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Prevalence of multidrug resistance in *Escherichia coli* strains isolated from river Yamuna, Delhi stretch

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Abstract: River water can act as a sink of multidrug resistant strains that may have serious public health implications. The aim of the study was to assess the antibiotic susceptibility and multidrug resistance character of twenty-eight *Escherichia coli* strains that have been isolated from water samples collected from the River Yamuna in Delhi stretch, India. The isolates were subjected to antibiotic sensitivity test by Kirby Bauer disc diffusion method as per Clinical and Laboratory Standard Institute (CLSI) guidelines using 24 different antibiotics belonging to three different modes of action namely β-lactams, aminoglycosides, phenicols, tetracyclines and quinolones. Most evident results of the study were that none of antibiotics used in the study was 100% effective. Findings have revealed that 100% of the isolates exhibited multi drug resistance (MDR) character and all the isolates were having a very high multiple antibiotic-resistance (MAR) index, suggesting the origin of the isolates to be of high antibiotic usage. Presence of multi drug resistant *E. coli* in river water can act as a medium to spread antibiotic resistance to other bacteria. The current attempt towards identification of existence of multidrug resistant bacteria isolated from the river Yamuna is a rather timely event which may find utility in the development of strategies to counteract this problem by incorporating the use of antibiotic resistance as a bacteriological water quality parameter.

Key words: River Yamuna; Coliforms; *Escherichia coli*; Antibiotics; Multidrug resistance

INTRODUCTION

Antibiotics are the biologically active compounds of natural or synthetic origin, which are widely used to prevent or treat infections in humans, animals and food-producing insects and plants[43]. However, the emergence and spread of antibiotic resistance has emerged as an issue of major concern worldwide[29] as the development of resistance for antibiotics in bacteria will make the use of these antibiotics ineffective. A wide range of biochemical and physiological mechanisms may be responsible for resistance. The abuse of antibiotics in human medicines, animal treatment and agriculture combined with inadequate wastewater treatment has led to the presence of antibiotics and antibiotic resistant bacteria in the environment particularly in the surface waters[20, 7]. Subsequently, it has led to the development of multiple drug resistance in many bacterial species[15, 38].

Bacterial contamination of surface waters has long been a major water quality issue due to potential for disease transmission. River water acts as a medium where bacteria (both pathogenic and non-pathogenic) from different sources like human, animal and environment (wastewater plants, urban or industrial effluents, agricultural run-off) mix together and this may result in exchange of antibiotic resistance genes among them. Furthermore, it may act as a source of dissemination of antibiotic resistant microorganisms among human and animal populations, if contaminated water is used for drinking purpose[17, 7].

The microbiological safety of drinking water is generally assessed using microbial indicators called fecal coliform bacteria and in particular *E. coli*. *E. coli* is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms. It is also found in water, soil and vegetation. The genus is a member of the Enterobacteriaceae family of the Gammaproteobacteria[13]. *E. coli* cells are able to survive outside the body for a limited amount of time, which makes them ideal indicator organisms to test environmental conditions for fecal contamination. The presence of *E. coli* in water is an indicator of fecal contamination and also represents the existence of all possible types of pathogenic bacteria transmittable through feces[22].

It must be noted that commensal *E. coli* in human gut can act as reservoirs of antibiotic resistance genes which might be rapidly transferred to other commensal or pathogenic bacteria[42, 9]. During the fecal contamination of water, antibiotic resistant bacteria harboring resistance genes present a potent threat to human health. Therefore, *E. coli* is a useful indicator of the spread of acquired antibiotic resistance genes in both commensal as well as in the environmental bacterial communities[11].

Several studies have reported the growing antibiotics resistance in clinical as well as environmental isolates of *E. coli* in both developed and developing countries[19, 2, 46, 18]. These studies have indicated that *E. coli* exhibits high rate of resistance as well as MDR against clinically significant antibiotics. The studies also suggested that the MDR is not congenital feature of *E. coli* but high and uncontrolled use is closely related to resistance development. Some studies have also provided the mechanisms of development of antibiotic resistance[21, 1, 28, 5].

Occurrence and prevalence of antibiotics and multidrug resistant *E. coli* from various sources has been investigated in India by a few researchers[13, 45, 41, 40]. However, resistance status of *E. coli* in the River Yamuna in Delhi stretch is inadequately studied so far. The present investigation was aimed to determine the antibiotic resistance pattern of the *E. coli* isolated from the River Yamuna, and to determine the existence of Multidrug resistant strains based on the criteria established by Magiorakos *et al.,* [31].

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MATERIALS AND METHODS

Study Area

The River Yamuna is the largest tributary of the River Ganga that originates from Yamunotri glacier and has a total length of 1,376 kilometers till its confluence with the River Ganga in Allahabad, India. Delhi stretch of the River Yamuna is 22 km long and it is located between Wazirabad and Okhla Barrage. Central location of the river corresponds to 28°36´ N and longitude 77°12´ E, at an altitude of 216 m above mean sea level[12]. The river is a major source of potable water to Delhi, which unfortunately also receives, untreated and partially treated domestic, agricultural and industrial wastes from the mega-metropolis city of Delhi[33].

Sampling Sites

Table 1: Description of study sites

Site Code	Site Name	Location	Description
A	Wazirabad Barrage	28° 43'23" Latitude north and 77°14'40" Longitude east	This site is the entry point of River Yamuna in Delhi
B	Najafgarh Drain (Outlet in the river)	28° 42'40" Latitude north and 77°13'55" Longitude east	It is the major polluting drain of River Yamuna in Delhi
C	Old Yamuna Iron Bridge	28° 39'36" Latitude north and 77°14'59" Longitude east	This is a place where there are many inhabitants by the riverbank and it reflects the impact of fecal discharge
D	ITO Barrage	28° 37'49" Latitude north and 77°15'20" Longitude east	It is the intermediate point having high amount of pollution
E	Nizamuddin Bridge	28° 36'20" Latitude north and 77°15'44" Longitude east	This is a place where there are few inhabitants with a visible presence of small houses
F	Okhla Barrage	28° 32'44" Latitude north and 77°18'57" Longitude east	It is the exit point of River Yamuna from Delhi
G	Shahdara Drain (Outlet in the river)	28° 31'44" Latitude north and 77°16'57" Longitude east	It is the second major polluting drain of River Yamuna in Delhi located at east of Okhla Barrage

All the media components, HiIMVic conventional biochemical test kit and the antibiotic octodiscs were procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India.

Sample collection and preservation

Samples were collected at seven different sampling locations (A-G) in 300 ml sterile autoclaved glass bottles in the month of December, April, August and November 2013-14. All the samples were maintained in vertical position at 4°C using ice packs until transported to the laboratory. Microbiological analysis was carried out within 5 h of sample collection[3].

Microbiological analysis of samples

Enumeration and Isolation of *E. coli:* *E. coli* was enumerated in the water samples collected from all the seven sampling sites by Most Probable Number (MPN) technique using three-tube test[3]. This included presumptive and confirmative tests. In presumptive test, serially diluted water samples were inoculated into single strength Lauryl Tryptose Broth medium. All the tubes were incubated at 37°C for 24-48 h. The positive tubes showing the formation of turbidity and gas were subjected to confirmative test. In confirmative test, the tubes showing positive results in presumptive test were inoculated into EC-MUG media followed by incubation at 44°C for 24 h in an incubator (Kuhner, Switzerland). All the tubes showing formation of blue color in EC-MUG media under UV light were recorded as MPN count of *E. coli*. Simultaneously, the cultures from positive lactose broth tubes were streaked onto Eosin Methylene Blue Agar. Isolates having green metallic sheen were further streaked and re-streaked onto MacConkey Agar till pure colonies were obtained. One bacterial isolate per site and per sampling was selected randomly from pure colonies growing on MacConkey Agar and was preserved on nutrient agar slants at 4°C for further studies.

Identification of *E. coli* by morphological and biochemical tests: The shape and color of each bacterial isolate was examined under the microscope after Gram staining according to Bergey's Manual of Systematic Bacteriology[16]. Further confirmation of *Escherichia* species was done using HiIMVic conventional biochemical test kit. This kit uses four conventional biochemical tests (Indole, Methyl red, Voges Proskauer's and Simmons citrate) and eight carbohydrate tests (Glucuronidase, Nitrate reduction, Ortho-Nitrophenyl-β-galactoside, Lysine utilization, Lactose, Glucose, Sucrose, Sorbitol).

Description of Antibiotics: As per the Clinical and Laboratory Standards Institute (CLSI) standards, twenty-four antibiotics belonging to ten different antibiotic classes were used to check the susceptibility and resistance pattern of *E. coli*. The antibiotics selected are those that are commonly used for medication against *E. coli*. Antibiotic concentration per disc included: Amikacin (AK) 30 µg; Ampicillin (AM) 10 µg; Aztreonam (AT) 30 µg; Co-Trimoxazole (COT) 25 µg; Cefepime (CPM) 30 µg; Cefotaxime (CTX) 30 µg; Cefoxitin (CX) 30 µg; Ceftazidime (CAZ) 30 µg; Cephalothin (CEP) 30 µg; Chloraphenicol (C) 30 µg; Ciprofloxacin (CIP) 5 µg; Gentamicin (GEN) 10 µg; Imipenem (IPM) 10 µg; Kanamycin (K) 30 µg; Levofloxacin (LE) 5 µg; Meropenem (MRP) 10 µg; Nalidixic Acid (NA) 30 µg; Netillin (NET) 30 µg; Nitrofurantoin (NIT) 300 µg; Norfloxacin (NX) 10 µg; Ofloxacin (OF) 5 µg; Streptomycin (S) 10 µg; Tetracyclin (TE) 30 µg; Tobramycin (TOB) 10 µg. *E. coli* ATCC 25922 was taken as the reference strain.

Determination of Antibiotic susceptibility of E. coli

The Antibiotic susceptibility pattern of all the bacterial isolates was determined by Kirby Bauer disc diffusion method[8] using Mueller Hinton agar as per the recommendations of CLSI[14]. A loopful culture of all the isolates were inoculated to 5.0 mL of Nutrient broth separately and grown overnight at 35°C in an incubator shaker (Kuhner, Switzerland). The turbidity of these overnight grown cultures was adjusted to a 0.5 McFarland standard[14]. Using sterile cotton swab, the bacterial suspension was spread on the surface of Muller Hinton agar plate. Antibiotic octodiscs were placed on the plates using sterile pointed forceps. Within 15-20 min of the application of the discs, the plates were inverted and incubated at 37°C

for overnight. The diameter of the inhibition zone for each antibiotic, thus obtained, was measured using a millimeter scale and expressed in mm. This zone size was used to classify the bacterial isolate as sensitive, intermediate or resistant. The susceptibility testing was done in triplicates and ATCC *E. coli* 25922 was used as positive control with each batch of antimicrobial susceptibility testing.

Multiple antibiotic resistance (MAR) indexing

MAR indices of individual isolates and sampling sites were calculated as per the method of Krumperman[27] using the equations given below:

$$MAR\ index\ of\ isolate = \frac{Number\ of\ antibiotics\ to\ which\ isolate\ was\ resistant}{Total\ number\ of\ antibiotics\ to\ which\ isolate\ was\ subjected\ for\ sensitivity\ test}$$

$$MAR\ Index\ of\ site = \frac{Average\ resistance\ index\ of\ all\ isolates\ from\ a\ site}{Number\ of\ antibiotics\ tested \times Number\ of\ isolates\ from\ the\ site}$$

An isolate with a value of MAR ≥0.2 is an indicator of the area with a high risk of contamination (e.g. animal farms, increased human population)[28].

RESULTS AND DISCUSSION

Enumeration and Isolation of E. coli from sampling sites

During the study, coliform contamination was detected in all the samples and the MPN count of *E. coli* at site A, B, C, D, E, F and G were found to be >400 MPN/100ml, >2300000 MPN/100ml, >480000 MPN/100ml, >2400000 MPN/100ml, >900000 MPN/100ml, >90000 MPN/100ml and >11000000 MPN/100ml respectively. The highest count was recorded at site G and the lowest count was recorded at site A. In this study, among the various strains isolated from the River Yamuna water, 28 strains of *E. coli* were selected after being subjected to morphological and biochemical confirmatory tests for further antibiotic studies.

Antibiotic susceptibility pattern of the Escherichia isolates

Numerous studies have been conducted to conclude the emergent concern of high levels of antibiotic resistance of *E. coli* isolated from the river water[4, 18, 40, 49]. Also, as majority of work has been conducted worldwide over clinical material[26, 39], there is a scope of understanding the mechanism of bacterial antibiotic resistance in natural environment. All the 28 *E. coli* strains isolated from the river Yamuna were subjected to antibiotic susceptibility tests in order to find out the bacteria behavior towards selected antibiotics as mentioned in Methods section.

Interpretation of results was carried out using the CLSI[14]. All strains showing "resistant" or "intermediate" behavior were subsumed under the category "resistant"(Reinthaler *et al.,* 2003). Table 2 shows that the highest (100%) resistance was recorded against Cefotaxime, Ceftazidime, Cephalothin, Ciprofloxacin, Imipenem, Meropenem and Nitrofurantoin. This was followed by Aztreonam (94.43%), Kanamycin (89.29%), Nalidixc Acid (89.28%), Amikacin (85.72%), Levofloxacin = Norfloxacin (85.71%), Ampicillin (82.14%) Ofloxacin (78.57%), Chloraphenicol (71.43%), Cefoxitin (63.28%), Cefemine = Streptomycin = Tetracycline (57.14%), Tobramycin (53.58%), Co-Trimoxazole (53.57%), Gentamicin (50%), and Netillin (25%). The recorded resistance of isolated strains to Nitrofurantoin was in same order of magnitude as that recorded for Beta-lactams.

Table 2: Antibiotic suceptibility profile of *E. coli* isolates

Antibiotics	Resistant (%)	Intermediate (%)	Sensitive (%)
Amikacin	28.57	57.14	14.29
Ampicillin	60.71	21.43	17.86
Aztreonam	60.71	35.71	3.58
Co-Trimoxazole	39.29	14.29	46.42
Cefepime	25.00	32.14	42.86
Cefotaxime	100.00	0.00	0.00
Cefoxitin	28.57	35.71	35.72
Ceftazidime	85.71	14.29	0.00
Cephalothin	96.43	3.57	0.00
Chloraphenicol	14.29	57.14	28.57
Ciprofloxacin	50.00	50.00	0.00
Gentamicin	32.14	17.86	50.00
Imipenem	100.00	0.00	0.00
Kanamycin	28.57	60.71	10.72
Levofloxacin	25.00	60.71	14.29
Meropenem	92.86	7.14	0.00
Nalidixic Acid	46.43	42.86	10.71
Netillin	7.14	17.86	75.00
Nitrofurantoin	96.43	3.57	0.00
Norfloxacin	39.29	46.43	14.28
Ofloxacin	25.00	53.57	21.43
Streptomycin	14.29	42.86	42.85
Tetracyclin	25.00	32.14	42.86
Tobramycin	10.71	42.86	46.43

Total number of test bacterial isolates = 28

These results clearly demonstrated that the bacterial strains isolated from the River Yamuna have a high degree of resistance to most of the antibiotics. The increasing resistance and multiple resistances of the bacterial strains in this river could be attributed to discharge resultant from fecal residues, household activities and hospital wastes from the areas adjoining the river, which overflowed in the River Yamuna through surface run off and sewage outlets. A study conducted to determine the fate of some commonly used antibiotics in a sewage treatment plant (STP) located in Delhi and the environmental concentration of these antibiotics in the Yamuna River has revealed that significant amounts of antibiotics were discharged in effluents and were also detected in the Yamuna waters showing the presence of antibiotics pollution[36]. These antibiotics as contaminants might also have played an important role in increasing the antibiotic resistance in microbes present in the river Yamuna.

MAR index of the isolates

Multiple resistance to antibiotics have been coded on plasmids, mutational events or on small mobile genetic elements called transposons[41,7]. Present study has shown that the isolated strains of *E. coli* exhibited multiple antibiotic resistance indicating that these bacteria are able to detoxify these antibacterial substances. The Multiple Antibiotic

Resistance (MAR) indexing was performed which is a cost effective method of bacteria source tracking[35]. Isolates with an MAR index of ≥0.2 are said to originate from high-risk sources of contamination like from humans, and animals sources where the antibiotics are frequently and erratically used.

In the current study, MAR index for the isolates was generated for antibiotics (Table 3) as well as for the sites (Table 4) and a high incidence of *E. coli* strains with MAR, was observed. None of the strains had MAR value < 0.2. All the strains were having the MAR value > 0.2 and up to 1, showing very high degree of resistance originating from high-risk source of contamination. This study has also brought out that there is a possibility that a large proportion of bacterial isolates had been exposed to a large number of antibiotics.

Table 3: MAR index of the *E. coli* isolates

Isolate	No. Of Antibiotics To Which The Isolate Was Resistant (A)	Mar Index (A/B)
A1	14	0.58
A2	9	0.37
A3	19	0.79
A4	14	0.58
B1	15	0.62
B2	16	0.66
B3	22	0.91
B4	18	0.75
C1	24	1
C2	23	0.95
C3	21	0.87
C4	22	0.91
D1	19	0.79
D2	24	1
D3	22	0.91
D4	22	0.91
E1	20	0.83
E2	22	0.91
E3	22	0.91
E4	23	0.95
F1	16	0.66
F2	14	0.58
F3	17	0.7
F4	17	0.7
G1	21	0.87
G2	16	0.66
G3	16	0.66
G4	18	0.75

Number of antibiotics to which the isolates were subjected = 24 (b)

Our results are corroborated by various studies that have also reported a high incidence of multiple resistance in terms of MAR index of *E. coli* in river water[48, 13, 18, 47]. The authors have reported high incidence of *E. coli* strains with MAR at all the sampling points

Table 4: MAR index of the sampling sites in the River Yamuna

SITE	MAR INDEX
Wazirabad Barrage	0.58
Najafgarh drain (Outlet in the river)	0.73
Old Yamuna Iron Bridge	0.93
ITO Barrage	0.9
Nizamuddin Bridge	0.9
Okhla Barrage	0.66
Shahdra Drain (Outlet in the river)	0.73

Based on the calculation of the MAR index of the sites, it can be deduced that at each sampling point, a high-risk source of fecal pollution, is present. The highest MAR values were recorded for Old Yamuna Iron Bridge >ITO barrage = Nizamuddin Bridge > Najafgarh drain outlet in River Yamuna = Shahdra drain outlet in River Yamuna > Okhla Barrage > Wazirabad Barrage.

Results of the research also speculate the fact that in the area along the river course, the antibiotics are largely used in the treatment of humans and domestic animals illnesses. The results are also suggesting that bacterial strains are becoming resistant intrinsically *in vivo* and fecal dissemination during rainfall or surface runoff into the river might have lead to the occurrence of these bacteria into the river system.

Multiple Drug Resistance (MDR) profile of the isolated strains

Multiple Drug resistance (MDR) has become an ominous evolving problem worldwide. The occurrence of MDR is usually predominant in Gram-negative bacteria[34]. Leverstein-van Hall *et al.,* [28] showed a very strong association between MDR and the presence of integrons in the members of Enterobacteriaceae, which plays a dominant role in the development of multi-resistance in them, independent of species or origin. Multidrug resistance in bacteria occurs by the accumulation, on resistance (R) plasmids or transposons, of genes, with each coding for resistance to a specific agent, and/or by the action of multidrug efflux pumps, each of which can pump out more than one drug type[38]. Also, administration of even single antibiotic along with long-term exposure of bacteria to high concentration of the antibiotic, can lead to the development of MDR strains[30].

In the current study, it was hypnotized that owing to high values of MAR, there is a possibility of presence of MDR strains of *E. coli* in River Yamuna water. If that's true, the River Yamuna can act as a sink of MDR strains, which have serious public health implications. The Multiple Drug Resistance (MDR) profile of all the 28 isolated bacterial strains is presented in Table 5.1 and Table 5.2.

All the bacterial isolates were resistant to one or more than one agent in three or more than three antimicrobial categories as per criteria described by Magiorakos *et al.,* [31]. Also, for all three definitions, non-susceptibility refers to either a resistant, intermediate or non-susceptible result obtained from in vitro antimicrobial susceptibility testing.

For the purpose of this paper, the bacterial isolate is designated as susceptible if they are sensitive to all and non-susceptible if they are resistant or intermediately resistant to any one of the antimicrobial agents in the given category.

Table 5.1: The Multiple Drug Resistance (MDR) profile of isolated bacterial strains

Antimicrobial Category	Mode Of Action	Antibiotics	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4	D1	D2
AMINOGLYCOSIDES	Inhibition of Protein Synthesis	Amikacin Gentamicin Tobramycin	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PHENICOLS	Inhibition of Protein Synthesis	Chloraphenicol	S	S	S	S	S	S	NS	S	NS	NS	NS	NS	NS	NS
TETRACYCLINS	Inhibition of Protein Synthesis	Tetracyclin	S	S	NS	NS	S	S	NS	S	NS	NS	NS	NS	NS	NS
β- LACTAM-CARBAPENEMS	Inhibition of Cell Wall Synthesis	Imipenem Meropenem	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
β- LACTAM-CEPHALOSPORIN	Inhibition of Cell Wall Synthesis	Cefepime Cefotaxime Ceftazidime	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
β- LACTAM-CEPHAMYCIN	Inhibition of Cell Wall Synthesis	Cefoxitin	NS	NS	NS	NS	S	S	S	NS	NS	NS	NS	NS	NS	NS
β- LACTAM-MONOBACTAMS	Inhibition of Cell Wall Synthesis	Aztreonam	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
β- LACTAM-PENICILLIN	Inhibition of Cell Wall Synthesis	Ampicillin	S	S	NS	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS
FLUOROQUINOLONES	Inhibition of DNA Synthesis	Ciprofloxacin	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 5.2: The Multiple Drug Resistance (MDR) profile of isolated bacterial strains

Antimicrobial Category	Mode Of Action	Antibiotics	D3	D4	E1	E2	E3	E4	F1	F2	F3	F4	G1	G2	G3	G4
AMINOGLYCOSIDES	Inhibition of Protein Synthesis	Amikacin Gentamicin Tobramycin	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PHENICOLS	Inhibition of Protein Synthesis	Chloraphenicol	NS	NS	NS	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	NS
TETRACYCLINS	Inhibition of Protein Synthesis	Tetracyclin	NS	NS	NS	NS	NS	NS	S	S	NS	S	S	S	S	S
β- LACTAM-CARBAPENEMS	Inhibition of Cell Wall Synthesis	Imipenem Meropenem	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
β- LACTAM-CEPHALOSPORIN	Inhibition of Cell Wall Synthesis	Cefepime Cefotaxime Ceftazidime	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
β- LACTAM-CEPHAMYCIN	Inhibition of Cell Wall Synthesis	Cefoxitin	NS	NS	NS	S	NS	NS	S	S	S	S	S	S	NS	NS
β- LACTAM-MONOBACTAMS	Inhibition of Cell Wall Synthesis	Aztreonam	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
β- LACTAM-PENICILLIN	Inhibition of Cell Wall Synthesis	Ampicillin	NS	NS	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	NS	S
FLUOROQUINOLONES	Inhibition of DNA Synthesis	Ciprofloxacin	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

An array of Multiple Drug Resistance was identified in the current study. No isolate was susceptible to all the antimicrobial agents used in the investigation. All the isolates were found to be non-susceptible to at-least three or more antibiotics of different classes. It was also found that all of these MDR strains were resistant to at least two of the tested members of β-lactam class of antibiotics. Highest level of drug resistance (100%) has been found for Carbapenems, Cephalosporins, and Flouroquinolones.

Monobactams, Aminoglycosides and Penicillin followed this. 96%, 93% and 82% of MDR *E. coli* were resistant to these classes of antibiotics respectively. Similarly, 74% and 61% of the MDR strains were resistant to Phenicols and Cephamycin respectively. Only 57% of the isolates were observed to be resistant to Tetracycline showing the highest sensitivity among all classes of antibiotics.

In present work, the resistance to β-lactam class groups gradually decreased in the following order; Newly used β-lactams- Carbapenems (Imipenem, Meropenem) = 3rd and 4th Generation Cephalosporins β-lactams- (Cefepime, Cefotaxime, Ceftazidime) > Newly used β-lactams- Monobactams (Aztreonam) > Conventional β-lactams- Penicillin (Ampicillin) > 2nd Generation Cephalosporins β-lactams- Cephamycin (Cefoxitin). Increasing resistance to this class of antibiotics have been reported by previous studies[32, 19, 6].

Flouroquinolones are mainstay and well-reputed broad-spectrum antibiotics that act against pathogenic strains of *E. coli*, however, the recorded resistance against them in the present study was exceedingly high (100%). Resistance to these antibiotics among gram-negative bacilli particularly *E. coli* is increasing due to increased use and abuse of this class of drugs, which is supported by various studies[2, 13, 25, 10, 29]. Recently, members of quinolones are being frequently prescribed in many developing countries as a treatment for enteric infections caused by Gram-negative bacteria[50]. This could explain the 100% resistance of detected MDR *E. coli* against quinolones.

93% isolates were found to be resistant against Aminoglycosides. Studies have reported mounting resistance of *E. coli* against this group of antibiotics[19, 2, 13, 46].

Substantial resistance was observed for Phenicols (74%) and Tetracycline (57%) although, the rate of resistance was relatively low compared to other classes of antibiotics. Results are in conformity with the studies showing high resistance developed by *E. coli* for Phenicols and Tetracycline[4, 48, 13, 23].

Various studies have shown MDR in different *E. coli* strains[37, 44, 2, 13, 40, 24]. Presence of MDR bacterial strains within River Yamuna water system represents a potential health risk as humans may become infected with MDR environmental bacteria through consumption of contaminated water and vegetables.

CONCLUSIONS

An ominous concern is extensive emergence of multiple drug resistance among microbes such as *E. coli*, which inhabit human intestine and readily contaminate the drinking water sources like rivers due to fecal contamination. Results of antimicrobial susceptibility test showed that all the isolated *E. coli* strains were resistant to most of the tested antibiotics, which may be explained by high and uncontrolled use of these antibiotics in humans, animals, pollution from pharmaceutical companies as well as heavy metals or biocides (antibiotic resistance co-selected by genes coding for metal resistance). Similarly, a high incidence of MAR and MDR has been detected in the collected samples from the river Yamuna, a lifeline of millions of people in India. Therefore, the analysis is highly informative in terms of assessing the fecal contamination of river water, determining resistance of *E. coli* against the commonly available antibiotics and existence of MDR strains in river water. Periodic monitoring of antimicrobial susceptibility should be the recommended strategy to counteract as the presence of high number of antibiotic resistant bacteria in river waters as this has serious ecological and public health implications.

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