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**Virulence gene and Antibigram profile as markers of pathogenic  
*Escherichia coli* in tropical beaches of North Western India:  
Implications for water quality and human health.**

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## Abstract

The purpose of the present study was to investigate the presence of pathogenic *Escherichia coli* in anthropogenically marred tropical beaches of Mumbai, India. Out of 200 presumptive *E. coli*, (n=125) isolates were analysed for presence of virulence genes specific to diarrheagenic pathotypes using a molecular approach. Fifty four percent of the isolates screened possessed at least one virulence gene. The presence of stx1, stx2, eaeA and hlyA genes was detected in 9%, 13%, 20% and 8% of isolates respectively, LT1 and ST1 genes was detected in 9% and 11% of isolates respectively. Also, Versova had higher prevalence of EPEC pathotype, Juhu and Girgaon had higher prevalence of EHEC and ETEC pathotypes, Dadar and Mahim had higher prevalence of ETEC pathotype. 100% of the isolates were Multiple Antibiotic Resistant with higher MAR indices stipulating an important human health concern due to a risk of developing water borne disease associated with exposure to contaminated waters.

**Keywords:** Fecal indicator bacteria; Virulence genes; Antibigram profile; Water quality; Tropical beaches.

## Introduction

Ocean dumping has emerged as a severe environmental issue worldwide. Unregulated disposal of wastes and other materials into the ocean degrades marine and natural resources and pose human health risk. The health hazards could be severe in heavily populated developing countries like India where, despite of several laws and regulations promulgated by the authorities of the Coastal Zone Management, Environmental Protection Agency and the Pollution Control Boards, waste water and sewage discharges from marine outfalls, sewer outflows and discharges of partially treated and untreated wastes from septic system and storm water runoff from urban and rural areas finds its way

in to the sea degrading the shoreline areas (Borade et al., 2015). However, few studies show that land based sources rather than the local outfall are the source of the higher counts of pollution indicator bacteria in coastal waters (Noble et al., 2006). With a view to keep timely check on coastal water pollution, water quality monitoring programmes are instituted by Indian Government bodies like the Ministry of Earth Sciences where microbiological water quality is assessed by determining the load of fecal indicator bacteria in marine waters according to guidelines of WHO, 2006. Due to vast diversity of fecal borne human pathogens the US-EPA has recommended the use of fecal indicator bacteria (FIB) commonly *Escherichia coli* (*E. coli*) and *Enterococci* to determine if fecal pollution is present. The indicator organism marks the proxy and serves as surrogate for pathogenic organisms.

*Escherichia coli*, being a commensal microorganism, is widely distributed in the intestinal tracts of warm blooded animals (Ishi et al., 2008). *E. coli* is often nonpathogenic, but certain strains possess virulence properties that allow them to cause a wide spectrum of infections in gastrointestinal, urinary, or central nervous systems (Nataro et al., 1998). On the basis of genes encoding for virulence factors, currently six categories of diarrheagenic *E. coli* have been acknowledged: Enterotoxigenic *E. coli* (ETEC) (Dalton et al., 1999), Enteropathogenic *E. coli* (EPEC) (Ruchaud-Sparagano et al., 2007), Enteroinvasive *E. coli* (EIEC) (Kaper et al., 2004), Enterohemorrhagic *E. coli* (EHEC) Shiga toxin-producing *E. coli* or STEC) (Takeda, 2011), Enteroaggregative *E. coli* (EAEC or EAaggEC) (Beauchamp and Sofos, 2010), and diffusely adherent *E. coli* (DAEC) (Scaletsky et al., 2002). These pathotypes are associated with various life threatening diseases both in humans and animals. The problem of coastal pollution is further aggravated if coastal waters used for recreational activities are found to be contaminated by these pathogenic organisms leading to major public health risk. Global estimates indicate that each year more than 120 million cases of gastrointestinal disease and 50 million cases of severe respiratory diseases are caused by swimming and bathing in wastewater-polluted coastal waters (Shuval et al., 2003). Moreover, environmental strains of bacteria are emerging as superbugs due to overgrowing resistance against antibiotics commonly used for treatment causing delay in appropriate therapy with subsequent increased morbidity and mortality (Schwaber and Carmeli, 2007; Tumbarello et al., 2007).

Findings of our recent study highlighted that Mumbai coastal waters are contaminated by Multiple Antibiotic resistant (MAR) serotypes of *E. coli* and is facing a great challenge of anthropogenic pollution (Maloo et al., 2017). This kind of coastal pollution could become a more significant public health issue if these isolates contain virulent genes (VGs) profile similar to EHEC, EPEC, ETEC or STEC. Despite several evidences that *E. coli* strains belonging to different pathotypes are isolated from sewage impacted coastal waters, there is a paucity of information elaborating the detection of

virulence genes specific to different pathotypes in fresh and marine waters. Therefore, the present study was designed to determine the presence of pathogenic *E. coli* using biomarkers. The specific objectives of the study were to (i) determine the frequency of occurrence of Virulent genes specific to pathotypes of *E. coli* (ii) characterize the VGs profile of *E. coli* isolates to determine the most common pathotype and its spatial distribution (iii) determine the antibiogram profile of isolated pathotypes of *E. coli*. The data generated from this study highlighted the extent of pollution and quality of water prevailing in the coast and associated health risk posed by prevalence of MAR diarrheagenic *E. coli* pathotypes in surface waters used for recreational activities.

## **2. Materials and methods**

### **2.1 Description of study area and collection of water samples**

Mumbai (18° 55' N 72° 54''E) is the most populous metropolitan city on the west coast of India. Being an economic capital of the country, its ever increasing population and infrastructure tend to take a toll on the environment. The state of Maharashtra accounts for 653 km long coastline with 17% of sandy beaches and many of them are lying within the Mumbai city (Jayasiri et al., 2013). The beaches here are extensively used for the recreational and holistic purpose. The Girgaon beach in the South west, Dadar and Mahim beach in Central, and Juhu and Versova beach in the North West Mumbai were selected for the study as these are the most famous and highly visited parts of the Mumbai coast (Figure.1). Study sites and their brief description are provided in Table 1 (adapted from Maloo et al., 2017).

One liter water samples were collected in pre-sterilized PEP bottles once per month from May 2015 to April 2016 starting at the middle of the beach with two additional samples taken equidistant (200-400m) from the middle point (one sample from north end and other sample from south end of the beach). Water sample was collected at knee depth halfway between the water surface and beach bottom. Collected samples were transported to the laboratory on ice and processed within 6 to 8 h of collection. The standard membrane filtration method was used for the quantification of presumptive *E. coli* from the collected water samples (APHA, 1998). Briefly, 1-ml and 10-ml samples were filtered through 0.45 µm nitrocellulose (Millipore) filters (47 mm) and placed on Eosin Methylene Blue Agar (Himedia, India). Plates were incubated overnight at 37 °C for characteristic green metallic sheen colonies and thereafter counted to determine the average number of CFU per 100 ml. The isolates were further purified on Hicrome *E. coli* Agar incubated at 37 °C overnight and individual well-isolated blue *E. coli* colonies were selected and transferred onto nutrient agar slants

for further studies. A total of 200 presumptive *E. coli* colonies were isolated during the 12-month sampling phase.

## **2.2 PCR confirmation of *E. coli* and extraction of DNA**

The presumptive *E. coli* were confirmed by polymerase chain reaction using the housekeeping malate dehydrogenase (*mdh*) gene marker as previously described (Omar and Barnard, 2014) using primers described by Ivanetich(2006), and the positive isolates were preserved at  $-80^{\circ}\text{C}$  in 20 % glycerol. *E. coli* MTCC 443 (IMTECH, India) was used as a positive control. The bacterial genomic DNA was extracted using the boiling method as described elsewhere (Dashti et al., 2009; Gilmore and Ferretti, 2003) and the recovered DNA was used as template for amplification reactions.

## **2.3 Detection of virulence genes by conventional singleplex PCR**

The list of VGs and the corresponding pathotypes tested in this study are shown in Table 2. Each isolate was screened for the presence of adhesion, invasion and toxin genes to correctly identify them under four main pathotypes. PCR-confirmed *E. coli* isolates ( $n = 125$ ), i.e twenty five isolates from each site were randomly selected for detection of six diarrheagenic *E. coli* VGs. The primers used for PCR detection of the VGs and other relevant characteristics are listed in Table 3. For each PCR experiment, appropriate positive and negative controls were included. The PCR amplification was performed using a thermocycler system (EppendorfVapoprotect). A master-mix was prepared using the following components: nuclease-free  $\text{H}_2\text{O}$ , 10X buffer containing 15mM  $\text{MgCl}_2$  (1X final concentration), additional  $\text{MgCl}_2$  (8mM for *stx1*,*stx2*,*eaeA* and *hlyA*;25 mM for *LT1* and *ST1*), 1.2mM dNTP mix for *stx1*, *stx2*, *eaeA* and *hlyA* and 10 mM dNTP mix for *LT1* and *ST1*, 0.2-0.5 $\mu\text{L}$  of Taq polymerase (5U/ $\mu\text{L}$ ), 1 $\mu\text{L}$  of 10pM of each primers and 2-5 $\mu\text{L}$  of template DNA was added to each 25  $\mu\text{L}$  reaction. PCR amplifications were carried out for 35 cycles using the following temperature programs (annealing temperature varying with the type of gene as given in Table 3), Initial denaturation:  $94^{\circ}\text{C}$  for 5 min, Denaturation:  $94^{\circ}\text{C}$  for 1min, Annealing ( $44- 63.7^{\circ}\text{C}$ ), Extension:  $72^{\circ}\text{C}$  for 1.30 min, Final Extension:  $72^{\circ}\text{C}$  for 8mins. To detect the amplified product, 5  $\mu\text{L}$  of amplicons was visualized by electrophoresis through a 1.5 % agarose gel (Himedia, India) containing ethidium bromide (0.5 $\mu\text{g}/\text{mL}$ ) (Puregene, USA) at a voltage of 100 for 45 min in 1X TAE buffer using the gel documentation system (Protein simple). Identification of the bands was established by comparison of the band sizes with molecular weight markers of 100-bp (Thermo Scientific, EU, Lithuania). Samples were considered to be positive for a specific VG when the visible band was of the same size as that of the positive control DNA. To minimize PCR contamination, DNA extraction, PCR set up, and gel electrophoresis were performed in isolated rooms. The positive

controls used for this study includes Genomic DNA of *Escherichia coli* O157 (EDL 933, Sigma, Germany) for stx1, stx2, eaeA and hlyA; *Escherichia coli* MTCC 723 (IMTECH, India) for LT1 and ST1.

#### 2.4 Determination of Susceptibility to Antimicrobial agents

We tested the entire isolates positive for virulence genes from each site for susceptibility against 25 antimicrobials belonging to ten classes. The details of the antimicrobials with concentration used are given in Table 4. These tests were performed as previously described by Maloo et al (2017) using an agar-diffusion method and antimicrobial impregnated rings (Hi-Media Ltd., Mumbai, India) as described by the Clinical and Laboratory Standards Institute (CLSI, 2005). Each test was performed in duplicate for each *E. coli* isolate and antimicrobial. Data for susceptibility to antimicrobials tested for each bacterial isolate has been reported as resistant, intermediate (isolates with reduced susceptibility), and sensitive, based on Clinical and Laboratory Standards Institute break points (CLSI, 2005).

The results were used to calculate the Antibiotic Resistance Index (ARI) and Multiple Antibiotic Resistance index (MAR) for total number of isolates as

$$ARI = y/nx,$$

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

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

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

#### 2.5 Statistical analysis

Statistical analysis was performed using XL-STAT (version 6.0, Addinsoft). The datasets for total *E. coli* counts and number of VG bearing *E. coli* with respect to sites and seasons were log<sub>10</sub> transformed prior to statistical analysis. Pearson's correlation analysis was applied to investigate existence of any correlation between total *E. coli* counts and VGs occurrence for the sites and for three different seasons. The difference in VG distribution among the five sites and existence of variation among VG patterns were determined by one way analysis of variance (ANOVA) on the pooled VG bearing *E. coli* data from three seasons, with significance defined as P values of < 0.05. The Student t test was performed to determine the significance of difference in the number of VG bearing *E. coli* between all possible pairs of sites with P value set at <0.05. The data for

pathotypes distribution and occurrence of different virulence genes (in different combinations) with respect to five sites were also used to perform non-metric multidimensional scaling (n-MDS) (after square root transformation and Bray-Curtis similarity calculation) to verify how stations were distributed relatively to each other on basis of pathotypes and occurrence of different virulence genes. PRIMER 6® software (Software package from Plymouth Marine Laboratory, UK) was used for n-MDS.

In order to classify the response of antimicrobials belonging to different classes towards all four pathotypes of *E. coli* isolated from study sites, an Agglomerative Hierarchical Clustering (AHC) analysis was employed, using Euclidean distance and the Ward's method for the aggregation criterion employing XL-STAT (version 6.0, Addinsoft).

### 3. Results

The log<sub>10</sub> transformed results for the detection of *E. coli* are presented in Table S1 (Supplementary table). In general, *E. coli* numbers in water samples from all sites varied between 1.7 to 4.3 log<sub>10</sub> per 100 ml (details given in supplementary table S1) during overall sampling phase. The mean *E. coli* numbers in monsoon period were significantly higher ( $p < 0.05$ ) as compared to postmonsoon period. Samples collected from site 1 (Versova), site 3 (Mahim) and site 4 (Dadar) has significantly higher ( $p < 0.05$ ) *E. coli* counts than site 2 (Juhu) and site 5 (Girgaon).

#### 3.1 *E. coli* confirmation

Out of 200 presumptive *E. coli* isolates recovered from the sampling sites 150 were confirmed to be *E. coli* out of which 125 isolates made up of 25 from each sampling site were pooled together for further analysis. Figure 2 shows the gel electrophoresis image of the PCR products of the *mdh* gene amplification.

#### 3.2 Prevalence of virulence genes (VGs) among *E. coli* isolates

Among 125 confirmed *E. coli* isolates screened for the presence of various VGs, 67 (54%) possessed at least 1 VG and 58 (46%) isolates possessed none. 70% of the isolates showed the presence of one VG while 30% of the isolates showed the presence of 2 VGs. None of the isolates possessed more than two virulence genes. The prevalence of 2 VGs in the *E. coli* isolates was equally higher in Dadar and Mahim than other sites particularly in monsoon. Also, a significant positive correlation was observed ( $r = 0.904$ ,  $p = 0.03$ ) between total number of *E. coli* isolates from each site and *E. coli* isolates that were carrying VGs in water samples from five sites and no correlation was found in occurrence of VG during three seasons. As presented in Table 5, among 125 *E. coli* isolates, the adhesion virulence gene *eaeA* which codes for intimin protein both in EPEC and EHEC pathotypes

was the single most prevalent gene (20%). Out of which, 10% were found to possess only *eaeA* gene, and 3% of the isolates showed presence of *eaeA* and *hlyA* gene. Among the toxin genes, *ST1* which codes for heat stable toxin of ETEC pathotype was the most prevalent (12%), 10% of the isolates possessed only *ST1* gene while 2% of the isolates possessed *ST1* and *LT1* (heat labile toxin), *LT1* alone was found to be present in 7% of the isolates with *ST1* gene being more prevalent in Monsoon (24%). Another toxin gene, *stx2* carried by STEC/EHEC pathotype was prevalent in 13% of the isolates followed by *stx1* (9%) with *stx2* gene least prevalent in Monsoon (7%). Also 11% of the isolates were found to belong to the EHEC category wherein *eaeA* gene in combination with *stx1*, *stx2* was found to be present in 2% and 4% of the isolates respectively. The toxin gene hemolysin (*hlyA*) was found to be present in combination with toxin genes *stx1* (2%) and *stx2* (3%) of the isolates, but none of the isolates screened scored positive for *hlyA* gene alone. The representative gel electrophoresis profile of amplified gene products of the investigated diarrheagenic pathotypes are shown in Figure.3.

In order to further explore the distribution of six VGs among all five sites, an ANOVA was performed on the pooled data of overall isolates collected throughout the sampling phase. There was a highly significant difference ( $p < 0.05$ ) between the occurrence of *stx1* gene and *eaeA* gene, *eaeA* and *hlyA* gene, *LT1* and *eaeA* gene. However, no significant difference was observed between the occurrences of other genes.

### 3.3 Comparative prevalence of *E. coli* pathotypes

The percentage of *E. coli* isolates with defined pathotypes from five sampling sites is shown in Figure. 4 on the basis of combination of VGs, *E. coli* isolates were grouped into four different pathotypes namely EHEC, EPEC, ETEC and STEC. The n-MDS plot as shown in Figure. 5(A-E), clearly reflects the spatial distribution of sampling sites on basis of prevailing pathotypes of *E. coli* (stress: 0). Two groups can be identified in n-MDS plot wherein Versova and Dadar are aggregated together and Juhu, Girgaon and Mahim are aggregated together due to their major contribution towards pathotypes. Percentage distribution of the *E. coli* pathotypes with respect to five sampling sites is shown in Figure 5. Overall, isolates belonging to ETEC pathotype were the most commonly detected (18%) followed by EPEC (14%), EHEC (11%) and the lowest was STEC (10%). All the four different pathotypes were found to be present at five sampling sites. Isolates belonging to ETEC pathotype were dominant at site1 (Versova), site 3 (Mahim) and site 4 (Dadar), while isolates belonging to EPEC pathotype were also dominant at site1 (Versova), whereas Juhu and Girgaon showed equal prevalence of EHEC and ETEC pathotypes and majority of STEC isolates were from Dadar which was also evidenced through n-MDS plot.



### 3.4 Comparison of *E. coli* VG profiles from 5 sampling sites

Based on functional characteristics of genes, VGs are further classified into toxin and adhesin (Table 2). The n-MDS plot (Figure 6A-6K) clearly reflects the spatial distribution of the sampling sites on basis of presence of different virulence genes bearing *E. coli* (stress: 0.03). Three groups can be identified in n-MDS plot: site 3 (Mahim) and site 4 (Dadar) are aggregated together forming one group, while site 1 (Versova) and site 5 (Girgaon) forms another group indicating similarity in the presence of virulence genes; site 2 (Juhu), less polluted appears isolated. Sites 1, 3, 4 and 5 are located quite apart from site 2 indicating distinct difference in the occurrence of VGs. An overall comparative analysis of the distributions of six VGs observed throughout the year across all the five sites is presented in Figure 7. In general the frequency of occurrence of VGs in *E. coli* isolates collected during monsoon period was higher than that during pre monsoon and post monsoon (Table 5). Also the highest frequency of VGs in *E. coli* isolates was obtained at site 4 Dadar (88%) followed by site 1 Versova (72%) and the lowest at site 2 Juhu (24%). The VGs stx 2, ST 1 and eae A were the most frequently detected across all the sites while stx 1 was not detected at site 5 Girgaon. Among the toxin genes screened in this study stx 2 and ST 1 genes were the two most commonly detected genes across all sites after the eaeA gene which was highly prevalent (29%). Out of the five sites, the toxin gene hlyA was least detected in Dadar (10%). A comparison between the sites was made (ANOVA) to determine if the sites were similar or different on the basis of occurrence of VGs. Overall, all the five sites were significantly different ( $p=0.014$ ) in the occurrence of genes with higher significant difference between site 2 (Juhu), site 4 (Dadar) and site 5 (Girgaon) as compared to other sites.

### 3.5 Susceptibility to antimicrobial agents

Distribution of multi-antimicrobial resistance in *E. coli* isolates was almost similar and not varied significantly ( $\chi^2$ :  $p<0.001$ ) across the five sites. The key observation was that 100% of the isolates tested for antimicrobial susceptibility were Multiple Antimicrobial Resistant (Table 5) with MAR index ranging from 0.24-0.92. The major issue of concern here was that the isolates showing resistance to more than twelve antibiotics were isolated from all the five sites with a MAR index of above 0.3 (except two isolates marked in bold Table 5). Figure 8 gives the percentage of resistant isolates from each site isolated in different seasons and overall MAR index with respect to sites. AR index of all sampling sites exceeded the high risk level (0.25) and revealed that Dadar has the highest AR index (0.75) followed by Versova (0.71), Mahim (0.70), Juhu (0.65) and Girgaon (0.55).

It was observed that all the isolates tested against antimicrobials from five sites along the Mumbai coast possess different levels of susceptibility towards antimicrobials tested. All the isolates from the study area were resistant to Nalidixic acid (NA) and Amoxyclav (AMC) as highlighted in Table 4. 91% of the isolates were resistant to Ticarcillin/Clavulanic acid (TCC), Imipenem (IMP) and Ampicillin (AMP), 81-88% of the isolates were resistant to Cefoperazone (CPZ), Aztreonam (AT) Ceftazidime/Clavulanic acid (CAC) and Gatifloxacin (GAT); 70-78% of the isolates were resistant to Cefepime (CPM), Cefotaxime (CTX), Ceftriaxone (CTR), Ceftizoxime (CZX), Ofloxacin (OF) and Ampicillin/Sulbactam (A/S); 67-68% of the isolates were resistant to Ceftazidime (CAZ), Cefuroxime (CXM) and Co-Trimoxazole (COT); 52-58% of the isolates were resistant to Ciprofloxacin (CIP) Meropenem (MRP), Levofloxacin (LE), Cefoxitin (CX) and Piperacillin/Tazobactam (PIT) and 15-27% of the isolates were resistant to Amikacin (AK) and Gentamicin (GEN).

### 3.5 Pathotype clustering

Antimicrobials belonging to different classes were clustered into groups according to similarity in response towards different pathotypes of *E. coli* as given in Figure. 9. Ten classes of antimicrobials were grouped into three major clusters [Cluster A (green), Cluster B (red), Cluster C (blue)] which were further divided to form subclusters. Antibiotics belonging to Penicillin combinations (PC) and Cephalosporins (C) showed the most similar response to *Escherichia coli* belonging to different pathotypes as reflected in subcluster of Cluster A. Similarly, antimicrobials from penicillin (P) and cephalosporins combinations (CC) class showed similar response to pathotypes of *E. coli* (subcluster of Cluster C). Further in cluster A, monobactams and Fluoroquinolones forms subclusters at higher ED, indicating increased dissimilarity as compared to the above mentioned subclusters. Cluster B consists of Quinolones and sulphonamides, showing similarity in response to pathotypes of *E. coli* but are dissimilar as compared to antimicrobials in Cluster C. Cluster C and Cluster D are linked together at 6.3 ED which is much higher than the cut-off point 2.5 ED, indicating increasing dissimilarity among the response of antimicrobials towards pathotypes of *E. coli*. Also, Cluster A and Cluster B are linked together at 2.8 ED (near the cut off point 2.5 ED), indicating similarity in response to antibiotics, more than antibiotics belonging to Cluster C and Cluster D.

### 4. Discussion

In the present study, VGs were detected in the multiple antimicrobial resistant *E. coli* isolates indicating the presence of pathogenic *E. coli* strains in surface waters. This may be due to persistent input of these bacteria from a common source or a transition of *E. coli* between virulent and non-

virulent forms by acquiring or losing virulence genes through Horizontal gene transfer or a combination of both. A potential cause of higher counts of *E. coli* in the monsoon period could be fresh human sewage input from sewage leakage and overflow or inputs from animal and bird sources (Davies and Bavor, 2000), mobilization of *E. coli* in the water column from sand and sediments (Brennan et al., 2010; Czajkowska et al., 2005). The results showed significant higher levels of *E. coli* bearing VGs ( $p=0.02$ ) isolated during the monsoon as compared to postmonsoon; however no correlation could be found between the *E. coli* numbers and the occurrence of VGs during the three seasons. This exhibits a potential risk of infection from *E. coli* bearing VGs in surface water even when *E. coli* numbers in water are not high.

Out of 125 isolates screened for the presence of virulence genes, 67 isolates tested positive for the presence of virulence genes. The adhesion gene *eaeA* associated with EPEC and EHEC strains was the most prevalent of all (20%) and this gene is necessary for intimate attachment to host epithelial cells in both the pathotypes. Similar observations were reported previously in watersheds of Baltimore (Shelton et al., 2006), and surface water of Brisbane (Sidhu et al., 2013). However *eaeA* alone was detected in 10% of isolates indicating wide prevalence of this gene within *E. coli* found in ecosystems. Similarly, high prevalence of the *eaeA* gene in surface water has been reported in other studies (Shelton et al., 2006; Masters et al., 2011). This is a cause of concern, as an atypical EPEC pathotype which carries only *eaeA* gene has been found to be a major cause of gastroenteritis worldwide (Obi et al., 2004). Further studies need to be carried out to check the presence of *bpf* gene to confirm the identity of these strains to be atypical EPEC. The relatively high occurrence of the combination of both typical and atypical EPEC genes in environmental waters is alarming due to its possible significance in the cause of severe diarrhea in humans.

*E. coli* belonging to EHEC pathotype cause hemorrhagic colitis and haemolytic uremic syndrome in humans, and the key virulence factors include intimin (*eaeA* gene), and shiga toxin genes (*stx1* and *stx2*) (Petit et al., 2017). Thus, occurrence of these genes in *E. coli* from surface waters of beaches is a matter of concern. In this study, *stx2* gene belonging to STEC/EHEC pathotype was second most prevalent gene (13%), and was also found to be present in combination with *eaeA* gene in 4% of the isolates and with another virulence factor *hlyA* in 3% of the isolates. Co-occurrence of *stx1* with *eaeA* and *stx1* with *hlyA* was found to be present in 2% of the isolates. *E. coli* carrying a combination of the *eaeA* and *stx2* genes was more common than the combination of *eaeA* gene and *stx1* gene. This observation is of concern as the former combination of genes is known to cause more severe diarrhea in humans (Paton and Paton, 1998). The estimated annual occurrence of waterborne and food-borne infection due to this pathogen is 37,000 cases, with 1,000 hospitalizations and 30

deaths, and it is as dangerous as *E. coli* O157 (Hunter, 2003; Rasmussen and Casey, 2001). Though, none of the isolates possessed a combination of *eaeA*, *hlyA*, *stx1* and *stx2* together, there is a high probability of occurrence of these combinations due to common mechanism of horizontal gene transfer enabling *E. coli* evolution and diversification (Oh et al., 2017). Moreover, in this study, isolates belonging to STEC pathotype were also encountered with relatively high occurrence of *stx2* gene (6%) compared to *stx1* (5%) suggesting that the capability of each gene in causing acute diarrhea in humans. Also, it has been documented that the *stx2* gene is considered to be most important virulence factor associated with severe human disease than was *stx1* indicating the importance of *stx2* in human infection (Naidu et al., 2011). *Stx2* gene is about 400 fold more toxic to mice than *stx1* and also has been shown to induce feto-placental reabsorption, intrauterine haematoma, fibrin deposition and neutrophil infiltration. Similar observation was reported by Sidhu et al. 2013, wherein *stx2* gene (10%) predominated *stx1* gene (6%) in the storm water *E. coli* isolates. However, our results contradicted with the relatively high occurrence if the *stx1* gene (6%) compared to *stx2* (1%) in the waters of South western Nigerian rivers (Titilawo et al., 2015).

The toxin gene ST1 associated with ETEC pathotype was the third most prevalent gene (12%) after *stx2* and *eaeA* gene. This finding is worrisome considering the fact that it is the most common agent of traveller's diarrhea with food and water implicated as the mode of transmission (Vidal et al., 2005). LT1 gene was found to be present in 9% of the isolates. Genes for LT and ST enterotoxins (LT1 and ST1) could be encoded together or separately on large variable plasmids called Entplasmid (Turner et al., 2006). Also, LT1 and ST1 together were found to be present in 3% of the isolates which is similar with the findings of Obi et al. (2004) wherein only 4% of the isolates recovered from South African river possessed both LT1 and ST1 genes. However some other studies from Indian rivers showed that 75% of ETEC isolates from waters of Ganga possessed both LT1 and ST1 (Ram et al., 2009) and 67% of ETEC isolates from waters of Saryu rivers possessed both LT1 and ST1 (Ram et al., 2008).

In this study, some of the genes (LT1, ST1 and *hlyA*) observed in *E. coli* isolates were plasmid encoded; therefore it is likely that exposure of the human population with surface waters in recreational beaches will enrich the environmental gene pool of the virulence genes and lead to emergence of new pathogenic variants. Although, the presence of a single or multiple VGs in an *E. coli* strain does not necessarily indicate that a strain is pathogenic unless that strain has the appropriate combination of VGs to cause disease in the host (Stange et al., 2016). VGs are considered ideal targets for determining the pathogenic potential of a given *E. coli* isolate (Kuhnert et al., 2000) and therefore, it was suggested that the direct screening of VGs in surface waters can be used as a

tool to indicate water quality (Masters et al., 2011). Therefore, in the present study, we evaluated the presence of VGs in *E. coli* isolates obtained from water, indicating the actual presence of *E. coli* strains carrying VGs in the water, which is more important from a public health point of view than detecting only the VGs directly from water without culturing the bacteria. However, in the absence of actual isolates, no conclusions could be drawn regarding the relative prevalence of pathotypes carrying VGs or the prevalence of potentially pathogenic *E. coli* strains (Sidhu et al., 2013).

On the basis of the combination of VGs, 67 isolates from this study were grouped into four main pathotypes. Pathotyping of *E. coli* isolates may also provide useful information to identify potential sources of pollution, as the principal reservoirs of ETEC and EPEC pathotypes are majorly humans and ruminants, whereas the bovine intestinal tract is the main source of the EHEC pathotype (Kaper et al., 2004; Levine, 1987). The lower prevalence of the EHEC pathotype compared to other pathotypes suggests that human fecal contamination is the main source of diarrheagenic *E. coli* pathotypes in the surface water as opposed to contamination from animals. This highlights the importance of controlling sources of human fecal pollution such as municipal wastewater sources, sewage leaks and overflows, wastewater treatment plant discharge to reduce potential threats to human health. Among the 54% of the isolates, 18% of the isolates belonged to ETEC pathotype, 14% of the isolates belonged to EPEC pathotype, 11% of the isolates belonged to EHEC pathotype followed by STEC (10%). Spatial distribution of pathotypes as depicted in n-MDS plot (Figure 4) reflects that Versova and Dadar had almost similar distribution of pathotypes, while Juhu, Girgaon and Mahim are aggregated together as they had similar distribution of pathotypes. As shown in Figure 4B, EPEC pathotype was predominant in Versova and there is high likelihood that the isolates were mainly from human and animal excreta (particularly dogs) since during sampling periods human and animal excreta were sighted at the beach area exposed during low tides. This further implicates both humans and animal as potential sources for the recovered *E. coli* pathotype. ETEC pathotype was predominant in Dadar, followed by Versova and Mahim (Figure 4C) indicating that contamination sources point or non point sources were potentially similar across these sites and this agrees with the fact that all these three sites have creeks and rivers carrying domestic discharge, treated and untreated sewage, waste from nullahs (sewerage), and a fishing community emptying into near shore waters. Higher prevalence of EHEC pathotype (Figure 4D) in Dadar followed by Mahim and Girgaon indicates the probability of non-human fecal contamination of the water and is the main source of diarrheagenic pathotypes in the surface water as compared to contamination from human sources. In these sites, there is a high possibility of fecal contamination derived from birds as during our sampling period pigeons; crows and seagulls were sighted at the beach areas. STEC

pathotype is the subset of EHEC, therefore almost similar pattern of spatial distribution (prevalent in Dadar and Versova) was observed. Though cattle and sheep are regarded as principle reservoir of STEC, it is also prevalent in gastrointestinal tract of other animals like pigs, dogs, and cats. Also, previous study from India (Farooq et al., 2009) showed presence of STEC in pigeon droppings.

Figure(6A-6K) depicts the further distribution of individual VGs across the sites indicating mixed sources of contamination. Aggregation of Mahim and Dadar in one group may be attributed to occurrence of *E. coli* bearing LT1 and ST1 together and co-occurrence of stx1 with eaeA. Similarly, aggregation of Versova and Girgaon in one group may be attributed to *E. coli* bearing stx2, ST1, stx2 coupled with hlyA gene. Juhu had a lower prevalence of VGs as compared to other sites as evidenced in n-MDS plot. Further, predominance of stx1, eaeA, and LT1 genes bearing *E. coli* at Versova may be attributed to domestic sewage, treated and untreated effluents originating from Malad creek, human and animal excreta, and waste from fishing boat and community. Similarly, predominance of LT1, stx2 coupled with hlyA, stx1 coupled with eaeA genes bearing *E. coli* at Mahim may be attributed to point source of contamination like waste from Mahim creek, sewage from nullahs, and excreta of birds. Predominance of stx1 coupled with hlyA, eaeA bearing *E. coli* at Juhu may be attributed to non point sources like excreta of dogs and birds as there is no known point source of pollution present in the vicinity. Predominance of stx2, ST1, stx1 gene coupled with hlyA gene and eaeA, stx2 coupled with eaeA at Dadar may be attributed to sewage, discharges from nullahs in the vicinity, excreta from humans, animals and birds and domestic sewage from Mithi river. Predominance of stx2 gene coupled with eaeA gene bearing *E. coli* at Girgaon may be attributed to sewage from nullahs in the vicinity, excreta from humans, birds and animals (particularly dogs). Overall, it was evident that the point and non-point sources of contamination were potentially similar across the sampling sites in their characteristics features. However, it should be noted that sources mentioned above were sighted during sampling, but there can be other factors responsible for fecal contamination in surface waters of these beaches. Further, Microbial Source Tracking (MST) studies should be conducted to confirm the source of contamination in these beaches, and therefore, future studies will focus on MST to find the source of *E. coli* pathogens in the water.

The occurrence of *E. coli* in an environment particularly used for recreation is a global health concern. The problem has been recently exacerbated by the emergence of multiple antimicrobial resistant *E. coli* strains in aquatic environments worldwide. One hundred percent of the isolates from this study exhibited resistance against multiple antimicrobials. This can probably be attributed to *E. coli* and other enteric bacteria that survived the extensive antibiotic treatment in the gut of humans or animals and enter aquatic systems through discharge from poultry and livestock production and hospital and

municipal waste water. Apart from its usage in human medicine, antibiotics are also exploited as therapeutics, prophylactics, and metaphylactics in animal and aquaculture farms (Romero et al., 2012). Overuse or misuse of non-therapeutic antibiotic for animals such as prophylaxis for growth promotion or increased feed efficiency, are leading to a 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 a person recreating at a beach when the bacteria enters the intestinal tract and passes the resistance to other flora in the system. The multiple antimicrobial resistance among 100% of the virulence genes bearing *E. coli* isolated from this study is worrisome because disease caused by these organisms will be very difficult to treat and they are the main cause of childhood diarrhea worldwide causing over 2 million deaths each year (Farthing et al., 2013). It is therefore imperative that the determination of antibiotic susceptibility patterns of isolated microbes is a part of the microbial monitoring process of coastal waters (Chandran et al., 2008). According to Krumperman (1983), the choice of a 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 in the present study exceeded the arbitrary level which revealed that all stations were highly polluted with fecal bacteria originating from high risk sources. Also MAR indexing of individual isolates ranged from 0.24-0.92 (Table 6), indicating high risk sources of contamination. Worldwide, Amoxycylav is commonly used to treat urinary tract infections caused by Uropathogenic *E. coli* (Rahnama, 2009), but surprisingly one hundred percent of the isolates from this study were found to be resistant against it, which can be a cause for concern. Moreover, resistance to broad spectrum first-generation Quinolone antibiotics is reported to be slowly emerging in Asian, South American, and African countries (Sreela et al., 2011) and is also evidenced in this study, since almost 100% of the isolates studied here expressed resistance to Nalidixic acid. 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). Around 91% of the isolates from this study were found to be resistant to Ampicillin and Ticarcillin/Clavulanic acid which may be due to  $\beta$ -lactamase producing *E. coli*. There are over 200  $\beta$ -lactamase genes some of which are carried on transmissible plasmids that can result in dissemination of resistance genes to pathogens (Brinas, 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). Therapeutic options have become somewhat limited because of the emergence of organisms carrying extended-spectrum  $\beta$ -lactamases (ESBL) and plasmid-mediated AmpC  $\beta$ -lactamases. The carbapenems (imipenem, meropenem, and ertapenem) are sometimes the only effective agents for treatment of severe infection caused by ESBL-positive *E. coli* as Carbapenem resistance has been rarely reported in *E. coli* (Hong et al., 2005). On the contradiction, 91% of the isolates from this study exhibited resistance against Imipenem from class carbapenems, 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  $\beta$ -lactamase (ESBL). Third-generation Cephalosporins are broad-spectrum drugs with high intrinsic activity against Gram-negative species. Surprisingly, in present study it was observed that 55-88% of the isolates were resistant to Cephalosporins and Cephalosporins combinations. 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).

Due to all of the aforementioned issues, emphasis should be placed on environment and coastal management in India. This will provide many general opportunities to increase beach environmental management. To achieve successful management outcomes, broadening of India's perspective of health, working to improve the health of beaches is first required. Collaborations between researchers and environmental managers will provide correct guidance and direction for planning of management strategies.

## 5. Conclusion

Coastal waters of Mumbai beaches were found to be contaminated with fecal matter confirmed by detection of heavy loads of *Escherichia coli*. Presence of virulence genes specific to EHEC, EPEC, ETEC and STEC pathotypes in *E. coli* indicated health risks to the public exposed to contaminated water during recreational activities. Diarrheagenic pathotypes ETEC and EPEC were found to be predominant among the studied sites, particularly in the monsoon, attributable to sewage overflows and land runoffs. Moreover, these pathogenic *E. coli* were found to be MDR which can be considered as a direct threat to human health. High Antibiotic resistance indices of isolates from all sampling sites indicated high risk sources of contamination. Overall, it can be concluded that waters prevailing in the beaches of Mumbai are of microbiologically poor quality and therefore effective measures (e.g. proper sanitation, efficient disposal facilities, and wastewater treatment) should be taken. Thus, future studies on other famous beaches of Maharashtra are recommended to avoid serious health consequences for locals and visitors.



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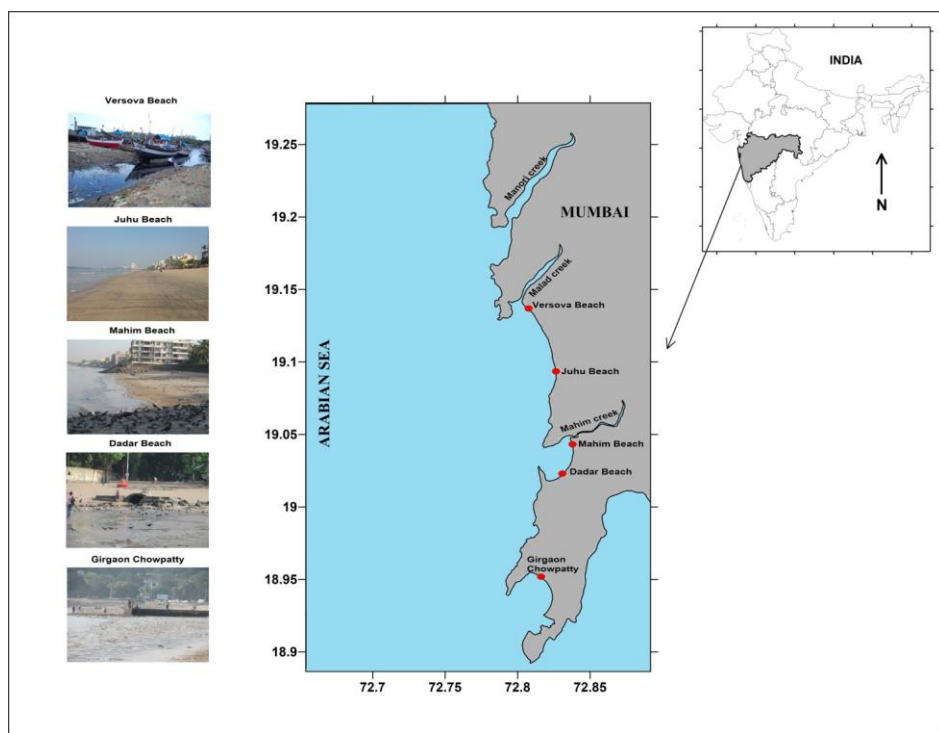
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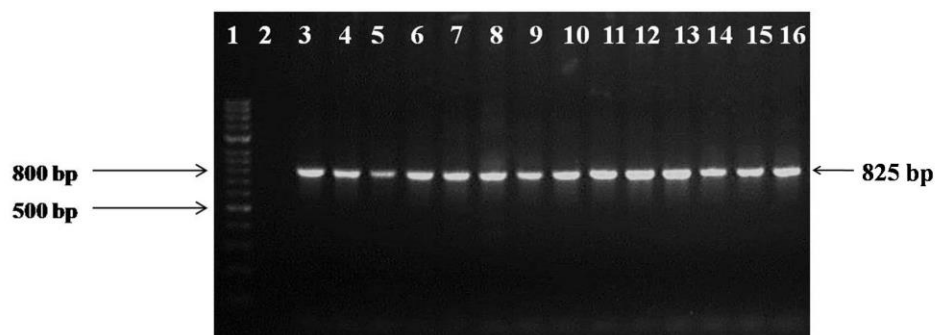
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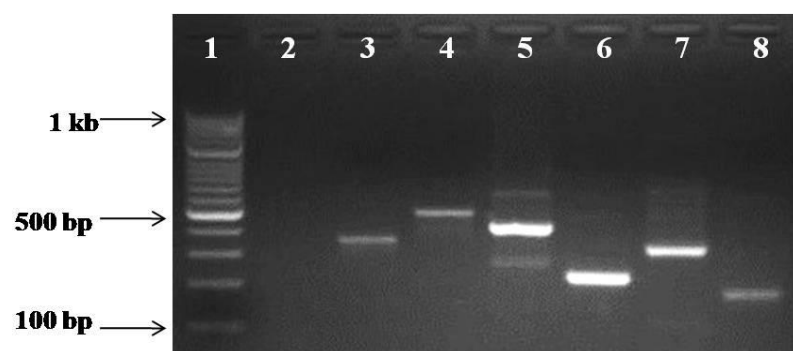
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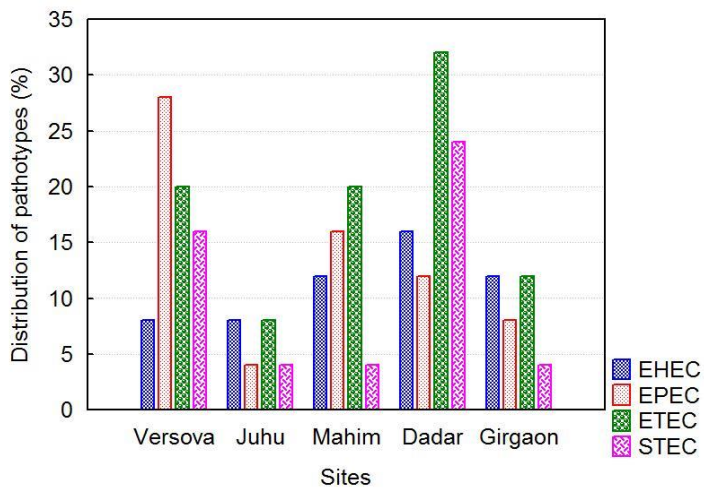
**Fig.1.** Map showing the sampling sites



**Fig.2.** PCR products of the amplification of *mdh* gene. Lane1: Molecular weight marker (100bp); Lane 2: negative control; Lanes 3-15: positive isolates; Lane 16: positive control MTCC 443



**Fig.3.** A representative gel electrophoresis image showing different virulence genes profile of *E. coli* isolates. Lane 1: molecular weight marker (GENESTA™ 100 bp DNA ladder), Lane 2: negative control, Lane 3: stx1 (348 bp), Lane 4: stx2 (478 bp), Lane 5: eae A (413bp), Lane 6: hlyA (224 bp), Lane 7: LT1 (322 bp), Lane 8: ST1 (175 bp).



**Fig.4.** Comparative distribution of *E. coli* pathotypes from all five sites in Mumbai, India

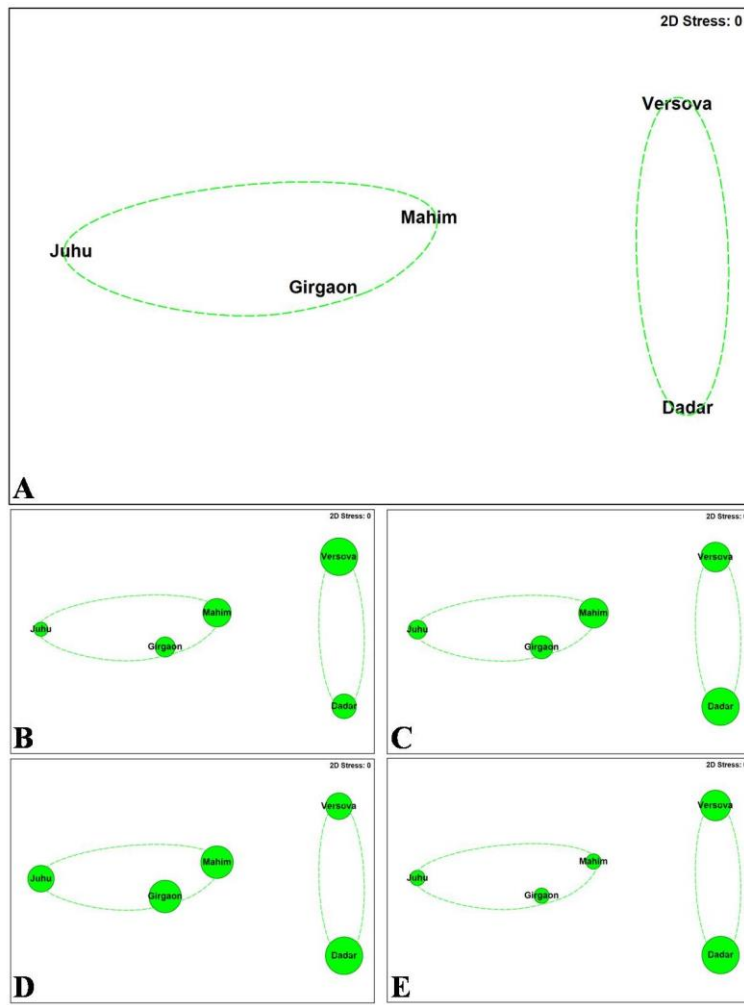
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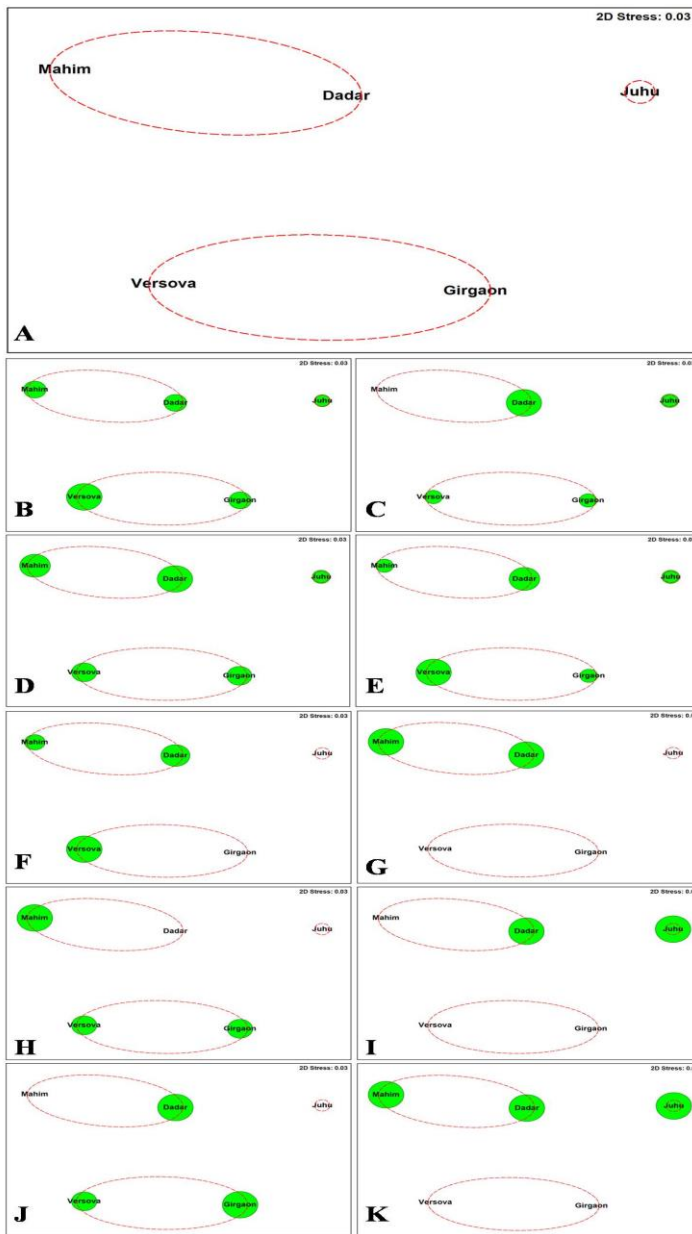
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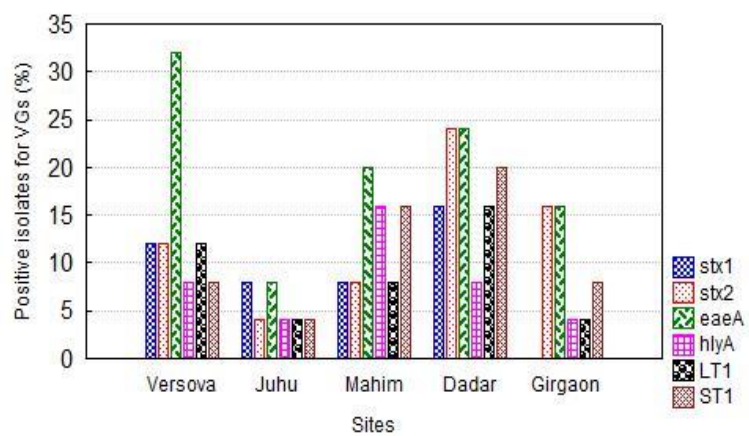




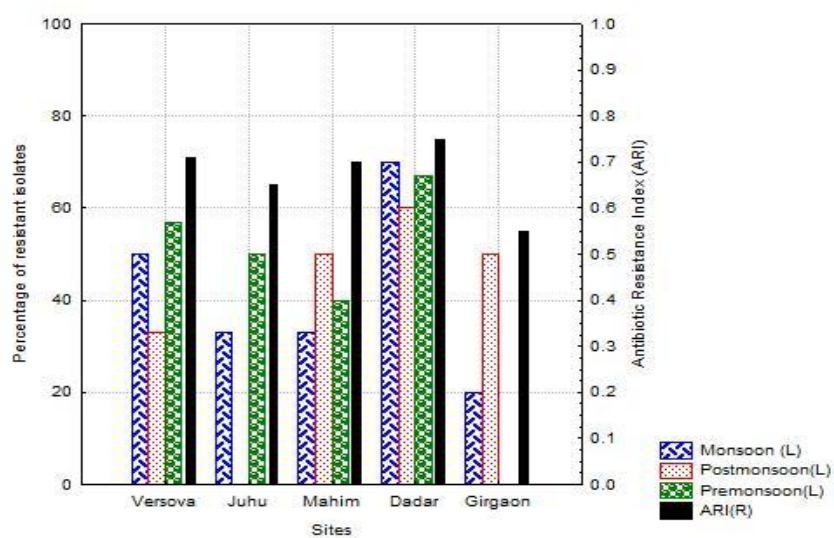
**Fig.5.** n-MDS ordination based on the following variable obtained from pathotypes data: number of *E. coli* isolate grouped in a particular pathotype (stress: 0). Stations are grouped as a function of occurrence of different pathotypes.



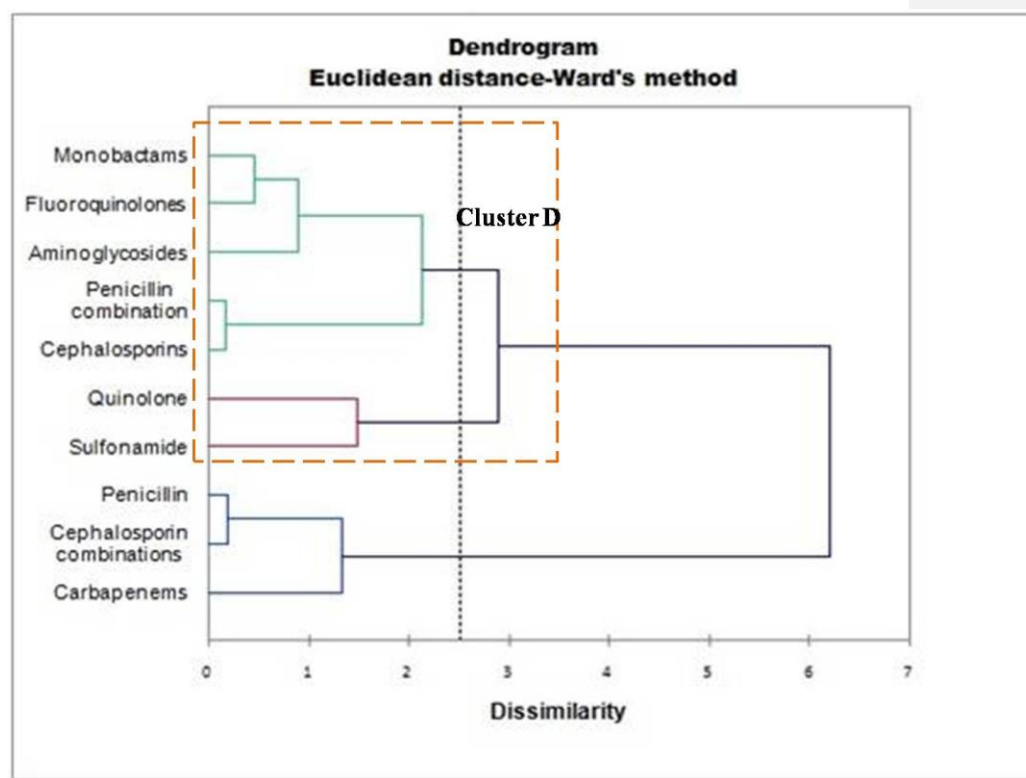
**Fig.6.** n-MDS ordination based on the following variables obtained from virulence genes data: number of isolates positive for virulence genes (stress:0.03).stations are grouped as a function of occurrence of different virulence genes bearing *E. coli*.



**Fig.7.** Comparative distribution of VGs in *E. coli* from all five sites in Mumbai, India



**Fig.8.** Susceptibility of *E. coli* isolates to antimicrobials from five sites and site specific Antibiotic Resistance Index (ARI)



**Fig.9.** Cluster illustrating the similarity among different class of antibiotics and their responses against different pathotypes of *E. coli* isolates.

**Fig.8.** Susceptibility of *E. coli* isolates to antimicrobials from five sites and site specific Antibiotic Resistance Index (ARI)

**Fig.6.** Susceptibility of *E. coli* isolates to antimicrobials from five sites and site specific Antibiotic Resistance Index (ARI)



**Table 1:** Sampling sites and their description

<u>Site name</u>	<u>Latitude</u> <u>Longitude</u>	<u>Description of site</u>
<u>1.Versova</u>	<u>19° 08' 37.62"N</u> <u>72° 48' 12.54"E</u>	<u>Continuation of Juhu beach, home to Mumbai's largest fishing community - the Kolis.</u> <u>Pollution source: Drainage from Malad creek and Versova fishing harbour, domestic sewage and discharge from sewage treatment plant located at Versova, animal and birds fecal input.</u>
<u>2.Juhu</u>	<u>19° 05' 58.02"N</u> <u>72° 49' 30.00"E</u>	<u>Famous beach of Mumbai and highly visited by tourist.</u> <u>Pollution source: No point source of pollution, Domestic sewage, discharge from tourist resorts located aside, fecal input from animal such as dogs and horses, birds input such as pigeons, crows etc.</u>
<u>3.Mahim</u>	<u>19° 02' 04.4"N</u> <u>72° 50' 17.3"E</u>	<u>Less visited beach as highly polluted by domestic sewage through nallahs, hospital wastes, unpleasant odour, animal input (dogs),birds input (seagulls, pigeons and crows).</u>
<u>4.Dadar</u>	<u>19° 01' 17.5"N</u> <u>72° 49' 49.1"E</u>	<u>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.</u>
<u>5.Girgaon</u>	<u>18° 57' 15.4"N</u> <u>72° 48' 53.3"E</u>	<u>Well known as Charni road chowpatty and is an extension of Marine drive, famous and highly visited tourist spot. Sandy stretch of coastline interspersed with boulders. Except during monsoon this beach is also famous for recreational water sports. It is affected by domestic effluent discharged through nallahs (sewage outlets) and direct fecal input from human, birds and animals.</u>

**Table 5:** Occurrence of VGs in *Escherichia coli* isolated from surface water samples across five sampling sites from North western coast of India.

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**Table 2: *Escherichia coli* pathotypes and associated Virulence genes (VGs) tests**

Pathotype	Virulence genes		Gene product	Function	Disease associated
	Toxin gene	Adhesin/Invasion			
STEC	stx1		Shigatoxin I	Inhibit protein synthesis of host cells leading to cell death	
	stx2		Shigatoxin II		
EHEC	stx1		Shigatoxin I	Inhibit protein synthesis of host cells leading to cell death	
	stx2		Shigatoxin II		
	hly A		Enterohemolysin	Pore forming cytotoxin on eukaryotic cells	bloody diarrhoea, thrombocytopenia, acute renal failure
		eae A	Intimin	Responsible for attaching and effacing lesions	Haemorrhagic colitis, Hemolytic uremic syndrome
EPEC		eaeA	Intimin	Responsible for attaching and effacing lesions	Acute Infantile Diarrhoea
ETEC	LT1		Heat labile toxin 1	Intestinal colonization due to secretions of enterotoxins	Infantile and traveller's Diarrhoea, porcine and bovine diarrhoea
	ST1		Heat stable toxin 1		

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	Primer sequence (5' → 3')	Annealing temp (°C)	Product size (bp)	Reference
Target gene				

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Table 3: Primer sequences, annealing temperatures and their expected product sizes

Table 3: Primer sequences, annealing temperatures and their expected product sizes

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	1. Mdh	<u>mdh F: TGAAGTCGCAGTCCTCGG</u>	60	825	<u>Ivanetich et al 2006</u>	Formatted: Font: 12 pt, Complex Script Font: 12 pt		
		<u>mdh R:TCCACGCCGTTTTTACCC</u>				Formatted: Font: 12 pt, Complex Script Font: 12 pt		
Class of antimicrobial	Antimicrobials	Sampling sites					% of resistance isolate	Formatted Table
		Versova	Juhu	Mahim	Dadar	Girgaon		Formatted: Font: 12 pt, Complex Script Font: 12 pt
		(n=18)	(n=6)	(n=13)	(n=21)	(n=9)		Formatted: Font: 12 pt, Complex Script Font: 12 pt
Cephalosporins	CXM(Cefuroxime)	12(0)	3(0)	8(1)	18(0)	4(0)	67	Formatted: Font: 12 pt, Complex Script Font: 12 pt
	<u>slx2 R: GGATGCACTCTGGTCATTG</u>							
	CAZ(Ceftazidime)	13(1)	5(1)	9(3)	13(3)	4(1)	66	Formatted: Font: 12 pt, Complex Script Font: 12 pt
4. EaeA	<u>eaeA F: GAAGCCAAAGCGCACAAAGACT</u>	44		413		<u>Ram et al 2007</u>	Formatted: Font: 12 pt, Complex Script Font: 12 pt	
	CPM(Cefepime)	13(1)	4(1)	11(1)	16(1)	3(2)	70	Formatted: Font: 12 pt, Complex Script Font: 12 pt
	<u>eaeA R: CTCCGCGGTTTACGAGACAC</u>							
5. hly A	<u>hlyA F: GCTATGGGCCTGTTCTCCTCTGC</u>	*50.9	224			<u>Ram et al 2007</u>	Formatted: Font: 12 pt, Complex Script Font: 12 pt	
	<u>hlyA R: ACCACTTTCTTTCTCCGACATCC</u>						Formatted: Font: 12 pt, Complex Script Font: 12 pt	
6. LT1	<u>LT1 F: TCTCTATGTGCATACGGAGC</u>	49	322			<u>Olive 1989</u>	Formatted: Font: 12 pt, Complex Script Font: 12 pt	
	<u>LT1 R: CCATACTGATTGCCGAAT</u>						Formatted: Font: 12 pt, Complex Script Font: 12 pt	
7. ST1	<u>ST1 F: CTTCCCCTCTTTTAGTCAG</u>	49	175			<u>Kong et al 1999</u>	Formatted: Font: 12 pt, Complex Script Font: 12 pt	
	<u>ST1 R: TAACATGGAGCACAGGCAGG</u>							

\*Annealing temperature for respective primers was optimized in this study.

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**Table 4.** Distribution of *E. coli* Resistant to antimicrobial agents in surface water at five sampling sites from tropical beaches of North western India.

		<a href="#">CTX(Cefotaxime)</a>	<a href="#">12(6)</a>	<a href="#">4(2)</a>	<a href="#">11(2)</a>	<a href="#">16(4)</a>	<a href="#">5(4)</a>	<a href="#">72</a>
		<a href="#">CTR(Ceftriaxone)</a>	<a href="#">13(4)</a>	<a href="#">4(1)</a>	<a href="#">11(0)</a>	<a href="#">17(2)</a>	<a href="#">4(1)</a>	<a href="#">73</a>
		<a href="#">CX(Cefoxitin)</a>	<a href="#">11(5)</a>	<a href="#">4(2)</a>	<a href="#">6(2)</a>	<a href="#">11(6)</a>	<a href="#">5(2)</a>	<a href="#">55</a>
		<a href="#">CPZ(Cefoperazone)</a>	<a href="#">14(4)</a>	<a href="#">5(1)</a>	<a href="#">11(1)</a>	<a href="#">19(2)</a>	<a href="#">5(3)</a>	<a href="#">81</a>
		<a href="#">CZX(Ceftizoxime)</a>	<a href="#">14(4)</a>	<a href="#">4(2)</a>	<a href="#">10(3)</a>	<a href="#">16(3)</a>	<a href="#">6(2)</a>	<a href="#">75</a>
<a href="#">Cephalosporins combinations</a>		<a href="#">CAC(Ceftazidime/Clavulanic acid)</a>	<a href="#">18(0)</a>	<a href="#">5(0)</a>	<a href="#">11(2)</a>	<a href="#">20(1)</a>	<a href="#">5(3)</a>	<a href="#">88</a>
<a href="#">Fluoroquinolones</a>		<a href="#">CIP(Ciprofloxacin)</a>	<a href="#">10(7)</a>	<a href="#">2(3)</a>	<a href="#">7(6)</a>	<a href="#">13(4)</a>	<a href="#">3(3)</a>	<a href="#">52</a>
		<a href="#">OF(Ofloxacin)</a>	<a href="#">14(2)</a>	<a href="#">4(1)</a>	<a href="#">10(2)</a>	<a href="#">17(0)</a>	<a href="#">3(2)</a>	<a href="#">71</a>
		<a href="#">GAT(Gatifloxacin)</a>	<a href="#">16(2)</a>	<a href="#">5(1)</a>	<a href="#">12(1)</a>	<a href="#">18(3)</a>	<a href="#">8(1)</a>	<a href="#">88</a>
		<a href="#">LE(Levofloxacin)</a>	<a href="#">9(9)</a>	<a href="#">1(5)</a>	<a href="#">8(5)</a>	<a href="#">13(8)</a>	<a href="#">5(4)</a>	<a href="#">54</a>
<a href="#">Aminoglycosides</a>		<a href="#">GEN(Gentamicin)</a>	<a href="#">5(3)</a>	<a href="#">1(0)</a>	<a href="#">3(5)</a>	<a href="#">6(10)</a>	<a href="#">3(1)</a>	<a href="#">27</a>
		<a href="#">AK(Amikacin)</a>	<a href="#">2(8)</a>	<a href="#">0(2)</a>	<a href="#">2(6)</a>	<a href="#">6(10)</a>	<a href="#">0(5)</a>	<a href="#">15</a>
<a href="#">Sulphonamides</a>		<a href="#">COT(Co-Trimoxazole)</a>	<a href="#">12(0)</a>	<a href="#">4(0)</a>	<a href="#">9(1)</a>	<a href="#">15(0)</a>	<a href="#">5(1)</a>	<a href="#">67</a>
<a href="#">Monobactams</a>		<a href="#">AT(Aztreonam)</a>	<a href="#">17(1)</a>	<a href="#">6(0)</a>	<a href="#">11(2)</a>	<a href="#">18(3)</a>	<a href="#">6(3)</a>	<a href="#">87</a>
<a href="#">Penicillin combinations</a>		<a href="#">A/S(Ampicillin/Sulbactam)</a>	<a href="#">13(4)</a>	<a href="#">3(2)</a>	<a href="#">10(1)</a>	<a href="#">20(1)</a>	<a href="#">6(2)</a>	<a href="#">78</a>
		<a href="#">TCC(Ticarcillin/Clavulanic acid)</a>	<a href="#">16(1)</a>	<a href="#">5(1)</a>	<a href="#">11(1)</a>	<a href="#">21(0)</a>	<a href="#">8(1)</a>	<a href="#">91</a>
		<a href="#">PIT(Piperacillin/Tazobactam)</a>	<a href="#">11(6)</a>	<a href="#">4(2)</a>	<a href="#">4(7)</a>	<a href="#">16(3)</a>	<a href="#">4(3)</a>	<a href="#">58</a>
		<a href="#">AMC (Amoxycylav)</a>	<a href="#">18(0)</a>	<a href="#">6(0)</a>	<a href="#">13(0)</a>	<a href="#">21(0)</a>	<a href="#">9(0)</a>	<a href="#">100</a>
<a href="#">Carbapenems</a>		<a href="#">IPM(Imipenem)</a>	<a href="#">13(1)</a>	<a href="#">5(1)</a>	<a href="#">6(4)</a>	<a href="#">15(2)</a>	<a href="#">4(3)</a>	<a href="#">91</a>
		<a href="#">MRP(Meropenem)</a>	<a href="#">11(5)</a>	<a href="#">2(3)</a>	<a href="#">8(3)</a>	<a href="#">10(8)</a>	<a href="#">4(4)</a>	<a href="#">52</a>
<a href="#">Penicillins</a>		<a href="#">AMP(Ampicillin)</a>	<a href="#">16(0)</a>	<a href="#">6(0)</a>	<a href="#">12(1)</a>	<a href="#">20(1)</a>	<a href="#">7(2)</a>	<a href="#">91</a>
<a href="#">Quinolones</a>		<a href="#">NA (Nalidixic Acid)</a>	<a href="#">18(0)</a>	<a href="#">6(0)</a>	<a href="#">13(0)</a>	<a href="#">21(0)</a>	<a href="#">9(0)</a>	<a href="#">100</a>

[Note: Number of isolates with reduced susceptibility is given in parenthesis](#)







**Table 6.** Antimicrobial resistance (with MAR index) and Virulence genes detected in *E. coli* isolated from surface waters of tropical beaches of North Western India.

<u>Isolate ID</u>	<u>Antibiogram profile (Sensitive to)</u>	<u>Virulence genes</u>						<u>MAR index</u>
		<u>stx1</u>	<u>stx2</u>	<u>eae A</u>	<u>hly A</u>	<u>LT</u>	<u>ST</u>	
<u>V1</u>	<u>CXM, CAZ, CPM, GEN, AK, COT, IPM, AMP</u>	=	=	=	=	±	=	<u>0.4</u>
<u>V2</u>	<u>CXM,GEN, AK, COT</u>	=	±	±	=	=	=	<u>0.64</u>
<u>V3</u>	<u>GEN</u>	=	±	=	±	=	=	<u>0.92</u>
<u>V4</u>	<u>GEN, AK</u>	=	=	±	=	=	=	<u>0.8</u>
<u>V5</u>	<u>CXM,GEN,A/S, TCC</u>	=	=	±	=	=	=	<u>0.68</u>
<u>V6</u>	<u>OF</u>	±	=	=	=	=	=	<u>0.8</u>
<u>V7</u>	=	=	=	=	=	±	=	<u>0.84</u>
<u>V8</u>	=	=	=	=	=	=	±	<u>0.88</u>
<u>V9</u>	=	=	=	±	=	=	=	<u>0.88</u>
<u>V10</u>	<u>CXM, CAZ, CPM,CTR,CX, GEN, K, COT, IPM</u>	=	=	=	=	±	=	<u>0.44</u>
<u>V11</u>	<u>MRP</u>	=	=	±	±	=	=	<u>0.76</u>
<u>V12</u>	<u>CXM,CAZ,CPM, GEN, AK, COT,IPM, AMP</u>	=	=	=	=	=	±	<u>0.4</u>
<u>V13</u>	<u>CPM, CX, OF,GEN, AK, COT,IPM</u>	=	=	±	=	=	=	<u>0.6</u>



<u>V14</u>	=	=	±	=	=	=	=	<u>0.8</u>
<u>V15</u>	<u>CXM, GEN, AK, COT</u>	=	=	±	=	=	=	<u>0.64</u>
<u>V16</u>	<u>GEN, AK</u>	=	=	±	=	=	=	<u>0.8</u>
<u>V17</u>	<u>CAZ, CIP,PIT, MRP</u>	±	=	=	=	=	=	<u>0.68</u>
<u>V18</u>	=	±	=	=	=	=	=	<u>0.88</u>
<u>J1</u>	<u>CXM, GEN, A/S, TCC</u>	=	±	=	=	=	=	<u>0.68</u>
<u>J2</u>	=	=	=	±	=	=	=	<u>0.8</u>
<u>J3</u>	<u>GEN, AK, MRP</u>	±	=	±	=	=	=	<u>0.76</u>
<u>J4</u>	<u>CXM, CAZ, CPM, CTR,CAC, CIP, OF, GEN, AK, COT</u>	=	=	=	=	=	±	<b><u>0.24</u></b>
<u>J5</u>	<u>GEN, AK</u>	±	=	=	±	=	=	<u>0.8</u>
<u>J6</u>	<u>CXM, GEN, AK,COT</u>	=	=	=	=	±	=	<u>0.64</u>
<u>M1</u>	=	±	=	±	=	=	=	<u>0.88</u>
<u>M2</u>	=	=	=	=	=	=	±	<u>0.64</u>
<u>M3</u>	<u>CXM, COT, A/S, TCC</u>	=	±	=	±	=	=	<u>0.68</u>
<u>M4</u>	=	=	=	=	=	±	±	<u>0.88</u>
<u>M5</u>	<u>CX, GEN, AK, IPM</u>	=	=	±	=	=	=	<u>0.72</u>
<u>M6</u>	<u>CX, GEN, AK, IPM</u>	=	=	±	±	=	=	<u>0.72</u>

<a href="#">M7</a>	<a href="#">OF</a>	=	=	=	=	±	±	<a href="#">0.8</a>
<a href="#">M8</a>	<a href="#">CXM, AK, COT,MRP</a>	=	±	=	±	=	=	<a href="#">0.72</a>
<a href="#">M9</a>	<a href="#">CX, IPM</a>	=	=	=	=	=	±	<a href="#">0.84</a>
<a href="#">M10</a>	<a href="#">CXM, CTR,CX, GEN, PIT</a>	=	=	=	=	±	=	<a href="#">0.32</a>
<a href="#">M11</a>	<a href="#">CXM, CAZ, CPM,CTR, CX, CPZ, OF, GEN, AK, COT, A/S, PIT</a>	±	=	=	=	=	=	<a href="#">0.36</a>
<a href="#">M12</a>	=	=	=	±	±	=	=	<a href="#">0.8</a>
<a href="#">M13</a>	<a href="#">GEN, AK, MRP</a>	=	=	±	=	=	=	<a href="#">0.76</a>
<a href="#">D1</a>	<a href="#">CTX, CX, CZX</a>	=	±	±	=	=	=	<a href="#">0.84</a>
<a href="#">D2</a>	<a href="#">MRP</a>	=	=	=	=	±	=	<a href="#">0.8</a>
<a href="#">D3</a>	<a href="#">COT</a>	=	±	=	=	=	=	<a href="#">0.84</a>
<a href="#">D4</a>	<a href="#">CXM, CAZ, CPM, CIP, OF, GEN, AK, IPM</a>	=	=	=	=	=	±	<a href="#">0.4</a>
<a href="#">D5</a>	<a href="#">GEN, AK</a>	=	=	=	=	=	±	<a href="#">0.8</a>
<a href="#">D6</a>	<a href="#">COT</a>	±	=	±	=	=	=	<a href="#">0.84</a>
<a href="#">D7</a>	<a href="#">CPM, CX, OF, GEN, AK, COT, IPM</a>	±	=	=	=	=	=	<a href="#">0.6</a>
<a href="#">D8</a>	=	=	±	=	=	=	=	<a href="#">0.8</a>
<a href="#">D9</a>	<a href="#">CXM, CAZ, CPM,CTR, CZX, CIP, OF, GEN, AK, COT,IPM</a>	=	=	=	=	=	±	<a href="#">0.44</a>

<a href="#">D10</a>	=	=	=	=	=	±	=	<a href="#">0.92</a>
<a href="#">D11</a>	<a href="#">CX, COT</a>	=	±	±	=	=	=	<a href="#">0.84</a>
<a href="#">D12</a>	=	=	=	=	=	±	±	<a href="#">0.88</a>
<a href="#">D13</a>	=	=	=	=	=	=	±	<a href="#">0.76</a>
<a href="#">D14</a>	=	=	=	±	±	=	=	<a href="#">0.84</a>
<a href="#">D15</a>	<a href="#">CAZ, CIP, PIT, MRP</a>	=	±	=	=	=	=	<a href="#">0.68</a>
<a href="#">D16</a>	=	=	=	±	=	=	=	<a href="#">0.92</a>
<a href="#">D17</a>	<a href="#">CAZ, CIP, PIT, MRP</a>	±	=	=	±	=	=	<a href="#">0.68</a>
<a href="#">D18</a>	<a href="#">OF</a>	=	±	=	=	=	=	<a href="#">0.8</a>
<a href="#">D19</a>	=	=	=	=	=	±	=	<a href="#">0.92</a>
<a href="#">D20</a>	<a href="#">CXM, CAZ, CPM, CTR, CX, GEN, AK, COT, IPM</a>	±	=	=	=	=	=	<a href="#">0.44</a>
<a href="#">D21</a>	=	=	=	±	=	=	=	<a href="#">0.88</a>
<a href="#">G1</a>	<a href="#">CXM, CAZ, CPM, CTR, CZX, CIP, OF, GEN, AK, COT, IPM</a>	=	±	=	=	=	=	<a href="#">0.44</a>
<a href="#">G2</a>	<a href="#">MRP</a>	=	±	±	=	=	=	<a href="#">0.76</a>
<a href="#">G3</a>	<a href="#">CXM, CTR, CX, GEN, PIT</a>	=	=	±	=	=	=	<a href="#">0.32</a>
<a href="#">G4</a>	=	=	=	=	=	=	±	<a href="#">0.76</a>

<a href="#">G5</a>	<a href="#">CXM, CAZ, CPM, CIP, OF, GEN, AK,IPM</a>	=	=	=	=	=	±	<a href="#">0.4</a>
<a href="#">G6</a>	<a href="#">CXM, CAZ, CPM, CTR, CX, CPZ,OF, GEN, AK, COT,A/S, PIT</a>	=	=	=	=	±	=	<a href="#">0.36</a>
<a href="#">G7</a>	=	=	=	±	=	=	=	<a href="#">0.8</a>
<a href="#">G8</a>	=	=	±	±	=	=	=	<a href="#">0.92</a>
<a href="#">G9</a>	<a href="#">CXM, CAZ, CPM, CTR, CAC, CIP, OF, GEN, AK, COT</a>	=	±	=	±	=	=	<a href="#">0.24</a>
		<a href="#">9*</a>	<a href="#">13*</a>	<a href="#">20*</a>	<a href="#">8*</a>	<a href="#">9*</a>	<a href="#">11*</a>	<a href="#">†0.24-0.92</a>

▲  
[\\*Percentage prevalence of each gene](#)

[† Overall range of MAR index](#)

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**Table 4.** Distribution of *E. coli* Resistant to antimicrobial agents in surface water at five sampling sites from tropical beaches of North western India.

Class of antimicrobial	Antimicrobials	Sampling sites					% of resistant isolate
		Versova (n=18)	Juhu (n=6)	Mahim (n=13)	Dadar (n=21)	Girgaon (n=9)	
Cephalosporins	CXM(Cefuroxime)	12(0)	3(0)	8(1)	18(0)	4(0)	67
	CAZ(Ceftazidime)	13(1)	5(1)	9(3)	13(3)	4(1)	62
	CPM(Cefepime)	13(1)	4(1)	11(1)	16(1)	3(2)	70
	CTX(Cefotaxime)	12(6)	4(2)	11(2)	16(4)	5(4)	72
	CTR(Ceftriaxone)	13(4)	4(1)	11(0)	17(2)	4(1)	73
	CX(Cefoxitin)	11(5)	4(2)	6(2)	11(6)	5(2)	55
	CPZ(Cefoperazone)	14(4)	5(1)	11(1)	19(2)	5(3)	81
	CZX(Ceftizoxime)	14(4)	4(2)	10(3)	16(3)	6(2)	75
Cephalosporins combinations	CAC(Ceftazidime/Clavulanic acid)	18(0)	5(0)	11(2)	20(1)	5(3)	88
Fluoroquinolones	CIP(Ciprofloxacin)	10(7)	2(3)	7(6)	13(4)	3(3)	55
	OF(Ofloxacin)	14(2)	4(1)	10(2)	17(0)	3(2)	71
	GAT(Gatifloxacin)	16(2)	5(1)	12(1)	18(3)	8(1)	88
	LE(Levofloxacin)	9(9)	1(5)	8(5)	13(8)	5(4)	54
Aminoglycosides	GEN(Gentamicin)	5(3)	1(0)	3(5)	6(10)	3(1)	27
	AK(Amikacin)	2(8)	0(2)	2(6)	6(10)	0(5)	15
Sulphonamides	COT(Co-Trimoxazole)	12(0)	4(0)	9(1)	15(0)	5(1)	67
Monobactams	AT(Aztreonam)	17(1)	6(0)	11(2)	18(3)	6(3)	81
Penicillin combinations	A/S(Ampicillin/Sulbactam)	13(4)	3(2)	10(1)	20(1)	6(2)	71
	TCC(Ticarcillin/Clavulanic acid)	16(1)	5(1)	11(1)	21(0)	8(1)	91
	PIT(Piperacillin/Tazobactam)	11(6)	4(2)	4(7)	16(3)	4(3)	58



Table S1. *Escherichia coli* count per 100ml detected in surface waters of tropical beaches of North Western India.

Sites	<i>E. coli</i> counts detected from surface waters in CFU/100ml												Average of <i>E. coli</i> counts detected in each season			log <sub>10</sub> transformed values of <i>coli</i> counts/100ml		
	Monsoon				Postmonsoon				Premonsoon				Mons	Postmon	Premons	Mons	Postmon	Premon
	Jun e	Jul y	Aug ust	Septem ber	Octo ber	Novem ber	Decem ber	Janu ary	Febru ary	Mar ch	Ap ril	Ma y						
Versova	700 0	18 00	1200	1700	2400	330	950	330	1300	110 0	320 0	244 0	2925	1003	2010	3.5	3.0	3.3
Juhu	240 0	10 00	1900	620	90	30	20	75	50	100	230	120	1480	54	125	3.2	1.7	2.1
Mahim	410 00	28 00	7500	3300	3900	1700	720	350	240	170 0	950 0	135 0	13650	1668	3198	4.1	3.2	3.5
Dadar	688 00	80 00	1470	3870	2400	1200	350	200	600	160 0	147 0	105 00	20535	1038	3543	4.3	3.0	3.5
Girgaon	108 0	20 30	1980	1430	350	150	75	89	250	90	120 0	960	1630	166	625	3.2	2.2	2.8



