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Multidrug Resistant Escherichia coli Isolated from Delhi Region with One Health Perspective

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ABSTRACT

Background: The constant increase in antimicrobial resistance (AMR) may lead to reversal of the world to the pre-antibiotic era, so it has become paramount to characterize the AMR prevalent in different ecosystems to study its various drivers and its possible impact on public health with one health perspective. The river Yamuna flowing in Northern India serve a large population of Delhi region. The river water is polluted by many sources and harbor infectious bacteria of public health importance. Escherichia coli are one such bacteria present in river water and is of utmost public health importance that are needed to be explored for emergence of AMR. **Methods:** In present study the samples for isolation of Escherichia coli were randomly collected from different ecosystems of Delhi region comprising human, animals and the environment. Environmental samples were collected from different locations of river Yamuna, animals samples were collected from livestock faeces (Cattle and Buffaloes) living in near vicinity of the river and human samples from establishments in the surrounding areas and processed for isolation and identification of E.coli as per standard procedures and guidelines. **Results:** A total of 84 isolates of E.coli obtained from human, animals (Cattle and Buffaloes) and the river Yamuna being the environmental samples were studied for their AMR pattern and all were found to be resistant for atleast 5 drugs. In human isolates Cefotaxime, Cefpodoxime, Clindamycin, Nalidixic acid was found 100% resistant whereas Tigecycline was found absolutely sensitive. High level of resistance was encountered for Cefotaxime in isolates obtained from all the three ecosystems under study that was of serious concern being third generation cephalosporin however not much resistance was observed for last resort antibiotics like carbapenems and Colistin. **Conclusion:** All the isolates obtained from human, animals and the environment were multidrug resistant E.coli that is of serious public health concern and further raise the threat of horizontal transmission of AMR genes to the unexposed population of human and animals that come in contact with the water of river Yamuna flowing through the three states.

Keywords: AMR, Escherichia coli, MDR, Yamuna, One Health.

INTRODUCTION

The constantly rising Antimicrobial resistance in pathogens is posing a great threat to the existence of mankind. The deaths from AMR are predicted to rise drastically by 2050.[1] The situation is more worsening due to the involvement of different ecosystems that harbour pathogenic, non-pathogenic and commensal bacteria that may possess and transmit the resistance genes among the different ecosystems. The present study is conducted to explore the emergence of multidrug-resistant bacteria in different ecosystems of Delhi region that are overlapping in certain extents and may disseminate the resistance genes among themselves via horizontal transmission. The different ecosystems comprising water of the river Yamuna, livestock (Cattle & Buffaloes) that come in contact with the river water or live in the close vicinity and from the human establishments in the surrounding areas.

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River Yamuna is a major tributary of the River Ganges in India (20.59370 N, 78.96290 E) and flows in Delhi region through three major states of Northern India comprising Delhi (28.70410N, 77.10250 E), Haryana (28.99310 N, 77.01510 E) and Uttar Pradesh (29.04680 N, 77.33240 E). The water of the river is considered holy and is also used by people for religious purpose and for daily chores and thus they are exposed to high risk of acquiring the dreadful bacterial infections due to the presence of multidrug-resistant bacteria in it. It was thus of paramount importance to study the pathogen (Escherichia coli) present in the river and associated ecosystems in reference to its possible impact on the public health of the Delhi region.

MATERIALS AND METHODS

Samples: Water samples were collected in sterile containers of 250 ml from barrages and banks of river Yamuna located in Delhi, Haryana and Uttar Pradesh (India). A total of 168 samples from 14 different locations of river Yamuna were collected and processed for isolation of E.coli as per the guidelines of fssai Manual of Methods of Analysis of Food- Water, 2016,[2] whereas 250 Faecal samples from Cattle and Buffaloes were processed as per standard microbiological procedures for E.coli The

clinical samples obtained from human were processed in Vitek system for E.coli and isolates were reconfirmed in the laboratory using standard microbiological techniques. All the isolates obtained were re-confirmed by cultural, biochemical and molecular test.

Antimicrobial susceptibility testing

Disk diffusion tests were performed as per Manual of antimicrobial susceptibility testing and Clinical Laboratory and Standard Institute using disks (Himedia Pvt. Ltd., Mumbai) impregnated with Ampicillin (AMP; 10), Amoxyclav (AMC;30), Azithromycin (AZM; 15), Aztreonam (AT; 30), Cefotaxime (CTX; 30), Cefotaxime/Clavulenic acid (CAC;30/10), Cefoxitin (CX;30), Cefpodoxime (CPD;10), Ceftazidime (CAZ;30), Ceftazidime/ Clavulenic acid (CEC; 30/10), Ceftriaxone (CTR;30), Chloramphenicol (C;30), Ciprofloxacin (CIP;10), Clindamycin (CD;2), Colistin (CL;10), Co-Trimoxazole (COT;25), Gentamicin (GEN;10), Imipenem (IPM; 10), Levofloxacin (LE;5), Meropenem (MRP;10), Nalidixic acid (NA; 30), Nitrofurantoin (NIT;300), Piperacillin/Tazobactum (PIT;100/10), Polymyxin B (PB;300units), Streptomycin (S;10), Tetracycline (TE;30), Tigecycline (TGC;15), Tobramycin (TOB;10).[3,4] European Committee on Antimicrobial Susceptibility Testing guidelines were used where CLSI guidelines were indeterminate. For quality control of the culture media and antimicrobial disks,[5] Escherichia coli (ATCC 25922), Salmonella enterica subsp. enterica serovar Enteritidis (ATCC13076), Salmonella enterica subsp. enterica serovar Typhimurium (ATCC 51812) were tested under the same conditions as was suggested by CLSI.

Isolation of E. coli

E. coli were isolated from water as per the guidelines of fssai Manual of Methods of Analysis of Food-Water, 2016 with minor modifications.[2] The samples were collected in sterile containers and brought to the laboratory in cold chain and stored at 4^0C till further processing. 250 ml of water samples were collected from each spot of different locations of the Yamuna flowing through Delhi, Haryana and Uttar Pradesh. The samples were filtered using 0.45µm filter (Millipore) and transferred the filter in a test tube containing 50 ml of Buffered peptone water (BPW) for further processing on differential and selective media. The human stool and animal faecal samples were processed in a similar manner. E. coli isolates obtained were further subjected to morphological characterization,[6] biochemical characterization comprising TSI, Sugars, IMVIC test and for molecular confirmation by duplex and multiplex PCR.[7-10] The clinical samples were obtained from the Department of Microbiology, BPS Government Medical College for Women, Gohana, Sonipat, Haryana which comprised samples from different sources and isolation was carried out in ViTek system and then was further processed on selective media and subjected to Biochemical Tests for confirmation. The stool samples obtained from the District Hospital Baghpat were processed as per standard laboratory procedures for isolation of E. coli from faecal samples.

Identification of E. coli

Cultural identification

The samples present on Nutrient Agar (NA) are greyish white with smooth convex colonies, that may be translucent to opaque. The single colony present on NA was streaked on the MacConkeys Lactose Agar to identify the status of the bacteria being a lactose fermenter. The lactose fermenting bacteria were further streaked on Eosin Methylene Blue (EMB) Agar that is a selective media for the E. coli on which the samples positive for E. coli give the growth of bacteria with the green metallic sheen characteristic of E. coli.

Morphological identification

Morphological characterization of E. coli isolates was carried out as per the methods described by Ewing (1996).[6] All E. coli isolates were subjected to the Gram's staining for their morphological characterization.

Biochemical identification

The biochemical tests comprised oxidase, TSI and citrate. All E. coli were oxidase negative. The test culture along with ATCC 25922, ATCC 13076 and one un-inoculated culture slant of TSI was streaked on the surface and also stabbed up to the butt of the agar medium. Inoculated TSI agar slants were incubated at 37^0C for 24 hours. The culture medium showing acidic reaction (yellow) in the slant and acidic reaction (yellow) in the butt and gas production on TSI agar medium was interpreted as a positive test for the presence of E. coli. The test culture of the organism was streaked over the surface of the Simmons citrate agar medium along with a positive control ATCC 13076 and a negative control ATCC25922 and incubated at 37^0C for 24 hours. The positive test was indicated by the change of green colour medium to blue colour and presence of growth, while a negative test was indicated by the absence of growth and colour change.

Molecular Identification

Molecular identification of different pathotypes of E.coli was conducted by targeting genes comprising eae (480bp) and fimA (324 bp) genes. These were identified using multiplex /duplex PCR.[8,9] PCR was performed in the Eppendorf Mastercycler® pro-Thermal Cycler (Eppendorf, Germany). Two sets of multiplex PCR (mPCR) and one duplex PCR (dPCR) were used for the identification of different E.coli pathotypes viz., enteropathogenic (EPEC),

shigatoxic (STEC), enterohemorrhagic (EHEC), enterotoxigenic (ETEC), and enteroaggregative (EAEC). First multiplex PCR (mPCR1) was used for the detection of stx1, stx2 and eae genes, whereas second one (mPCR2) was used for detection of bfp, lt and stII genes. A duplex PCR was also used for the detection of fimA and aggR genes. In brief, the standardized PCR protocol for 25 µl reaction mixture included 12.5 µl of 2 x PCR master mixes (Thermo Scientific), 10 pmol of a primer set containing forward and reverse primers, 2µl of DNA template and nuclease free water to make up the reaction volume. PCR cyclic conditions for mPCR1 were Initial denaturation at 94°C for 5 min. with 1 cycle, denaturation at 94°C for 1.5 min. with 5 cycles, annealing at 62°C 1.5 min. with 35 cycles, extension at 72°C 1.5 min with 35 cycles and elongation at 72°C 10 min. with 1 cycle. The PCR cyclic conditions for m PCR2 were initial denaturation at 94°C for 5 min. with 1 cycle, denaturation at 94°C for 1.5 min. with 5 cycles, annealing at 62°C 1.5 min. with 35 cycles, extension at 72°C 1.5 min with 35 cycles and elongation at 72°C 10 min. with 1 cycle. The PCR cyclic conditions for d PCR were initial denaturation at 95°C for 2 min. with 1 cycle, denaturation at 95°C for 40 sec. with 40 cycles, annealing at 58°C 1.0 min. with 40 cycle, extension at 72°C 1.5 min with 40 cycle and elongation at 72°C 10 min. with 1 cycle.

Table 1: Details of primer sequence for virulence genes

Gene	Primer sequence, 5′-3′	Size of product (bp)	Reference
eae	F- TCAATGCAGTTCCGTTATCAGTT R- GTAAAGTCCGTTACCCCAACCTG	482	[9]
bfp	F- GGAAGTCAAATTCATGGGGGTAT R- GGAATCAGACGCAGACTGGTAGT	254	[9]
stx1	F- CAGTTAATGTGGTGGCGAAGG R-CACCAGACAATGTAACCGCTG	348	[10]
stx2	F- ATC CTATTCCCG GGA GTTTACG R- GCGTCATCGTATACA CAGGAG C	584	[10]
lt	F- GCA CAC GGA GCT CCT CAG TC R- TCC TTC ATC CTT TCA ATG GCT TT	218	[9]
stII	F- AAA GGA GAG CTT CGT CAC ATT TT R- AAT GTC CGT CTT GCG TTA GGA C	129	[9]
aggR	F- CCTAAAGGATGCCCTGATGATAA R-TAACGCTGGACATGAGATAACC	663	[8]
fim A	F- TTAGTAGCCTGATGTTGCTGGGCA R- ATGTGCCTGCCGCAGTTTCAATAC	324	[8]

RESULTS

E.coli isolates

Out of the total isolates obtained from various samples only 84 isolates comprising 40 human isolates, 22 water isolates and 22 animal isolates were selected for AMR studies.

Figure 1: E.coli on MLA- decrease the size

Cultural characterization of E.coli

E. coli produced pink colonies [Figure 1] on Mac Conkeys Lactose Agar (MLA) and on Eosin Methylene Blue (EMB) media it exhibited green metallic sheen [Figure 2] which led to the presumption of it being an E.coli isolate.

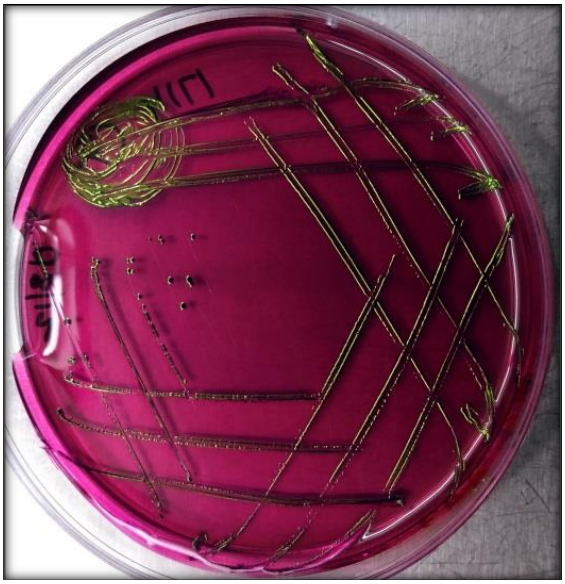

Figure 2: E.coli on EMB agar- decrease the size

Figure 3: TSI Slant (Uninoculated)- decrease the size

Figure 4: TSI slant inoculated with E.coli (A/A) - decrease the size.

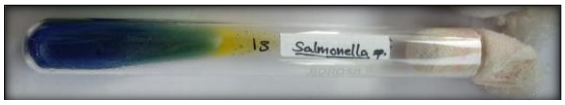

Figure 5: Citrate Slant inoculated with Salmonella Typhimurium-Citrate positive)- decrease the size.

Figure 6: Citrate Slant inoculated with Escherichia coli- Citrate negative) - decrease the size

Molecular identification and characterization of E.coli

Molecular characterization of E.coli isolates into different strains based on the detection of the various virulence genes in their genomes. The gene eae (480bp) i.e. Enteropathogenic E.coli (EPEC) was present in 17 isolates and the gene fim A (324bp) was found in 16 isolates whereas stx1, stx2, lt, stII were not identified in any of the isolates.[8] No isolate was found positive for Shiga toxins genes stx1 and stx2.

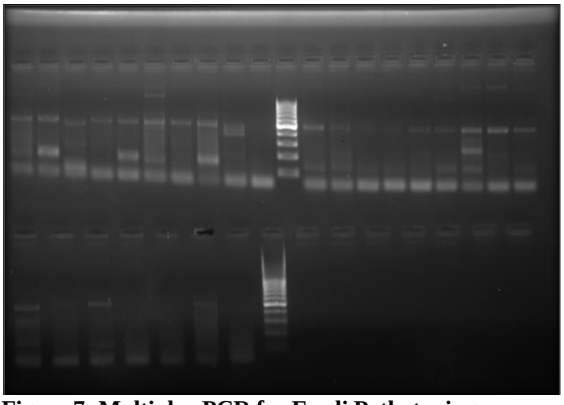

Figure 7: Multiplex PCR for E.coli Pathotyping

eae- 1-9, 11, 12, 15-20- are positive for eae (480bp) i.e. EPEC.
fimA : 3, 9,10,12,15,18,19,20,22 are negative. Rest all are positive (duplex PCR)
All sample were negative for second multiplex (mPCR2)

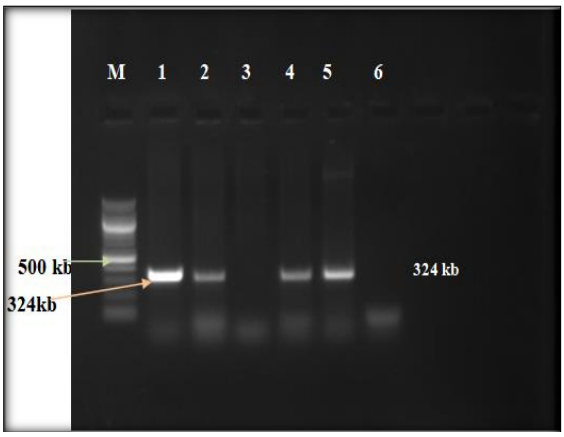

Figure 8: Duplex PCR for E.coli pathotypes

Lane 1,2, 4,5 : for fimA (324 bp)
Lane 3: negative samples for fimA (324 bp)
Lane 6: Negative control
M: 100 bp DNA ladder

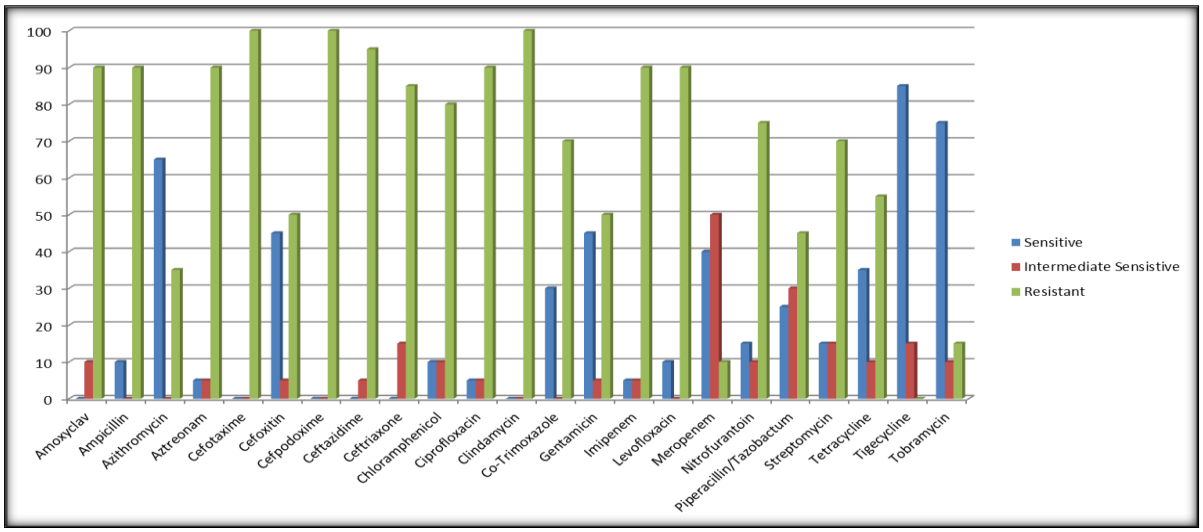

Figure 9: Resistance Profile of Isolates of E. coli (Source: Human)

Biochemical characterization of E. coli

All the selected E.coli isolates were subjected to Biochemical characterization with different biochemical tests comprising oxidase, catalase indole MR, VP, Citrate, TSI and Urease. All isolates showed negative for oxidase test. The isolates exhibiting oxidase negative, catalase-positive, indole positive, MR positive, VP negative, Citrate negative

and TSI positive (A/A) and urease negative were only processed further. All the isolates produced A/A reaction [Figure 4] in TSI test that indicated Acidic slant/Acidic butt. All the sugars comprising lactose, sucrose and glucose were fermented producing acid that turned the pH indicator colour to yellow from red. There was no colour change in un-inoculated TSI slant [Figure 3]. All the isolates were citrate negative. ATCC13076 turned blue [Figure 5] being positive control and ATCC25922 remained unchanged [Figure 6] being negative control of citrate test.

Table 2: Resistance profile of isolates of E. coli (source: Human)

S.No.	Antibiotic	Symbol	Concentration (mcg)	Sensitive (%)	Intermediate (%)	Resistant (%)
1	Amoxyclav	AMC	30	0	10	90
2	Ampicillin	AMP	10	10	0	90
3	Azithromycin	AZM	15	65	0	35
4	Aztreonam	AT	30	5	5	90
5	Cefotaxime	CTX	30	0	0	100
6	Cefotaxime/Clavulenic acid	CAC	30	25	75	0
7	Cefoxitin	CX	30	45	5	50
8	Cefpodoxime	CPD	10	0	0	100
9	Ceftazidime	CAZ	30	0	5	95
10	Ceftazidime/ Clavulenic acid	CEC	30	32.5	67.5	0
11	Ceftriaxone	CTR	30	0	15	85
12	Chloramphenicol	C	30	10	10	80
13	Ciprofloxacin	CIP	10	5	5	90
14	Clindamycin	CD	2	0	0	100
15	Colistin	CL	10	100	0	0
16	Co-Trimoxazole	COT	25	30	0	70
17	Gentamicin	GEN	10	45	5	50
18	Imipenem	IPM	10	7.5	7.5	85
19	Levofloxacin	LE	5	10	0	90
20	Meropenem	MRP	10	40	50	10
21	Nalidixic acid	NA	30	0	0	100
22	Nitrofurantoin	NIT	300	15	10	75
23	Piperacillin/Tazobactum	PIT	100/10	25	30	45
24	Polymyxin	PB	300units	100	0	0
25	Streptomycin	S	10	15	15	70
26	Tetracycline	TE	30	35	10	55
27	Tigecycline	TGC	15	85	15	0
28	Tobramycin	TOB	10	87.5	0	12.5

Table Summary: It describes the percentage sensitivity and resistance in human isolates. Cefotaxime, Cefpodoxime, Clindamycin, Nalidixic acid are depicted as 100% resistant whereas Cefotaxime/Clavulanic acid, Ceftazidime/ Clavulanic acid and Tigecycline appears to be absolutely sensitive.

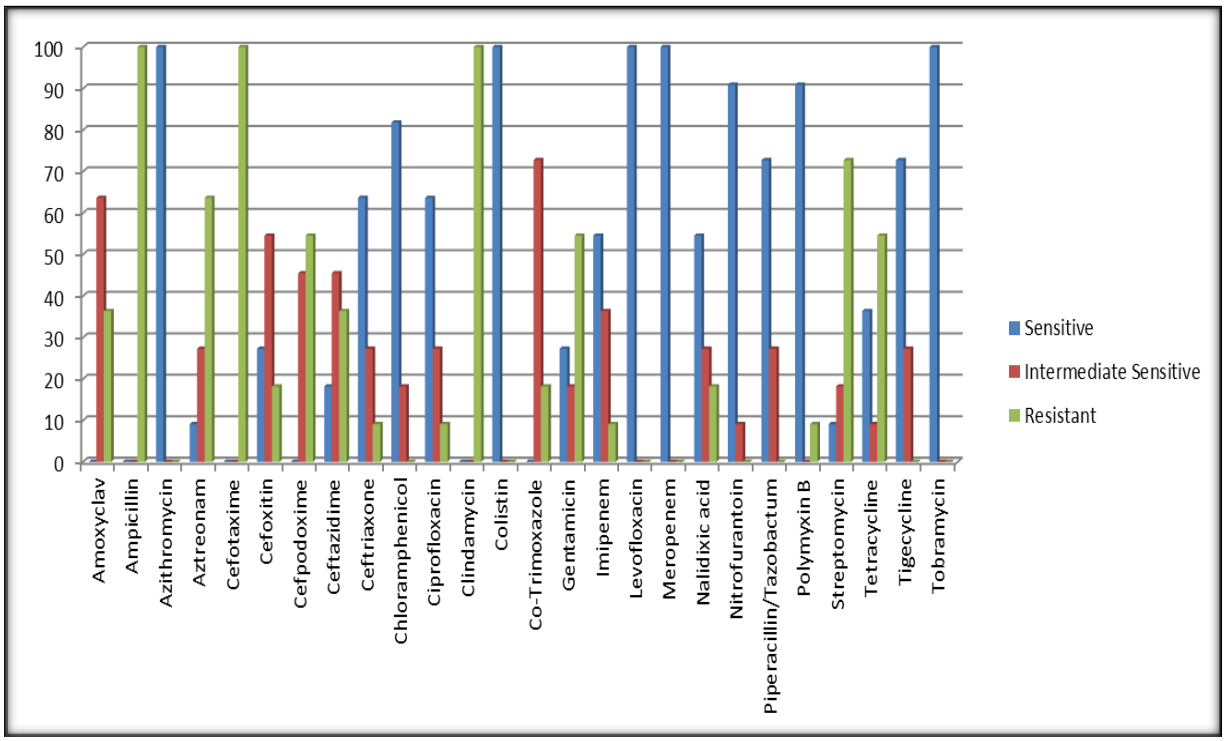

Figure 10: Resistance Profile of Isolates of E. coli (Source: Cattle and Buffaloes)

Table 3: Resistance Profile of Isolates of E. coli (Source: Animals)

S.No.	Antibiotic	Symbol	Concentration (mcg)	Sensitive (%)	Intermediate (%)	Resistant (%)
1	Amoxyclav	AMC	30	0	63.63	36.36
2	Ampicillin	AMP	10	0	0	100
3	Azithromycin	AZM	15	100	0	0
4	Aztreonam	AT	30	9.09	27.27	63.63
5	Cefotaxime	CTX	30	0	0	100
6	Cefoxitin	CX	30	27.27	54.54	18.18
7	Cefpodoxime	CPD	10	0	45.45	54.54
8	Ceftazidime	CAZ	30	18.18	45.45	36.36
9	Ceftriaxone	CTR	30	63.63	27.27	9.09
10	Chloramphenicol	C	30	81.81	18.18	0
11	Ciprofloxacin	CIP	10	63.63	27.27	9.09
12	Clindamycin	CD	2	0	0	100
13	Colistin	CL	10	100	0	0
14	Co-Trimoxazole	COT	25	0	72.72	18.18
15	Gentamicin	GEN	10	27.27	18.18	54.54
16	Imipenem	IPM	10	54.54	36.36	9.09
17	Levofloxacin	LE	5	100	0	0
18	Meropenem	MRP	10	100	0	0
19	Nalidixic acid	NA	30	54.54	27.27	18.18
20	Nitrofurantoin	NIT	300	90.90	9.09	0
21	Piperacillin/Tazobactum	PIT	100/10	72.72	27.27	0
22	Polymyxin B	PB	300 units	90.90	0	9.09
23	Streptomycin	S	10	9.09	18.18	72.72
24	Tetracycline	TE	30	36.36	9.09	54.54
25	Tigecycline	TGC	15	72.72	27.27	0
26	Tobramycin	TOB	10	100	0	0

Table Summary: It describes the percentage sensitivity and resistance in animal isolates. Ampicillin, Cefotaxime is depicted as 100% resistant whereas Azithromycin, Chloramphenicol, Levofloxacin, Meropenem, Nitrofurantoin, Piperacillin/Tazobactum, Tigecycline and Tobramycin showed no resistance.

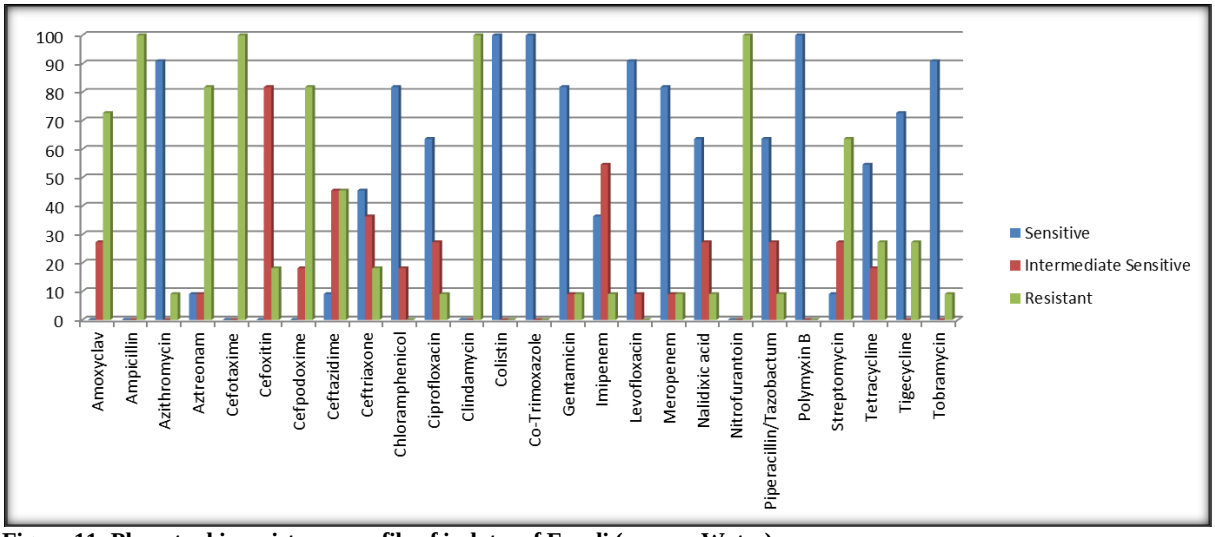

Figure 11: Phenotyphic resistance profile of isolates of E. coli (source: Water)

Table 4: Resistance profile of isolates of E. coli (source: Water)

S.No.	Antibiotic	Symbol	Concentration (mcg)	Sensitive (%)	Intermediate (%)	Resistant (%)
1	Amoxyclav	AMC	30	0	27.27	72.72
2	Ampicillin	AMP	10	0	0	100
3	Azithromycin	AZM	15	90.90	0	9.09
4	Aztreonam	AT	30	9.09	9.09	81.81
5	Cefotaxime	CTX	30	0	0	100
6	Cefoxitin	CX	30	0	81.81	18.18
7	Cefpodoxime	CPD	10	0	18.18	81.81
8	Ceftazidime	CAZ	30	9.09	45.45	45.45
9	Ceftriaxone	CTR	30	45.45	36.36	18.18
10	Chloramphenicol	C	30	81.81	18.18	0
11	Ciprofloxacin	CIP	10	63.63	27.27	9.09
12	Clindamycin	CD	2	0	0	100
13	Colistin	CL	10	100	0	0
14	Co-Trimoxazole	COT	25	100	0	0
15	Gentamicin	GEN	10	81.81	9.09	9.09
16	Imipenem	IPM	10	36.36	54.54	9.09

17	Levofloxacin	LE	5	90.90	9.09	0
18	Meropenem	MRP	10	81.81	9.09	9.09
19	Nalidixic acid	NA	30	63.63	27.27	9.09
20	Nitrofurantoin	NIT	300	0	0	100
21	Piperacillin/Tazobactum	PIT	100/10	63.63	27.27	9.09
22	Polymyxin B	PB	300 units	100	0	0
23	Streptomycin	S	10	9.09	27.27	63.63
24	Tetracycline	TE	30	54.54	18.18	27.27
25	Tigecycline	TGC	15	72.72	0	27.27
26	Tobramycin	TOB	10	90.90	0	9.09

Table Summary: It describes the percentage sensitivity and resistance in water isolates. Ampicillin, Cefotaxime, Clindamycin and Nitrofurantoin are depicted as 100% resistant whereas Chloramphenicol, Co-Trimoxazole and Levofloxacin showed no resistance.

Table 5: Resistance Profile of drugs in different ecosystems- One Health

S.No.	Antibiotic	Symbol	Concentration (mcg)	Human isolates	Animals isolates (Cattle & Buffaloes)	Water isolates
1	Amoxyclav	AMC	30	90	36.36	72.72
2	Ampicillin	AMP	10	90	100	100
3	Azithromycin	AZM	15	35	0	9.09
4	Aztreonam	AT	30	90	63.63	81.81
5	Cefotaxime	CTX	30	100	100	100
6	Cefoxitin	CX	30	50	18.18	18.18
7	Cefpodoxime	CPD	10	100	54.54	81.81
8	Ceftazidime	CAZ	30	95	36.36	45.45
9	Ceftriaxone	CTR	30	85	9.09	18.18
10	Chloramphenicol	C	30	80	0	0
11	Ciprofloxacin	CIP	10	90	9.09	9.09
12	Clindamycin	CD	2	100	100	100
13	Colistin	CL	10	0	0	0
14	Co-Trimoxazole	COT	25	70	18.18	0
15	Gentamicin	GEN	10	50	54.54	9.09
16	Imipenem	IPM	10	85	9.09	9.09
17	Levofloxacin	LE	5	90	0	0
18	Meropenem	MRP	10	10	0	9.09
19	Nalidixic acid	NA	30	100	18.18	9.09
20	Nitrofurantoin	NIT	300	75	0	100
21	Piperacillin/Tazobactum	PIT	100/10	45	0	9.09
22	Polymyxin B	PB	300 units	0	0	0
23	Streptomycin	S	10	70	72.72	63.63
24	Tetracycline	TE	30	55	54.54	27.27
25	Tigecycline	TGC	15	0	0	27.27
26	Tobramycin	TOB	10	12.5	0	9.09

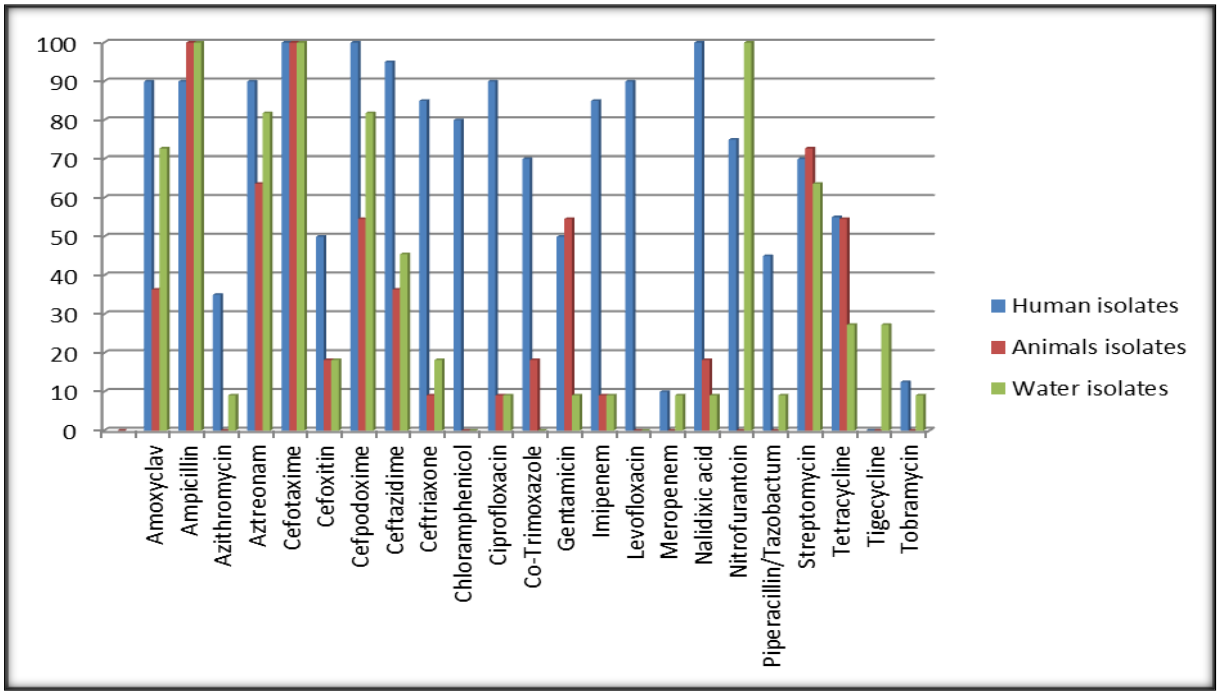

Figure 12: Resistance Profile of drugs in different ecosystems- One Health

On examining resistance pattern in E.coli isolates from different ecosystems, all the isolates showed resistance for drugs ranging from minimum 5 to maximum 19 out of at least 25 antibiotics tested. The resistance towards Amoxyclav a combination of Amoxycillin and Clavulanic acid was observed highest in human with 90% followed by the isolates from the Yamuna with 72.72% and least was recorded in animals with 36.36%. The possible reason is the excessive exposure of human to the Amoxyclav due to its indiscriminate use. Ampicillin is noticed to be 100% resistant in animals and environmental isolates followed by human with 90% resistance but it showed that bacteria have become resistant to Ampicilin when used alone. Azithromycin showed good results and was 0% resistant in animals and 0.09% in the environment. Aztreonam showed high resistance and cefotaxime is 100% resistant in all the isolates obtained from the three ecosystems. Cefoxitin showed 50% resistance in human and 18.18% in animal and water isolates. Human isolates showed absolute resistance for Cefpodoxime followed by water with 81.81% and animal isolates showed the least resistance among all with 54.54%. Resistance for Ceftazidime was highest in human with 95% followed by water isolates as 45.45% and least was in animals with 36.36%. Human isolates showed the highest resistance for Ceftriaxone with 85% followed by isolates from water as 18.18% followed by animal isolates 9.09%. Human isolates showed 80% resistance for Chloramphenicol where animal and water isolates showed zero per cent resistance similarly human isolates exhibited high resistance for Ciprofloxacin as 90% whereas showed 9.09% in animals and water isolates. Clindamycin was resistant as it is not effective against gram-negative bacteria and there were no mutant isolates that presented any sensitivity for the drug. Colistin appeared sensitive for most of the isolates in all the ecosystems as per the zone of inhibition prescribed by for ATCC25922 as there were no criteria described for Enterobacteriaceae in as per CLSI.[11] Human isolates showed 70% resistance for Co-trimoxazole whereas it was 18.18% in animals and zero in water samples. Gentamicin showed 50% in human 54.54% in animals and 9.09% in water isolates. Human isolates showed the highest resistance towards Imipenem with 90% whereas animals and water isolates showed little resistance with 9.0%. The extensive use of the drug in human patient seems the main reasons for the rise in resistance in human isolates. Levofloxacin too was not effective in human isolates and exhibited 90% resistance whereas no resistance for Levofloxacin was observed in animal and human isolates. Meropenem was an effective drug with little resistance of 10% in human isolates followed by water isolates with 9.09% while no resistance was observed in animals isolate the possible reason may be differential use and exposure of different ecosystems with the drug. The highest resistance for Nitrofurantoin was observed in water samples with 100% resistance followed by human isolates exhibiting 75% while there was no resistance observed in the animal isolate. The combination of Piperacillin and Tazobactum appeared effective with 45% resistance in human isolates, 9.0% in water isolates and no resistance in the animal isolate. Animal isolates showed the highest resistance of 72.72% for streptomycin that was similar to human isolates with 70% resistance followed by water isolates with 63.63%. Tetracycline was most effective in water isolates with least resistance i.e.27.27% followed by animal isolates with 54.54% that was almost equal to the resistance exhibited by human isolates with 55%. Tigecycline being an advanced form of Tetracycline showed no resistance in human and animal isolates but it showed 27.27% of resistance in environmental isolates that is of concern as the human and animal isolates can receive the resistant gene from environmental isolates through horizontal gene transmission (HGT) and thus increasing the risk of AMR in human and animals many folds. Tobramycin being effective in animal isolate with no resistance but 9.09% resistance was observed in environmental isolates and 15% resistance in human.

DISCUSSION

This study comprised the isolation and identification of the E.coli from the different ecosystems that are presumed to be integrated and may spread the AMR genes in the pathogens among them through horizontal gene transmission. There are very fewer studies that are conducted with One Health approach to study the multidrug-resistant (MDR) bacteria that may spread AMR in interconnected ecosystems, though the studies are conducted to know the bacteria present in the river Yamuna no organised study was conducted to study its role in the dissemination of MDR bacteria to the other ecosystems. In this study, the isolates were obtained from three ecosystems of Delhi region and were subjected to AMR studies. All the isolates obtained from different ecosystems were found to be pathogenic and MDR E.coli. Animal and environmental isolates were found positive for eae (480 bp) which is indicative of Enteropathogenic E.coli (EPEC) that is considered to be a virulent subgroup of E.coli possessing genetic factors which are absent from commensals and are responsible for disease production.[12] EPEC produce a cytolytic toxin and it is known to cause of diarrhoea in both children and animals though a study also stated that EPEC appears to be exclusively human pathogens responsible for infant diarrhoea especially in developing countries.[13,14] The selected isolates of E.coli were subjected to AMR studies to analyze the

pattern and prevalence of AMR in different ecosystems. Resistance pattern in human isolates reveals that Cefotaxime, Cefpodoxime, Clindamycin, Nalidixic acid as 100% resistant whereas Cefotaxime/Clavulenic acid, Ceftazidime/ Clavulenic acid and Tigecycline appeared absolutely sensitive. Resistance pattern in animal isolates depicted Ampicillin, Cefotaxime as 100% resistant whereas Azithromycin, Chloramphenicol, Levofloxacin, Meropenem, Nitrofurantoin, Piperacillin/Tazobactum, Tigecycline and Tobramycin exhibited no resistance. Resistance pattern of isolates obtained from the river Yamuna reveals 100% resistance for Ampicillin, Cefotaxime, Clindamycin and Nitrofurantoin. The resistance pattern of isolates obtained from the river Cauveri has also been observed to monitor the faecal contamination where 48 antibiotics were used and the isolates were resistant to most of them revealing 93.51% multidrug-resistant isolates.[15] The comparative study of AMR in all ecosystems with One Health approach revealed that least resistance was observed for Meropenem followed by Colistin in all the isolates. The human clinical isolates showed high resistance for most of the common antibiotics compare to the isolates obtained from other two ecosystems, the possible reason for higher resistance in human isolates is the indiscriminate use of the antibiotics. The presence of multidrug-resistant E.coli in the water of river Yamuna pose the risk of dissemination of resistance genes via horizontal transmission to different ecosystems of different states and geographical locations.

CONCLUSION

According to this study, all the three ecosystems of Delhi region comprising the water of the river Yamuna, the livestock in the near vicinity and the human establishment in the surrounding areas were found to harbour pathogenic and multidrug-resistant E.coli, the resistance can further disseminate through horizontal transmission raising the problem of AMR to the higher levels, so there is an urgent need of formulation of effective strategies under one health approach to contain the spread of AMR in different ecosystems.

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