Public health implications of plasmid-mediated quinolone and aminoglycoside resistance genes in *Escherichia coli* inhabiting a major anthropogenic river of India

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Abstract

Presence of antimicrobial resistance (AMR) genes in *Escherichia coli* inhabiting anthropogenic rivers is an important public health concern because plasmid-mediated AMR genes can easily spread to other pathogens by horizontal gene transfer. Besides β-lactams, quinolones and aminoglycosides are the major antibiotics against *E. coli*. In the present study, we have investigated the presence of plasmid-mediated quinolone resistance (PMQR) and aminoglycoside resistance genes in *E. coli* isolated from a major river of northern India. Our results revealed that majority of the strains were phenotypically susceptible for fluoroquinolones and some aminoglycosides like amikacin, netilmicin, tobramycin and gentamicin. However, 16.39% of the strains were resistant for streptomycin, 8.19% for kanamycin and 3.30% for gentamicin. Of the various PMQR genes investigated, only *qnrS1* was present in 24.59% of the strains along with *ISEc2*. Aminoglycoside-resistance genes like *strA-strB* were found to be present in 16.39%, *aphA1* in 8.19% and *aacC2* in only 3.30% of the strains. Though, no co-relation was observed between phenotypic resistance for fluoroquinolones and presence of PMQR genes, phenotypic resistance for streptomycin, kanamycin and gentamicin exactly co-related with the presence of the genes *strA-strB*, *aphA1* and *aacC2*, respectively. Moreover, all the AMR genes discerned in aquatic *E. coli* were found to be situated on conjugative plasmids and, thus easily transferrable. Our study accentuates the importance of routine surveillance of urban rivers to curtail the spread of AMR genes in aquatic pathogens.

Introduction

*Escherichia coli* is a diverse bacterial species, strains of which might be commensal or pathogenic in nature. It is primarily an inhabitant of the lower intestinal tract of humans and warm-blooded animals and is discharged in the environment through faeces and wastewater treatment plants [1]. Among all the members of the family *Enterobacteriaceae*, *E. coli* has a remarkable capability to serve as a donor and recipient of antimicrobial resistance (AMR) genes. It is therefore regarded as a major reservoir of AMR genes which can be disseminated to other bacteria by horizontal gene transfer. Thus, AMR in *E. coli* is considered as one of the major challenges in both humans and animals, worldwide [2–4].

Besides β-lactams, quinolones and aminoglycosides are the major antibiotics which are used for treatment of infections caused by *E. coli*. Quinolones target the bacterial DNA gyrase or the topoisomerase IV enzyme, thereby inhibiting DNA replication and transcription. In *E. coli*, mutations in the quinolone resistance-determining region of the chromosomal DNA gyrase and DNA topoisomerase IV are regarded as an important mechanism underlying fluoroquinolone resistance [5, 6]. Besides chromosomal gene mutations, several plasmid encoded resistance mechanisms have been identified in *E. coli* like, (i) genes encoding pentapeptide repeat family proteins which protect bacterial DNA gyrase and topoisomerase IV from inhibitory effect of quinolones (*qnrA to qnrD* and *qnrS*), (ii) *aac(6′)-Ib-cr* which encodes acetyltransferases that modify fluoroquinolones like ciprofloxacin and ofloxacin, and (iii) genes encoding efflux pump proteins like *qepA* and *qexAB* [7]. Since the probability of spontaneous multiple mutations is quite less (10−14 to 10−18 for fluoroquinolones) the plasmid-mediated quinolone resistance (PMQR) determinants might play a major role in enabling bacterial survival in the presence of quinolones [8]. A research study indicated that although the PMQR genes cannot confer a high level of resistance for quinolones and fluoroquinolones, they might reduce the susceptibility of *E. coli* for these antibiotics [9]. Several researchers have reported that insertion sequences (IS) play an important role in the mobilisation of PMQR genes [10, 11]. IS like *ISEc2* and *IS26* have been associated with the mobilisation of PMQR...
genes like qnrS and aac(6’)-Ib-cr, respectively [11]. However, most of the studies regarding PMQR genes and their genetic environment have been conducted on clinical isolates [10–12] and only a few studies have been conducted on aquatic E. coli [13–15].

Aminoglycosides bind to the 30S ribosomal subunit and interfere with the bacterial protein synthesis. In E. coli, resistance to aminoglycosides can develop by mutations in the 16S rRNA, modification of the drug target(s) or by enzymatic inactivation of aminoglycosides by the bacterial enzymes. Modification of the aminoglycoside target site is performed by 16S rRNA methylases like ArmA, RmtA to RmtH and NmpA which methylate some amino acids of the 16S rRNA, resulting in resistance for amikacin, gentamicin, tobramycin and netilmicin [16]. In E. coli, three types of aminoglycoside modifying enzymes are known viz., phosphotransferases, acetyltransferases and nucleotidyltransferases. Genes encoding these enzymes are frequently located on plasmids facilitating their transfer to other pathogens. The linked strA-strB genes which encode for aminoglycoside phosphotransferases are the most common streptomycin resistance genes prevalent in E. coli, worldwide. In E. coli, phosphotransferase gene aphA1 which confers kanamycin resistance and aacC2 (an acetyltransferase) that confers gentamicin resistance are also reported [17, 18].

Most of the studies regarding distribution and characterisation AMR genes have been conducted on clinical isolates of E. coli. Studies regarding the prevalence and characterisation of AMR and virulence genes in E. coli inhabiting natural water bodies, especially urban rivers, are quite less [19–25]. It is important to study the AMR genes of E. coli, a prominent faecal indicator bacteria residing in urban rivers because these rivers are impacted by various anthropogenic activities. Thus, urban rivers represent a reservoir of diverse E. coli and an ideal ecosystem for transfer and dissemination of AMR genes from aquatic E. coli to other pathogens [26–30]. In an earlier study, we had reported the distribution and characteristics of β-lactamase genes of E. coli isolated from Yamuna, a prominent river of northern India [31]. A collection of 61 strains representing the four phylogroups of E. coli viz. A, B1 (non-pathogenic phylogroups) and B2, D (pathogenic phylogroups) was investigated [31]. Here, we have studied the phenotypic susceptibilities of these E. coli strains for quinolones and aminoglycosides, and the presence of PMQR and plasmid-mediated aminoglycoside resistance genes. We have also tried to discern a correlation between AMR genes and phylogroups, if any. The genetic elements associated with the PMQR genes were also studied to understand their role in the spread of PMQR genes in the environment.

Materials and methods

Bacterial strains

A total of 61 strains of E. coli collected from various sites along the entire stretch of river Yamuna across the National Capital Region of India were used in this study. These strains were transported to the laboratory on ice in sterile screw-capped bottles and processed within 6 h. The details of the sampling sites, year of isolation, seasonality and the methods used for enrichment and isolation of E. coli have been described in detail, previously [31]. All the strains were confirmed by biochemical testing and sequencing of the gene encoding 16S rDNA. The strains were serotyped at the National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, Himachal Pradesh, India. The phylogenetic profiles of the strains were determined by triplex PCR which revealed that these strains belonged to the phylogroups A, B1, B2 and D [32]. The azide-resistant E. coli strain J53 used as the recipient during conjugation experiments was a gift from Dr George A. Jacoby and was provided to us by Dr Sulagna Basu (National Institute of Cholera and Enteric Diseases, Kolkata, India).

Antimicrobial susceptibility testing for quinolones and aminoglycosides

Antimicrobial susceptibilities of all the 61 E. coli strains for quinolones and aminoglycosides were determined by Kirby-Bauer disk diffusion test, using antibiotic disks (Himedia, Mumbai, India) and following the guidelines of Clinical Laboratory Standards Institute [33]. The antibiotic disks which were used in this study (charge in μg/disk) included nalidixic acid (30 μg), ciprofloxacin (5 μg) and ofloxacin (5 μg) for quinolone susceptibility, and streptomycin (10 μg), kanamycin (30 μg), tobramycin (10 μg), netilmicin (30 μg) and amikacin (30 μg) for aminoglycoside susceptibility.

Detection of PMQR genes

For PCR-based detection of PMQR genes, qnrA, qnrB, qnrC, qnrD, qnrS, qepA, oqxA, oqxB and aac(6’)-Ib-cr, DNA of the E. coli strains was isolated by boiling lysis procedure [34]. The 25 μL PCR-reaction mixture contained 2.5 μL of 1× buffer, 200 μM of each dNTP, 20 pmol of the forward and reverse primers, 1 U of Taq DNA polymerase and 10 μL of template DNA. PCR was carried out in My Cycler™ Thermal Cycler, using the published primers and PCR conditions (Table 1). PCR amplicons were purified and sequenced using the methods described earlier [35]. Briefly, PCR amplicons were visualised after electrophoresis on 1% agarose gels at 80 V and purified using Hi-Yield™ extraction kit (RBC Bioscience, New Taipei City, Taiwan) following the manufacturer’s protocol. The purified amplicons were submitted for sequencing to a commercial facility (Invitrogen BioServices India Pvt. Ltd., Bangalore, India) where they were sequenced using Sanger’s method. Similarity search of the nucleotide sequences was performed using NCBI-BLASTn.

Analysis of the genetic environment of qnrS

Genetic environment of qnrS was analysed by PCR amplification using the published primers and PCR protocol [10]. Insertion sequences IS26 and ISecl2 were targeted in the upstream region of qnrS gene. The components of the PCR reaction mixture were the same as used for PCR amplification of PMQR genes. The PCR conditions and the details of the primers are mentioned in Table 1. The PCR amplicons were purified and sequenced as described earlier, using the respective forward primers. Similarity search was performed for the sequences obtained using NCBI-BLAST.

Detection of plasmid-mediated aminoglycoside resistance genes

PCR-based detection of plasmid-mediated aminoglycoside resistance genes encoding 16S rRNA methylases – armA, rmtA, rmtB, rmtC and rmtD and aminoglycoside modifying enzymes – aacC, strA-strB, aphA1, aphA2 was carried out using published primers [17, 36]. The components of the PCR reaction mixture and the
PCR conditions were the same as for amplification of PMQR genes, except the annealing temperatures, which have been mentioned in Table 1. The amplicons were purified and sequenced as described earlier and the similarity search was performed using NCBI-BLASTn.

Transferability of AMR genes by conjugation and plasmid analysis

To confirm if the PMQR and aminoglycoside resistance genes were transferrable, conjugal transfer of these genes was assessed with a broth culture mating assay using an azide-resistant E. coli J53 as recipient, as also described earlier [21]. Briefly, the donor and the recipient E. coli J53 strains were separately grown in LB broth at 37 °C, 200 rpm for 12–14 h. Conjugal transfer was carried by mixing each donor and recipient in a 1:1 ratio, followed by incubation at 37 °C for 12–14 h under static conditions. Subsequently, appropriate culture dilutions were spread plated on LB agar containing sodium azide (100 μg/ml) supplemented with ampicillin (100 μg/ml) and incubated at 37 °C for 16–18 h. The transferability of the PMQR (qnrS1, aac(6)’-Ib) and aminoglycoside resistance (strA-strB, aphA1 and aacC2) genes was confirmed by PCR amplification of the plasmid DNA isolated from the transconjugants.

Accession numbers

The partial coding DNA sequence (CDS) of qnrS1 genes including their genetic environment were identical in all the 15 strains, therefore the DNA sequence of only one representative strain

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**Table 1.** Details of primers and PCR conditions used for analyses of plasmid-associated quinolone and aminoglycoside resistance genes, and genetic environment of qnrS in E. coli isolated from a major urban river of India

| Primers   | Nucleotide sequence | Target genes | Amplicon size (bp) | Annealing temperature (°C) | References |
|-----------|---------------------|--------------|--------------------|-----------------------------|------------|
| RMTA-F    | 5'-CTAGCGTCATCCGTTTCCTC-3' | rmtA          | 653                | 57                          | [36]       |
| RMTA-R    | 5'-TTTCTCCATGCGTCCCGCC-3'     |              |                    |                             |            |
| RMBT-F    | 5'-GGTCTTGCGCCGTAGTGA-3' | rmtB          | 173                | 60                          | [36]       |
| RMBT-R    | 5'-ATGCAATGCGCCTCTGTT-3'     |              |                    |                             |            |
| RMTC-F    | 5'-CGAAGAAGTAACAGCAAGA-3' | rmtC          | 711                | 55                          | [36]       |
| RMTC-R    | 5'-ATCCCAACTCTCCTCCACT-3'     |              |                    |                             |            |
| ARMA-F    | 5'-ATTCTGCTATCCTCAAATGG-3' | armA          | 315                | 46                          | [36]       |
| ARMA-R    | 5'-ACC TACTACCTATAGTCTG-3'     |              |                    |                             |            |
| str-F     | 5'-TATCTGCGATGAGCCCTC-3' | strA-strB     | 538                | 62                          | [54]       |
| str-R     | 5'-ATTATCATTGCGAGGTT-3'     |              |                    |                             |            |
| aacC2-F   | 5'-TAGAGAATGGATACGCGATG-3' | aacC2         | 861                | 55                          | [18]       |
| aacC2-R   | 5'-ATTATCATTGCGAGGTT-3'     |              |                    |                             |            |
| AphA1-F   | 5'-ATGGCGTCGGATAATGC-3' | aphA1         | 600                | 55                          | [17]       |
| AphA1-R   | 5'-CTCAGCGGAGCATTCC-3'     |              |                    |                             |            |
| QA-F      | 5'-TGCGCGCTGGGCTTTAT-3' | qnrA          | 517                | 60                          | [55]       |
| QA-R      | 5'-TTGCAAGTGACCGCTCTG-3'     |              |                    |                             |            |
| QB-F      | 5'-AACCTGAAAGATGCTC-3' | qnrB          | 405                | 50                          | [55]       |
| QB-R      | 5'-AAGCGCTGTTAATAC-3'     |              |                    |                             |            |
| QC-F      | 5'-GGGTGTTACATTTAGTACAT-3' | qnrC          | 447                | 50                          | [55]       |
| QC-R      | 5'-TCCACCTTAGGAGCTTC-3'     |              |                    |                             |            |
| QD-F      | 5'-CGAGATCAATTACGGGGAA-3' | qnrD          | 582                | 57                          | [56]       |
| QD-R      | 5'-CGAGATCAATTACGGGGAA-3' |              |                    |                             |            |
| QS-F      | 5'-GAGCTGCTACAATGGTGAT-3' | qnrS          | 456                | 55                          | [31]       |
| QS-R      | 5'-GACCACTCCTCGATCGA-3'     |              |                    |                             |            |
| ACC-F     | 5'-TGCGATGCTGCTGATGGCT-3' | aacC2         | 482                | 60                          | [57]       |
| ACC-R     | 5'-CTCAGTGTCCTCGGTTT-3'     |              |                    |                             |            |
| QEP-F     | 5'-CTCGAAGTGCTCGTATG-3' | qepA          | 403                | 55                          | [37]       |
| QEP-R     | 5'-CTGTTGCTCGATTCC-3'     |              |                    |                             |            |
| XA-F      | 5'-GACAGCGGCGACAGAATG-3' | oxqA          | 339                | 62                          | [57]       |
| XA-R      | 5'-GGGACAGGGATTGTATGGA-3' |              |                    |                             |            |
| XB-F      | 5'-CGCAAAAGATTCTCCCCCAACC-3' |               | 240                | 62                          | [57]       |
| XB-R      | 5'-GCGGGCGCAATTGAGATA-3' |              |                    |                             |            |
| Pre-qnrs1-F | 5'-CTGATAAAGGACCTCTCACCATC-3' |               |                    |                             |            |
| Pre-qnrs1-R | 5'-CTGTTT1TATAAATTTTGACG-3' |               |                    |                             |            |
| IS26-F    | 5'-GTTCTCCGGCGGAATACTG-3' | IS26, qnrs1   | 2381               | 50                          | [57]       |
| Pre-qnrs1-L | 5'-CTGTTT1TATAAATTTTGACG-3' |               |                    |                             |            |
| IS26-L    | 5'-GTTCTCCGGCGGAATACTG-3' | IS26, qnrs1   | 2381               | 50                          | [57]       |
| AAC-R     | 5'-CTGTTT1TATAAATTTTGACG-3' |               |                    |                             |            |
| Pre-qnrs1-R | 5'-CTGTTT1TATAAATTTTGACG-3' |               |                    |                             |            |

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Present in river Yamuna E. coli representative strain was sequenced and similarity search by BLASTn genes like qnrA genes which encode for the pentapeptide repeat family proteins, strains were susceptible to these antibiotics, as also recommended E. coli. The zone diameters (in mm) of the bacterial growth around antibiotic disks of nalidixic acid, ciprofloxacin and ofloxacin ≤ 19, ≥ 31 and ≥ 16, respectively, indicating that all the E. coli strains were susceptible to these antibiotics, as also recommended by the CLSI 2018 guidelines [33]. However, the growth zone diameters (in mm) of two E. coli strains IP24 and IPE around the antibiotic disks of nalidixic acid, ciprofloxacin and ofloxacin ≤ 14, ≤ 20 and ≤ 12, respectively, indicate that both these strains were resistant for these antibiotics. The results of the antibiotic susceptibility testing are presented in Table 2, PCR-based testing revealed that none of the E. coli strains harboured the PMQR genes which encode for the pentapeptide repeat family proteins, qnrA, qnrB, qnrC, qnrD and qepA. Similarly, PMQR efflux genes like oqxA and oqxB were not detected in any strain. However, the PMQR gene qnrS was detected in 24.59% (n = 15) of the strains. The 456 bp amplicon of the qnrS gene of one representative strain was sequenced and similarity search by BLASTn revealed that it shared 99% homology with the qnrS1 gene. Thus, it can be inferred that qnrS1 was the predominant PMQR gene present in river Yamuna E. coli isolates. Earlier studies also reported that qnrS type genes were highly prevalent in E. coli isolated from the waterbodies of the world [37, 38].

The PMQR gene aac(6′)-Ib-cr was detected in only one E. coli strain (IPE). The aac(6′)-Ib-cr gene encodes for a common aminoglycoside acetyltransferase AAC(6′)-Ib-cr which is involved in acetylation of quinolones. Earlier studies have also reported that qnrS and aac(6′)-Ib-cr were the most frequent PMQR genes in E. coli isolated from environmental sources, worldwide [14, 19, 40]. The PMQR gene qnrS1 was present in strains of both pathogenic and non-pathogenic phylogroups.

Phenotypic resistance to quinolones did not co-relate with the presence of the PMQR genes because several strains of E. coli which were phenotypically susceptible to fluoroquinolones harboured the qnrS1 gene. An earlier study reported that PMQR genes provide a low-level of resistance for fluoroquinolones which though usually do not surpass the clinical breakpoints; they make the treatment difficult [9]. This suggests that clinical breakpoints should be reassessed in the context of PMQR genes, which results in reduced susceptibility and consequent therapeutic failures, despite going undetected by traditional phenotypic methods [41]. Thus, phenotypic methods for testing fluoroquinolone susceptibilities might result in underestimation of prevalence of quinolone resistance, and confirmation of PMQR genes by PCR is necessary to assess the true fluoroquinolone susceptibilities of E. coli. Moreover, due to the presence of PMQR genes in aquatic E. coli, these could serve as potential reservoirs for undetected spread and dissemination of PMQR genes to other waterborne pathogens.

Genetic environment associated with qnrS1 and aac(6′)-Ib-cr genes

PCR mapping was used to identify the genetic structures associated with qnrS1 in all the qnrS1-positive E. coli strains. PCR amplification resulted in 1113 bp amplicon in each strain. Similarity search revealed that in all the strains, ISEcl2 was present at 303 bp upstream of the start codon of qnrS1 (Table 2). The insertion sequence ISEcl2 belongs to the IS6 family of insertion sequences. The genetic environment associated with qnrS1 was similar to that reported for E. coli isolated from Vietnam and France [10, 42]. However, the role of ISEcl2 in the mobilisation of qnrS1 gene is not known, so far. The PCR mapping from earlier studies suggested that mobilisation of qnrS1 gene might have occurred as an independent event [11].

The genetic elements surrounding the aac(6′)-Ib-cr gene detected in the E. coli strain IPE were also investigated by PCR mapping and similarity search at NCBI. None of the IS, particularly the IS26 which has been widely reported to be present upstream of aac(6′)-Ib-cr, was found in the aac(6′)-Ib-cr-positive E. coli strain of river Yamuna [11].

Aminoglycoside susceptibilities and plasmid-mediated aminoglycoside resistance genes

The bacterial growth zone diameters (in mm) around the antibiotic disks of amikacin, netilmicin and tobramycin were ≥ 17, ≥ 15 and ≥ 15, respectively. Thus, all the E. coli strains were considered phenotypically susceptible for amikacin, netilmicin and tobramycin as also recommended by the CLSI 2018 guidelines [33]. However, zone diameters of 16.39% (n = 10) of the E. coli strains around the antibiotic disks of streptomycin were ≤ 11, of 8.19% (n = 5) strains around kanamycin disks were ≤ 13 and of 3.30% (n = 2) of the strains around gentamicin disks were ≤ 12 indicating that these strains were resistant for these antibiotics. The results of the antibiotic susceptibility testing are presented in Table 2. Thus, our results are similar to an earlier study which also reported that E. coli isolated from waterbodies of Malaysia exhibited lower levels of resistance for aminoglycosides [43]. However, another study from India reported that E. coli strains isolated from coastal waters of India were highly resistant for streptomycin and gentamicin [44].

Though 16S rRNA methylase genes have been reported in clinical strains [45], very few studies have reported the distribution of these genes in aquatic strains of E. coli [46]. Our results revealed that plasmid-mediated 16S rRNA methylase genes (armA, rmtA, rmtB, rmtC and rmtD) were not present in E. coli strains of river Yamuna. It has been proposed that rRNA methylases that confer resistance to aminoglycosides have not disseminated widely in E. coli for reasons related to fitness [47, 48]. The linked strA-strB genes which encode for phosphotransferases and are reportedly the most prevalent streptomycin resistance genes in E. coli worldwide [49] were found to be present in only 16.39% (n = 10) of the E. coli isolates (Table 2). A study from Capetown, South Africa also reported a high prevalence of strA-strB in E. coli strains isolated from wastewater effluents [50]. The aminoglycoside phosphotransferase gene aphA1 which confers resistance to kanamycin was present in 8.19% (n = 5) of the strains. The aminoglycoside acetyltransferase gene aacC2 that confers resistance to gentamicin was present in only 3.3% (n = 2) of the strains (Table 2). Notably, unlike the quinolones, phenotypic resistance to a particular aminoglycoside antibiotic exactly co-related with the presence of its corresponding resistance gene. It has been reported that genes encoding aminoglycoside-modifying enzymes have disseminated globally [49]. This might be due to the fact that these genes are frequently found on transposons, which might have played an important role. 

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Table 2. Resistance phenotypes and plasmid-associated genes encoding fluoroquinolone and aminoglycoside resistance in E. coli strains isolated from a major urban river of India

| Strain designation | Phylogroup | Antibiotic resistance | Resistance gene(s) | Genetic environment of qnrS1 |
|--------------------|------------|------------------------|--------------------|-----------------------------|
| IPH                | A          | –                      | –                  | –                           |
| IPS                | A          | –                      | –                  | –                           |
| IS5                | A          | –                      | –                  | –                           |
| IS57               | A          | –                      | –                  | –                           |
| IS58               | A          | STM                    | strA-strB          | –                           |
| IS76               | A          | –                      | –                  | –                           |
| IP1                | A          | –                      | –                  | –                           |
| KK36               | A          | –                      | qnrS1             | ISEcl2                      |
| KK30               | A          | –                      | qnrS1             | ISEcl2                      |
| NG28               | A          | –                      | –                  | –                           |
| NG9                | A          | –                      | –                  | –                           |
| PA18               | A          | –                      | –                  | –                           |
| IP11               | A          | –                      | –                  | –                           |
| IP18               | A          | –                      | qnrS1             | ISEcl2                      |
| IS40               | A          | –                      | –                  | –                           |
| MKNJ               | A          | –                      | qnrS1             | ISEcl2                      |
| WB23               | A          | –                      | qnrS1             | ISEcl2                      |
| WB31               | A          | –                      | qnrS1             | ISEcl2                      |
| DND14              | A          | –                      | –                  | –                           |
| KP5S               | A          | –                      | –                  | –                           |
| KP21               | A          | –                      | qnrS1             | ISEcl2                      |
| IST                | A          | KAN                    | qnrS1, aacA1      | ISEcl2                      |
| NG3                | A          | –                      | –                  | –                           |
| PA21               | A          | –                      | –                  | –                           |
| WB28               | A          | GEN                    | aacC              | –                           |
| IPG                | A          | –                      | –                  | –                           |
| IS54               | A          | –                      | –                  | –                           |
| IP24               | B1         | NAL, CIP, OFX          | qnrS1             | ISEcl2                      |
| ISD                | B1         | –                      | qnrS1             | ISEcl2                      |
| KK32               | B1         | STM                    | strA-strB         | –                           |
| NG29               | B1         | –                      | –                  | –                           |
| PA4                | B1         | –                      | –                  | –                           |
| MKNE               | B1         | –                      | –                  | –                           |
| SVI                | B1         | –                      | –                  | –                           |
| DND6               | B1         | –                      | –                  | –                           |
| DND1               | B1         | –                      | –                  | –                           |
| WB4                | B1         | –                      | –                  | –                           |
| NG32               | B1         | –                      | –                  | –                           |
| IPSN               | B1         | –                      | –                  | –                           |
| PA32               | B1         | –                      | –                  | –                           |
| KK46               | B1         | –                      | –                  | –                           |

(Continued)
in the dissemination of aminoglycoside resistance across inter- and intra-species boundaries [51]. Interestingly, the aminoglycoside phosphotransferases strA-strB and aphA1 were more prevalent in strains of the pathogenic phylogroup D, than in the strains of the non-pathogenic phylogroups (Table 2).

Co-occurrence of fluoroquinolone and aminoglycoside resistance genes was observed in only six E. coli strains, viz. KK16, WB6, KKA, PA12, IST and IPE.

Analysis of the transconjugants

Analysis of the plasmid DNA isolated from the transconjugants revealed that qnrS1 in the 14 strains and aac(6′)-Ib detected in one E. coli strain were plasmid-mediated and transferrable. Previous studies have indicated that conjugative plasmids were highly transferable and played a key role in conferring a multi-resistance phenotype to waterborne E. coli [14, 20, 52, 53].

Conclusion

Of the various PMQR genes investigated, qnrS1 was present in 25% of the strains, and ISEcI2 was present in its upstream region. Among the aminoglycoside resistance genes, genes encoding for strA-strB and aphA1 were present in 16.39% and 8.19% of the E. coli strains, respectively. Though no co-relation was observed between phenotypic resistance for quinolones and PMQR genes, phenotypic resistance for streptomycin, kanamycin and gentamicin co-related well with the presence of plasmid-mediated aminoglycoside resistance genes strA-strB, aphA1 and aacC2, respectively. Since PMQR and aminoglycoside resistance genes were situated on conjugative plasmids they could be easily disseminated to other pathogens. Thus, our study highlights the importance of routine surveillance of microbial population of urban waterbodies to check the wide spread dissemination of antibiotic resistance determinants.

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