	AkKNvOSTVa
O157**	0.66	AAkKNvOSTVa
O157**	0.66	AAkKNvOSTVa
O8*	0.66	AAkKNvOSTVa
O8*	0.83	AAkCCfGKNvOTVa
O101	0.91	AAkCfGKNaNvOSTVa
O101	0.91	AAkCfGkNaNvOSTVa
O101	0.91	AAkCfGKNaNvOSTVa
O101	0.91	AAkCfGKNaNvOSTVa
O101	0.5	ACfKNaNv ST
O101	0.5	KNvOSTVa
O91+	0.58	AAkKNvOTVa
O165	0.66	A AkKNvOSTVa
O165	0.58	AKNvOSTVa
O106	0.66	AAkKNvOSTVa
O104	0.83	AAkCfKNaNvOSTVa
O107	0.58	AKNvOSTVa
O14	0.41	KNvSTVa
O69	0.66	AAkKNvOSTVa
O69	0.75	AAkKNaNvOSTVa
O19	0.58	ANaNvOSTVa
O51	0.66	AKNaNvOSTVa
O51	0.66	AKNaNvOSTVa

 # Uropathogenic E. coli (UPEC). *** Enterohaemorrhagic E. coli (EHEC),*

** Enterotoxigenic E. coli, + Enteropathogenic E. coli.*

Table 2. MAR index and resistance pattern of *E. coli* serotypes isolated from station 2 and 3.

Station No./ Name	E. coli serotypes	MAR Index	Resistance pattern
Station 2/ Bolgatty	O25*	0.58	AKNv OSTVa
	O25*	0.33	NvOSVa
	O25*	0.33	NvOSVa
	O25*	0.5	KNvOSTVa
	O25*	0.5	KNvOSTVa
	O25*	0.58	AKNvOSTVa
	O25*	0.58	AKNvOSTVa
	O139*	0.41	Nv OSTVa
	O139*	0.25	Nv Ova
	O86+	0.58	AKNvOSTVa
Station 3/ Off			
Marine Science	O33	0.75	AkCfGKNvOSTVa
Jetty	O33	0.58	AkNaNv OSTVa
	O33	0.41	KNv OTVa
	O33	0.75	AkCKNaNvOSTVa
	O33	0.75	AkCCfKNvOSTVa
	O33	0.58	AKNvOSTVa
	O63	0.83	ACCfGKNaNvOTVa
	O63	0.58	AAkKNv OTVa
	O156	0.5	KNv OSTVa
	O156	0.75	AAkCfKNaNvOSVa
	O156	0.75	AAkCfKNv OSTVa
	O113**	0.5	AkKNvOTVa

*** Enterohaemorrhagic E. coli *Enterotoxigenic E. coli, +Enteropathogenic E. coli*

Table 3. MAR index and resistance pattern of *E. coli* serotypes isolated from station 4 and 5.

Station No./ Name	E. coli serotypes	MAR Index	Resistance pattern
Station 4/ Thevara	O22#	0.66	A Ak K Nv OSTVa
	O22#	0.66	Ak C K Nv OSTVa
	O22#	0.66	A C K Nv OSTVa
	O22#	0.5	C Nv OSTVa
	O102	0.83	AAk C K Na Nv OSTVa
	O117**	0.83	AAkCfKNaNvOSTVa
	O2#	0.66	AAkKNvOSTVa
	O105	0.5	KNvOSTVa
Station 5/ Barmouth	O60	0.5	KNvOSTVa
	O116*	0.58	ANaNvOSTVa
	O150	0.75	AAkNvKNvOSTVa
	O132	0.75	AAkGKNvOSTVa
	O135	0.5	ANaNvOTVa
	O15**	0.75	AAkKNvOSTVa
	O29	0.75	ACfKNaNvOSTVa
	O9	0.75	ACfKNaNvOSTVa
	O9	0.75	AkCfKNaNvOSTVa
	O9	0.5	AKNaNvOVa
	O88	0.5	AKNvOTVa
	O88	0.58	ACfKNvOTVa
	O80	1.0	AAkCCfGKNaNvOSTVa
	O131	0.75	ACfkNaNvOSTVa
	O66	0.5	KNvOSTVa
	O30	0.75	ACCfKNaNvOSVa
	O20+	0.66	AAkKNvOSTVa
	UT***	0.5	KNvOSTVa
	UT	0.58	KNaNvOSTVa
	UT	0.5	KNvOSTVa

*# Uropathogenic E. coli * Enterotoxigenic E. coli **Eneterohaemorrhagic E. coli, +Enteropathogenic E. coli ,***Uuntypable*

Figure 1. Cochin estuary map showing sampling locations

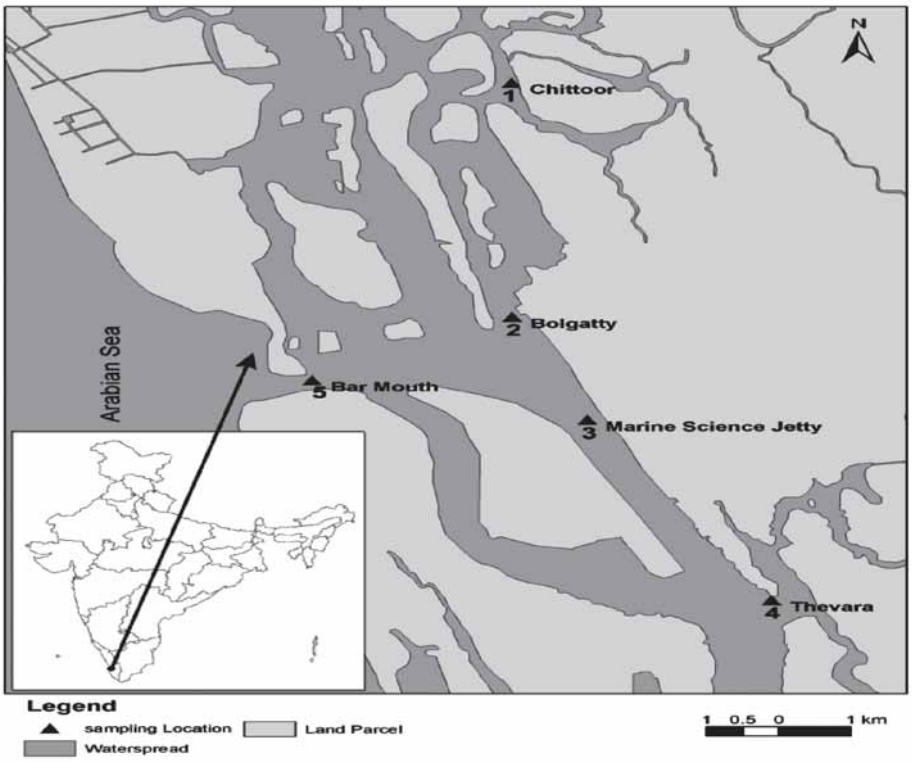

Table 4. Multiple antibiotic resistance index of different sampling stations

Sampling station No./ Name	No. of isolates	% of isolates resistant to seven or more antibiotic	MAR index
1. Chittoor	31	90.32	0.68
2. Bolgatty	10	40.00	0.46
3. Off Marine Science Jetty	12	75.00	0.64
4. Thevara	8	75.00	0.66
5. Bar mouth	20	65.00	0.64

Table 5. Numbers and percentage of *E. coli* from each station resistant to the antibiotic tested.

Sampling Site	No. of isolates	Percentage of resistance to Antibiotics											
		*A	Ak	C	Cf	G	K	Na	Nv	O	S	T	Va
1	31	70.96	64.5	3.22	41.93	19.35	96.77	41.93	100	87.09	87.09	90.3	90.3
2	10	40.00	0	0	0	0	60	0	100	100	90	70	100
3	12	41.66	66.66	25	41.66	16.66	91.66	33.33	100	100	66.66	91.66	100
4	8	62.5	62.5	50	12.5	0	87.5	25	100	25	100	100	100
5	20	70.00	25.00	15	40	15	75	50	65	85	65	65	85
Total	81	62.	50	10	30	14	85	36	91	84	80	83	93

*A (Ampicillin), Ak (Amikacin),C (Chloramphenicol),Cf (Ciprofloxacin), G (Gentamycin) K(Kanamycin), Na (Nalidixic acid), Nv(Novobiocin), O (Oxytetracyline), S (Streptomycin), T (Tetracycline), Va (Vancomycin).