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Occurrence and distribution of multiple antibiotic-resistant bacteria of Enterobacteriaceae family in waters of Veraval coast, India

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Abstract

Current investigation was aimed to the assess occurrence and distribution of multiple antibiotic-resistant bacteria of the Enterobacteriaceae family in surface and bottom waters along the Veraval coast. Comparative prevalence of drug-resistance pattern among the isolates was determined using a battery of antibiotics. Fecal coliforms, *Escherichia coli, Enterobacter* spp., *Salmonella* spp. and *Klebsiella* spp. were isolated on specific media and tested for their resistance to specific antibiotics commonly used for treating human infections. Antibiotic resistance of bacteria was determined by the disc diffusion method. Of 235 isolates, 99 fecal coliforms, 30 *Escherichia coli*, 43 *Enterobacter* spp., 56 *Klebsiella* spp., and seven *Salmonella* spp. were examined. Multiple resistance, i.e. resistance to more than two antibiotics, occurred in almost 100% of *Enterobacter* spp., 86.8 % of fecal coliforms, 85.7% of *Klebsiella* spp., 56.6% of *Escherichia coli*, and 42.8% of *Salmonella* spp. In total, 97% of the isolates exhibited multi drug resistance character and all the isolates had a very high multiple antibiotic-resistance index, suggesting the origin of the isolates to be of high antibiotic usage. Antibiotic resistance indices were found to be highest for *Enterobacter* spp. and lowest for *Salmonella* spp. The study revealed that imprudent use of antibiotics in humans, aquaculture, poultry and livestock may pose high ecological risk to the Veraval coastal waters. There is a need to control anthropogenic activities in coastal water bodies to avert the occurrence of multiple antibiotic resistant bacteria.

Key words: antibiotic resistance, Enterobacteriaceae, multiple drug resistance.
Abbreviations: ARI, antibiotic resistance indices; AMR, antimicrobial resistance; ED, Euclidian distance; MAR, multiple antibiotic resistance; MDR, multidrug resistance.

Introduction

The presence of antibiotic resistance bacteria associated with coastal water has been a major public health concern (Servais et al. 2009). The occurrence of antibiotic resistant bacteria is increasing in aquatic environments (Al-Bahry et al. 2009). Many studies have been carried out to investigate the occurrence and distribution of antibiotic resistant bacteria in water basins (Mudryk et al.1998; Chuanwu et al. 2009; Yin et al. 2013). The sources of fecal indicator bacteria to marine regions consist of both human waste (Gerba 2000) and animal waste including wildlife (Dunlap, Thies 2002) and domestic animals (Meals, Broun 2006).

Overuse of antibiotics in human and animals for treatment leads to release of antibiotics and antibiotic resistant strains into the environment (Silbergeld et al. 2008; Ghafur et al. 2010). The main cause of multi drug resistance (MDR) bacteria is the overuse and misuse of antibiotics in human medicine, veterinary medicine, agriculture and aquaculture (McManus, Stockwell 2001). Microbial indicators have been used worldwide as a tool to indicate the contamination of water by human wastes (Sivanandham et al. 2012). The anthropogenic impacts on coastal areas through inflow of domestic effluents can be evaluated by measuring fecal bacteria levels present in water and the occurrence of bacterial resistance to antimicrobial agents (Fernandes et al. 2010). Veraval is the one of the largest fish landing sites and is surrounded by number of small to medium scale industries. Coastal waters of Veraval appears to be pink in colour due to heavy marine pollution as effluents from fish processing industries, and as municipal sewerage from the towns discharged intothe nearby harbor area without prior treatment. The present study investigates the occurrence and distribution of multiple antibiotic-resistant bacteria of Enterobacteriaceae family in waters of the Veraval coast, India.

Materials and methods

Description of the study site

Veraval lies on the south western coastal strip of Saurashtra, Gujarat state, at the intersection of latitude 20°55.062'N and longitude 70°21.037'E of the Arabian Sea, India. The main activity at Veraval includes the fish processing, which

provides a significant economic source to its residents and is one of the largest fishing ports in India. The Gujarat Industrial Development Corporation and Indian Rayon (a unit of Aditya Birla Nuvo Ltd.) a multi-product industrial conglomerate is located in the surrounding area of Veraval. Seven sampling stations with distance covering 0 to 5.0 km from the shore were chosen for this study to check the microbial contamination spread: station-1 (0 km from shore i.e. jetty area), station-2 (0.5 km inside to mouth harbor), station-3 (0.5 km outside to mouth harbor), station-4 (2 km right from station-3), station-5 (5 km left from station-3), station-6 (2 km from left side of shore), and station-7 (2 km from right side shore). The stations represented areas of high human impact, domestic sewage and industrial discharge, fishing and shipping activity (Fig. 1).

Sample collection

Two samples each from surface and bottom water samples were collected from seven stations at a distance 0 km to 5 km from the shoreline and 2 km from either side of shore during April 2012 (Fig. 1). Water samples for microbiological analysis were collected from surface and bottom using a Niskin water sampler (Dinesh Kumar et al. 1991) and aseptically transferred into sterilized glass bottles and transported to the laboratory by keeping them in icebox and processed within 4 h. Analysis was performed in triplicate for each station.

Bacterial counts

Counting of bacteria was carried out by using standard procedures. Water sample and sediment samples were prepared in 50% of aged sea water separately and then serially diluted using the same diluents. Diluents 10^2 to 10^4 were spread plated for enumeration of total viable count, fecal pollution indicator (total and fecal coliforms), *Escherichia coli*, *Shigella*, *Salmonella*, *Proteus* and *Klebsiella*, *Vibrio cholerae*, *Vibrio parahemolyticus*, *Pseudomonas aeruginosa* and *Streptococcus fecalis* like organisms. Nutrient agar prepared in 10% seawater was used for determining of total viable count. Total coliforms were counted on MacConkeys agar, which was incubated for 24 h at 37 °C. All colonies showing pink-red colour were counted as total coliforms. Fecal coliforms were counted on m-Fc agar with rosolic acid as an indicator and incubated at 44.5 °C for 24 h. Typical blue-green colonies were counted for fecal coliforms. *Escherichia coli* were counted as yellow colour colonies on M7Hrfc agar. For counting of *Shigella*, *Salmonella,* as well as *Proteus* and *Klebsiella*, xylose lysine deoxycholate agar for 24 h at 37 °C was used. Colonies with pink/red coloration were counted as *Shigella*, pink with black centered colonies as *Salmonella*, and yellow mucoid colonies as *Proteus* and *Klebsiella,* respectively. Yellow colonies with < 2 mm diameter were quantified as *Vibrio cholerae* and green colonies as *Vibrio parahemolyticus* on thiosulphate citrate bile salts sucrose agar. All colonies growing on Pseudomonas agar with glycerol were counted as *Pseudomonas aeruginosa* and maroon colonies on M-Enterococcus agar were considered as *Streptococcus fecalis*. Isolated strains were re-streaked and pure cultures stored at 4 °C, and used for further studies in order to determine biochemical characteristics and antibiotic resistance. All dehydrated media used in this study for microbiological analysis were obtained from Himedia, Mumbai, India and prepared according to manufacturer's instructions.

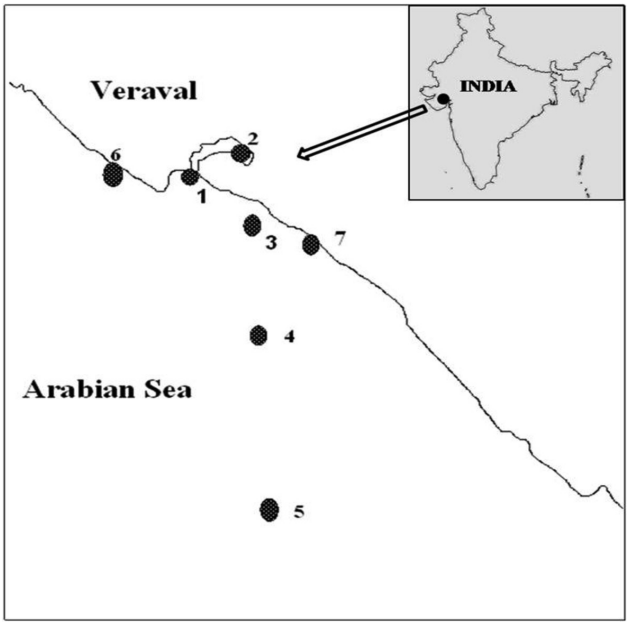

Fig. 1. Map showing the sample location of the study in the Veraval coast.

Biochemical characterization

To carry out biochemical analysis, purified isolates were grown in Nutrient broth for 18 h and then used for further biochemical identification. Purified isolates were subjected to a series of 25 biochemical tests for the identification and differentiation of commonly encountered genera and species of Enterobacteriaceae within 24 h using the KB003 Hi25TM Enterobacteriaceae Identification kit (Hi-Media, Mumbai; Khan et al. 2011). Further morphological and biochemical properties of the isolates were also studied according to Bergey's manual of determinative bacteriology (Holt et al. 1997) for identification of the isolates.

Antimicrobial susceptibility testing

Antibiotic resistance of bacteria of Enterobacteriaceae family was determined by the disc diffusion method (Bauer et al. 1996). The isolates were challenged with the following antibiotics (concentrations given in parentheses): ampicilin (10 µg), bacitracin (10 units), cefotaxime (30 µg), cefpodoxime (10 µg), cephalothin (30 µg), co-trimoxazole (23 µg), chloramphenicol (30 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), neomycin (30

μg), ofloxacin (5 μg), oxacillin (1 μg), penicillin G (10 units), polymyxin B (300 μg) and vancomycin (30 μg). A bacterial suspension of overnight grown cultures was prepared and turbidity was adjusted to a 0.5 McFarland standard. A sterile cotton swab was used to inoculate the bacterial suspension on the surface of a Mueller Hinton Agar plate. Antibiotic impregnated disc (Hi-Media, Mumbai) Dodeca Universal-1 (eight discs) and Hexa Universal-1 (six discs) were dispensed on the surface of inoculated agar plate and incubated overnight at 37 °C. The isolates were scored as susceptible, intermediate or resistant to a given antibiotic in duplicate by the inhibition zone diameter around the disc and according to the recommendations of the National Committee for Clinical Laboratory Standards for antimicrobial susceptibility tests (NCCLS 2002).The results were used to calculate the ARI and MAR index for total number of isolates as

$$\text{ARI} = y / nx,$$

where y is a number of resistant isolates, n is a number of isolates and x is a number of antibiotics (Hinton, Linton, 1983) and

$$\text{MAR index} = a/b,$$

where a is a number of antibiotics to which the isolates are resistant and b is a total number of antibiotics exposed.

Statistical analysis

Statistical analysis was performed using Statistica 7 software. Correlations between bacterial contamination and frequency of multiple antibiotic resistant bacteria with respect to different stations, and variability of multiple antibiotic resistances with respect to types of bacteria from the study area, were calculated using the Pearson's correlation coefficient (at 5% significance level). Variation in resistance among the types of bacteria against sixteen antibiotics and also between the different classes of antibiotics was determined using ANOVA. A dendrogram was constructed to organize observed data into meaningful structures to illustrate similarities among antibiotic resistance profile pattern for gram negative organisms (Rose et al. 2009). A dissimilarity matrix of antibiograms was expressed in Euclidian distance (ED), which estimated differences in antibiotic sensitivity pattern.

Results

Bacterial counts

The distribution of microbial populations among stations is shown in Table 1. Of 235 isolates, the maximum 113 isolates was recorded at station-3, while minimum from station-7. The overall occurrences of microbial population were larger 0.5 km from shore (station-3) and lower 5 km offshore (station-7). Fecal coliforms and *Escherichia coli* were recorded at station-1 (0 km) from shore. At station-2, there were populations of fecal coliforms and *Enterobacter* spp., *Escherichia coli, Klebsiella* spp. and *Salmonella* spp. At station-4 there were only fecal coliforms and Klebsiella spp. Station-5, station-6 and 7 had only fecal coliforms. Of 235 total isolates, 99 fecal coliforms, 30 *Escherichia coli*, 43 *Enterobacter* spp., 56 *Klebsiella* spp. and seven *Salmonella* spp. were examined in the antibiotic sensitivity test by Kirby Bauer disc diffusion method.

Biochemical characterization

With the help of morphological as well as biochemical criteria using the KB003 Hi25TM Enterobacteriaceae Identification kit, different genera and species which constitute total coliform, fecal coliform, *Escherichia coli, Salmonella* spp. and *Klebsiella* spp. were identified and confirmed (Table 2).

Antibiotic sensitivity test

An antibiotic is a kind of ubiquitous contaminant in the aquatic environment and not surprisingly, antibiotics will enter the coastal environment with river water, industrial effluents and sewage discharge. Table 3 describes resistance of isolates to different antibiotics. Resistance to bacitracin and penicillin G was observed in 100% of the isolates, followed by oxacillin (82.1%), ampicilin (80.0%) and vancomycin (62.1%). No resistance to polymyxin B, gentamicin, ofloxacin, co-trimoxazole and cefpodoxime was observed. Multiple resistance to more than two antibiotics occurred in 100% *Enterobacter* spp., 86% fecal coliforms, 85.7% *Klebsiella* spp., 56.6% *Escherichia coli*, and 42.8% *Salmonella* spp. (Table 4). *Escherichia coli* possessed maximum 100% resistance to β-lactam, polypeptides

Table 1. Distribution of bacteria belonging to Enterobacteriaceae family among stations. Values in brackets indicate percentage of total count

Station No.	Total number of isolates	Fecal coliforms	*Escherichia coli*	*Enterobacter* spp.	*Klebsiella* spp.	*Salmonella* spp.
1	22	10.0 (4.2)	5.0 (2.1)	7.0 (2.9)	–	–
2	54	20.0 (8.5)	10.0 (4.2)	15.0 (6.3)	6.0 (2.5)	3.0 (1.2)
3	113	33.0 (14.2)	15.0 (6.3)	21.0 (8.9)	40.0 (17.0)	4.0 (1.7)
4	20	10.0 (4.2)	–	–	10.0 (4.2)	–
5	10	10.0 (4.2)	–	–	–	–
6	15	15.0 (6.3)	–	–	–	–
7	1	1.0 (0.4)	–	–	–	–

Table 2. Biochemical properties confirming the status of the respective pathogenic groups using the Enterobacteriaceae Identification Kit. +, indicates positive (more than 90%); –, indicates negative (more than 90%); v, indicates 11 to 89% positive

Test	Fecal coliforms	*Enterobacter* spp.	*Escherichia coli*	*Klebsiella* spp.	*Salmonella* spp.
ONPG	+	+	+	+	–
Lysine	–	+	+	+	+
Ornithnine	+	+	v	–	–
Urease	–	–	–	+	–
TDA	v	–	–	–	–
Nitrate	+	+	–	+	+
H_2S	–	–	–	–	+
Citrate utilization	+	+	–	+	–
Voges Proskauer's	+	+	–	+	–
Methyl Red	–	–	+	v	+
Indole	v	–	+	–	–
Malonate	v	+	–	+	–
Esculin hydrolysis	+	+	v	+	–
Arabinose	+	+	+	+	–
Xylose	+	+	+	+	v
Adonitol	–	v	–	+	–
Rhamnose	+	+	v	+	–
Cellobiose	+	+	–	+	–
Melibiose	+	+	v	+	+
Saccharose	+	+	v	+	–
Raffinose	+	+	v	+	–
Trehalose	+	+	+	+	–
Glucose	+	+	+	+	+
Lactose	+	v	+	+	+
Oxidase	+	–	+	+	–

Table 3. Number of bacterial isolates from the Veraval coast and proportion (%) of resistance to different antibiotics. *, percentage calculated as from the total 235 isolates checked for AST

Antibiotic used (amount)	Class of antibiotics	Zone size interpretation (mm)#			Number of resistant isolates	Isolates with resistance (%)*
		Resistant	Intermediate	Sensitive		
Gentamicin (10 µg)	Aminogylcoside	12	13-14	15	0	0
Neomycin (30 µg)	Aminogylcoside	12	13-16	17	31	13.2
Ampicilin (10 µg)	β-lactamase	13	14-16	18	188	80.0
Penicillin G (10 units)	β-lactamase	16	–	17	235	100
Oxacillin (1 µg)	β-lactamase	10	11-12	13	193	82.1
Erythromycin (15 µg)	Macrolide	13	14-22	23	152	65.0
Cefotoxime (30 µg)	Cephalosporin	23	15-22	14	0	0
Cephalothin (30 µg)	Cephalosporin	14	15-17	18	130	55.3
Cefpodoxime (10 µg)	Cephalosporin	17	18-20	21	0	0
Vancomycin (30 µg)	Glycopeptide	14	15-16	17	146	62.1
Clindamycin (2 µg)	Lincosamide	15	16-18	19	135	57.4
Polymyxin-B (300 µg)	Polypeptide	11	–	12	0	0
Bacitracin (10 units)	Polypeptide	–	12-22	-	235	100
Co-trimoxazole (23 µg)	Sulphanamide	10	11-15	16	0	0
Ofloxacin (5 µg)	Quinolone	12	13-15	16	0	0
Chloramphenicol (30 µg)	Chloramphenicol	12	13-17	18	20	8.5

Table 4. Incidence of resistance and multiple resistances among bacteria isolated from the Veraval coast. Multi-resistant isolates were resistant to more than two antibiotics. *, expressed as mean ± standard deviation

Type of bacteria	Total number of isolates	Isolates resistant to single antibiotic		Multi-resistant isolates		Antibiotic resistance index
		(number)*	(%)	(number)	(%)	
Fecal coliforms	99.0	3.0 ± 1.15	3.0	86.0	86.8	0.05
Enterobacter spp.	43.0	0.0	0.0	43.0	100	0.07
Escherichia coli	30.0	12.0 ± 1.0	40.0	17.0	56.6	0.04
Klebsiella spp.	56.0	8.0 ± 0.0	14.2	48.0	85.7	0.06
Salmonella spp.	7.0	4.0 ± 1.0	57.1	3.0	42.8	0.03
Total	235.0	27.0	–	197.0	–	–

and macrolides and *Salmonella* spp. possessed maximum resistance against macrolides and polypeptides (Table 5). The highest ARI value 0.07 was calculated for *Enterobacter* spp., followed by 0.06 for *Klebsiella* spp. then ARI decreased to 0.05, 0.04 and 0.03 for fecal coliforms, *Escherichia coli* and *Salmonella* spp., respectively. Moreover, 97% of the isolates showed more than 0.2 MAR index.

Statistical analysis

A positive correlation between the degree of bacterial contamination and frequency of multiple antibiotic resistant bacteria ($r = 0.99$, $p < 0.0001$) and between the degree of bacterial contamination and variability of multiple antibiotic resistance ($r = 0.98$, $p = 0.0016$) was observed. Significant differences between the population means was found (particularly between fecal coliforms and *Salmonella* spp.) at 0.05 level of significance (F value 3.55, probability value 0.01, q value 4.800)

Antibiotic clustering

Statistical comparison of similarity between different taxonomic groups of bacteria with respect to resistance against different class of antibiotics was done using cluster analysis (Fig. 2). Solid lines in the cluster diagram indicate significant differences between taxonomic groups of bacteria and solid lines with a rectangle at the centre and asterisks at the node indicate insignificant differences in resistance patterns between two groups of bacteria against a class of antibiotics. The five taxonomic groups (fecal coliforms, *E. coli, Enterobacter, Klebsiella* and *Salmonella*) were grouped into four clusters. *E. coli* and *Salmonella* spp. were linked together in cluster 1 at an approximate linkage distance of 0.52 ED, indicating maximum similarity in the resistance pattern between these two taxonomic groups. Fecal coliforms and *Klebsiella* spp. were linked in cluster 2 at an approximate linkage distance of 1 ED. *Enterobacter* spp. joined this cluster at a linkage distance of 1.1 ED forming cluster 3 indicating significant dissimilarity between groups of bacteria present in cluster 1 and cluster 3. We also observed that cluster A joined the cluster 3 at a linkage distance of 3 ED which is greater than distances of other clusters, indicating that there is significant dissimilarity between different taxonomic groups of bacteria in cluster A versus taxonomic group of bacteria in cluster B with respect to resistance against different class of antibiotics. These observations indicated that many isolates of Enterobacteriaceae family isolated from Veraval coastal waters have emerged into multi drug resistant phenotypes (Table 4).

Discussion

This study revealed the occurrence of multiple antibiotic resistant bacteria in Veraval coast. Presence of antibiotic resistance bacteria in a given environment may be an indication that an area is contaminated with antibiotics (Gunaseelan, Ruban 2011). Environmental antibiotic concentrations may exert selective pressure on environmental bacteria and may also foster the transfer of resistance genes, helping create the "resistome" mixing pot of genetic AMR traits (WHO 2002). Antibiotic resistance of fecal bacteria in surface waters has been studied by

Table 5. List of bacteria resistant to different classes of antibiotics. Values in bracket indicates percentage of resistant isolate to mentioned antibiotic. ND, not determined

Type of bacteria	Amino-glycosides	β-lactamase	Macro-lides	Cephalo-sporin	Glyco-peptides	Lincos-amides	Poly-peptides	Sulphan-amides	Quino-lones	Chloram-phenicol
Fecal coliforms	0 (0)	89 (90)	49 (50)	66 (67)	63 (64)	47 (47)	ND	ND	ND	13 (13)
Enterobacter spp.	9 (21)	43 (100)	43 (100)	16 (37)	43 (100)	43 (100)	43 (100)	ND	ND	9 (21)
Escherichia coli	9 (30)	30 (100)	30 (100)	0 (0)	ND	ND	30 (100)	0(0)	0(0)	0 (0)
Klebsiella spp.	0 (0)	32 (57)	48 (86)	16 (29)	8 (14)	48 (86)	ND	ND	ND	8 (14)
Salmonella spp.	ND	ND	7 (100)	0 (0)	ND	ND	7 (100)	0 (0)	0 (0)	0 (0)

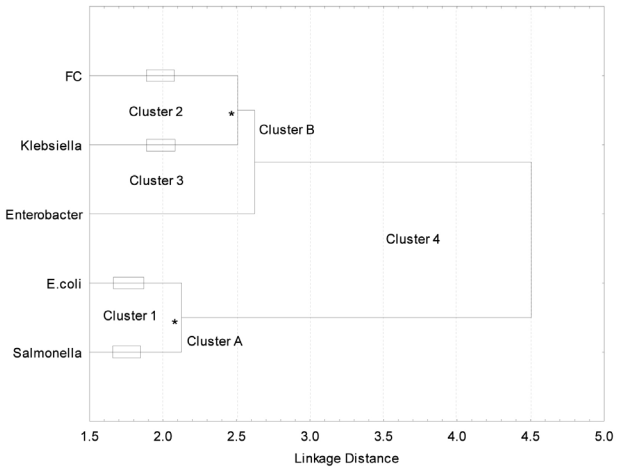

Fig. 2. Cluster dendrogram illustrating linkage distances of different taxonomic groups of bacteria with respect to resistance against different classes of antibiotics.

various researchers from different types of surface waters, rivers (Watkinson et al. 2007), estuaries (Parveen et al. 1997), lakes (Edge, Hill 2005) and coastal waters (Kimiran-Edrem et al. 2007). This is mainly because the natural characteristics of the coastal ecosystem have faced dreadful changes, as untreated sewage discharges and industrial effluents carry high loads of pathogenic bacteria, especially enteric groups, and pose a potential threat to human health (Reeves et al. 2004). Several studies have used the antibiotic resistance pattern of fecal indicator bacteria to investigate the source of fecal pollution in the given marine environment (Webster et al. 2004; Moore et al. 2005). *E. coli* are normally present in human and animal intestines and are the most reliable indicator of fecal contamination in waters. The population of *Enterobacter* spp. and *E. coli* were absent in offshore stations. These observations were quite similar with findings of Patra et al. (2009), who investigated the occurrence and distribution of bacterial indicators and pathogens in the coastal waters of Orissa. In the present study, *Klebsiella* spp. were observed in the range of 2.5 and 17.0% at 0 to 0.5 km from shore, and *Salmonella* spp. with 1.7% at 0.5 km from shore. In the offshore area, microbial populations were observed less and the results were comparable to the southern coast of Kerala, India (Sudhanadh et al. 2012).

The main reasons causing the marine environmental contamination were improper and unnecessary use of antimicrobial drugs by human and animals (Al-Bahry et al. 2009). The present assessment of the microbial population for antibiotic resistance profile to different classes of antibiotics showed a high proportion of strains resistance to β-lactam antibiotics, followed by resistant to macrolides. These results complied with the finding of Foster (1983) and Nair et al. (1992). All the isolates were susceptible to sulphanamides and fluoroquinolone antibiotics. Bacterial isolates showed resistance to bacitracin and penicillin (100% of cases), oxacillin (82.1%), ampicilin (80%) and vancomycin (62.1%). Interestingly, the lowest frequencies of antibiotic resistance were against ofloxacin (0%), chloramphenicol (8.5%) and neomycin (13.2%). No isolates showed resistance to polymyxin B, gentamicin, co-trimoxazole and cefpodoxime. Harakeh et al. (2006) showed the bacterial resistance to common antibiotics in the coastal waters and sediment.

The increased concentration of multi drug resistant bacteria in the aquatic environment creates selective pressures on natural bacterial strains (Alpay-Karaoglu et al. 2007). This could be due to the fact that terrestrial bacteria entering into seawater with antibiotic resistant plasmids may be responsible for the prevalence of the resistant genes in the marine environment (Chandrasekaran et al. 1998). The β-lactam antibiotics tested in this study were ampicilin, penicillin and oxacillin, 100% resistances were observed in more than two antibiotics tested. Maximum sensitivity was shown by *E. coli* (43.4%). Apart from β-lactam, isolates showed resistance to macrolides, lincosamides, glycopeptides, cephalosporin, polypeptides, chloramphenicol and aminoglycoside in a decreasing manner. *Salmonella* spp. had 100% resistance to macrolide and polypeptide classes of antibiotics, and the very low antibiotic resistance index (0.03) for *Salmonella* spp. could be the result of a low number of isolates resistant to multiple number of antibiotics or may be due to the efficacy of tested antibiotics against them. The highest ARI (0.07) was calculated for *Enterobacter* spp., as a higher number of isolates showed resistance against multiple numbers of antibiotics, similar to results obtained by Marina et al. (2012).

In conclusion, the present study underscores the widespread distribution and persistence of multiple antibiotic-resistant bacteria of Enterobacteriaceae family, which are known to be indicators of water contamination along the Veraval coast, India. Bacterial populations in all transects were higher in stations which were close to the shore. The majority of microbial populations isolated from this area were resistant to more than two antibiotics. The Veraval coast and fishing harbor had the highest fecal contamination, which was mainly due to human, animal waste, shorebirds and domestic animals. The high levels of antibiotic resistance in marine bacteria might be the result from the non-indigenous bacteria with antibiotic resistant plasmids entering the sea water. Further studies are necessary to establish the role of antibiotics substances in control of marine bacterial populations and subsequent management of these problems are vital to prevent the emergence of drug-resistant bacteria.

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