Lentic and effluent water of Delhi-NCR: a reservoir of multidrug-resistant bacteria harbouring \textit{bla}CTX-M, \textit{bla}TEM and \textit{bla}SHV type ESBL genes

Asghar Ali, Insha Sultan, Aftab Hossain Mondal, Mohammad Tahir Siddiqui, Firdoos Ahmad Gogry and Qazi Mohd. Rizwanul Haq*

Department of Biosciences, Jamia Millia Islamia, New Delhi 110025, India
*Corresponding author. E-mail: haqqmr@gmail.com; qhaque@jmi.ac.in

**ABSTRACT**

Antimicrobial resistance is not restricted to clinics but also spreading fast in the aquatic environment. This study focused on the prevalence and diversity of extended-spectrum \(\beta\)-lactamase (ESBL) genes among bacteria from lentic and effluent water in Delhi-NCR, India. Phenotypic screening of 436 morphologically distinct bacterial isolates collected from diverse sites revealed that 106 (∼24%) isolates were ESBL positive. Antibiotic profiling showed that 42, 60, 78 and 59% ESBL producing isolates collected from Ghazipur slaughterhouse, Lodhi garden pond, Hauz Khas lake and Jasola wastewater treatment plant, respectively, were multidrug-resistant (MDR). The multiple antibiotic resistance (MAR) index varied from 0.20 to 0.32 among selected locations. The prevalence of ESBL gene variants \textit{bla}SHV, \textit{bla}TEM and \textit{bla}CTX-M were found to be 17.64, 35.29 and 64%, respectively. Furthermore, the analysis of obtained gene sequences showed three variants of \textit{bla}CTX-M (15, 152 and 205) and two variants of \textit{bla}TEM (TEM-1 and TEM-116) among ESBL producers. The co-existence of 2–3 gene variants was recorded among 48% ESBL positive isolates. New reports from this study include the \textit{bla}CTX-M gene in \textit{Acinetobacter lwofii}, \textit{Enterobacter ludwigi}, \textit{Exiguobacterium mexicanum} and \textit{Aeromonas caviae}. Furthermore, the identification of \textit{bla}TEM and \textit{bla}SHV in an environmental isolate of \textit{A. caviae} is a new report from India.

**Key words:** AMR, aquatic environment, \textit{bla}CTX-M, \textit{bla}SHV, \textit{bla}TEM, ESBLs

**HIGHLIGHTS**

- **Occurrence of ESBL genes from \textit{Exiguobacterium mexicanum} was first reported.**
- **\textit{bla}CTX-M gene from environmental isolate of \textit{Aeromonas caviae} is the first report.**
- **Co-resistance of \(\beta\)-lactam and non-\(\beta\)-lactam classes of antibiotics was observed among a high proportion of ESBL positive bacterial isolates.**
- **This study emphasizes the need for more comprehensive genetic research of diverse microorganisms from environmental settings.**

**INTRODUCTION**

Extensive use of antibiotics in developed as well as in developing countries had led to a rapid increase in their concentration in the aquatic environment, as a significant fraction of antibiotics is unutilized and released into wastewater or effluent from treated wastewater (Zhou et al. 2011; Sultan et al. 2020). Due to the frequent use of third-generation cephalosporins for the treatment of infections caused by members of Enterobacteriaceae, the upshot has been recorded in resistance to these antibiotics. Extended-spectrum \(\beta\)-lactamases (ESBLs) are recognized to provide resistance to most of the \(\beta\)-lactam antibiotics along with the other type of antibiotics like aminoglycosides, monobactam, carbapenem, ansamycin, tetracycline and polymyxins (Taco et al. 2014; Gogry et al. 2019; Siddiqui et al. 2020). The presence of various classes of antimicrobials in the wastewater contributes as an important factor towards the emergence of resistance and their dissemination (Aminov & Mackie 2007). There are numerous reports of antibiotic-resistant bacteria (ARB) in environmental settings such as wastewater treatment plants (WWTPs), rivers, hospital effluents, lakes and ponds (Falodun & Ikusika 2019; Bhattacharyya et al. 2020; Smyth et al. 2020). The emergence and distribution of antibiotic resistance genes (ARGs) among pathogenic organisms can impair human and animal fitness. In the environment, ARG transmission occurs through the horizontal gene transfer (HGT) mechanism (Zhang et al. 2011). Reuse of wastewater for different domestic as well as agricultural purposes favours the uptake

This is an Open Access article distributed under the terms of the Creative Commons Attribution Licence (CC BY 4.0), which permits copying, adaptation and redistribution, provided the original work is properly cited (http://creativecommons.org/licenses/by/4.0/).
of ARGs by plants, humans and animals (Fatta-Kassinos et al. 2011). Wastewater and treated effluents have contributed towards an increase in antibiotic-resistant microorganisms in the environment and thereby enhanced the ARG load into the water bodies (Karthikeyan & Meyer 2006). The WWTPs are measured as significant reservoirs for ARGs since activated sludge facilitates the richness of microbial community and also supports the HGT using mobile genetic elements (MGEs) of ARGs (Zhang et al. 2011; Moura et al. 2012).

To prevent the probable health risks originating from ARB in the aquatic environment, the evaluation of such infectious and harmful bacteria is of high importance. Therefore, the present study was carried out to determine the prevalence and diversity of ESBL genes among bacterial isolates from lentic and effluent water in Delhi-NCR, India.

**MATERIALS AND METHODS**

**Inspection of sites and sample collection**

In this study, four different sites were selected to investigate the occurrence and distribution of ARGs among bacterial isolates from lentic and effluent water in Delhi-NCR, one of the largest metropolitan cities of India with a population size of approximately 24 million. Sampling site Hauz Khas lake is a 125 acres 14th-century water body in New Delhi, now shrunk in size to 15 acres and has a maximum depth of 1–2.5 m. The lake is a site of recreation for people, and its water is used for irrigation, animal use in a nearby park (Deer Park). The second site, the Lodhi garden pond, present in Lodhi estate – a tourist site in Delhi, is a duck-fish integrated aquaculture pond. The pond is land-locked and does not receive any sewage water. The third site, Ghazipur slaughterhouse, has a capacity of 1,000 head of cattle to be slaughtered per day. A bio-methanation plant converts digested and undigested stomach contents of ruminants into gas and slurry. The blood released during the slaughtering process is treated before being released into the river Yamuna. The samples were collected from effluent water of the wastewater treatment plant. The fourth site, Jasola wastewater treatment plant, is the biggest treatment plant in South Delhi having the potential to impact the environment to a large extent. Out of four sites, two sites were effluent treated water, viz. Ghazipur slaughterhouse, Ghazipur U.P. India (28°62’N, 77°28’E) and Jasola wastewater treatment plant, Jasola Vihar, New Delhi (28° 54’N, 77°28’E), and the other two sites were lentic water bodies, viz. Hauz Khas lake, Hauz Khas, New Delhi, Delhi (28°55’N, 77°19’E) and Lodhi garden pond, Lodhi Estate, New Delhi, Delhi (28°59’N, 77°21’E). Water samples were aseptically collected in a 100 mL sterile glass bottle, stored in an icebox (−4 °C) and transported within 4 h from the sampling sites to the Microbiology Research Laboratory. Before transportation, temperature and pH were recorded for each sampling site. The samples were collected during the year 2015–2016.

**Isolation and identification of ESBL positive bacteria**

For isolation, samples were serially diluted up to $1 \times 10^{-4}$, after dilution of the samples 100 μL of each dilution were spread on Luria Agar (LA) plates and kept for 16–18 h incubation at 37 °C (Siddiqui et al. 2019). After incubation, colonies with different morphology (based on size, appearance, colour, texture, etc.) were picked and streaked on LA plates for further detection of ESBL producing isolates. Morphologically distinct isolates from all four sites were screened by a preliminary test. It was performed using third-generation antibiotics cephalosporins and monobactam by the disk diffusion method. Suspected ESBL producers in the preliminary test were further confirmed by the phenotypic disc confirmatory test (PDCT) which was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2016) for ESBL production. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700605 were used as ESBL negative and positive control, respectively.

**Antibiotic profiling and multiple antibiotic resistance index**

Antibiotic profiling of ESBL positive bacterial isolates was performed by the disk diffusion method on Muller Hinton Agar (MHA) plate using 12 different antibiotic classes as per CLSI guidelines (CLSI 2016). Twenty different antibiotics associated with 12 classes include Aminoglycosides: amikacin; Penicillin: ampicillin; Penicillin + inhibitor: piperacillin/tazobactam; ampicillin/subbactam, Cephalosporins: ceftriaxone, cefotaxime, ceftazidime, and cefazolin, cefoxitin; Quinolone: ciprofloxacin, levofoxacin, Carbenem: imipenem, ertapenem; Polypeptide: polymyxin B and colistin, Folate pathway inhibitor: trimethoprim, tetracyclin: tetracycline, Phenicol: chloramphenicol, ansamycins: rifampicin, Monobactam: aztreonam (Himedia India) included in the study. After spreading of inoculum on MHA, plates were incubated overnight at 37 °C and the susceptibility pattern was determined by the zone inhibition test. Isolates showing resistant phenotype against three (equal or more than three) different classes of antibiotics were categorized as multidrug-resistant (MDR) (Magiorakos et al. 2005).
The multiple antibiotic resistance (MAR) index of isolates against different classes of antibiotics was calculated. MAR index is calculated by formula $a/b$, where ‘$a$’ signifies the number of antibiotics to which the isolate was resistant and ‘$b$’ represents the number of antibiotics to which the isolate was subjected. MAR index was inferred for each location and specific isolate as detailed by Krumperman (1983).

Minimum inhibitory concentration (MIC) of eight antibiotics, i.e., ceftazidime (CAZ), ciprofloxacin (CIP), ampicillin (AMP), rifampicin (RIF), cefotaxime (CTX), chloramphenicol (C), colistin (CL) and trimethoprim (TR), against ESBL positive isolates was determined by the broth microdilution method as per CLSI guidelines. Luria Broth (LB) was used for the culture of bacterium and as a reference. 10.24 mg/mL stock solution of antibiotics were prepared and inoculated into the first well of each column onto the polystyrene plate. The progressive serial broth dilution was performed to obtain the required concentrations of 1,024, 512, 256, 128, 64, 32, 16, 8, 4 and 2 μg/mL in 96 well microtitre plate, and medium was inoculated with 10 μL of 1,000 times diluted 0.1 OD600 culture and incubated overnight at 37 °C. The optical density (OD) was measured at 600 nm using a microplate reader (Thermo Scientific MultiscanGo).

**Isolation of genomic and plasmid DNA**

For isolation of genomic and plasmid DNA, a pure bacterial colony was suspended into sterile LB media and incubated overnight to obtain mid to late log phase of the bacterial culture. The cells were pelleted by centrifugation. Further, the genomic DNA from ESBL samples was extracted by Phenol:Chloroform:Isoamyl alcohol (PCI) procedure and plasmid DNA isolated using alkaline lysis methods. Extracted DNA was visualized over the ultraviolet (UV) transilluminator (GeNei-India). These genomic and plasmid DNA were used as a template in a polymerase chain reaction (PCR) for amplification of various ESBL genes. For amplification of genes, 50 μL master mix were prepared that comprises of 39.5 μL PCR grade water, 5 μL of 10× buffer (with 25 mM MgCl2), 1 μL of deoxynucleotide triphosphate mixture (10 mM dNTP), 1 μL of forward primer, 1 μL of reverse primer, 0.5 μL Taq polymerase (5 U/μL, GeNei-India) and 2 μL of extracted DNA template. PCR conditions were kept as follows: Initial denaturation for 5 min at 95 °C, followed by 30 cycles of cyclic denaturation for 1 min, annealing for 45 s at respective annealing temperature (Tm) of different genes, an extension for 1 min at 72 °C and a final extension for 10 min at 72 °C. After that amplified PCR products were loaded in well (1% agarose gel). After running of gel, DNA bands were visualized over UV transilluminator (GeNei-India). Primers used in PCR amplification for various genes are mentioned in Table 1.

| Targeted gene | Primer | Sequences | Amplicon length | References |
|---------------|--------|-----------|----------------|------------|
| 16S rRNA      | RRF    | 5’GGCGGAGGGGTGTAATGT-3’ | 1,252         | Azam et al. (2016) |
|               | RRR    | 5’CGATTACTAGCGATTCCGACTCA-3’ |             |           |
| blaCTX-M      | CTX-F  | 5’TGTGAGYACCAGTAAGGTKAT-3’ | 610          | This study |
|               | CTX-R  | 5’TARGTCAACCAGACVCAGG-3’ |             |           |
| blaCTX-M-1    | C F -1 | 5’AGGAAGTGTCGCCGTGATG-3’ | 750          | This study |
|               | C R -1 | 5’GTTTGAGGTGGTGGAAGTAA-3’ |             |           |
| blaCTX-M-2    | CM2F   | 5’ATGATGACTCAGAGCATCCG-3’ | 742          | Azam et al. (2016) |
|               | CM2R   | 5’TCGTTTGGTGGTGCATAATCC-3’ |             |           |
| blaCTX-M-8    | CM8F   | 5’AAGCGACAGACGCTACACC-3’ | 517          | Azam et al. (2016) |
|               | CM8R   | 5’GTTAGGCCCAACCTGAAT-3’ |             |           |
| blaCTX-M-9    | CT-9 F | 5’ATGGTGACAAGAGAGTGC-3’ | 811          | Siddiqui et al. (2019) |
|               | CT-9 R | 5’GTTCGTCGGGCTGGAAT-3’ |             |           |
| blaCTX-M-25   | CTX-25F| 5’ATGATGAAAGGAGTCGAGGCGG-3’ | 876         | Azam et al. (2016) |
|               | CTX-25R| 5’TTCAAATCCGTCGGATCAGT-3’ |             |           |
| blaTEM        | TEMF   | 5’ATGAGTATMACATTTTCGYGTG-3’ | 861         | Azam et al. (2016) |
|               | TEMR   | 5’TACCACATGTTTAAATCAGTGG-3’ |             |           |
| blaSHV        | S-F    | 5’ATGCGTTATATCTCCTGTGAT-3’ | 861         | Siddiqui et al. (2019) |
|               | S-R    | 5’GGTTGCCASTGCTGATCAGC-3’ |             |           |
Molecular characterization of CTX-M type ESBL genes

Phenotypically ESBL positive isolates were screened for the presence of different ESBL genes using specific primers (Table 1). PCR products of various blaCTX-M, blaTEM and blaSHV genes were purified and sequenced with corresponding primers. Sequencing was carried out by Agrigenome, India. The obtained gene sequence data were examined using FinchTV and Bio-Edit. Nucleotide sequences were subject to Basic Local Alignment Search Tool (BLAST) to identify different gene variants.

Bacterial identification using 16S rRNA approach

All the CTX-M positive samples were characterized by 16S rRNA sequencing. To perform it, 16S rRNA-specific primers were used for PCR amplification of 16S rRNA gene and the obtained products were sequenced. The obtained gene sequence data were analyzed, and homology was searched using BLAST at NCBI.

Conjugation transfer experiment

Conjugation was performed to learn about the capacity of ESBL positive bacteria to move their resistant factors (blaCTX-M, blaTEM and blaSHV) to recipient bacteria. Four isolates, i.e., JST71, HK106, SH52 and LG1, from different sampling sites harbouring ESBL genes and having MDR phenotype were selected as a donor, and the E. coli J53 (Sodium Azide resistant) was selected as recipient. The primary culture of donors was grown in cefotaxime (CTX 2 μg/mL) supplemented LB media and the recipient grown in azide (100 μg/mL) supplemented LB medium. Secondary culture of both donor and recipient were mixed in equal volume in LB and kept at 37 °C for 24 h (HiMedia, India). After that, serial dilutions were prepared in LB medium and spread on LA plates containing NaN3 (100 μg/mL) and CTX (2 μg/mL). Plasmid DNA was isolated from trans-conjugant and used as a template for PCR amplification to detect the presence of ESBL genes.

RESULTS

Screening of ESBL producers

A total of 436 non-duplicate bacterial isolates were isolated from Ghazipur slaughterhouse (103), Lodhi garden pond (98), Hauz Khas lake (140) and Jasola wastewater treatment plant (95). Based on the disc diffusion method, the preliminary and PDCT depicts that 106 isolates were ESBL positive. Thus, the prevalence of ESBLs producing bacteria in Ghazipur slaughterhouse, Lodhi garden pond, Hauz Khas lake and Jasola wastewater treatment plant was found to be 32, 23, 20 and 23%, respectively (Table 2).

Antibiotic profiling

Non-β-lactam

Among non-β-lactam, polymyxins class of antibiotics exhibited less effective towards phenotypically ESBL positive isolates. 39.62 and 33.01% ESBL positive isolates were resistant towards colistin and polymyxin B, respectively (Figure 1(a)). Resistance to rifampicin was found in 26.41% of ESBL positive isolates, followed by amikacin (19.81%), trimethoprim (18.86%) and chloramphenicol (6.60%). 1.88% of ESBL positive isolates were found to be resistant towards ciprofloxacin, levofloxacin and tetracycline (Table 3).

Table 2 | ESBL producers from different locations

| S. no | Sampling sites                  | Site code | Location          | Number of isolates | ESBL producers (%) |
|-------|---------------------------------|-----------|-------------------|--------------------|--------------------|
| 1     | Ghazipur slaughterhouse         | SH        | 28°62'N 77°32'E   | 103                | 32                 |
| 2     | Lodhi garden pond               | LG        | 28°59'N 77°21'E   | 98                 | 23                 |
| 3     | Hauz Khas lake                  | HK        | 28°55'N 77°19'E   | 140                | 20                 |
| 4     | Jasola wastewater treatment plant | JST    | 28°54'N 77°28'E   | 95                 | 23                 |
Among ESBL positive isolates, highest resistance (73.85%) was observed for cefotaxime, followed ceftazidime (70.75%), ceftriaxone (52.83%), monobactam (aztreonam) (51.88%), ampicillin (37.73%), cefazolin (34.90%) and cefoxitin (21.69%). Furthermore, some of these isolates showed resistance to the combination of drugs, ampicillin/Sulbactam (8.94%) and piperacillin/tazobactam (4.71%). 10.38% of isolates were resistant towards carbapenem class of antibiotics, viz. ertapenem and imipenem (Figure 1(b)). Site-wise resistance pattern of ESBL positive isolates against different beta-lactam antibiotics is shown in Table 3.

### Table 3 | Showing site-wise resistance pattern

| Antibiotics          | Ghazipur slaughterhouse (n = 33) | Lodhi garden pond (n = 23) | Hauz Khas lake (n = 28) | Jasola wastewater treatment plant (n = 22) |
|----------------------|----------------------------------|---------------------------|------------------------|------------------------------------------|
|                      | S     | I  | R  | S     | I  | R  | S    | I  | R  | S     | I  | R  |
| Non β-lactam         |       |    |    |       |    |    |       |    |    |       |    |    |
| Amikacin             | 24    | 8  | 1  | 16    | 4  | 3  | 11   | 6  | 11 | 10    | 6  | 6  |
| Colistin             | 17    | 4  | 12 | 16    | 2  | 5  | 12   | 2  | 14 | 8     | 3  | 11 |
| Polymyxin B          | 15    | 10 | 8  | 19    | 1  | 3  | 13   | 2  | 13 | 11    | 0  | 11 |
| Trimethoprim         | 25    | 3  | 5  | 22    | 0  | 1  | 15   | 6  | 7  | 14    | 1  | 7  |
| Rifampicin           | 28    | 3  | 2  | 10    | 4  | 9  | 14   | 6  | 8  | 9     | 4  | 9  |
| Ciprofloxacin        | 30    | 2  | 1  | 23    | 0  | 0  | 20   | 7  | 1  | 18    | 4  | 0  |
| Tetracycline         | 33    | 0  | 0  | 23    | 0  | 0  | 27   | 0  | 1  | 18    | 3  | 1  |
| Chloramphenicol      | 30    | 2  | 1  | 20    | 3  | 0  | 21   | 2  | 5  | 19    | 2  | 1  |
| Levofloxacin         | 32    | 1  | 0  | 20    | 3  | 0  | 26   | 1  | 1  | 21    | 0  | 1  |
| β-lactam             |       |    |    |       |    |    |       |    |    |       |    |    |
| Ampicillin           | 24    | 2  | 7  | 14    | 1  | 8  | 12   | 2  | 14 | 10    | 1  | 11 |
| Ampicillin/Sulbactam | 33    | 0  | 0  | 21    | 1  | 1  | 25   | 0  | 3  | 15    | 2  | 5  |
| Piperacillin/Tazobactam | 30  | 3  | 0  | 21    | 1  | 1  | 20   | 7  | 1  | 15    | 4  | 3  |
| Aztreonam            | 7     | 5  | 21 | 9     | 1  | 13 | 13   | 2  | 13 | 11    | 3  | 8  |
| Imipenem             | 33    | 0  | 0  | 18    | 3  | 2  | 22   | 0  | 6  | 17    | 2  | 3  |
| Ertapenem            | 29    | 3  | 1  | 20    | 3  | 0  | 20   | 2  | 6  | 14    | 4  | 4  |
| Cefazolin            | 23    | 4  | 6  | 14    | 4  | 5  | 15   | 0  | 13 | 9     | 0  | 13 |
| Cefoxitin            | 25    | 5  | 3  | 16    | 2  | 5  | 16   | 4  | 8  | 10    | 5  | 7  |
| Ceftazidime          | 3     | 1  | 29 | 5     | 2  | 16 | 5    | 3  | 20 | 9     | 3  | 10 |
| Cefotaxime           | 0     | 4  | 29 | 4     | 3  | 16 | 8    | 3  | 17 | 4     | 2  | 16 |
| Ceftriazone          | 20    | 4  | 9  | 6     | 3  | 14 | 7    | 2  | 19 | 1     | 7  | 14 |

n, number of ESBL positive isolates; S, sensitive; I, intermediate and R, resistant.

### β-lactam

Among ESBL positive isolates, highest resistance (73.85%) was observed for cefotaxime, followed ceftazidime (70.75%), ceftriaxone (52.83%), monobactam (aztreonam) (51.88%), ampicillin (37.73%), cefazolin (34.90%) and cefoxitin (21.69%). Furthermore, some of these isolates showed resistance to the combination of drugs, ampicillin/Sulbactam (8.94%) and piperacillin/tazobactam (4.71%). 10.38% of isolates were resistant towards carbapenem class of antibiotics, viz. ertapenem and imipenem (Figure 1(b)). Site-wise resistance pattern of ESBL positive isolates against different beta-lactam antibiotics is shown in Table 3.
MAR index and MIC
42, 60, 78 and 59% isolates had MDR phenotype from Ghazipur slaughterhouse, Lodhi garden pond, Hauz Khas lake and Jasola wastewater treatment plant, respectively, among all tested ESBLs producing isolates. The MAR index value of Jasola wastewater treatment plant and Hauz Khas lake was found similar 0.32, followed by Lodhi garden pond 0.22 and Ghazipur slaughterhouse 0.2 water. MIC (μg/mL) values for tested antimicrobials of ESBL positive isolates were ascertained as CTX (<2–1.024), AMP (<2– > 1.024), CAZ(2– > 1.024), RIF (<2–16), C (<2–256), CL (<2– > 1.024) and TR (<2– > 1.024) (Table 4).

Identification of ESBL determinant
Among 106 ESBLs producers, 68 (64%) isolates were harbouring the blaCTX-M gene. Further, site-wise 77.27, 73.91, 66 and 57.14% isolates from Jasola wastewater treatment plant, Lodhi garden pond, Ghazipur slaughterhouse and Hauz Khas lake harbouring blaCTX-M gene, respectively. Analysis based on particular groups showed that blaCTX-M-group-1 was the predominant type of ESBL gene (52/68) and blaCTX-M-group-25 (22/68) was the second most predominant type. Three different variants of blaCTX-M viz. blaCTX-M-15 associated with group-1, blaCTX-M-205 and blaCTX-M-152 from group-25 were identified among isolates. Among the blaCTX-M gene, the most prevalent was blaCTX-M-15 (70.5%) followed by blaCTX-M-205 (22.05%) and blaCTX-M-152 (4.41%) (Table 4). The most prevalent blTEM type was blTEM-116 and detected in 62% of isolates, whereas 33% of isolates possessed non-ESBL blTEM-1 (Table 4). Moreover, the blaSHV gene was successfully amplified from 17.64% of phenotypically ESBL positive isolates. 35.29% of the isolates possessed both blTEM and blaCTX-M genes among the ESBLs producing isolates (Table 4).

Molecular characterization of ESBL producing isolates
16S rRNA gene-based characterization of bacterial isolates showed diversity (21 different types) and belonged to six different families (Enterobacteriaceae, Bacillaceae, Moraxellaceae, Aeromonadaceae, Brucellaceae and Pseudomonadaceae). The isolates were identified as Enterobacter sp. (15), E. coli (13), Acinetobacter sp. (9), Citrobacter sp. (7), Bacillus sp. (3), Enterobacter cloacae (3), Acinetobacter Iwofii (2), Serratia marcescens (2), Acinetobacter calcoaceticus (2) and one isolates each of Aeromonas caviae, Acinetobacter beijerinckii, Citrobacter wernkannii, Ochrobactrum anthropi, Exiguobacterium mexicanum,Ralstonia sp., Kluyvera georgiana, K. pneumoniae, Pantoea agglomerans, Enterobacter ludwigi, Citrobacter freundii and Pantoea sp. (Table 4).

Conjugation experiment
The conjugation assay revealed that donor bacterial isolates including LG1, HK 106, JST 71 and SH52 from different sites were able to transfer their resistance genes to the recipient bacteria (Figure 2). The blaCTX-M genes were successfully amplified in all four trans-conjugants and blTEM from HK106 and SH52. None of the four transconjugant exhibited amplification of the blaSHV gene.

Accession numbers of the analyzed nucleotide sequence
Accession numbers of 16S rRNA genes (partial sequences) deposited in GenBank database are MN093322-MN093328, MT672728-MT672743, MN327103, MN327105, MN327106, MN327109, MN327111, MN327112, MT581480-MT581484, MT577527-MT581484, MT577527-MT577533, MT579677, MT577960-MT577363, MT576960-MT577367, MT576700, MN267572, MT267575, MT267576, MT267577, MT267579, MN267554-MN267556, MT376690-MT376693 and MT576698. The partial sequences of blaCTX-M genes were deposited in GenBank database under the accession number of MT636756-MT636759, MT636769-MT636782 and MT640044-MT640050. blaCTX-M gene complete gene sequences deposited under the accession numbers MT636760 and MT636783-MT636801. Complete gene sequences of blTEM were deposited under the accession numbers MT636802-MT636822.

DISCUSSION
Our study established the substantial occurrence of ARB in the surface water of Hauz Khas lake and Lodhi garden pond along with effluent water from WWTPs, i.e., Ghazipur slaughterhouse and Jasola from Delhi-NCR India. There are several reports of the prevalence of ARB in many freshwater sources from India (Siddiqui et al. 2019; Sultan et al. 2020), and this study thoroughly documented the prevalence and diversity of ESBL genes among ESBL producers in lentic and effluent water from Delhi-NCR, India. In both lentic as well as lotic water bodies, antimicrobial resistance has significantly increased due to the heavy load of
Table 4 | Antibiotic resistance phenotype and genotype along with MAR index and MIC of the different isolates

| Isolates                  | 16S rRNA sequence-based identification | β-lactamase gene/s | Minimum inhibitory concentration (μg/mL) | Antibiotic resistance                              | MAR Index |
|---------------------------|----------------------------------------|-------------------|------------------------------------------|---------------------------------------------------|-----------|
| HK1 Enterobacter sp.      | blaCTX-M-205, blaTEM-116               |                   | 1024 8 2 <2 256 128 <2 128              | CL, PB, TR, RIF, AMP, A/S, AT, CZ, CX, CAZ, CTX, CTR | 0.6       |
| HK4 Enterobacter sp.      | blaCTX-M                               | <2 256 <2 2 <2 1024 <2 <2             | RIF, CAZ                                             | 0.1       |
| HK8 Ochrobactrum anthropl | blaCTX-M                               | <2 <2 <2 4 16 8 <2 <2               | RIF, AT, CAZ, CTX, CTR                              | 0.25      |
| HK24 Acinetobacter       | blaCTX-M-15, blaCTX-M-205, blaTEM, blaSHV | 1024 <2 64 16 1024 8 <2 8             | PB, IPM, ETP, CZ, CX, CTX, CTR                       | 0.3       |
| HK32 Enterobacter cloacae| blaCTX-M-205, blaTEM-116               | 1024 256 2 32 1024 128 <2 256         | CL, PB, TR, RIF, AMP, A/S, AT, CZ, CX, CAZ, CTX, CTR | 0.6       |
| HK41 Enterobacter cloacae| blaCTX-M-205, blaTEM-116               | 1024 16 16 <2 256 128 <2 256          | AK, CL, TR, AMP, CZ, CX, CTX, CTR                     | 0.4       |
| HK43 Exiguobacterium     | blaCTX-M-15, blaCTX-M-205, blaTEM-116, blaSHV | >1024 64 64 8 1024 256 <2 1024    | AK, CL, PB, AMP, AT, ETP, CZ, CAZ, CZ, CX, CTR | 0.5       |
| HK48 Citrobacter sp.     | blaCTX-M-205                           | 256 <2 8 <2 1024 <2 1024             | AK, CL, PB, AMP, IPM, ETP, CZ                         | 0.35      |
| HK52 Acinetobacter       | blaCTX-M-15, blaCTX-M-205, blaTEM-116, blaSHV | <2 <2 <2 <2 256 <2 1024 <2 <2     | AK, CL, PB, AMP, AT, IPM, ETP, CZ, CX, CAZ, CTX, CTR | 0.55      |
| HK91 Ralstonia sp.       | blaCTX-M, blaTEM-116                  | 2 128 4 <2 8 4 <2 8                 | AK, C, RIF, AMP, AT, CZ, CAZ, CTX, CTR              | 0.45      |
| HK106 Citrobacter sp.    | blaCTX-M-15, blaCTX-M-205, blaTEM-116, blaSHV | <2 >1024 4 <2 <2 >1024 32 512  | AK, CL, PB, C, TE, AMP, CAZ, CTX, CTR              | 0.75      |
| HK108 Citrobacter freundii| blaCTX-M-205, blaTEM-116              | <2 <2 <2 <2 <2 4 <2 <2             | AK, CL, PB, C, TE, AMP, CAZ, CTX, CTR              | 0.45      |
| HK110 Enterobacter sp.   | blaCTX-M-205, blaTEM-116              | 128 4 4 <2 16 <2 4                  | AK, CL, C, CAZ                                       | 0.2       |
| HK111 Pantoea sp.        | blaCTX-M-205, blaSHV                   | 8 4 2 <2 8 16 <2 16                 | CL, LE, AT, CAZ, CTX, CTR                           | 0.3       |
| HK117 Enterobacter sp.   | blaCTX-M-205                           | 4 1024 16 4 <2 1024 <2 128          | AK, PB, C, RIF, AMP, CAZ, CTX, CTR                  | 0.35      |
| HK132 Acinetobacter      | blaCTX-M-205, blaTEM-116              | <2 1024 128 4 128 16 <2             | AK, TR, CZ, CX, CAZ                                 | 0.25      |
| SH1 Serratia marcescens  | blaCTX-M-15                            | 2 64 4 <2 4 8 <2 8                 | CAZ, CTX, CTR                                       | 0.15      |
| SH14 Kluyvera georgiana  | blaCTX-M-15                            | <2 128 4 <2 8 4 2 8                | CAZ, CTX, CTR                                       | 0.15      |
| SH17 Acinetobacter sp.   | blaCTX-M-15                            | <2 256 <2 <2 <2 1024 <2 <2         | CAZ, CTX, CTR                                       | 0.15      |
| SH23 Citrobacter sp.     | blaCTX-M-15                            | 2 128 4 <2 8 4 <2 8                | PB, CTX, CTR                                        | 0.15      |
| SH25 Bacillus sp.        | blaCTX-M-15                            | <2 16 8 <2 8 <2 8 <2 8             | CL, PB, AT, CAZ, CTX                                | 0.50      |
| Sample ID | Organism Name | Gene | MIC Values | Resistance Patterns |
|-----------|---------------|------|------------|---------------------|
| SH26      | Acinetobacter lwoffi | blaCTX-M-15 | <2, 8, 4, <2, 2, 8 | <2 | AMP, 0.05 |
| SH28      | Aeromonas caviae | blaCTX-M-15 | >1024, 128, 64, <2, 16, 16 | <2, 2 | CL, C, TR, AMP, AT, CAZ, CTX, CTR, 0.40 |
| SH34      | Serratia marcescens | blaCTX-M-15 | 4, 128, 4, <2, 8, 4 | <2, 8 | CL, AT, CAZ, CTX, 0.20 |
| SH36      | Acinetobacter beijerinckii | blaCTX-M-15 | <2, 128, 4, <2, 8, 8 | <2, 8 | TR, AMP, CAZ, CTX, 0.20 |
| SH42      | Acinetobacter sp. | blaCTX-M | <2, 256, <2, <2, 1024 | <2 | CL, AT, CAZ, CTX, 0.20 |
| SH51      | Acinetobacter sp. | blaCTX-M | <2, 16, 8, <2, <2, 8 | <2, 2 | AT, CAZ, CTX, 0.15 |
| SH52      | Citrobacter werkmanii | blaCTX-M-15, blaTEM-1, blaSHV | <2, 256, 2, <2, <2, 1024 | <2, 1024 | CL, PB, TR, RIF, AT, ETP, CZ, CX, CAZ, CTX, 0.50 |
| SH53      | Citrobacter sp. | blaCTX-M | 2, 128, 4, <2, 8, 4 | <2, 8 | CL, PB, AT, CAZ, CTX, 0.25 |
| SH68      | Citrobacter sp. | blaCTX-M | 8, 4, 2, <2, 8, 16 | <2, 2 | AT, CAZ, CTX, 0.15 |
| SH71      | Citrobacter sp. | blaCTX-M | 4, 64, 4, <2, 8, 8 | <2, 8 | AT, CZ, CAZ, CTX, 0.20 |
| SH74      | Acinetobacter sp. | blaCTX-M | 2, 256, 4, <2, 8, 8 | <2, 16 | AMP, CTX, 0.10 |
| SH77      | Citrobacter sp. | blaCTX-M | 4, 1024, 4, <2, <2, 512, 16 | 256 | AT, CAZ, CTX, 0.15 |
| SH78      | Acinetobacter sp. | blaCTX-M | 8, 4, 2, <2, 8, 16 | <2, 16 | CL, AT, CAZ, CTX, CTR, 0.25 |
| SH80      | Acinetobacter sp. | blaCTX-M | 4, 64, 4, <2, 8, 4 | <2, 8 | CL, PB, AT, CAZ, CTX, 0.25 |
| SH85      | Acinetobacter sp. | blaCTX-M, blaTEM | 2, 256, 4, <2, 8, 8 | <2, 16 | CL, PB, CIP, AT, CX, CAZ, CTX, CTR, 0.40 |
| SH86      | Acinetobacter lwoffi | blaCTX-M | 4, >1024, 4, <2, <2, >1024, 4, 256 | AK, CL, AT, CZ, CAZ, CTX, 0.30 |
| SH98      | Acinetobacter sp. | blaCTX-M-152, blaTEM | <2, 16, 8, <2, <2, 8 | <2, <2 | CL, PB, TR, CX, CAZ, CTX, CTR, 0.35 |
| LG1       | Bacillus sp. | blaCTX-M-15, blaTEM, blaSHV | 1024, 512, 2, <2, 128, 64 | <2, 16 | AK, CL, AT, CZ, CAZ, CTX, 0.35 |
| LG6       | Escherichia coli | blaCTX-M-15 | 16, 64, 2, 8, 16, 16 | <2, 4 | CL, PB, RIF, AT, CAZ, CTX, 0.30 |
| LG11      | Enterobacter sp. | blaCTX-M | 2, 8, 4, <2, <2, 2 | <2, <2 | AK, CL, RIF, P/T, CAZ, CTX, CTR, 0.35 |
| LG20      | Enterobacter sp. | blaCTX-M, blaTEM | <2, 512, 8, <2, 32, 1024 | <2, 32 | RIF, AMP, CZ, CX, CAZ, CTX, 0.30 |
| LG24      | Escherichia coli | blaCTX-M-15 | <2, 64, 8, <2, <2, 512 | <2, 64 | AMP, AT, CAZ, CTX, CTR, 0.25 |
| LG29      | Enterobacter sp. | blaCTX-M | 2, 128, 2, <2, 6, 4 | <2, 8 | CL, AT, CAZ, CTX, CTR, 0.25 |
| LG32      | Enterobacter sp. | blaCTX-M | 2, 8, 4, <2, 8, 4 | <2, 8 | AK, CL, PB, AT, CAZ, CTX, CTR, 0.35 |
| LG33      | Enterobacter sp. | blaCTX-M | <2, 128, 4, <2, 8, 8 | <2, 8 | AK, CL, PB, AT, CAZ, CTX, CTR, 0.35 |
| LG45      | Escherichia coli | blaCTX-M | 2, 128, 4, <2, 8, 4 | <2, 8 | AK, CL, PB, AT, CAZ, CTX, CTR, 0.30 |
| LG51      | Escherichia coli | blaCTX-M, blaTEM, | 64, 4, 256, 2, 1024, 64 | <2, 64 | AK, RIF, AM, IPM, CAZ, CTX, CTR, 0.35 |
| LG55      | Escherichia coli | blaCTX-M | 2, 8, 2, <2, 8, 4 | <2, 8 | CL, PB, RIF, AT, CAZ, CTX, CTR, 0.30 |
| LG71      | Enterobacter cloacae | blaCTX-M-15 | 1024, <2, 16, 4, 512 | >1024, <2, 1024 | AK, RIF, AMP, CZ, CX, 0.25 |

(Continued)
| Isolates | 16S rRNA sequence-based identification | \(\beta\)-lactamase gene/s | Minimum inhibitory concentration (\(\mu\)g/mL) | Antibiotic resistance | MAR Index |
|--------|----------------------------------|-----------------|-----------------|------------------|---------|
| LG80   | *Escherichia coli* | blaCTX-M | | | |
| JST14  | *Escherichia coli* | blaCTX-M | < 2 2 4 | < 2 4 4 | < 2 8 | AT, CAZ, CTX, CTR |
| JST15  | *Escherichia coli* | blaCTX-M | 2 8 4 | < 2 4 8 | < 2 8 | CZ |
| JST21  | *Enterobacter ludwigii* | blaCTX-M-152 | 8 4 2 | < 2 8 16 | < 2 16 | AT, CAZ, CTR |
| JST28  | *Escherichia coli* | blaCTX-M-15, blaSHV | 1024 < 2 2 8 1024 2 2 8 | AK, TR, RIF, AMP, AT, CZ, CAZ, CTX, CTR |
| JST33  | *Klebsiella pneumoniae* | blaCTX-M-15, blaTEM-1, blaSHV | 256 64 4 | < 2 < 2 256 < 2 256 | AK, CL, PB, AMP, AT, IPM, CZ, CAZ, CTX, CTR |
| JST36  | *Enterobacter sp.* | blaCTX-M, blaTEM-1, blaSHV | 512 64 4 | < 2 < 2 1024 < 2 256 | AK, CL, PB, RIF, AMP, AT, CZ, CAZ, CTX, CTR |
| JST61  | *Pantoea agglomerans* | blaCTX-M-15, blaCTX-M-205, blaTEM-1 | 1024 512 256 | < 2 128 256 < 2 256 | AK, PB, TR, RIF, AMP, A/S, CZ, CX |
| JST62  | *Enterobacter sp.* | blaCTX-M, blaTEM | > 1024 < 2 64 16 | > 1024 256 < 2 512 | RIF, AMP, P/T, AT, IPM, ETP, CZ, CX, CAZ, CTX, CTR |
| JST63  | *Bacillus sp.* | blaCTX-M-15 | 1024 1024 | < 2 < 2 128 128 < 2 128 | AK, CL, PB, RIF, AMP, A/S, CZ, CX, CTX |
| JST68  | *Escherichia coli* | blaCTX-M-15, blaCTX-M-205, blaTEM-1, blaSHV | 1024 1024 | < 2 < 2 64 256 < 2 251 | BL, PB, TR, RIF, AMP, A/S, CZ, CX, CAZ, CTX, CTR |
| JST69  | *Enterobacter sp.* | blaCTX-M-205 | 2 8 4 | < 2 8 4 | < 2 8 | CTX, CTR |
| JST70  | *Escherichia coli* | blaCTX-M-205 | 8 4 2 | < 2 8 16 | < 2 16 | CL, PB, CZ, CTX |
| JST71  | *Enterobacter sp.* | blaCTX-M-152, blaTEM, blaSHV | 256 < 2 < 2 1024 128 < 2 1024 | AK, CL, PB, C, TE, RIF, AMP, A/S, ETP, CZ, CX, CTX, CTR |
| JST72  | *Escherichia coli* | blaCTX-M | 1024 4 4 | < 2 8 16 | < 2 32 | AK, CL, TR, RIF, AMP, A/S, P/T, CZ, CTX, CTR |
| JST73  | *Enterobacter sp.* | blaCTX-M | 128 8 4 | < 2 8 4 | < 2 16 | PB, AT, CTX, CTR |
| JST78  | *Escherichia coli* | blaCTX-M-15 blaTEM | > 1024 8 4 8 | > 1024 1024 < 2 128 | AK, RIF, AMP, IPM, ETP, CZ, CX |
| JST79  | *Enterobacter sp.* | blaCTX-M | 256 128 4 | < 2 8 4 | < 2 8 | CL, PB, LE, TR, RIF, AMP, P/T, AT, ETP, CZ, CX, CAZ, CTX, CTR |

AMP, Ampicillin; A/S, Ampicillin/Sulbactam; CX, Cefoxitin; AT, Aztreonam; IPM, Imipenem; P/T, Piperacillin/Tazobactam; CZ, Cefazolin; CAZ, Cefazidime; CTX, Cefotaxime; CTR, Ceftriaxone; ETP, Ertapenem; AK, Amikacin; CL, Colistin; PB, Polymyxin B; CIP, Ciprofloxacin; LE, Levofloxacin; C, Chloramphenicol; TE, Tetracycline; TR, Trimethoprim; RIF, Rifampicin.
industrialization, overpopulation and mismanagement of the metropolitan cities (Devarajan et al. 2015; Yang et al. 2017). ESBL phenotype was observed in 32, 25, 20 and 23% of bacterial isolates from Ghazipur slaughterhouse, Lodhi garden pond, Hauz Khas lake and Jasola wastewater treatment plant Delhi-NCR, respectively. Other studies from the aquatic environment of Switzerland and Taiwan have similar reports where the prevalence of ESBL positive Enterobacteriaceae was reported to be 36.2 and 30%, respectively (Zurfluh et al. 2013; Chen et al. 2016). There are few reports of a higher percentage of ESBL producers with MDR bacteria from freshwater bodies (Taco et al. 2014; Bajaj et al. 2015). The occurrence of the high percentage of ESBL producing bacteria worldwide is challenging and worrisome. In our study, the majority of ESBL positive isolates exhibited resistance towards cefotaxime, ceftazidime, ceftriaxone, aztreonam, ampicillin and cefazolin similar to report from China urban river sediment (Lu et al. 2010). Furthermore, resistance towards colistin was found at a higher scale than earlier reports of clinical and environmental samples (Ansari et al. 2015). Thereby, a heightened resistance towards colistin which is the last drug available in the current pipeline to treat infections caused by MDR bacteria is alarming. Among ESBL producers isolates 42, 60, 78 and 59% from the Ghazipur slaughterhouse, Lodhi garden pond, Hauz Khas lake and Jasola wastewater treatment plant, respectively, were found to be MDR. From the site Hauz Khas lake, the resistance pattern was found similar to those reported from surface water and wastewater by Taco et al. (2014). The MAR index value for each site was >0.2 which suggests a deleterious situation and a high pollution load in a particular sampling area indicating that the antibiotic exposure of a specific area could be higher (Krumpelman 1983). High MIC value (≥ 1,024 μg/mL) for ceftazidime, cefotaxime, ampicillin, colistin and trimethoprim showed elevated resistance to β-lactam and folate pathway inhibitors among ESBL producers. Our findings are in line with previous reports from the freshwater of Delhi-NCR (Bajaj et al. 2015; Azam et al. 2016).

The blaCTX-M gene was detected among 64% ESBL positive isolates which corroborates previous reports from India and Australia (Reinthaler et al. 2010; Bajaj et al. 2015). In this study, we observed that blaCTX-M-15 was the most prevalent type which is similar to other reports (Azam et al. 2016; Maravić et al. 2016). The present study also justifies that the blaCTX-M-15 is the frequently dispersed ESBL among Enterobacteriaceae (Bevan et al. 2017). The co-occurrence of ESBL variants (blaCTX-M, blaTEM and blaSHV) identified in this study is in line with findings from the river Yamuna in Delhi-NCR (Siddiqui et al. 2019). blaTEM-116 was the most prevalent type among blaTEM similar to earlier reports of environmental isolates of Enterobacteriaceae (Maravić et al. 2016). blaSHV was found to be the least prevalent type of ESBL. The blaSHV gene is mainly dominant in clinical isolates (Maravić et al. 2016); however, it has been reported in freshwater from Switzerland (Zurfluh et al. 2013). The test isolates predominantly include members of Enterobacteriaceae (70.5%) followed by Moraxellaceae (20.5%), Bacillaceae (4.4%) and others (4.5%, i.e., Aeromonadaceae, Brucellaceae and Pseudomonadaceae). Our data demonstrate a high percentage of E. coli similar to other samples of environmental origin followed by Acinetobacter spp., which is in line with other reports (Lu et al. 2010; Taco et al. 2014; Maravić et al. 2016). New reports from this study include the blaCTX-M gene in A. lwofii, E. ludwigi, E. mexicanum and A. caviae. Furthermore, the identification of blaTEM and blaSHV in an environmental isolate of A. caviae is a new report from India.

Results of our conjugation experiment revealed the successful transfer of ESBL genes from donor to recipient. The results of conjugation assay are in line with previous reports where movement of TEM, CTX-M and SHV genes were possible through HGT like the phenomenon of conjugation (Siddiqui et al. 2020).
CONCLUSION
This research spotlights the high prevalence of ESBL producing bacteria in lentic and wastewater efluents of Delhi-NCR, India. With a low level of hygiene and discharge of antimicrobial compounds into the aquatic environment, leading to not only building up of resistance among inhabitants of water bodies but also the transfer of resistance genes to human pathogenic and non-pathogenic bacteria. 42–78% of ESBL positive isolates from different sampling sites showed MDR phenotype, which is a matter of concern. The ESBL positive isolates harboured blaCTX-M-15, blaCTX-M-152, blaCTX-M-205 and blaTEM-116 gene variants. blaCTX-M-15 was found to be the most prevalent type. Furthermore, this study reports occurrence of blaCTX-M gene in A. loiiii, E. ludwigii, E. mexicanum and A. caviae. The findings of this study showed that the urban aquatic environment (both lentic and lotic) of Delhi-NCR is crammed with ESBL producing bacteria. This study highlights the need for strong measures for positive actions to control the emergence and dissemination of MDR in the urban aquatic environment. Detailed studies are required to understand the emergence and transmission of ESBL producing bacteria in anthropogenically influenced aquatic environments.

ACKNOWLEDGEMENTS
The authors AA, AHM and MTS acknowledge UGC, India, for financial support in the form of fellowship. IS and FAG are extremely grateful to ICMR, India, for providing RA and SRF, respectively.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

FUNDING
No external funding was acquired for this project.

DATA AVAILABILITY STATEMENT
All relevant data are included in the paper or its Supplementary Information.

REFERENCES
Aminov, R. I. & Mackie, R. I. 2007 Evolution and ecology of antibiotic resistance genes. FEMS Microbiol. Lett. 271, 147–161. https://doi.org/10.1111/j.1574-6968.2007.00757.x.
Ansari, S., Nepal, H. P., Gautam, R., Shrestha, S., Neopane, P., Gurung, G. & Chapagain, M. L. 2015 Community acquired multi-drug resistant clinical isolates of Escherichia coli in a tertiary care center of Nepal. Antimicrob. Resist. Infect. Control 4, 2–9. https://doi.org/10.1186/s13756-015-0059-2.
Azam, M., Jan, A. T. & Haq, Q. M. R. 2016 blaCTX-M-152, a novel variant of CTX-M-group-25, identified in a study performed on the prevalence of multidrug resistance among natural inhabitants of river Yamuna, India. Front. Microbiol. 7, 1–13. https://doi.org/10.3389/fmicb.2016.00176.
Bajaj, P., Singh, N. S., Kanaujia, P. K. & Virdi, J. S. 2015 Distribution and molecular characterization of genes encoding CTX-M and AmpC β-lactamases in Escherichia coli isolated from an Indian urban aquatic environment. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2014.09.084.
Bevan, E. R., Jones, A. M. & Hawkey, P. M. 2017 Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. J. Antimicrob. Chemother. 72, 2145–2155. https://doi.org/10.1093/jac/dkx146.
Bhattacharyya, D., Saha, S., Banerjee, J., Bandyopadhyay, S. & Sarkar, K. 2020 Molecular characterization of multi drug resistant (MDR) extended spectrum β-lactamase (ESBL) producing Klebsiella spp. from pond water in West Bengal. J. Sci. Res. 64, 80–86. https://doi.org/10.37398/jsr.2020.640210.
Chen, P., Hung, C., Huang, P., Chen, J., Huang, I., Chen, W., Chiou, Y., Hung, W., Wang, J., Cheng, M., Protection, E. & Rivers, S. 2016 Escherichia coli strains isolated from multiple rivers in southern. Appl. Environ. Microbiol. 82, 1889–1897. https://doi.org/10.1128/AEM.03222-15.
CLSI 2016 Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. 24, M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA.
Devarajan, N., Lafitte, A., Graham, N. D., Meijer, M., Prabakar, K., Mubedi, J. I., Elongo, V., Mpiana, P. T., Ibelings, B. W., Wildi, W. & Poté, J. 2015 Accumulation of clinically relevant antibiotic-resistance genes, bacterial load, and metals in freshwater lake sediments in central Europe. Environ. Sci. Technol. 49, 6528–6537. https://doi.org/10.1021/acs.est.5b01031.
Falodun, O. I. & Ikusika, E. O. 2019 Extended spectrum beta-lactamase and metallo beta-lactamase producing pseudomonas species isolated from fish pond water in Ibadan, Nigeria. *Int. J. Environ. Stud.* 77, 1–11. https://doi.org/10.1080/00207233.2019.1705044.

Fatta-Kassinos, D., Kalavrouziotis, I. K., Koukoulakis, P. H. & Vasquez, M. I. 2011 The risks associated with wastewater reuse and xenobiotics in the agroecological environment. *Sci. Total Environ.* 409, 3555–3563. https://doi.org/10.1016/j.scitotenv.2010.03.036.

Gogry, F. A., Siddiqui, M. T. & Haq, Q. M. R. 2019 Emergence of *mcr-1* conferred colistin resistance among bacterial isolates from urban sewage water in India. *Environ. Sci. Pollut. Res.* 26, 33715–33717. https://doi.org/10.1007/s11356-019-06561-5.

Karthikeyan, K. G. & Meyer, M. T. 2006 Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. *Sci. Total Environ.* 361, 196–207. https://doi.org/10.1016/j.scitotenv.2005.06.030.

Krumperman, P. H. 1983 Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of faecal contamination of water. *Environ. Sci. Pollut. Res.* 22, 10969–10980. https://doi.org/10.1007/s11356-014-3887-3.

Lu, S. Y., Zhang, Y. L., Geng, S. N., Li, T. Y., Ye, Z. M., Zhang, D. S., Zou, F. & Zhou, H. W. 2010 High diversity of extended-spectrum beta-lactamase-producing bacteria in an urban river sediment habitat. *Appl. Environ. Microbiol.* https://doi.org/10.1128/AEM.00711-10.

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C., Grundmann, H., Harbarth, S., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Suetens, C., Stelling, J., Struelens, M. J., Vatopoulos, A., Todd, J. & Monnet, D. L. 2012 Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection* 18 (3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x.

Maravić, A., Skočiškić, M., Predotović, Ž., Šamanić, I., Cvjetan, S., Knežović, M. & Pužina, J. 2016 Urban riverine environment is a source of multidrug-resistant and ESBL-producing clinically important *Acinetobacter* spp. *Environ. Sci. Pollut. Res.* 23, 3525–3535. https://doi.org/10.1007/s11356-015-5386-0.

Moura, A., Pereira, C., Henriques, I. & Correia, A. 2012 Novel gene cassettes and integrons in antibiotic-resistant bacteria isolated from urban wastewaters. *Res. Microbiol.* 165, 92–100. https://doi.org/10.1016/j.resmic.2011.10.010.

Reinthaler, F. F., Feierl, G., Galler, H., Haas, D., Leitner, E., Mascher, F., Melkes, A., Posch, J., Winter, I., Zarfel, G. & Marth, E. 2010 ESBL-producing *E. coli* in Austrian sewage sludge. *Water Res.* 44, 1981–1985. https://doi.org/10.1016/j.watres.2009.11.052.

Siddiqui, M. T., Mondal, A. H., Sultan, I., Ali, A. & Haq, Q. M. R. 2019 Co-occurrence of ESBLS and silver resistance determinants among bacterial isolates inhabiting polluted stretch of river Yamuna, India. *Int. J. Environ. Sci. Technol.* 16, 5611–5622. https://doi.org/10.1007/s13762-018-1939-9.

Siddiqui, M. T., Mondal, A. H., Gogry, F. A., Husain, F. M. & Alsalme, A. 2020 Plasmid-mediated ampicillin, quinolone, and heavy metal co-resistance among ESBL-producing isolates from the Yamuna river, New Delhi, India. *Antibiotics* 9, 826. https://doi.org/10.3390/antibiotics9110826.

Smyth, C., O’Flaherty, A., Walsh, F. & Do, T. T. 2020 Antibiotic resistant and extended-spectrum β-lactamase producing faecal coliforms in wastewater treatment plant effluent. *Environ. Pollut.* 262, 114244. https://doi.org/10.1016/j.envpol.2020.114244.

Sultan, I., Ali, A., Gogry, F. A., Rather, I. A., Sabir, J. S. M. & Haq, Q. M. R. 2020 Bacterial isolates harboring antibiotics and heavy-metal resistance genes co-existing with mobile genetic elements in natural aquatic water bodies. *Saudi J. Biol. Sci.* 27, 2660–2668. https://doi.org/10.1016/j.sjbs.2020.06.002.

Taco, M., Moura, A., Correia, A. & Henriques, I. 2014 Co-resistance to different classes of antibiotics among ESBL-producers from aquatic systems. *Water Res.* https://doi.org/10.1016/j.watres.2013.09.021.

Yang, Y., Xu, C., Cao, X., Lin, H. & Wang, J. 2017 Antibiotic resistance genes in surface water of eutrophic urban lakes are related to heavy metals, antibiotics, lake morphology and anthropic impact. *Ecotoxicology* 26, 831–840. https://doi.org/10.1007/s10646-017-1814-3.

Zhang, T., Zhang, X. X. & Ye, L. 2011 Plasmid metagenome reveals high levels of antibiotic resistance genes and mobile genetic elements in activated sludge. *PLoS One* 6, e1–7. https://doi.org/10.1371/journal.pone.0026041.

Zhou, L. J., Ying, G. G., Zhao, J. L., Yang, J. F., Wang, L., Yang, B. & Liu, S. 2011 Trends in the occurrence of human and veterinary antibiotics in the sediments of the Yellow River, Hai River and Liao River in northern China. *Environ. Pollut.* 159, 1877–1885. https://doi.org/10.1016/j.envpol.2011.03.004.

Zurfluh, K., Hächler, H., Nüesch-Inderbinen, M. & Stephan, R. 2013 Characteristics of extended-spectrum β-lactamase- and carbapenemase-producing Enterobacteriaceae isolates from rivers and in reservoirs in Switzerland. *Appl. Environ. Microbiol.* 79, 3021–3026. https://doi.org/10.1128/AEM.00054-13.

First received 15 March 2021; accepted in revised form 15 May 2021. Available online 28 May 2021