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Research Article

Multidrug resistant and extended spectrum β-lactamase producing *E.coli* isolated from coastal waters of Bay of Bengal

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Keywords

Marine *E. coli*,
Mutlidrug resistant,
Cell hydrophobicity test,
ESBL,
Precipitation.

Abstract

The study deals with distribution of mutlidrug resistant *E.coli* in coastal waters of Bay of Bengal at Parangipettal to Cuddalore stretch. 150 strains isolated from water and sediment samples were tested against 11 antibiotics namely Ampicillin (AMP) - 25µg, Cefuroxime (CXM) - 30µg, Amoxcillin (AMC) - 30µg, Cefpodoxime (CPD) - 10µg, Cephalexin (CN) - 30µg, Doxycycline (DO) - 30µg, Levofloxacin (LE) - 5µg, Gentamicin (GEN) - 10µg, Ciprofloxacin (CF) - 5µg, Norfloxacin (NX) - 10µg and Ofloxin (OF) - 5µg. In the present study for marine *E. coli* isolates the highest resistance was observed against AMP (66%) followed by CPD (45.3%), CN (38.6%), CXM (34%), LE, CF and OF (26.6%), GEN and NX (19.3%), AMC (15.3%) and low resistance was observed to DO (0%). Cell hydrophobicity test for the marine isolates showed 32% of the isolates showed precipitation to least concentration of ammonium sulphate *i.e* at 0.2M concentration itself. Whereas 20%, 44.1% and 4% of the isolates showed precipitation to ammonium sulphate at a concentration of 0.4M, 1.0M and1.2M respectively out of a total of 150 isolates tested. Regarding ESBL production in the marine isolates 24 out of 150 strains (16%) were found to be positive.

Introduction

Coliform bacteria are described and grouped, based on their common origin or characteristics, as either Total or Fecal Coliform. The Total group includes Fecal Coliform bacteria such as *Escherichia coli (E .coli)*, as well as other types of Coliform bacteria that are naturally found in the soil. Fecal Coliform bacteria exist in the intestines of warm blooded animals and humans, and are found in bodily waste, animal droppings, and naturally in soil.

Substantial population of fecal coli forms and *E. coli* are harbored in freshwater bottom sediments, bank soils, and beach sands as well as marine sediments. Testing sediments to evaluate bacterial pollution was first proposed more than 100 years ago (Savage, 1905). *Escherichia coli* strains have been isolated 20 m above the ground from rain forest epiphytic plants (Bermudez and Hazen, 1988). More importantly, some of these environmental strains have been found to resist several antibiotics (Rivera *et al*., 1988).

Relatively little is known about the diversity of *E. coli* inhabiting sediments and *E. coli* population dynamics in that part of ecosystem. Since the gastrointestinal tract of warm-blooded animals is the primary habitat for *E. coli*, all sediment-borne *E. coli* are assumed as initially derived from fecal runoff and deposition. However, the relative diversity of *E. coli* strains found in sediments may be different from those in the overlying water column or feces of local animal populations (Atwill *et al.*, 2007). Numerous authors have observed that concentration of fecal coliforms in sediments is multiple-fold higher than in the water column. The concentrations of sediment fecal coli form were 100–1000 times greater than that of overlying waters in various aquatic environments (Van Donsel and Geldreich, 1971). Goyal *et al.*, 1977 found that fecal coliforms in sediment were from 1 to 383 times higher than in water with a median value of 10.

The dissemination of fecal matter into the water bodies by dumping of sewage dominantly from hospitals or public defecation also adds to the propagation of antibiotic resistance (Ahmed *et al*., 2010). The extensive use of antimicrobial agents has invariably resulted in the development of antibiotic resistance, which, in recent years, has become a major problem worldwide. Resistant bacteria can be transferred to humans via livestock and through wastewater from slaughterhouses, as well as wastewater from hospitals and pharmaceutical plants (Carlet *et al*., 2012). Discharge of human waste is another major reservoir of antibiotic resistant bacteria (Jayalakshmi, 1992).

Materials and Methods

Collection of samples

Marine water and sediment samples were taken from 5 sampling sites in coastal areas of Parangipettai. Samples were collected using sterile containers and were brought to the laboratory and stored at 4 °C. All the samples were brought to the laboratory immediately and analyses were made within two hours of collection. The analysis was done using composite samples.

Bacteriological analysis
Total viable count

Water and sediment samples were appropriately diluted with phosphate-buffer (pH-7) after proceeding as per guidelines mentioned above. For the enumeration of total viable bacteria, 0.1 ml portions of diluted samples were spread-plated in surface dried Zobell marine agar medium in triplicates. Plates were incubated at room temperature (Ca.27^{o}C) for 48 hrs. Bacterial colonies were counted in plates containing 30 to 300 colonies and the bacterial density was expressed as CFU/ml or CFU/g depending on the type of sample used.

Estimation of total coliforms (TC)

Water and sediment samples were monitored for coliforms as per the procedures given in USEPA (1978).

In this method, enumeration of coliforms included a presumptive, a confirmative and a completed test. 10 ml, 1 ml and 0.1 ml of appropriately diluted samples were inoculated into fermentation tubes containing Lauryl Tryptose (LT) broth (10ml of sample in to 10ml double strength medium, and 1 ml and 0.1 ml samples into 10 ml single strength medium). Tubes were incubated for 24 hrs and 48 hrs at 37^{o}C in a thermostatically controlled incubator. Formation of gas at the end of the incubation period constituted a positive presumptive test for members of the total coliform group.

In the confirmed test, a loopful of growth from each positive presumptive tube was transferred to tubes of Brilliant Green Lactose Bile (BGLB) broth. As this medium contains selective and inhibitory agents to suppress the growth of all noncoliform groups, gas production within 48 hrs at 37^{o}C was taken as positive. The density was calculated from MPN table based on number of positive tubes and represented as MPN/100 ml or MPN/g.

Occasionally the confirmed data were verified employing completed test in which a loopful of growth in the medium was streaked on Eosin Methylene Blue (EMB) agar plates and incubated at 37^{o}C for 24 hrs. Typical (Golden green metallic sheen or reddish purple colour with nucleation) and atypical (red, pink or colourless, unnucleated and mucoid) colonies were tested for gas production in LT broth fermentation tubes. For gram negative rods, gas production constitutes a positive completed test. The density of total coliforms was calculated from MPN table.

Faecal coliforms (FC)

About 2-3 loopful of growth medium from positive tubes of LT broth was inoculated in to EC broth and incubated at 44.5^{o}C for 24 hrs. Formation of gas in any quantity in the inverted vial was recorded as confirmed faecal coliforms (FC) for the purpose of MPN counting (Geldreich, 1966).

E.coli

About 2-3 loopful of culture from positive EC fermentation tubes were streaked on EMB agar plates and incubated at 35^{o}C for 24 and 48 hrs. 1 or 2 well isolated typical colonies (nucleated with or without sheen) were tested for IMVIC test series (Indole-Methylred-Vokes Proskauer- Citrate utilization) and plates with IMVIC pattern ++--/ -+-- were considered positive for *E.coli*. To avoid errors due to false results isolates were again examined for gas production in LT fermentation tubes and the positive results were used to calculate *E.coli* density from an appropriate MPN table. Further confirmation of *E.coli* was done in the biochemical characteristics given by Edwards and Ewing (1972) (Table 1).

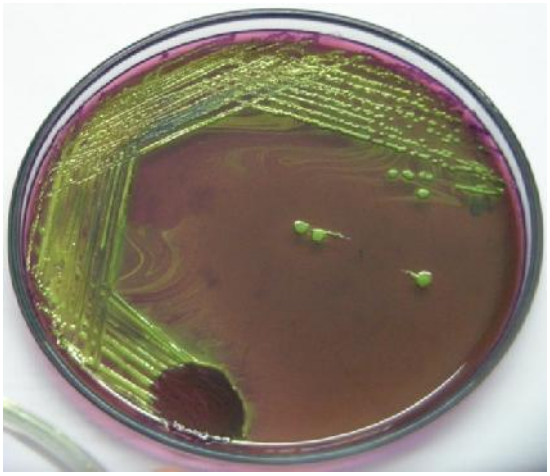

Fig. 1: Isolation of marine E. coli on EMB agar plate

Antimicrobial susceptibility testing

Antibiotic susceptibilities of the isolates were determined by the well diffusion method using Muller Hinton agar. 150 isolates from water and sediment samples were tested against 11 antibiotics namely Ampicillin (AMP) - 25µg, Cefuroxime (CXM) - 30µg, Amoxcillin (AMC) - 30µg, Cefpodoxime (CPD) - 10µg, Cephalexin (CN) - 30µg, Doxycycline (DO) - 30µg, Levofloxacin (LE) - 5µg, Gentamicin (GEN) - 10µg, Ciprofloxacin (CF) - 5µg, Norfloxacin (NX) - 10µg and Ofloxin (OF) - 5µg. The results were explained using Clinical and Laboratory Standards Institute criteria (CLSI, 2006).

Cell surface hydrophobicity

Cell surface hydrophobicity of the environmental (marine) isolates was determined by using Salt Aggregation Test (SAT) (Raksha *et al*., 2003; Siegfried *et al*., 1994 and Siegfried *et al*., 2007). Ten µl of the log phase culture suspension made in phosphate buffer saline pH – 7.4 was mixed with equal amount of ammonium sulphate solution of different molar concentrations (0.2, 0.4, 1, 1.4, 2m) on a glass slide, and the visible clumping or aggregation of the organism was observed for one min. while rotating. The highest dilution of ammonium sulphate solution giving visible clumping of isolates was considered as SAT value (Siegfried *et al*., 1994). Strains that had SAT value less than or equal to 1.4 m were considered hydrophobic. But strains showing aggregation in 0.002 m phosphate buffer alone (PH 6.8) were considered as auto agglutination (Sharma *et al*., 2007).

Disc susceptibility test to screen for ESBL

All environmental (marine) isolates were screened for ESBL production using three indicator cephalosporins, namely ceftazidime (30µg), cefotaxime (30µg) and cefpodoxime (30µg). The isolates were considered to be resistant if the diameter of the inhibition zone for ceftazidime, cefotaxime or cefpodoxime was ≤22 mm, ≤27 mm or ≤17 mm, respectively. The strains that showed resistance to at least one of the three cephalosporins were tested further using phenotypic confirmation methods as per Srisangkaew and Vorachit (2004) and CLSI (2006).

Double disc synergy test (DDST)

E. coli isolates showing resistance to any of the three indicator cephalosporins were tested for ESBL production by the DDST. Cefotaxime (30µg), cefuroxime (30µg), cefpodoxime (30µg) and amoxicillin/clavulanic acid (Amoxicillin 20µg + clavulanic acid 10µg) (Hi-Media Laboratories Ltd., Mumbai, India) were used for ESBL detection (Duttaroy and Mehta (2005). Amoxicillin/clavulanic acid and third generation cephalosporin discs were placed at a distance of 20 mm from center to center on lawn cultures on Muller-Hinton agar plates. The plates were incubated at 37^{o}C overnight. Any enhancement in zone of inhibition of cephalosporins towards the amoxicillin/clavulanic acid disc was considered a positive result for an ESBL.

Results and Discussion

Coliforms in water samples

Monthly variations in TC, Fc and *E.coli* (per 100ml) in the surface water during the period of investigation are shown in Figs. 2 and 3. During the first year of study density of TC was from a minimum value of 2.23x 10^2 MPN/100ml in April, to a maximum of 4.0 x 10^4MPN/100ml in November. The population level of FC was in the range of 1.4 x 10^1(Apr) –3.5x10^3 MPN/100 ml (Nov) whereas that of *E.coli* ranged from 0.8 (Apr) to 2.9 x10^2 MPN/100 ml (Nov). In the second year minimum counts in TC, FC and *E.coli* were recorded in April 2014 and the maximum values in October 2014. Maximum TC, FC and *E. coli* population were 4.4 x 10^4, 3.2 x 10^3 and 2.5 x 10^2 MPN/100 ml respectively. Whereas the minimum density of TC, FC and *E. coli* were 2.0x 10^2, 1.1 x 10^1 to and 0.3 to MPN/100 ml respectively.

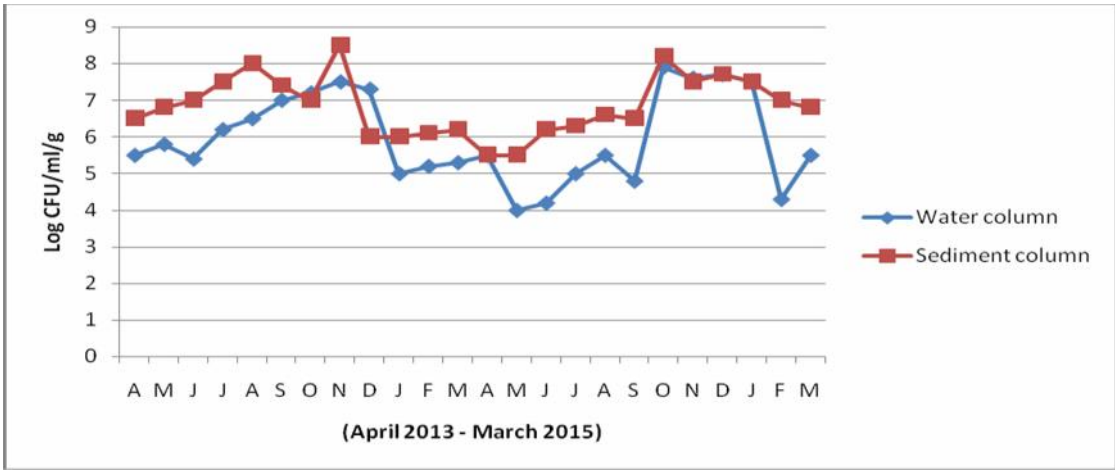

Fig. 2: Total Viable Count

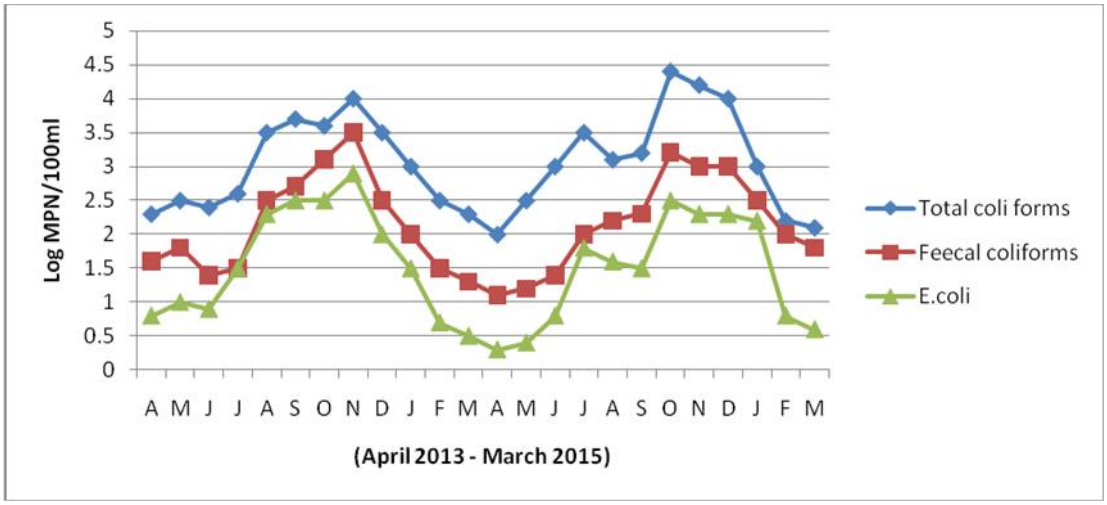

Fig. 3: Seasonal variations of coliforms in the water column

Coliforms in sediment samples

Monthly fluctuations in different coliform bacteria in the sediments are shown in figs. 4-6. During the first year lowest TC counts were recorded in May (1.0×10^3 MPN/g). The highest count was registered in November 9.0×10^4 MPN/g. whereas the maxima and minima of FC was 2.5×10^4 MPN/g and 1.0×10^2 MPN/g respectively. EC was minimum in April (8.0×10^1 MPN/g) and the maximum of 3.3×10^3. In the second year the highest densities of TC, FC and *E.coli* were 8.0×10^4 (Oct), 4.0×10^4 (Oct) and 3.4×10^3MPN/g (Oct) respectively. Similarly the minimum TC, FC and EC were 1.0×10^3 MPN/g (May), 1.0×10^2 MPN/g (May) and 8.0×10^1 MPN/g (April) respectively. It was found that the density in the sediment sample was higher compared to the water sample. Gerba (1976) through an experimental study found that longer survival of *E. coli* in the sediment was attributed to the higher content of organic matter present in the sediment than the water. The study proved that the coastal waters under study harbor coliforms including *E. coli* at a higher level indicating storm water, river water and sewage input contaminating the environment.

Garzio (2009) observed an increase in sediment *E. coli* concentrations with increasing silt content in the sediment of a Maryland creek. The same trend was observed by Niewolak (1998) across 10 observation sites on a river in Poland. On the other hand, Cinotto (2005) reported the highest median concentration of *E. coli* (2160 CFU g−1 wet) in the 0.125–0.5 mm size range of natural sediments. The effect of sediment composition was not studied in the present investigation. However same thing might be true in the present study also.

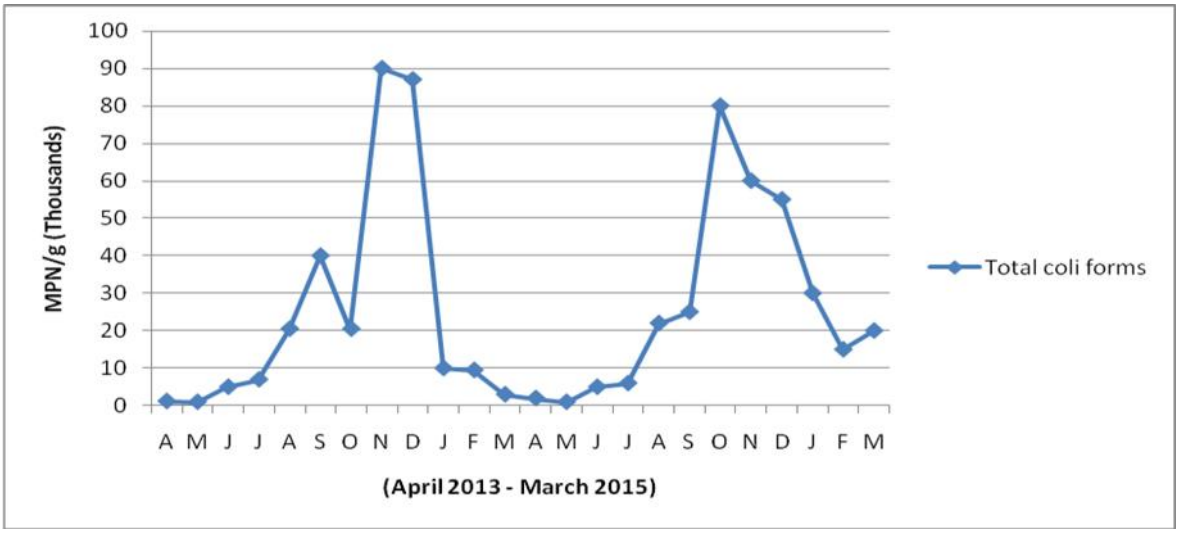

Fig. 4: Seasonal variations of total coliform (in sediment)

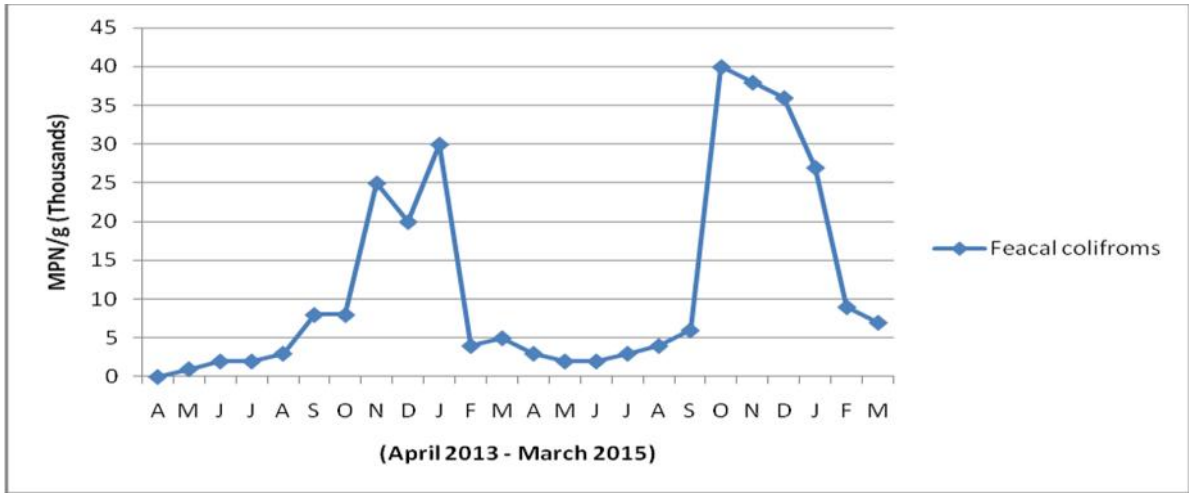

Fig. 5: Seasonal variations of faecal coliform (in sediment)

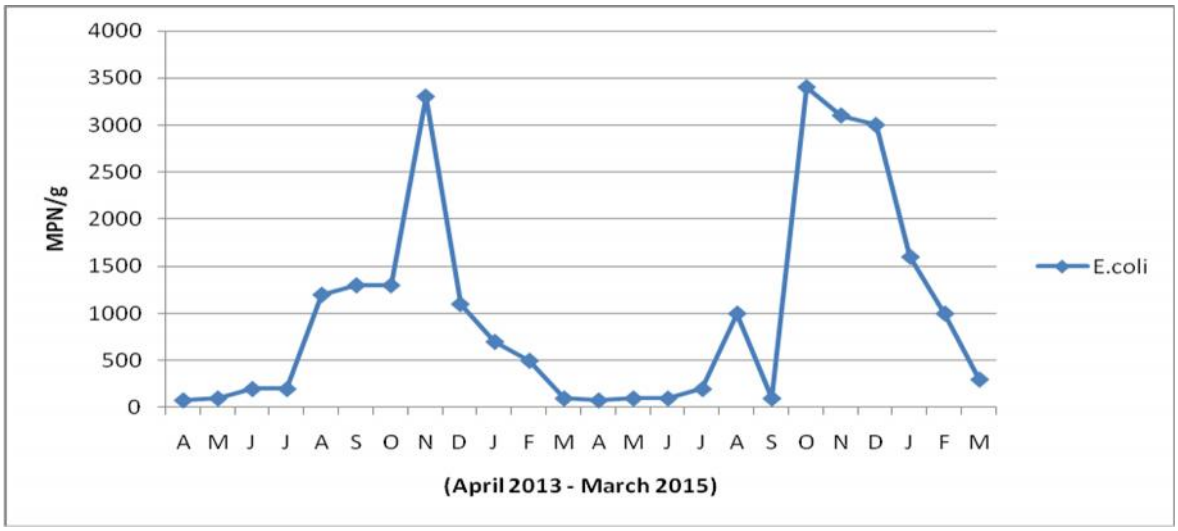

Fig. 6: Seasonal variations of *E.coli* (in sediment)

Fecal coliforms (FC) and enterococci have been reported from marine sediments (Anderson *et al*., 1997; Ferguson *et al*., 2005 and Obiri-Danso and Jones, 2000), and it has been also proposed that fecal indicator bacteria accumulated in the sediments have the potential to contaminate the overlying waters by resuspension of sediment particles (Le Fevre and Lewis, 2003). There is evidence available for fecal indicator bacteria and pathogenic bacteria can survive longer in aquatic sediments than in the overlying water column (Crabill *et al.*, 1999 and LaLiberte and Grimes. 1982).

Van Donsel and Geldreich (1971) noted that concentrations of sediment FC were 100–1000 times greater than that of overlying waters in various aquatic environments. Goyal *et al.*, (1977) found that FC in sediment was from 1 to 383 times higher than in water with a median value of 10. Similar difference was observed by Erkenbrecher (1981) in an estuary in the Chesapeake Bay. Doyle *et al.*, 1992 reported ratios of mean sediment FC densities to mean water FC densities from between 10 and 100 to 1.

Antibiotic Resistance

In the present study for marine *E. coli* isolates tested the highest resistance was observed against AMP (66%) followed by CPD (45.3%), CN (38.6%), CXM (34%), LE, CF and OF (26.6%), GEN and NX (19.3%), AMC (15.3%) and low resistance was observed to DO (0%) (Table1).

Table 1: Antibiotics resistance percentage of marine isolates

S.no	Antibiotic tested	No. of strains positive	Percent of resistance
1.	AMP	99	66 %
2.	CXM	51	34 %
3.	AMC	23	15.3 %
4.	CPD	68	45.3 %
5.	CN	58	38.6 %
6.	DO	0	0 %
7.	LE	40	26.6 %
8.	GEN	29	19.3 %
9.	CF	40	26.6 %
10.	NX	29	19.3 %
11.	OF	40	26.6 %

Regarding the antibiotic resistance pattern, 12.5% of the strains showed resistance to single antibiotic (i.e) 5.3 % to AMP, 3.3% to CPD 2.6% to CXM and 1.3% towards CN. Regarding resistance to two antibiotics 40.5%of strains showed 6 different patterns. AMP – CXM (16%), CPN – CN (10.6%), CXM-AMC (5.3%) AMP- CN (4.6%), AMP-CP (2.6%) and CXM – CPD (1.3%) 16.6 % strains were found to be resistant towards three antibiotics combination in which the pattern AMP-CPN-CN shared the highest percentage incidence (10%), followed by AMP-CXM-CPD (4%) and CXM-CPN-CN (1.3%). Likewise two patterns were found in 3.3% of strains resistance towards four antibiotics AMP-CXM-CPD-CN (2%), AMP-CXM-AMC-CPD (1.3%). Five antibiotics (2.6) by AMP-CXM-AMC-CPD-CN (1.3%), LE, GEN-CF-NX-OF (1.3%). Six antibiotics 17.9 % by AMP-LE-GEN-CF-NX-OF (11.3%), CN-GEN-LE-CF-NX-OF (6.6) and seven antibiotics by AMP-AMC-CPD-CN-LE-CF-OF (7.3%) strains (Table 2).

Table 2: Antibiotic resistance pattern and percent resistant to antibiotics of *E. coli* isolates from marine samples

S.no	Antibiotic resistant pattern of marine strains	No. of strains	Percentage of resistance
1.	AMP	8	5.3%
2.	CPD	5	3.3%
3.	CN	2	1.3%
4.	CXM	4	2.6%
5.	AMP-CPD	4	2.6%
6.	AMP-CN	7	4.6%
7.	CXM-CPD	2	1.3%
8.	CPD-CN	16	10.6%
9.	CXM-AMC	8	5.3%
10.	AMP-CXM	24	16%
11.	AMP-CPD-CN	15	10%
12.	AMP-CXM-CPD	6	4%
13.	CXM-CPD-CN	2	1.3%
14.	AMP-CXM-CPD-CN	3	2 %
15.	AMP-CXM-AMC-CPD	2	1.3%
16.	AMP-CXM-AMC-CPD-CN	2	1.3%
17.	LE-GEN-CF-NX-OF	2	1.3%
18.	AMP-LE-GEN-CF-NX-OF	17	11.3%
19.	CN-GEN-LE-CF-NX-OF	10	6.6%
20.	AMP-AMC-CPD-CN-LE-CF-OF	11	7.3%

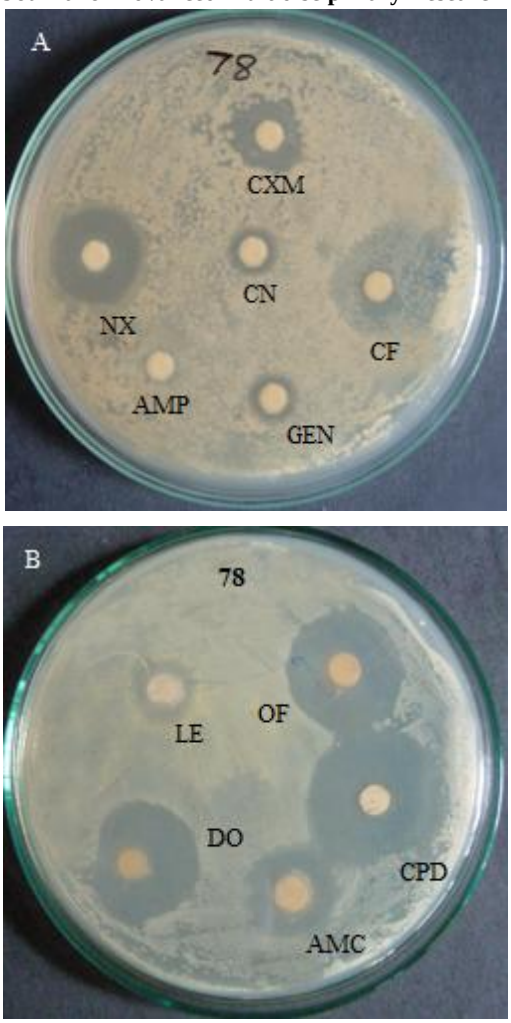

Fig. 7: Marine strain antibacterial susceptibility testing for marine isolate no.78 (Plate A and B) for tested 11 antibiotics viz. Ampicillin (AMP) - 25µg, Cefuroxime (CXM) - 30µg, Amoxcillin (AMC) - 30µg, Cefpodoxime (CPD) - 10µg, Cephalexin (CN) - 30µg, Doxycycline (DO) - 30µg, Levofloxacin (LE) - 5µg, Gentamicin (GEN) - 10µg, Ciprofloxacin (CF) - 5µg, Norfloxacin (NX) - 10µg and Ofloxin (OF) - 5µg.

Chandran *et al.*, 2013 evaluated the survival response of multi-drug resistant enteropathogenic *E. coli* and *Salmonella paratyphi* to the salinity fluctuations induced by a saltwater barrier constructed in Vembanadu lake, India showed that an enhanced survival exhibited by the multi-drug resistant enteropathogenic *E. coli* and *S. paratyphi* over a wide range of salinity levels suggested that they are able to remain viable for a very long time at higher densities in all seasons of the year in Vembanadu lake irrespective of saline concentrations. Doughari et al., 2011 reported a multidrug resistance index (MDRI) showed that the MDRI values ranged between 7.00 to 33.00%, with isolates from wastewater samples exhibiting the highest MDRI values. The present study also endorsed these results.

Cell surface hydrophobicity

In the present study cell hydrophobicity test for the marine isolates showed 32% of the isolates showed precipitation to least concentration of ammonium sulphate i.e at 0.2M concentration itself. Whereas 20%, 44.1% and 4% of the isolates showed precipitation to ammonium sulphate only at a concentration of 0.4M, 1.0M and1.2M respectively out of a total of 150 isolates tested (Table -3).

Table 3: Cell surface hydrophobicity test for marine strains

Concentration of Ammonium sulphated tested (in Molar)	**0.2**	**0.4**	**1.0**	**1.4**	**2**
Percentage of cell precipitated at the concentration	32%	20%	44.1%	4%	-
Strains	48 strains	30 strains	66 strains	6 strains	-

Cell surface hydrophobicity enhances the adherence of bacterial cells to host cell surfaces including mucosal epithelial cells and confers them with resistance to phagocytosis by host cells. In this study, greater number of the *E. coli* strains demonstrated hydrophobicity. Sunman et al., 2001 and Raksha et al., 2003 studies on urinary tract infection cases reported high rate of exhibition of cell surface hydrophobicity by some pathogenic strains of *E. coli*. The presence of hydrophobic strains of *E. coli* in this water sources is an indication that the water could be a potential source of agents of urinary tract infections or gastroenteritis if consumed. Doughari et al., 2011 reported 81% exhibited cell surface hydrophobicity, from *E. coli* isolated from water and wastewater samples in Cape Town, South Africa.

ESBL production

In the present study ESBL production in the marine isolates was 24 out of 150 strains (i.e.) 16% were found to be positive. There have been varied prevalence reports of ESBL from Indian hospitals, ranging from 31.7% - 81.0% by many researchers. Umadevi *et al*., 2011 reported 81%. Suryawanshi *et al.,* 2011 observed 61.76% ESBL mediated resistance. β-lactamase are enzymes that open β-lactamase ring of antibiotics like pencillum and cephalosporins and destroy their activity (Danel et al., 1997). According to Medeiros et al., 1997, a new β-lactamase causing resistance to that antibiotic to emerged. Due to increased spectrum of activity especially against expanded spectrum cepholosporins these enzymes were called as extended spectrum β-lactamase (ESBL) (Bradford, 2001). The study indicated higher level of multiple antibiotic resistance acquired by *E. coli* cells. Cell surface hydrophobicity and ESBL production might confer them resistance besides many other possible mechanisms. The study indicated the threat the coastal environ as well as the stakeholders are facing.

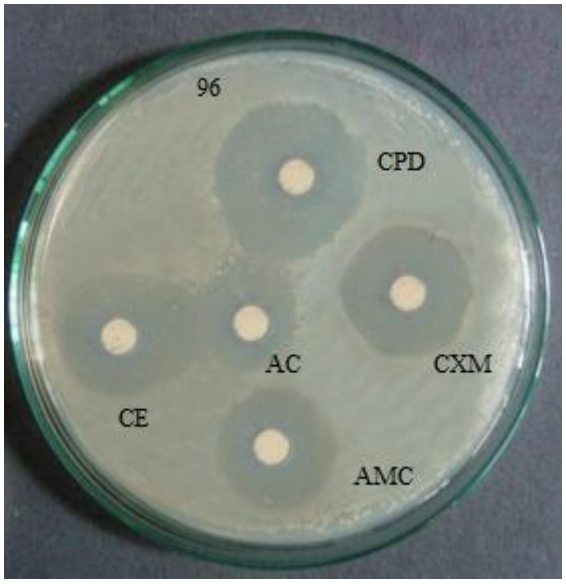

Fig. 8: Screening for extended spectrum of beta lactamase (ESBL) production by marine *E.coli* isolate No. 96 using double disk synergy test (DDST) – Cefotaxime (CE), Amoxicillin + Clavulanic acid (AC), Amoxicillin (AMC), Cefuroxime (CXM), Cefpodoxime (CPD).

References

Ahmed, M.O., P.D. Clegg, N.J. Williams, K.E. Baptiste and M. Bennett, 2010. Antimicrobial resistance in equine faecal *Escherichia coli* isolates from North West England. *Ann. Clin. Microbiol. Antimicrob.*, 9:12.

Anderson, S. A., S. J. Turner and G. D. Lewis, 1997. Enterococci in the New Zealand environment: implications for water quality monitoring. *Water Sci. Technol*., 35:325–331.

Atwill, R., D. Lewis, M. Pereira, M. Huerta, R. Bond, S. Ogata and P. Bass, 2007. Characterizing freshwater inflows and sediment reservoirs of fecal coliforms and *E. coli* at five estuaries in Northern California. University of California School of Veterinary Medicine and Cooperative Extension in Sonoma and Marin Counties, Davis, CA. http://ucce.ucdavis.edu/files/filelibrary /2161/41255. pdf (accessed February 20, 2011).

Carlet, J., V. Jarlier and S. Harbarth, 2012. Ready for a world without antibiotics? the pensieres antibiotic resistance call to action. In : Antimicrobial Resistance and Infection Control. 1:11.

Chandran, A., P. S. Suson, A. P. Thomas, M. Hatha and A.Mazumder, 2013. Survival of multi-drug resistant enteropathogenic *Escherichia coli* and *Salmonella* paratyphi in Vembanadu lake as a function of saltwater barrier along southwest coast of India. *J. Water Health*, 324-332.

Cinotto, P. J., 2005. Occurrence of fecal-indicator bacteria and protocols for identification of fecal contamination sources in selected reaches of the West Branch Brandywine Creek, Chester County, Pennsylvania. U.S. Geological Survey Scientific Investigations Report 2005–5039.

Clinical and Laboratory Standards Institute (CLSI), 2006. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; Approved guideline (M45-A). Clinical and Laboratory Standards Institute, Wayne, PA.

Crabill, C., R. Donald, J. Snelling, R. Foust and G. Southam, 1999. Impact of sediment fecal coliform reservoirs on seasonal water quality in Oak Creek, Arizona. *Water Res.*, 33: 2163–2171.

Doughari, H. J., P. A. Ndakidemi, I. S. Human and S. Benade, 2011. Virulence factors and antibiotic susceptibility among verotoxic non O157: H7 *Escherichia coli* isolates obtained from water and wastewater samples in Cape Town, South Africa. *Afr. J. Biotechnol.*, 10(64): 14160-14168.

Doyle, J. D., B.Tunnicliff, K. Kramer, R. Kuehl and S.K. Brickler, 1992. Instability of fecal coliform populations in waters and bottom sediments at recreational beaches in Arizona. *Water Res.*, 26: 979–988.

Duttaroy, B., Mehta, S. 2005. Extended- spectrum beta lactamases (ESBL) in clinical isolates of *Klebsiella pneumonia* and *Escherichia coli. Indian J. Pathol. Microbiol.*, 48: 45 48.

Edwards, R. and W.N. Ewing, 1972. Identification of *Enterobacteriaceae*. (3rd edn.), Burgess Publishing Co., Minnesota.

Ferguson, D. M., D. F. Moore, M. A. Getrich and M. H. Zhowandai, 2005. Enumeration and speciation of enterococci found in marine and intertidal sediments and coastal water in southern California. *J. Appl. Microbiol.*, 99: 598–608.

Garzio, A., 2009. Survival of *E. coli* delivered with manure to stream sediment. Environmental Science and Policy Honors Thesis. University of Maryland, College Park.

Geldreich, E.E. 1966. Sanitary significance of fecal coliforms in the environment. FWPCA Publ. WP-20-3 (Nov.). U.S. Dep. Interior, Washington, D.C.

Gerba, C. P., and J. S. McLeod, 1976. Effect of sediments on the survival of *Escherichia coli* in marine waters. *Appl. Environ. Microbiol.*, 32: 114–120.

Goyal, S. M., C. P. Gerba and G. L. Melnick, 1977. Occurrence and distribution of bacterial indicators and pathogens in canal communities along the Texas Coast. *Appl. Environ. Microbiol.*, 43: 139–149.

Goyal, S. M., C. P. Gerba and G. L. Melnick, 1977. Occurrence and distribution of bacterial indicators and pathogens in canal communities along the Texas Coast. *Appl. Environ. Microbiol.*, 43: 139–149.

LaLiberte, P. and D. J. Grimes. 1982. Survival of *Escherichia coli* in lake bottom sediment. *Appl. Environ. Microbiol.*, 43: 623–628.

Le Fevre, N. M. and G. D. Lewis, 2003. The role of resuspension in enterococci distribution in water at an urban beach. *Water Sci. Technol.*, 47: 205– 210.

Niewolak, S., 1998. Total viable count and concentration of enteric bacteria in bottom sediments from the Czarna Ha´ncza River, Northeast Poland. *Pol. J. Environ. Stud.*, 7: 295–306.

Obiri-Danso, K. and K. Jones. 2000. Intertidal sediments as reservoirs for hippurate negative campylobacters, salmonellae, and fecal indicators in three EU recognized bathing waters in north west England. *Water Res.*, 34: 519–527.

Raksha, R., H. Srinivasa, R.S. Macaden, 2003. Occurrence and characterization of uropathogenic *Escherichia coli* in urinary tract infections. *Indian J. Med. Microbiol.*, 21: 102-107.

Rivera, S., T. C. Hazen and G. A. Toranzos. 1988. Isolation of fecal coliforms from pristine sites in a tropical rain forest. *Appl. Environ. Microbiol.*, 54: 513–517.

Savage, W. G., 1905. Bacteriological examination of tidal mud as an index of pollution of the river. *J. Hygiene*, 5, 146–174.

Sharma S, G.K. Bhat and S. Shenoy 2007. Virulence factors and drug resistance in *Escherichia coli* isolated from extraintestinal infections. *Indian J. Med. Microbiol.*,; 25: 369-73.

Sharma, S., G.k. Bhat and S. Shenoy, 2007. Virulence factors and drug resistance in Escherichia coli isolated from extraintestinal infections. *Indian J. Med. Microbiol.*, 25(4): 369-373.

Siegfried L, M. Kmetova, H. Puzove, M. Molokacova and J. Filka. 1994. Virulence- associated factors in *Escherichia coli* strains isolated from children with urinary tract infections. *J Med. Microbiol.*, 41: 127-132.

Singhal, S., T.Mathur,A. Rattan, D.J. Upadhyay, S.Chugh, and R.Gaind, 2005.. Evaluation of methods for AmpC beta-lactamase in Gram negative clinical isolates from tertiary care hospitals. *Indian J. Med. Microbiol.*, 23 : 120-4.

Sunman, E., G. Bhat K, B.M. Hegde, 2001. Bacterial adherence and immune response in recurrent UTI. *Int. J. Gynaecol. Obstet.*, 75: 263-268.

Van Donsel, D. J. and E. E. Geldreich, 1971. Relationships of salmonellae to fecal coliforms in bottom sediments. *Water Res.*, 5: 1079–1087.

Van Donsel, D. J. and E. E. Geldreich, 1971. Relationships of salmonellae to fecal coliforms in bottom sediments. *Water Res.*, 5: 1079–1087.