Environ Sci Pollut Res (2017) 24:11504–11517
DOI 10.1007/s11356-017-8760-8

RESEARCH ARTICLE

Pathogenic multiple antimicrobial resistant *Escherichia coli* serotypes in recreational waters of Mumbai, India: a potential public health risk

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Received: 29 August 2016 / Accepted: 7 March 2017 / Published online: 18 March 2017

Abstract Globally, coastal waters have emerged into a pool of antibiotic resistance genes and multiple antibiotic resistant microorganisms, and pathogenicity of these resistant microorganisms in terms of serotypes and virulence genes has made the environment vulnerable. The current study underscores the presence of multiple antibiotic resistant pathogenic serotypes and pathotypes of *Escherichia coli*, the predominant faecal indicator bacteria (FIB), in surface water and sediment samples of famous recreational beaches (Juhu, Versova, Mahim, Dadar, and Girgaon) of Mumbai. Out of 65 faecal coliforms (FC) randomly selected, 38 isolates were biochemically characterized, serotyped (for 'O' antigen), antibiogram-phenotyped (for 22 antimicrobial agents), and genotyped by polymerase chain reaction (for virulence factors). These isolates belonged to 16 different serotypes (UT, O141, O2, O119, O120, O9, O35, O126, O91, O128, O87, O86, R, O101, O118, and O15) out of which UT (18.4%), O141 (15.7%), and O2 (13.1%) were predominant, indicating its remarkable diversity. Furthermore, the generated antibiogram profile revealed that 95% of these isolates were multiple antibiotic resistant. More than 60% of aminoglycoside-sensitive *E. coli* isolates exhibited resistance to penicillin, extended penicillin, quinolone, and cephalosporin classes of antibiotic while resistance to other antibiotics was comparatively less. Antibiotic resistance (AR) indexing indicated that these isolates may have rooted from a high-risk source of contamination. Preliminary findings revealed the presence of enterotoxin-encoding genes (stx1 and stx2 specific for enterohaemorrhagic *E. coli* and Shiga toxin-producing *E. coli*, heat-stable toxin enterotoxin specific for enterotoxigenic *E. coli*) in pathogenic serotypes. Thus, government authorities and environmental planners should create public awareness and adopt effective measures for coastal management to prevent serious health risks associated with these contaminated coastal waters.

Keywords Faecal indicator bacteria · Multiple antibiotic resistant *Escherichia coli* · Pathogenic serotypes · Anthropogenic stress · Recreational beaches · Environmental pollution · India

Introduction

Swimming at recreational water sites such as the beach is one of the most popular activities during holidays. However, population explosion and inadequate infrastructure to properly treat and dispose sewage, lack of sanitary condition, poverty, and overexploitation of natural waters has resulted in the discharge of considerable quantities of untreated waste into the natural waters (Chandran et al. 2008a). Pathogenic microorganisms and toxic compounds are added to the coastal waters by sewage contamination rendering these water bodies unsafe for bathing. Microbial source tracking studies on polluted environmental waters in Southeast Queensland, Australia using nifH gene marker of *Methanobrevibacter smithii* detected the presence of sewage contamination (Ahmed et al. 2011). Exposure to these pathogens can occur during swimming or other recreational activities through ingestion, inhalation, or direct skin contact with polluted beach water (Praveena et al. 2015). Global estimates indicate that each year more than 120

Responsible editor: Diane Purchase

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million cases of gastrointestinal disease and 50 million cases of severe respiratory disease and 50 million cases of severe respiratory diseases are caused by swimming and bathing in waste water-polluted coastal waters (Abdelzaher et al. 2013). Antibiotics belonging to classes fluoroquinolones, quinolones, and cephalosporins are considered as first-line agents to cure these diseases (Pitout and Laupland 2008; Pitout 2012; Shepherd and Pottinger 2013), but overgrowing resistance to these agents is causing delay in appropriate therapy with subsequent increased morbidity and mortality (Schwaber and Carmeli 2007; Tumbarello et al. 2007).

Antibiotic resistance has been recognized as a global threat to humans and veterinary medicine both in developed and developing countries (Chandran et al. 2008b), but nowhere is it as stark as in India (Ganguly et al. 2011). In 2010, India was the world's largest consumer of antibiotics for human health (Laxminarayan and Chaudhury 2016) as there is no strict monitoring programme regarding the use of antibiotics in animals and humans; more drugs are available in private clinics and medical shops than in public hospitals, even without prescription. Antibiotic pollution in the form of overuse in humans, animals, and agriculture encourages the transfer of resistance genes to human commensal and pathogenic bacteria and release of antibiotic resistant strains in the environment (Rutgersson et al. 2014). Waste generated in the form of industrial effluents and domestic sewage after treatment is discharged into the coastal waters for dilution creating a reservoir for pathogenic bacteria exhibiting resistance to multiple antimicrobials. The severity of this problem increases multifold when these bacteria account for life-threatening diseases.

For microbiological quality assessment of surface waters, traditional as culture-based methods are employed. Culture-based methods are important in investigating the microbial ecology of natural and anthropogenically impacted environment. However, with recent advancement in genomics and sequencing technologies, there is an increasing realization that comprehensive understanding of microbial ecology requires a combination of culture-based and molecular-based assays, and this is especially true for antibiotic resistance in various ecosystems. Molecular methods are extremely useful for obtaining information on diversity and presence of antibiotic-resistant genes (Luby et al. 2016). Also, advances in next generation sequencing technologies have made it possible to develop genetic-based subtyping and molecular serotyping methods for pathogen, which are more discriminatory compared to phenotypic typing methods (Fratamico et al. 2016).

E. coli, the predominant faecal indicator bacteria, have been traditionally used as indicators of faecal contamination and health status of coastal waters. Emergence of *E. coli* isolates with multiple antibiotic resistant phenotypes, involving co-resistance to four or more unrelated families of antibiotics, is a serious matter of concern (Maynard et al. 2003) because in spite of being commensal, some strains have acquired specific virulence traits that allow them to cause a wide spectrum of intestinal and extraintestinal infections such as diarrhoea, urinary tract infections, and both community- and hospital-acquired bacteraemia (Salvadori et al. 2004). Pathogenic strains of *E. coli* in clinical specimens, foods, and environmental samples can be detected by serotyping wherein according to the modified Kauffman and White scheme, *E. coli* is serotyped on the basis of its O (somatic), H (flagellar), and K (capsular) surface antigen profiles (Nataro and Kaper 1998). Occurrence of pathogenic serotypes like O2, O15, O25, O86, O91, O141, O157, and untypeable (UT) belonging to enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), Shiga toxin-producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC), and uropathogenic *E. coli (*UPEC) pathotypes have been previously reported in the waters of Cochin Estuary (Sukumaran et al. 2012; Chandran et al. 2008b) and Bhavani River (Hatha et al. 2004). Similar studies on fresh waters of famous Indian rivers like Gomti, Sarayu, and Ganga revealed that they are contaminated with multiple antimicrobial resistant diarrheagenic strains of *E. coli* belonging to EHEC, STEC, and ETEC pathotypes demanding regular surveillance and formulation of effective strategies to protect public health (Ram and Shanker 2005; Ram et al. 2007, 2008). However, no such study has been carried out on recreational marine waters of the metropolitan city Mumbai. Thus, the present study was designed with a view to determine the health status of famous recreational beaches of Mumbai, India affected by anthropogenic pressure. These beaches are frequently visited by the people of Mumbai as well as tourists. Hence, in this study, microbiological water quality assessment of surface water and sediment of beaches was performed and predominant faecal pollution indicator bacteria *E. coli* isolated were serotyped and screened for resistance to antimicrobial agents commonly used to treat infections caused by them, thereby, helping in knowing the effective treatment/medication in case of disease outbreak. Combining the data of water quality assessment, serotyping and antibiogram profile of faecal indicator bacteria (FIB) will provide a basis for robust and graded classification of beaches. The results of classification can be used to grade beaches in order to support informed personal choice, provide onsite guidance to users on relative safety, assist government authorities in the identification and promotion of effective management intervention, and provide an assessment of regulatory compliance.

Materials and methods

Description of sampling sites

Mumbai (18°55′ N, 72°54′ E) is the most populous metropolitan city on the west coast of India and capital of the state Maharashtra. The state of Maharashtra accounts for a 653-

km-long coastline with 17% sandy beaches, many of them lying within the city of Mumbai. The increase in urbanization and industrialization has led to an increase in marine discharges. The city generates 2.2×10^6 m^3 day^{-1} of domestic sewage out of which about 2.0×10^6 m^3 day^{-1} (partially treated or largely untreated) enters the marine waters including creeks and bays and tides, and the current brings these pollutants to the beaches, exacerbating the problem (Jayasiri et al. 2014). Also, being a metropolitan city, major industries of the country are located in and around Mumbai. Largely untreated industrial effluents and domestic sewage from the city enter the continental shelf in the region. There are a number of ports wherein the ship and cargo boarding activities contribute to marine pollution. This has resulted in the degradation of the coastal water quality and contamination of adjoining beaches and sea fronts. As a coastal city, beaches for recreation such as Girgaon, Mahim, Juhu, Aksa, Marine Drive, and Versova are few of the major attractions for tourists. Out of the nine beaches located along the city coast, five beaches namely Versova, Juhu, Mahim Bay, Dadar, and Girgaon which show wide geographic coverage and degree of beach usage for recreation and tourism activities were selected for this study. Details of sampling sites are shown in Table 1 and Fig. 1.

Collection and transportation of samples

For microbiological analyses, water and sediment samples were collected for a period of 4 months (April 2015 to July 2015) from five recreational beaches of Mumbai in presterilized polypropylene bottles (Tarsons, India) and in sterile 50-ml Falcon tubes (Tarsons, India), respectively, during high tide and low tide in triplicates. All samples were collected by taking precautions required for microbiological analysis, held in an icebox, and processed within 6–8 h of collection.

Assessment of faecal indicator bacteria

The total viable bacterial counts (TVC) from the surface water samples were estimated via the spread plating method on marine agar plates with 0.1 mL of suitable dilutions. The enumeration of total coliforms (TC) and faecal coliforms (FC) was carried out using membrane filtration according to the standard method (APHA 1998). In brief, water samples were filtered through 0.45-μm pore-sized cellulose acetate filters (Millipore) and aseptically placed on selective media plates. All of the media plates, except M-FC agar plates, were incubated at 37 °C ± 1 °C for 24–48 h and final counts of colonies were noted. M-FC agar plates were incubated at 44.5 °C ± 1 °C for 24–48 h. All trials were performed in triplicates. Typical colony morphology characteristics of different bacterial groups were noted and an initial enumeration of faecal pollution indicator bacteria was completed according to the media manufacturer's guide. A total of 80 presumptive *E. coli* colonies (60 from water samples and 20 from sediment samples) showing various shades of blue on the M-FC medium were confirmed by streaking on Levine-eosin methylene blue agar (EMB Agar) and 65 isolates were identified as *E. coli* after streaking them on chromogenic agar (Hicrome *E. coli* agar) and biochemical characterization using Hi *E. coli* Identification Kit (Hi-Media, Mumbai, India). Purified colonies of *E. coli* were preserved as their 30% glycerol stock for further studies. Out of 65, 38 confirmed *E. coli* cultures were randomly selected and serotyped at the National Salmonella and Escherichia Centre, Kasauli, Himachal Pradesh, India.

Table 1 Sampling sites and their description

Site name	Description of site
1. Juhu	Famous beach of Mumbai and highly visited by tourists. Pollution source: domestic sewage, discharge from tourist resorts located beside, faecal input from animals such as dogs and horses, input from birds such as pigeons, crows, etc.
2. Versova	Continuation of Juhu beach, home to Mumbai's largest fishing community—the Kolis, pollution source: drainage from Malad Creek and Versova fishing harbour, domestic sewage and discharge from the sewage treatment plant located at Versova, animal and bird faecal input.
3. Mahim	Less visited beach as highly polluted by domestic sewage through nallahs, hospital wastes, unpleasant odour, animal input (dogs), bird input (seagulls, pigeons, and crows).
4. Dadar	Part of Mahim Bay and popular among Mumbaikars. Affected by anthropogenic activities. Receives sewage from nearby located marine outfalls (Bandra and Worli), domestic effluent from several sewage outlets opening near the coast and drainage from Mithi River and Mahim Creek.
5. Girgaon	Well known as Charni Road Chowpatty and is an extension of Marine Drive, a famous and highly visited tourist spot. Sandy stretch of coastline interspersed with boulders. Except during the monsoon, this beach is also famous for recreational water sports. It is affected by domestic effluent discharged through nallahs (sewage outlets) and direct faecal input from human, birds, and animals.

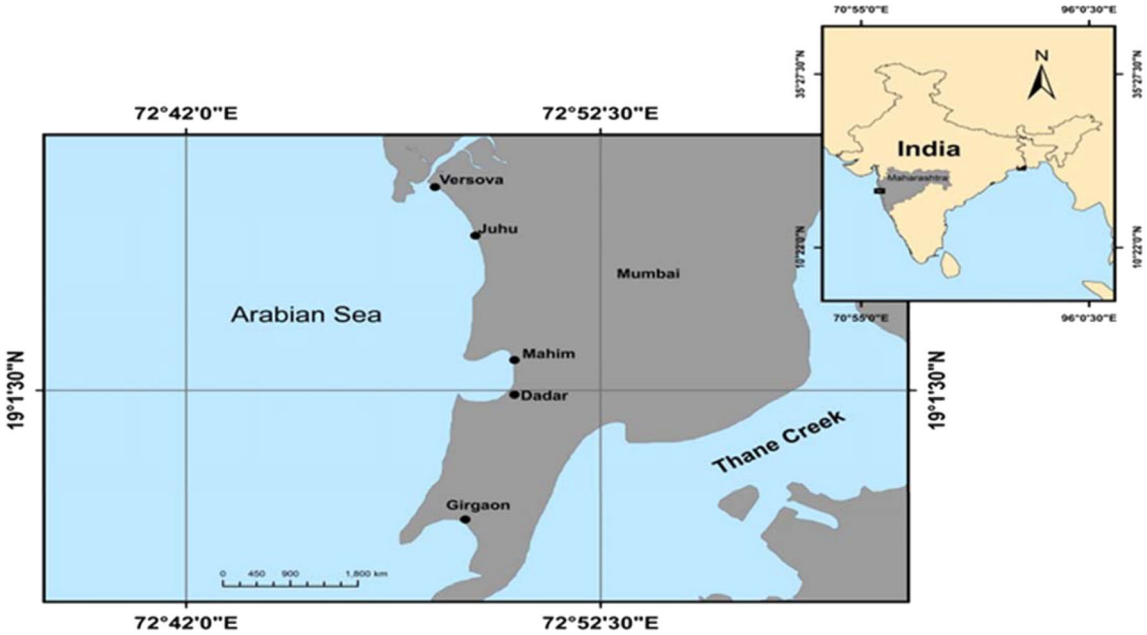

Fig. 1 Map showing the sampling sites

Typical colony characteristics of each bacterial group and specific media used for enumerating them are listed in Table 2.

Antibiotic resistance analysis

Purified colonies of *E. coli* isolated from both water and sediment samples from all the five beaches were tested for antimicrobial susceptibility. Antibiotic susceptibility testing was performed using a disk diffusion method as mentioned by Bauer et al. (1966). The isolates were challenged with 22 different antibiotics on Mueller Hinton Agar (Himedia, India), and results were interpreted based on recommendations of CLSI (Clinical and Laboratory Standards Institute, 2007). A sterile cotton swab was used to inoculate the standardized bacterial suspension matching an optical density of 0.5 McFarland standards corresponding to 10^8 CFU mL^{-1} on the surface of previously prepared sterile Mueller Hinton agar plates by rotating the plate every 60° to ensure homogenous growth, followed by addition of antibiotic-impregnated rings (Hi-Media, Mumbai, India). After 30 min, the plates were inverted and incubated at 37 °C for 18–24 h. This test was performed in duplicate for each *E. coli* strain and antimicrobial. In each experimental set, *E. coli* ATCC 25922 was used as negative control. The quality control was performed according to manufacturer's instructions. The list of antibiotics tested and data for antimicrobial resistance of each bacterial isolate have been reported as resistant (R), intermediates (I), and sensitive (S) based on CLSI break points as shown in Table 3. The results were used to calculate the antibiotic resistance index (ARI) and multiple Antibiotic Resistance Index (MAR) for total number of isolates as:

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

Where, y is the number of resistant isolates, n is the number of isolates, and x is the number of antibiotics (Hinton and Linton 1983);

Table 2 Specific media used for isolation and enumeration of different bacterial types

Serial no	Bacterial type	Culture medium	Positive colonies
1	Total viable count (TVC)	Marine agar	All colonies counted
2	Total coliforms (TC)	MacConkey agar	All colonies counted
3	Faecal coliforms (FC)	M-FC agar	Blue colonies counted
4	*Escherichia coli* (EC)	Levine-eosin methylene blue agar	Colonies with green metallic sheen
		Hicrome *E. coli* agar	Blue colonies

All culture media were purchased from Hi-Media Laboratories Limited, Mumbai, India

Table 3 Zone size break points described by CLSI used to interpret data in agar diffusion test performed through antibiotic rings

Class of antibiotic	Antimicrobial tested/code	Concentration (mcg) and generation		CLSI break points (zone of inhibition in mm)			Standard data for *Escherichia coli* ATCC (25922)	
				R	I	S	Quality control limits (CLSI)	As observed in our study
Aminoglycoside	Streptomycin (S)	10	–	≤11	12–14	≥15	12–20	18, S
	Gentamicin (GEN)	10	–	≤12	13–14	≥15	19–26	22, S
	Amikacin (AK)	30	–	≤14	15–16	≥17	19–26	25, S
Cephalosporin	Cefpodoxime (CPD)	10	Third	≤17	18–20	≥21	23–28	23, S
	Ceftriazone (CTR)	30	Third	≤ 13	14–20	≥21	29–35	32, S
	Cefixime (CFM)	5	Third	≤15	16–18	≥19	23–27	26, S
	Cefazolin (CZ)	30	First	≤19	20–22	≥23	21–27	25, S
	Cephalothin (CEP)	30	First	≤14	15–17	≥18	15–21	20, S
	Cefuroxime (CXM)	30	Second	≤14	15–17	≥18	20–26	22, S
	Cefepime (CPM)	30	Fourth	≤14	15–17	≥18	31–37	35, S
	Cefoperazone (CPZ)	75	Third	≤15	16–20	≥21	28–34	32, S
	Cefoxitin (CX)	30	Second	≤14	15–17	≥18	23–29	32, S
	Cefotaxime (CTX)	30	Third	≤14	15–22	≥23	29–35	32, S
Fluoroquinolone	Levofloxacin (LE)	5	Third	≤13	14–16	≥17	29–37	32, S
	Ciprofloxacin (CIP)	5	Second	≤15	16–20	≥21	30–40	34, S
Quinolone	Nalidixic acid (NA)	30	First	≤13	14–18	≥19	27–28	25, S
Penicillin	Ampicillin (AMP)	10	–	≤13	14–16	≥17	16–22	18, S
Extended penicillin	Ampicillin/sulbactam (A/S)	10/10	–	≤11	12–14	≥15	19–24	22, S
	Augmentin (AMC)	30	–	≤13	14–17	≥18	18–24	23, S
Tetracycline	Tetracycline (TE)	30	–	≤14	15–18	≥19	18–25	23, S
Other	Chloramphenicol (C)	30	–	≤12	13–17	≥18	21–27	22, S
	Rifampicin (RIF)	5	–	NM	NM	NM	8–10	8, S

NM not mentioned

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

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

Statistical analysis

All statistical analyses were performed using XL-STAT (version 6.0, Addinsoft) and a p value of 0.05 was considered to be significant. Fisher's exact test was performed to analyse associations between responses of *E. coli* isolates for two antimicrobials to assess the co-selection (Seigal and Catellan 1987). The significance of variation in occurrence of multiple antimicrobial resistance between the sites were also analysed using the Chi-square test (Seigal and Catellan 1987). In order to classify these occurrences in different serotypes, an agglomerative hierarchical clustering (AHC) analysis was employed, using Euclidean distance and the Ward method for the aggregation criterion.

Results

Quantitative enumeration of faecal indicator bacteria density

All the selected sampling sites exhibited bacterial contamination. Overall range (expressed in CFU 100 mL for water and CFU g^{-1} for sediment) of bacterial counts in dry as well as wet season is given in Table 4. In brief, the faecal coliform levels were higher in the wet season as compared to those in the dry season. In the dry season, the minimum levels of faecal coliforms were reported in water (35×10^2 CFU per 100 mL) and sediment (52×10^2 CFU g^{-1}) of Juhu beach; whereas, maximum levels were reported in water (730×10^2 CFU per mL) and sediment (1150×10^2 CFU g^{-1}) of Mahim beach. In wet season, the minimum levels of faecal coliforms were reported in water (20×10^2 CFU per 100 mL) and sediment (100×10^2 CFU g^{-1}) of Girgaon beach while maximum levels were reported in the water (850×10^2 CFU mL^{-1}) of Mahim beach and sediment (1000×10^2 CFU g^{-1}) of Mahim and Versova beach as illustrated in Table 4.

Table 4 Overall range (expressed in CFU per 100 mL and CFU per gram) of bacterial counts in surface waters and sediment samples at recreational beaches of Mumbai

Sampling sites	Dry Season (April–May)						Wet season (June–July)					
	Water (CFU per 100 mL)			Sediment (CFU per g)			Water (CFU per 100 mL)			Sediment (CFU per g)		
	TVC ($\times 10^4$)	TC ($\times 10^2$)	FC ($\times 10^2$)	TVC ($\times 10^4$)	TC ($\times 10^2$)	FC ($\times 10^2$)	TVC ($\times 10^4$)	TC ($\times 10^2$)	FC ($\times 10^2$)	TVC ($\times 10^4$)	TC ($\times 10^2$)	FC ($\times 10^2$)
Juhu	28–40	40–150	35–60	30–56	120–280	52–280	520–800	200–250	190–210	600–1000	280–400	250–360
Versova	28–220	80–140	70–110	45–800	90–280	80–280	120–450	180–520	100–480	280–900	400–1000	350–1000
Mahim	197–1250	210–800	200–730	1800–2025	950–1250	800–1150	150–3200	800–850	800–850	1500–6000	700–1000	620–1000
Dadar	1635–1680	150–600	130–480	1650–1714	400–900	320–850	150–4000	150–160	110–140	5800–10,000	150–200	130–180
Girgaon	820–1100	200–520	150–400	980–1430	580–700	360–500	200–1200	50–110	20–80	900–2000	120–190	100–180

The above table gives the overall range of counts obtained for water and sediment samples together

TVC total viable count, *TC* total coliform, *FC* faecal coliform

Susceptibility to antimicrobial agents and serotyping

All 38 isolates confirmed as *E. coli* by biochemical testing were tested for susceptibility to 22 antimicrobials and serotyped. It was observed that *E. coli* isolated from the five sites along the Mumbai coast possess different levels of susceptibility towards antimicrobials tested. All the isolates from the study area were sensitive to amikacin, gentamicin, levofloxacin, rifampicin, and streptomycin; while, on the contrary, all of the isolates were resistant to Augmentin. Further, 97, 73.6, 68.4, 65.7, and 55.2% of the isolates were resistant to nalidixic acid, cephalothin, ampicillin/sulbactam, cefpodoxime, ampicillin, and cefepime, respectively (Table 5). Figure 2a shows the percentage (%) of *E. coli* isolates (%) having resistance to multiple antibiotics. One hundred per cent of the isolates were resistant to cephalothin, ampicillin/sulbactam, ampicillin, nalidixic acid, and Augmentin from site 1 (Juhu); nalidixic acid and Augmentin from site 2 (Versova) and site 4 (Dadar); Augmentin from site 3 (Mahim); and ampicillin, cefixime, nalidixic acid, and Augmentin from site 5 (Girgaon); while more than 50% of the isolates were resistant to cefazolin, cefoxitin, cefuroxime, ciprofloxacin, cefepime, and cefpodoxime from site 1 (Juhu); cephalothin, cefuroxime, cefotaxime, ampicillin, ampicillin/sulbactam, and cefpodoxime from site 2 (Versova); cefpodoxime, cefixime, nalidixic acid, ampicillin, and ampicillin/sulbactam from site 3 (Mahim); cefpodoxime, cephalothin, ciprofloxacin, ampicillin, ampicillin/sulbactam, and cefoxitin from site 4 (Dadar); and cefpodoxime, cephalothin, ampicillin/sulbactam, and ceftriazone from site 5 (Girgaon). Figure 2b shows the percentage (%) of *E. coli* isolates having reduced susceptibility to multiple antibiotics. Thirty-three per cent of the isolates from site 1 showed reduced susceptibility to cefazolin and tetracycline; 22 and 11% the isolates from site 2 showed reduced susceptibility to (cefazolin, cefoxitin, cefixime) and (cefoperazone, tetracycline, cefpodoxime), respectively; 30, 20 and 10% of the isolates from site 3 showed reduced susceptibility to (cephalothin, cefpodoxime), cefixime, (cefuroxime, tetracycline), respectively; 18% of the isolates from site 4 showed reduced susceptibility to cefazolin, cefotaxime, tetracycline, and cefixime while 9% showed reduced susceptibility to ampicillin/sulbactam, cefoxitin, cefoperazone, ampicillin, and chloramphenicol; and 20% of the isolates from site 5 showed reduced susceptibility to cephalothin, cefoxitin, cefuroxime, and cefoperazone. The major issue of concern here was that the isolates showing resistance to more than seven antibiotics were isolated from all the five sites with a MAR index of above 0.3. Figure 2c gives the number of isolates showing resistance to two or more antibiotics and overall MAR index and with respect to stations. AR index of all sampling sites exceeded the high risk level (0.25) and revealed that Versova has the highest

AR index (0.404) followed by Juhu (0.378), Dadar (0.376), Girgaon (0.372), and Mahim (0.309); whereas, percentage of isolates resistant to eight or more antibiotics was found to be the highest in Dadar (73%) followed by Juhu (67%), Versova (56%), Mahim, and Girgaon (40%).

Serotyping results indicated remarkable diversity in *E. coli* isolates in the way that 38 isolates exhibited 16 different serological identities predominated by UT (18.4%), O141(15.7%), O2(13.1%), O119, and O120 (7.8%) as depicted in Fig. 3. Furthermore, it was observed that dry season (April and May) encountered the highest number of *E. coli* (26.3%) belonging to O141 serotype followed by O35 (10.5%) while from the wet season (June and July), 31.5% of the *E. coli* isolates were untypeable and 26.3% of the isolates belonged to O2. MAR index and resistance pattern of 38 isolates (comprising of 16 serotypes) is represented in Table 6. Interestingly, all the pathogenic serotypes belonging to probable pathotypes (EHEC, ETEC, EPEC, STEC, EAEC, and UPEC) were multiple antibiotic resistant, and the number of antibiotics to which these isolates were resistant ranged from 2 to 14. The most common pattern of resistance observed among all serotypes was cefazolin, cephalothin, ampicillin/sulbactam, cefuroxime, ampicillin, cefixime, nalidixic acid, Augmentin, and cefpodoxime (Table 6).

Table 5 Percentage antibiotic resistance of *E. coli* isolates (n = 38) isolated from recreational beaches of Mumbai

Antimicrobial	Percentage of resistant isolates
Cefazolin (CZ)	31.5
Cephalothin (CEP)	73.68
Ampicillin/sulbactam (A/S)	68.42
Cefepime (CPM)	34.2
Cefoxitin (CX)	42.1
Cefuroxime (CXM)	42.1
Cefotaxime (CTX)	42.1
Ciprofloxacin (CIP)	44.7
Cefoperazone (CPZ)	7.8
Ampicillin (AMP)	65.78
Tetracycline (TE)	10.52
Cefixime (CFM)	55.26
Nalidixic acid (NA)	97.36
Augmentin (AMC)	100
Ceftriazone (CTR)	28.94
Cefpodoxime (CPD)	65.78
Chloramphenicol (C)	2.7
Amikacin (AK)	0.0
Gentamicin (GEN)	0.0
Levofloxacin (LE)	0.0
Rifampicin (RIF)	0.0
Streptomycin (S)	0.0

Statistical analysis

The resistance to nalidixic acid was significantly associated with Augmentin (Fisher's exact test significant at 5% level). Similarly, resistance to cephalothin, cefixime, and cefpodoxime (belonging to the cephalosporin class of antibiotic) was observed to be significantly associated with resistance to ampicillin and ampicillin/sulbactam (belonging to the penicillin and extended penicillin class of antibiotic) (Fisher's exact test significant at 5% level). The distribution of multiantimicrobial resistance in *E. coli* varied significantly (X^2 p 0.001) between the sites. However, *E. coli* isolated from site 1 (Juhu) were resistant to seven or more antimicrobials followed by site 2 (Versova), site 4 (Dadar), and site 5 (Girgaon) exhibiting resistance to 5–14 antimicrobials while *E. coli* isolates from site 3 exhibited resistance to 5–11 antimicrobials. Surprisingly, it was noted that not a single isolate was sensitive to all the antimicrobials tested.

Antibiotic clustering

Serotypes were clustered into groups according to similarity in response against different classes of antibiotics tested as shown in Fig. 4. Solid lines in the figure indicates significant dissimilarity between groups of serotypes and their response against class of antibiotics and solid lines with an asterisk at the node indicate significant similarity between two individual serotypes and their response against the class of antibiotics. Sixteen serotypes were grouped into three major clusters (cluster A, cluster B, and cluster C) which were further divided to form subclusters. The cluster A comprised of isolates belonging to serotype O2, O128, O7, O101, and UT indicating that these serotypes showed a similar resistance pattern against the class of antibiotics. Similarly, O119, O118, O120, O9, R, O86, O35, and O15 were grouped together in cluster B, and O141, O126, and O91 were grouped together in cluster C. Cluster A, cluster B and cluster C are linked together at 18 ED which is higher than the cutoff point (16 ED) thus indicating significant dissimilarity among serotypes and their responses towards a class of antibiotics. Serotypes belonging to clusters formed below the cutoff point have a significant similarity in their response against a class of antibiotics.

Discussion

Microbial pollution from sewage is becoming an increasing threat to recreational water users and coastal ecosystem health as the human population expands along global coastlines. Thus, for public health reasons, beach water assessment is of prime importance. The faecal indicator bacterial counts observed in this study are much higher than the permissible limits and support the recent findings of CSIR-NEERI,

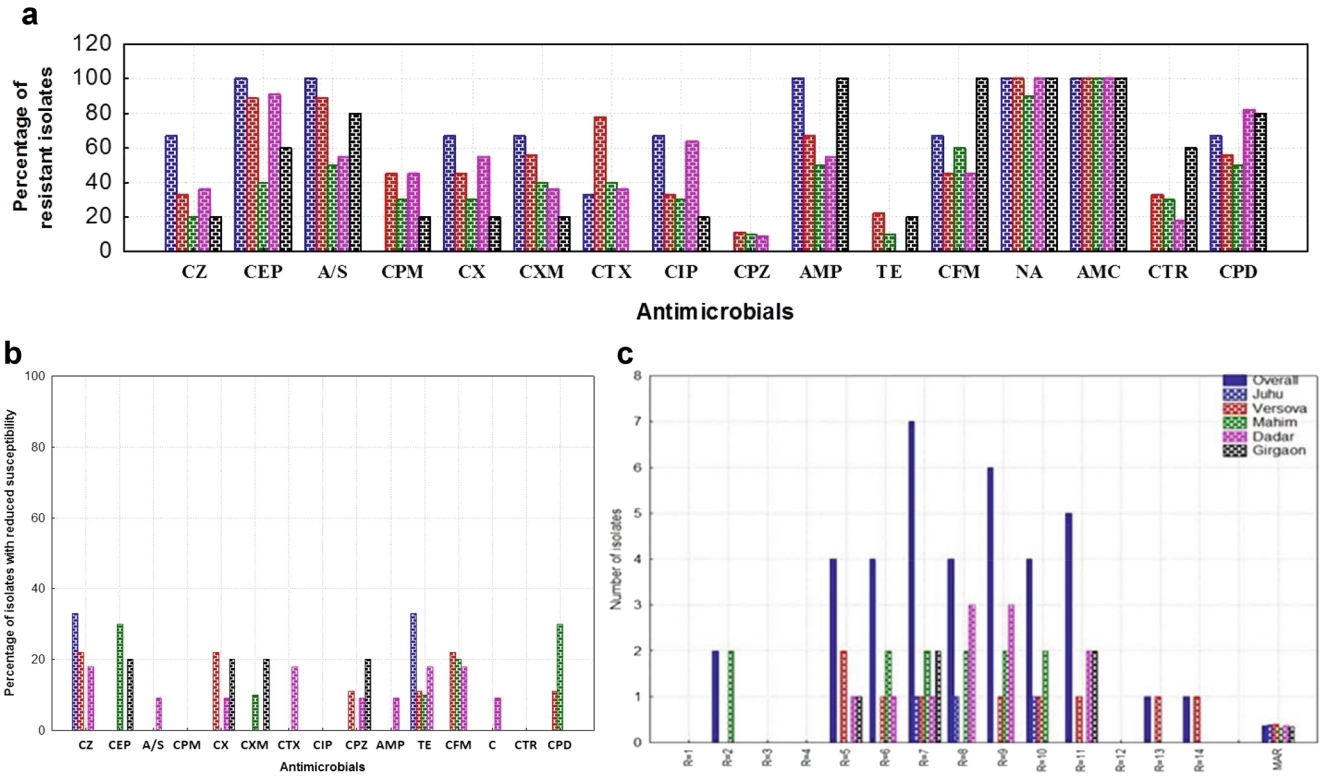

Fig. 2 Susceptibility of *E. coli* isolates towards antimicrobial agents at different recreational beaches of Mumbai. **a** *E. coli* isolates (%) having resistance to multiple antibiotics. **b** *E. coli* isolates (%) having reduced susceptibility to multiple antibiotics. **c** Number of isolates showing resistance to two or more antibiotics and overall MAR index with respect to stations. (Note: *Legends* are common for **a**, **b**, and **c**)

Nagpur, India (National Environmental Engineering Research Institute 2016) which states that faecal contamination at discharge points is very high. Although the sewage does get diluted when it mixes with the sea and the creek water, the FC count is still 100 to 1000 times higher than permissible limits at all the city beaches. However, this study gives the broader picture of pathogenic faecal coliforms prevailing in the coastal environment. Though a correlation of the infections due to contact polluted waters is not established, the possible associated risks have provided the basis for delineating standards for bathing and recreation waters. Hence, the Central Pollution Control Board (CPCB) specified Sea Water standards (*Criteria for classification and zoning of coastal waters (sea waters)—A coastal pollution control* series: COPOCS/6/ 1993) for designated best use like SWII for commercial fishing, contact recreation, and bathing activities; SWIII for industrial cooling; and SWIV for harbour water. The present study investigated the water quality at the beaches of Mumbai following the standards laid by CPCB in terms of faecal coliforms and *E. coli* levels indicating the poor health status of beaches. Higher and unacceptable counts of faecal coliforms in coastal waters may be attributed to the treated/ untreated sewages from marine outfalls, nallahs, slum discharges, and effluents from dairy and other industries. Water quality assessment studies conducted previously in the same regions denoted the same FIB pollution levels throughout the year irrespective of tide and do not satisfy the SWII or SWIII standards of FC 100FC/100 and 500FC/100 mL, respectively. Though the Municipal Corporation of Greater Mumbai (MCGM) has diverted waste water from Love Grove Pumping Station (LGPS) through a 3.4-km-long outfall and undertaken cleanliness drive for beaches, 196 MLD of waste water is discharged in near-shore region through non-point sources; slum sanitation programmes were inadequate and therefore no improvement in microbiological water quality was witnessed (Dhage et al. 2006).

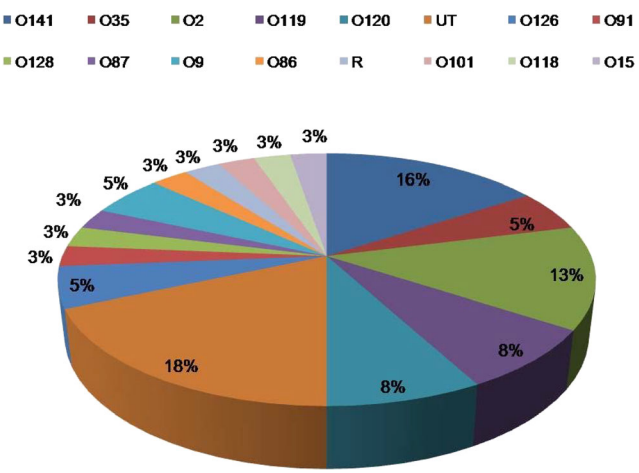

Fig. 3 Different serotypes and its percentage occurrence

Table 6 Serological identity and antibiotic resistance pattern with MAR index of *E. coli* isolates recovered from recreational beaches of Mumbai

Isolate ID	Serotype	Antimicrobial resistance pattern	MAR index	Isolate ID	Serotype	Antimicrobial resistance pattern	MAR index
EC1	O35	NA, AMC	0.09	EC45	UT	CEP, CX, CXM, CTX, CIP, NA, AMC	0.27
EC2	O15	NA, AMC	0.09	EC47	UT	CZ, CEP, A/S, CX, CXM, CIP, AMP, CFM, NA, AMC	0.32
EC6	O126	CZ, CEP, AMP, NA, AMC	0.22	EC48	UT	CZ, CPM, CPZ, NA, AMC, CTR, CPD	0.32
EC10	UT	CZ, CEP, A/S, CPM, CX, CXM, CTX, AMP, CFM, NA, AMC, CTR, CPD	0.59	EC50	O9	CEP, A/S, CXM, AMP, CFM, NA, AMC, CPD	0.41
EC12	O141	A/S, CPM, CTX, CIP, AMP, TE, CFM, NA, AMC, CTR, CPD	0.5	EC51	UT	CEP, A/S, CXM, AMP, CFM, NA, AMC, CPD	0.36
EC13	O120	CEP, A/S, CX, CTX, NA, AMC, CPD	0.32	EC53	O9	CEP, A/S, CPM, CX, CIP, AMP, NA, AMC, CPD	0.41
EC14	O141	CZ, CEP, A/S, CPM, CXM, CTX, CPZ, AMP, TE, CFM, NA, AMC, CTR, CPD	0.64	EC61	UT	CZ, CEP, CX, CXM, CTX, CIP, NA, AMC, CPD	0.41
EC15	O35	CEP, A/S, AMP, NA, AMC	0.23	EC62	O128	CEP, CPM, CX, CIP, NA, AMC, CTR, CPD	0.36
EC16	O119	CZ, CEP, A/S, NA, AMC, CFM	0.27	EC63	O126	CEP, CPM, CX, CIP, CFM, NA, AMC, CPD	0.36
EC17	O118	CZ, CEP, A/S, CPM, CXM, CTX, CPZ, AMP, NA, AMC	0.45	EC64	O2	CPM, CX, CXM, CTX, CIP, TE, CFM, NA, AMC, CTR	0.45
EC19	O119	AMP, CFM, NA, AMC, CTR, CPD	0.27	EC65	O119	A/S, CPM, CTX, AMP, AMC, NA	0.32
EC21	O141	CEP, A/S, AMP, CFM, AMC, CTR, CPD	0.32	EC71	O141	CZ, CEP, A/S, CX, CXM, CTX, CIP, AMP, NA, AMC, CPD	0.5
EC23	O101	CZ, CEP, A/S, CX, CTX, CIP, AMP, NA, AMC, CPD	0.45	EC72	O91	CZ, CEP, A/S, CFM, AMP, TE, CFM, NA, AMC, CTR, CPD	0.5
EC24	O120	CZ, CEP, A/S, CPM, CXM, CTX, AMP, NA, AMC	0.41	EC84	UT	CEP, A/S, CXM, CTX, CIP, CFM, NA, AMC, CPD	0.45
EC29	O141	CEP, A/S, CIP, CFM, NA, AMC	0.27	EC85	O87	CEP, A/S, CX, CXM, CIP, AMP, CFM, NA, AMC, CTR, CPD	0.5
EC31	O120	A/S, CIP, AMP, CFM, NA, AMC, CTR	0.32	EC89	O2	A/S, CX, CXM, CIP, AMP, CFM, NA, AMC, CPD	0.41
EC37	R	AMP, CFM, NA, AMC, CPD	0.23	EC91	O2	CEP, CX, CXM, CTX, CIP, CFM, NA, AMC, CPD	0.41
EC40	O141	CEP, A/S, AMP, CFM, NA, AMC, CPD	0.32	EC93	O2	CEP, A/S, CX, CXM, CTX, CIP, AMP, CFM, NA, AMC, CPD	0.5
EC41	O86	CEP, A/S, AMP, NA, AMC	0.23	EC103	O2	CEP, A/S, CXM, AMP, CFM, NA, AMC, CPD	0.36

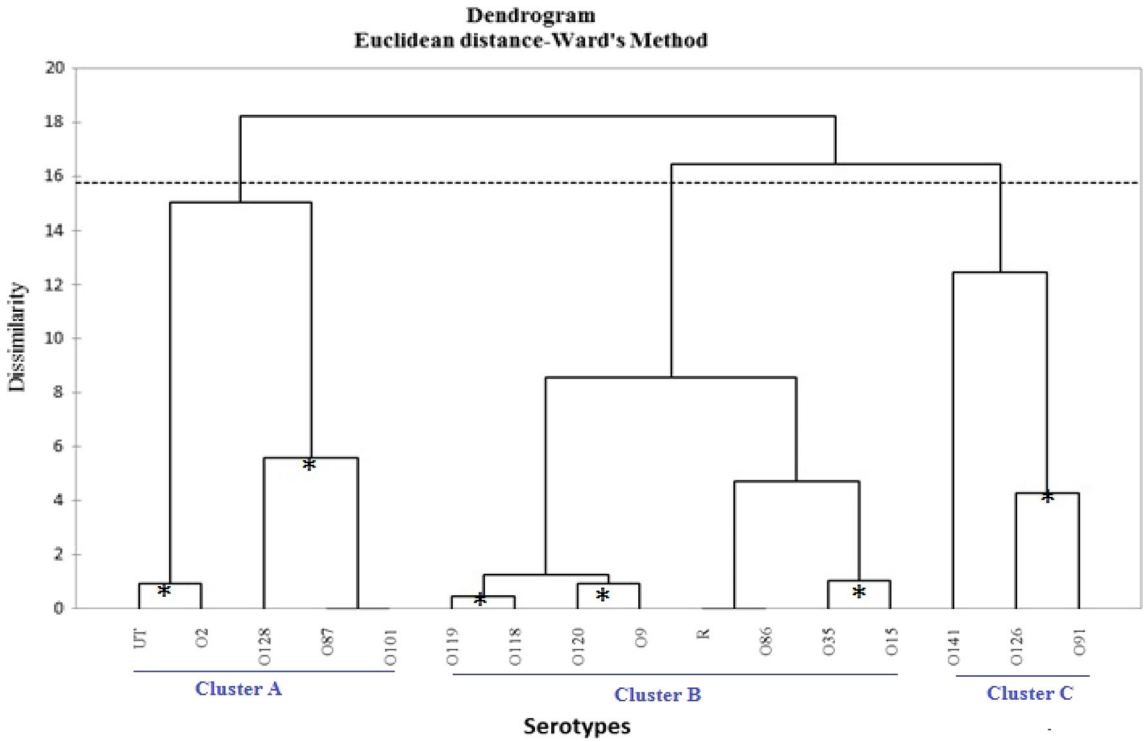

Fig. 4 Cluster illustrating the similarity among different serotypes of *E. coli* isolates and their responses against different class of antibiotics

E. coli were isolated consistently from all the sites, with differences in their abundance level. Molecular-based assays and a number of other investigations have shown that pathogenic FIBs are prevalent in marine waters being impacted by sewage. These faecal-derived pathogens can cause a broad range of asymptomatic to severe gastrointestinal, respiratory, eye, nose, ear, and skin infections in people exposed through recreational use of water. Moreover, their multiple drug resistance nature is worrisome as the circulation of these microorganisms in the environment not only affects the ability to treat the infection but also the cost and duration. Present investigation revealed the presence of 95% of the isolated FIBs as MAR. Likewise, similar studies have been reported by various researchers from different types of surface waters: rivers (Watkinson et al. 2007), estuaries (Sukumaran et al. 2012), lakes (Edge and Hill 2005), and coastal waters (Maloo et al. 2014). The ability of *E. coli* and *Enterococci* to acquire antibiotic resistance by horizontal gene transfer has exacerbated the problem of antibiotic resistance in bacteria (Huddleston 2014). Active population of beach-adapted faecal bacteria may play an intermediary role in mobilizing resistance genes between environmental bacteria and pathogens (Martinez 2009). Although mutations contribute to antibiotic resistance, most pathogens acquire resistance genes through horizontal transfer of mobile genetic elements such as plasmids and transposons. Worldwide, Augmentin is commonly used to treat urinary tract infection caused by uropathogenic *E. coli* (Rahnama et al. 2009), but surprisingly, 100% of the isolates from this study were found to be resistant against it which can be a cause for concern. Mumbai waters also encountered the presence of pathogenic serotype O2 which is a known cause of urinary tract infections and its resistance against the widely used drug Augmentin can be fatal. According to researchers, Augmentin-resistant *E. coli* is usually treated with a combination of gentamicin and ceftriaxone, but 29% of the isolates from this study were resistant to ceftriaxone. In urinary tract infections, previous treatment with Augmentin is a risk factor for the development of Augmentin resistance, and its resistance can be attributed to mechanisms like β-lactamase overproduction, AmpC, cephalosporinase hyperproduction, and inhibitor-resistant penicillinases (Oteo et al. 2008). Resistance to ciprofloxacins as well as broad-spectrum first-generation quinolone antibiotics is reported to be slowly emerging in Asian, South American, and African countries (Sreela et al. 2011). Almost 97% of the isolates studied here expressed resistance to nalidixic acid, the first-generation quinolone. Antibiotics from this class inhibit the activity of bacterial DNA gyrase and DNA topoisomerase enzymes, which are essential for replication. Single nucleotide polymorphisms (SNPs) in the quinolone resistance-determining regions (QRDR) of gyrA and parC, the two genes that encode DNA gyrase and topoisomerase IV, respectively, can lead to conformational changes in these enzymes that cause them to block quinolones from binding to the DNA-substrate

complex, yet still preserve their enzymatic function (Sreela et al. 2011). Resistance to penicillin and extended penicillin class of antibiotics was common among beach-isolated *E. coli* and can be attributed to production of β-lactamase by these organisms. Around 65–68% of the isolates from this study were found to be resistant to ampicillin and ampicillin/sulbactam which may be due to β-lactamase-producing *E. coli.* There are over 200 β-lactamase genes, some of which are carried on transmissible plasmids that can result in dissemination of resistance genes to pathogens (Brinas et al. 2002). High levels of ampicillin resistance in *E. coli* isolated from water sources can be attributed to sewage contamination (Amaya et al. 2012, Kappell et al. 2015).

Third-generation cephalosporins are broad-spectrum drugs with high intrinsic activity against Gram-negative species. Surprisingly, in the present study, it was observed that 58 and 13% of the isolates were resistant and showed reduced susceptibility to cephalosporins, respectively. The rising resistance to these drugs is worrisome because it could be a proxy for the emergence and spread of Enterobacteriaceae strains producing extended-spectrum β-lactamase (ESBL). Studies on antibiotics resistance revealed that F porin mutation in *E. coli* may confer resistance to the newer cephalosporins such as cefmenoxime, cefepime, ceftazidime, and cefuroxime (Moosdeen 1997).

Some of this growing resistance in *E. coli* and other bacteria are due to the fact that antibiotics are being overprescribed, handed out to patients who have no bacterial infections. There is also evidence that the genes that give bacteria resistance to drugs are being spread in livestock-farming operations, where antibiotics are a common ingredient in animal feed. Ciprofloxacin is one of those antibiotics and researchers have found that *E. coli* resistant to it are thriving in poultry farms. Overuse or misuse of antibiotics in animals for non-therapeutic use such as prophylaxis, growth promotion, or increase of feed efficiency are leading to the rise or emergence of antibiotic resistance (Paulson et al. 2015). Therefore, it is vital to limit therapeutic antibiotic use in animals and non-therapeutic use should be reduced. Spread of antibiotic resistance is amplified when this bacteria is acquired by the person recreating at beaches and when this bacteria enters his intestinal tract and passes on the resistance to other flora of the system. According to Krumperman (1983), the choice of MAR index of 0.2 to differentiate between low- and high-risk contamination is arbitrary. Indices between 0.2 and 0.25 are in a range of ambiguity, and samples in this range require careful scrutiny. Overall, indices at all stations exceeded the arbitrary level which revealed that all stations were highly polluted with faecal bacteria originating from a high-risk source. Also, MAR indexing of individual isolates ranged from 0.09 to 0.64, which is much higher than 0.25 (Table 6), indicating the high risk source of contamination.

Perelle et al. (2007) reported that food contaminated by pathogenic *E. coli* serotypes, including O103, represents a major public health concern. Isolation of a great number of isolates with serotypes O2, O91, O86, and O15 belonging to UPEC, EPEC, EHEC, and ETEC categories of pathotypes was a matter of concern. Literature review showed that most of the serotypes encountered in the current study are potential pathogens as they are the cause of various diseases associated with humans and animals such as birds mentioned in Table 7. It is well documented that hospital wastes are often contaminated by antimicrobial agents which even the subinhibitory concentration may promote selection and survival of resistant strains (Al-Ahmad et al. 1999; Kim et al. 2007). Occurrence of *E. coli* in coastal waters belonging to different serotypes can be attributed to hospital wastes released through sewage (Chandran et al. 2008b). Sukumaran et al. (2012) reported the presence of diverse serotypes in Cochin waters, and a similar observation was made in the current study wherein more diverse serotypes were isolated from site 3 (Mahim) which suggests the possible release of these organisms through waste derived from hospitals located in the vicinity. Infections caused by such serotypes may pose a significant public health risk in our country. Furthermore, serologic antigens are not directly involved in virulence but can provide crucial information about the prevailing serotypes on the environment and those involved in disease outbreak (Nataro and Kaper 1998). Pathogenecity of these serotypes also relies on the determination of virulence genes (VGs) present in their genome; however, the presence of single or multiple VGs does not necessarily indicate that the strain is pathogenic unless it expresses the appropriate combination of VGs to cause disease in the host (Sidhu et al. 2013). Thus, our future studies intend to focus on determination of virulent genes specific for ETEC, EPEC, EHEC, and STEC. Preliminary studies on this objective indicated the presence of shigatoxin genes (stx1 and stx2) specific for EHEC and STEC and heat-stable toxin gene specific for ETEC in a few of the pathogenic serotypes (data not presented here).

Emphasis should be placed on environment and coastal management in India. This will thereby provide many general opportunities to improve beach environmental management and to achieve successful management outcomes; broadening our perspective of health of beaches is required. Collaborations between researchers and environmental managers will provide correct guidance and direction for planning of management strategies.

Conclusion

Coastal waters of Mumbai have emerged into a dumping ground for the waste generated in the city. Deteriorated microbiological quality of recreational waters indicated the poor health status of the beaches monitored in this study. Elevated levels of *E. coli* in surface water and sediment of beaches represent a public health risk to those who frequently get

Table 7 *E. coli* of different serotypes encountered in this study and its relevant information from literature

Serotype	As per literature			Reference
	Tentative pathotypes	Probable reservoir	Disease associated	
UT	ETEC, EAEC, EPEC	Humans, dogs, cats, pigs	Acute diarrhoea, cholera-like disease, infant diarrhoea, traveller's diarrhoea	Boerlin et al. 1999, Nataro and Kaper 1998
O141	non-O157 STEC, EPEC, EHEC	Humans, dogs, cats, pig—post weaning	Mild and non-bloody to bloody stools with no leucocytes, haemorrhagic colitis, intestinal and renal disease, Edema	Benenson 1995, Boerlin et al. 1999
O2	STEC, UPEC	Humans, dogs, cats, newborn rabbit, pigeons	Haemorrhagic uremic syndrome (HUS), bloody diarrhoea, gastroenteritis, poultry cellulitis and septicaemia	Boerlin et al. 1999, Benenson 1995, Dutta et al. 2013, Hatha et al. 2004
O119	EPEC, STEC, EAEC, APEC	Humans, dogs, cats, cattles, birds	Diarrhoea (bloody/mucos), gastroenteritis	Boerlin et al. 1999, Nataro and Kaper 1998, Benenson 1995
O120	None reported	Calves	None reported	Sharma et al. 2006
O35	EPEC	Dogs, cats	Diarrhoea, gastroenteritis, calves' septicaemia	Benenson 1995, Nataro and Kaper 1998
O126	EAEC, EPEC	Humans, dogs, cats	Acute diarrhoea, bloody diarrhoea, cholera-like disease, gastroenteritis	Benenson 1995, Boerlin et al. 1999, Nataro and Kaper 1998
O91	STEC, EHEC	Humans, pigeons, cattles	Intestinal and renal disease, HUS	Boerlin et al. 1999, Benenson 1995, Dutta et al. 2013
O128	EHEC, ETEC	Humans, rabbit—weaned, dogs, cats, pigeons	Acute diarrhoea, bloody diarrhoea, cholera-like disease, gastroenteritis	Boerlin et al. 1999, Nataro and Kaper 1998, Benenson 1995, Franke et al. 1995, Dutta et al. 2013, Schmidt et al. 2000
O87	STEC, EPEC	Humans, ruminants like cows and cattle, pigeons	HUS, haemorrhage and congestion of kidneys in pigeons	Benenson 1995, Boerlin et al. 1999, Nataro and Kaper 1998, Dutta et al. 2013
O9	EPEC, ETEC	Pigs, calves, newborn, pigeons	Acute diarrhoea, cholera-like disease, gastroenteritis, travellers' diarrhoea	Nataro and Kaper 1998, Benenson 1995, Dutta et al. 2013, Sharma et al. 2006
O86	EAEC	Wild birds, crows, pigeons	Diarrhoea (traveller's diarrhoea), gastroenteritis	Nataro and Kaper 1998, Benenson 1995, Dutta et al. 2013, Wieler et al. 2000,
O101	EHEC, ETEC	Humans, porcine, intestinal tract of ruminants especially bovines	Non-bloody diarrhoea	Benenson 1995, Sharma et al. 2006, Franke et al. 1995
O118	EHEC, non O157 STEC	Humans, intestinal tract of ruminants especially bovines	Non-bloody diarrhoea, cattle haemorrhagic dysentery	Boerlin et al. 1999, Nataro and Kaper 1998, Wieler et al. 2000, Benenson 1995
O15	ETEC, EAEC, EPEC	Humans, newborn rabbit, dogs, cats, piglets	Watery or bloody diarrhoea, diarrhoea (traveller's diarrhoea), cholera-like disease	Benenson 1995, Boerlin et al. 1999, Nataro and Kaper 1998
R	EAEC	Humans, pigeons, cows	Traveller's diarrhoea	Dutta et al. 2013, Sharma et al. 2006

ETEC Enterotoxigenic *E. coli*, *EAEC* enteroaggregative E. *coli*, *EPEC* enteropathogenic *E. coli*, *STEC* Shiga toxin-producing *E coli*, *EHEC* enterohemorrhagic E. *coli*, *UPEC* uropathogenic *E. coli*, *APEC* avian pathogenic *E coli*

exposed to contaminated waters. Moreover, the presence of *E. coli* with defined multiple antibiotic resistance and pathogenic serotype is a matter of concern. Further analysis of virulence factors present in marine FIBs using molecular approach will be the most promising. This highlights the need for government authorities to take effective measures (e.g. proper sanitation, disposal facility, public awareness, and waste water treatment) to reduce pollution of beaches to avoid serious consequences of a health care management within localities and visitors. Also, data presented here may be useful for decision makers and resource managers working with environmental planning and management of coastal area to implement proper treatment procedure for waste water and effluents, before discharge into the sea to avoid various human diseases outbreaks.

Acknowledgements The authors are grateful to the Director, CSIR-National Institute of Oceanography (CSIR-NIO), Goa, India and CSIR-NIO, Scientist-in-Charge, Regional Centre, Mumbai for their encouragement and support. The funding for this work was provided by OLP1209. NIO contribution number: 6009.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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