

Original Research Article

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Isolation of Multidrug Resistant *Salmonella* spp. from the River Yamuna in Delhi Region of India

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ABSTRACT

The present study was conducted to explore the prevalence of multidrug resistant *Salmonella* spp. in the water of river Yamuna in Delhi region of India. The river Yamuna which is a major tributary of river Ganges flows through the three states of northern India comprising Delhi, Haryana and Uttar Pradesh. The samples of water for the isolation of *Salmonella* spp. were randomly collected from different locations of the river Yamuna flowing through the three states of Delhi NCR and processed as per standard guidelines of fssai, CDC and WHO for the isolation of the *Salmonellae*. The isolates were further subjected to molecular identification and serotyping. All the isolates obtained were positive for the *invA* gene that is highly conserved in *Salmonella* spp. and the major serovar found among the serotyped isolates was *Salmonella* Typhimurium. The isolates were subjected to AMR studies and all the isolates were found multidrug resistant for at least five drugs with multiple antibiotic resistance index (MARI) above 0.2 indicating their origin from high source of contamination. The result of this study revealed the presence of MDR *Salmonella* spp. in the water of river Yamuna in Delhi region which is a serious public health concern and emphasizes the need of containment of spread of MDR *Salmonella* spp. to the susceptible human and animals population that come in contact with the river water.

Keywords

AMR, *Salmonella*,
MDR, Yamuna

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Introduction

The continuous rise in antimicrobial resistance (AMR) is a great threat to public health and is expected to lead to drastic increase in mortalities by the year 2050 (Coates & Hu, 2018). The involvement of different ecosystems comprising human, animals and the environment further worsen the situation by harbouring pathogenic, non-pathogenic and commensal bacteria carrying

the resistance genes (González-Zorn & Escudero, 2012). There are 2,610 *Salmonella* serotypes, (Achtman *et al.*, 2012) of which the *Salmonella* Typhimurium is the most common serovar that cause human infection and the contamination of the environment (Wales & Davies, 2013). Salmonellosis is the most common foodborne illness which is self-limiting disease in human but may also lead to life threatening systemic infections. The emergence of the AMR in *Salmonella* strains

of human and animals is a major public health concern throughout the world (Vo, Van Duijkeren, Gaastra, & Fluit, 2010). Surveillance of the AMR is important for providing information on the extent and trends in AMR (WHO, 2001). It is reported that human and animals excrete *Salmonellae* in the environment during the illness and the convalescent phase and the *Salmonellae* have the potential to survive in water and soil for several years under favourable environment (Todar, 2016). Salmonellosis is generally transmitted through oro-faecal route and faecal contamination of water (Todar, 2016). The infections are also associated with factors like consumption of contaminated food of animals origin and water (Dechet *et al.*, 2006). In order to control and prevent the spread of AMR in *Salmonellae* it is of paramount importance to acquire the accurate information about the current status of circulating bacteria and its prevalence and resistance pattern in the ecosystem and since river water can transmit the infections caused by *Salmonella* spp. (Angulo *et al.*, 1997) so, the present study is conducted to explore the prevalence of multidrug resistant (MDR) *Salmonella* spp. in water (Ahhammad, Sreekrishnan, Hands, Knapp, & Graham, 2014) of the river Yamuna flowing through Delhi region, in Northern India that serve the need of a large of population and may spread the infections caused by *Salmonella* spp. in human and animal population coming in contact with the river water. The river Yamuna flows 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 river is polluted by several sources and harbour many multidrug resistant *Salmonella*, (Azam, Jan, & Haq, 2016) which bear the potential to transfer the resistance genes, (Walsh, C., 2003) to different bacteria via horizontal gene transmission, (Lavigne & Blanc-Potard, 2008) so the study of MDR

Salmonella and its AMR pattern becomes more important to contain its spread.

Materials and Methods

Isolation

A total of 168 samples were collected in sterile containers of 250 mL from 14 different locations comprising barrages, banks and bridges of river Yamuna located in Delhi, Haryana and Uttar Pradesh (India). Water samples were collected from six different locations of Delhi. Five to seven samples were from different spots of each location. 250 mL of water was collected in sterile containers from 1-2 meters of water from river bank. Samples were collected both from surface water and under the surface water. The samples were transported to laboratory in cold chain and maintained at 4 °C till further processing. *Salmonella* organisms were isolated from water as per the guidelines of *fssai* Manual of Methods of Analysis of Food-Water, 2016 (fssai, 2016). To check the stool and faecal contamination of water the samples were processed for both typhoidal and non-typhoidal *Salmonella* as per the methods described in Standard ISO 6579: (2002) and also in accordance with the new revised Guidelines of ISO 6579, 1:2017, (Mooijman, 2018) and CDC/WHO manual (Perilla *et al.*, 2003). *Salmonella* spp. organisms were identified using the methods described by Ewing (Ewing, 1986) and McCartney ("Mackie and McCartney Practical Medical Microbiology, 14th Edition," 1996).

Pre-enrichment of the samples was done in Buffered Peptone Water (Hi-media, Mumbai, India) whereas multiple selective enrichment media were used for isolation of *Salmonella* spp. from water and faecal samples. In this study at least 2 selective enrichment media were used for each sample in order to ensure the recovery of *Salmonellae*. Enrichment

media, (Mooijman, 2018) used were Tetrathionate broth (Hi-media, Mumbai, India) for isolation of non-typhoidal *Salmonella* (NTS) and Selenite F broth (Hi-media, Mumbai, India) for Typhoidal *Salmonella*. Three selective media Hoektoen Enteric Agar (Hi-media, Mumbai, India) and Xyline deoxycholate Agar medium (Hi-media, Mumbai, India) and Xylose lysine Tergitol-4 (Difco, USA) were used for isolation of *Salmonella* spp. Mac Conkey's Lactose Agar (Hi-media, Mumbai, India) was used as differential media, Nutrient Agar (Hi-media, Mumbai) and Trypticase Soy Agar (Difco, USA), Trypticase Soy broth (Difco, USA), were used for culture revival and storage.

Characterization of *Salmonella* spp.

Cultural: *Salmonella* spp. were isolated from different sources was subjected to cultural characterization. The cultures were inoculated on different selective and differential media, (Blood & Curtis, 1995) as per the methods described by Ewing and McCartney (Ewing, 1986; "Mackie and McCartney Practical Medical Microbiology, 14th Edition," 1996).

Morphological: Isolates were subjected to the Gram's staining for their morphological characterization as per the methods described by Ewing and McCartney (Ewing, 1986; "Mackie and McCartney Practical Medical Microbiology, 14th Edition," 1996).

Biochemical: The test culture was streaked on the surface of the TSI agar slant (Maness *et al.*, 1999) and also stabbed up to the butt of the agar medium. Inoculated TSI agar slant was incubated at 37 °C for 24 hours as per the methods described by Ewing and McCartney (Ewing, 1986; "Mackie and McCartney Practical Medical Microbiology, 14th Edition," 1996). The culture medium showing alkaline reaction (red) in the slant an acidic

reaction, (yellow) in the butt and with blackening of the medium along the stab line due to hydrogen sulphide gas production in TSI agar medium was interpreted as positive test. The test culture was streaked over the surface of the slope of the Christensen's urea medium and incubated at 37 °C for 24 hours. A positive test was indicated by the development of pink colour in the medium. In case of negative test, no change of colour was found in the medium.

The culture tube showing negative test after 24 hours of incubation was further incubated for 7 days to observe colour change in the medium. Isolates showing no development of pink colour in the medium even after 7 days of incubation were considered urease negative. The test culture of organism was also subjected to citrate test with change to blue colour and presence of growth in citrate slant as positive test while negative test was indicated by absence of growth and colour change.

Molecular identification

Molecular identification of *Salmonella* spp. isolates was done by PCR for *invA* gene (Chiu & Ou, 1996; Malorny, Hoorfar, Bunge, & Helmuth, 2003). The *invA* gene is highly conserved and used for the identification of the *Salmonella* spp., (Malorny, Huehn, Dieckmann, Krämer, & Helmuth, 2009). DNA was extracted by thermal lysis. The final concentration of DNA was adjusted to 50 ng/μL. PCR reaction mixture consisted of 12.5 μL of 2x PCR master mixtures (Thermo Scientific), 1 μL (10pmol/μL) of each primer (Eurofins, India), 2 μL of DNA template and nuclease-free water to make final volume up to 25 μL.

The cycling condition comprised an initial denaturation at 94 °C for 5 min, followed by 30 cycles each of denaturation at 94 °C for 1

min, primer annealing at 51°C for 1 min, elongation at 72°C for 1 min and finally a single extension step at 72°C for 7 min. The PCR products were resolved by agarose gel electrophoresis (1.5%) stained with 0.5% ethidium bromide and documented under gel documentation system (Bio-Rad).

Serotyping

The isolates of *Salmonella* spp. obtained from water samples were sent for serotyping to *Salmonella* Typing centre, Indian Veterinary Research Institute, Izatnagar, Bareilly, India.

Antimicrobial susceptibility testing

Disk diffusion tests were performed as per Manual of antimicrobial susceptibility testing, (Huehn *et al.*, 2010) and Clinical Laboratory and Standard Institute, (CLSI, 2016) using disks (Himedia Pvt. Ltd., Mumbai, India) impregnated with Ampicillin (AMP; 10), Amoxycylav (AMC;30), Azithromycin (AZM; 15), Aztreonam (AT; 30),Cefotaxime (CTX; 30),Cefotaxime/ Clavulanic acid (CAC;30/10), Cefoxitin (CX;30), Cefpodoxime (CPD;10), Ceftazidime

(CAZ;30),Ceftazidime/ Clavulanic 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/ Tazobactam (PIT;100/10), Polymyxin B (PB;300units), Streptomycin (S;10),Tetracycline (TE;30), Tigecycline (TGC;15), Tobramycin (TOB;10). European Committee on Antimicrobial Susceptibility Testing, (European Committee on Antimicrobial Susceptibility Testing, 2016) guidelines were used where CLSI guidelines were insufficient. (Arthur L. Barry, William A. Craig, Harriette Nadler, .: Barth reller, Christie C. Sanders, 2016) *Escherichia coli* (ATCC 25922), *Salmonella enterica subsp. enterica* serovar Enteritidis (ATCC13076), *Salmonella enterica subsp. enterica* serovar Typhimurium (ATCC 51812) were tested as controls under the same conditions as was suggested by CLSI (Arthur L. Barry, William A. Craig, Harriette Nadler, .: Barth reller, Christie C. Sanders, 2016).

Oligonucleotide Primer Sequences			
F:GTGAAATTATCGCCACGTTCCGGGCAA	<i>invA</i>	284bp	Rahn <i>et al.</i> , 1992 (Rahn <i>et al.</i> , 1992)
R: TCATCGCACCGTCAAAGGAACC	gene		

Results and Discussion

Isolation: A total of 11 isolates were obtained out of 168 samples (Table 1). The prevalence of *Salmonella* isolates obtained from the 14 different locations of water collection from the river Yamuna flowing through the three states comprising Delhi, Haryana and Uttar Pradesh was 6.55%. All the isolates were obtained from Yamuna Bank region of Delhi except one isolate was obtained from ITO Bridge of Delhi.

Cultural characterization

Salmonella spp. isolates showed different cultural characteristics on different media. Colonies of *Salmonella* spp. appeared as moderately large, moist, smooth and colourless with pink background on brilliant green agar (BGA), blue green colour colony with black center on Hektoen enteric agar and red colony with a black center on xylose lysine deoxycholate agar (XLD)/XLT4 Agar and colour less colonies on Mac Conkeys lactose agar. *Salmonella* on MLA that

appeared transparent and pale due to no acid production whereas the *E. coli* colonies on MLA appeared pinkish as the negative control *E. coli* (ATCC25922) being a lactose fermenter, it fermented the lactose present in the MLA and produced the acid that lowered the pH of the media due to which the indicator neutral red present in the media gave pink colouration. The XLD media contained phenol red indicator that was pink in alkaline medium and yellow in acidic medium. The medium had sugar xylose that was well utilised by the *Salmonella* but not by *Shigella* and acid is produced, but on exhaustion of xylose *Salmonella* utilised the lysine present in the media due to which the pH again increased to alkaline and then *Salmonellae* metabolised thiosulfate to produce hydrogen sulphide due to which a black centre in the colonies of *Salmonella* appeared on XLD media. The agar too turned red due to the presence of *Salmonella* type colonies whereas negative control *E. coli* produced yellow colonies with characteristics bright yellow media background due to acid production due metabolism of sugars present in the media by the negative control *E. coli*. The HEA media contained various sugar source viz. lactose, sucrose, and salicin none of which were used by the *Salmonella* but the medium also comprised peptone which was metabolised by *Salmonella* to produce the alkaline environment in the media turning the Bromothymol blue (pH indicator) from green to blue so *Salmonella* on HEA agar produced blue green colonies with black centre due to hydrogen sulphide gas with blue background of media whereas negative control *E. coli* produced pale yellow colonies and pinkish or yellowish background of media that was produced due to the production of acid on metabolism of various sugars present in the media by the *E. coli*. The XLT4 media had anionic surfactant Niaproof earlier known as Tergitol-4 and was highly selective for *Salmonella*, rest the mechanism of isolation

was similar to that of XLD with phenol as indicator. *Salmonella* produced pinkish colonies with black centre on XLT4 media with the pinkish background of media whereas negative control *E. coli* colonies appeared pale with a bright yellow background of media due to acid production.

Biochemical characterization of *Salmonella* spp.

All the *Salmonella* spp. isolates were subjected to different biochemical tests comprising Oxidase, Catalase, Indole, MR, VP, Citrate, TSI and Urease and all the isolates exhibited Indole negative, MR positive, VP negative, Citrate positive, TSI (K/A) with H₂S production and urease negative results.

Molecular characterization of *Salmonella* spp.

PCR assay for *invA*, followed by agarose gel electrophoresis revealed specific amplification of a 284 bp nucleotide segments indicating presence of *invA* gene (Fig.1).

Serological characterization of *Salmonella* spp.

The water of river Yamuna though showed highest prevalence of *Salmonella* spp. in the region of Yamuna Bank, Delhi. Selected isolates were subjected to serotyping to identify their antigenic characteristic. The results revealed that most of the samples belonged to Serovar *Salmonella* Typhimurium with antigenic structure as 4,5,12:i:1,2, one isolate S2 belonged to Serotype Group C2 with antigen 6,8 and one of the isolate S14 was untypable. *S. Typhi* was not isolated from any location. Isolates exhibited high MARI and thus indicate that they originated from the source of high risk of contamination.

Table.1 Details of source of *Salmonella* isolates

S.No.	Isolate I.D.	Location
1.	S2	Yamuna bank, Delhi
2.	S3	Yamuna bank, Delhi
3.	S6	Yamuna bank, Delhi
4.	S8	Yamuna bank, Delhi
5.	S9	Yamuna bank, Delhi
6.	S11	Yamuna bank, Delhi
7.	S12	Yamuna bank, Delhi
8.	S13	ITO Bridge, Delhi
9.	S14	Yamuna bank, Delhi
10	S15	Yamuna bank, Delhi
11	S16	Yamuna bank, Delhi

Table.2 *Salmonella* spp. Resistance Pattern

S.No.	Isolate No.	Resistance Pattern	Resistant Drugs
1)	S2	CTX-CD-NIT-PIT-S	5
2)	S3	AMC-AMP-CTX-CAZ-CD-NIT-S	7
3)	S6	AMC-AMP-CTX-CD-GEN-NIT-S	7
4)	S8	AMC-AMP-CTX-CD-GEN-NIT-S	7
5)	S9	AMC-AMP-CTX-CIP-CD-GEN-NIT-S	8
6)	S11	AMC-AMP-CTX-CD-GEN-NIT-S	7
7)	S12	AMP-AZM-CTX-CD-GEN-NA-NIT-S	8
8)	S13	AMC-AMP-CTX-CTR-CD-NA-NIT	7
9)	S14	AMP-CTX-CAZ-CD-NIT-S	6
10	S15	AMC-AMP-AZM-CTX-CAZ-CD-NA-NIT-S-TGC	10
11	S16	AMP-AZM-CTX-CD-NA-NIT	6

Note:

AMC= Amoxicillin+Clavulanic acid, AMP= Ampicillin, AZM= Azithromycin, AT= Aztreonam, CTX= Cefotaxime, CEC= Cefotaxime/ Clavulanic acid, CX= Cefoxitin, CPD= Cefpodoxime, CAZ= Ceftazidime, CAC= Ceftazidime/ Clavulanic acid, CTR= Ceftriaxone, C= Chloramphenicol, CIP = Ciprofloxacin, CD= Clindamycin, CL= Colistin, COT= Co-Trimoxazole, GEN= Gentamicin, IPM= Imipenem, LE= Levofloxacin, MRP= Meropenam, NA= Nalidixic acid, NIT=Nitrofurantoin, PIT= Piperacillin/Tazobactam, S=Streptomycin, TE= Tetracycline, TGC= Tigecycline, TOB= Tobramycin

Table.3 Multiple Antibiotic Resistance Index of *Salmonella* spp.

S.No.	Source	Resistance Pattern	No. of Isolate	MARI	Average MARI
1.	Yamuna water	CTX-CD-NIT-PIT-S	1	0.208	0.297
		AMC-AMP-CTX-CAZ-CD-NIT-S	1	0.292	
		AMC-AMP-CTX-CD-GEN-NIT-S	3	0.292	
		AMC-AZM-CTX-CD-GEN-NA-NIT-S	1	0.334	
		AMC-AMP-CTX-CIP-CD-GEN-NIT-S	1	0.334	
		AMC-AMP-CTX-CTR-CD-NA-NIT	1	0.292	
		AMP-CTX-CAZ-CD-NIT-S	1	0.25	
		AMC-AMP-AZM-CTX-CAZ-CD-NA-NIT-S-TGC	1	0.417	
		AMP-AZM-CTX-CD-NA-NIT	1	0.25	

Table.4 Resistance profile of isolates of *Salmonella* spp.

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



Fig.1 *Salmonella* on MLA media



Fig.2 *E. coli* ATCC 25922 on MLA media

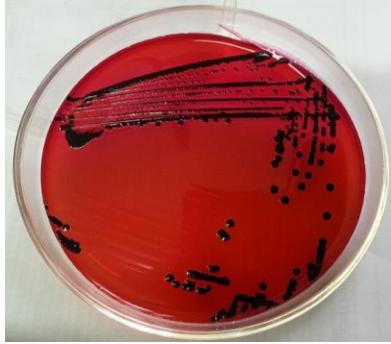


Fig.3 *Salmonella* Typhimurium on XLT4 plate



Fig.4 *E.coli* ATCC 25922 on XLT4 media



Fig.5 *Salmonella* on HEA media



Fig.6 *E. coli* ATCC 25922 on HEA media

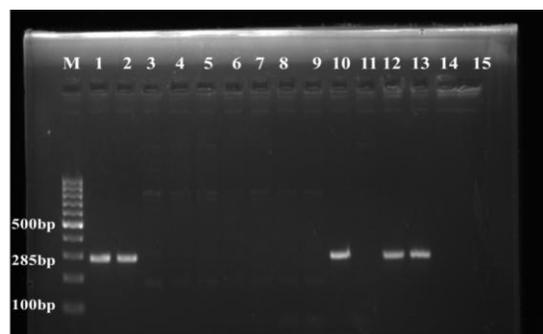


Fig.7 *Salmonella* on XLD media

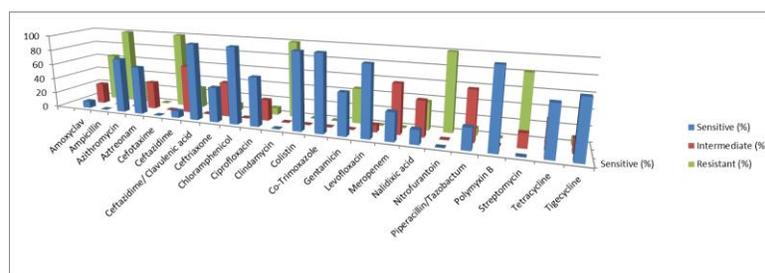


Fig.8 *E.coli* on XLD media

Fig.9 PCR for *invA* gene (284 bp) of *Salmonella* spp.



Lane 1,2,10,12,13:Positive for *invA* gene
Lane 3-9, 11, 14:Negative for *invA* gene (284bp)
Lane 15: Negative control
M: 100 bp DNA ladder

Fig.10 Resistance profile of isolates of *Salmonella* spp.

The present study was conducted to explore the existence of MDR *Salmonella* in the water of the river Yamuna that flows through the heart of city Delhi and surrounding regions and serve a large population of the human and animals that thrive on it and may acquire the deadly infections due to MDR *Salmonella*. To control and prevent the spread of AMR in *Salmonella* spp. it is must to have the accurate information on the prevalence of the circulating bacteria. Before the present study the information regarding the prevalence of the *Salmonella* spp. in the river Yamuna in Delhi region appeared insufficient, so the study was undertaken to study the prevalence of MDR *Salmonella* spp. in the water of river Yamuna that reaches to a larger section of human and animal population in Delhi region. Further to mention that the patients infected with MDR *Salmonella* are at higher risks of developing bacteraemia and deaths than those who are infected with the susceptible strains (Varma *et al.*, 2005). In the present study the Yamuna being a major tributary of river Ganges revealed the presence of MDR *Salmonella* in similar way as it was found in a study conducted in different locations of river Ganges and 24 MDR isolates of *Salmonella* were obtained (Kalaiyarasu, Saxena, & Gupta, 2013). All the isolates obtained from Ganges were 100% resistant to Ampicillin like in our study and also showed lower resistance to the Ciprofloxacin similar to our results where isolates obtained from Yamuna were less resistant to Ciprofloxacin. These studies

indicate the prevalence of MDR *Salmonella* spp. in the major rivers of Northern India. The water of the river Yamuna is polluted by various sources that leads to the selective pressure and increase in AMR in circulating bacteria. The water harbours a wide variety of *Salmonella* spp. that is both pathogenic and non-pathogenic in nature and could have been originated from human, animal or environmental source. The isolates obtained from the river Yamuna were multidrug resistant with varied resistance. Though samples were collected randomly in three seasons comprising winters, summers and rains with minimum of 5 samples from each location at different time intervals and different spots but Yamuna bank samples were found positive for *Salmonella* spp. in all seasons of sample collection, the samples were positive for *Salmonella* spp. even during flood when water from Hathini Barrage of Haryana was released flooding the river. Hence it showed the constant prevalence of *Salmonella* spp. in Yamuna Bank area of Yamuna river of Delhi region. Though water of Okhla barrage was much polluted raising expectation of finding *Salmonella* spp. and other pathogenic bacteria but no specific genera were identified except unidentified coliforms. Out of *Salmonella* spp. isolated from Yamuna water maximum were isolated from Yamuna Bank area with exception of one that was obtained from ITO Bridge. No *Salmonella* spp. could be isolated from the water samples collected from other location including all the locations of Haryana and

Uttar Pradesh. Though the major serovars isolated from the river Yamuna were *S. Typhimurium* but on serotyping one belonged to Group C2 that is a non typhoidal *Salmonella* (NTS) which can cause septicaemia and focal infections like meningitis, endocarditis or osteomyelitis. This isolate of Group C2 may be *Salmonella Bovismorbificans* (Manning, Gole, & Chousalkar, 2015), *Salmonella* Newport (Frye & Fedorka-Cray, 2007) or any other serovar of group C. In human non-typhoidal serovars cause 93 million enteric infections and 155,000 diarrhoeal deaths each year. (Majowicz *et al.*, 2010) *Salmonella* Newport, (Kumar, Gupta, Vaish, & Gupta, 2016) cause enteritis in Cattle and has also been reported as a major cause of non-typhoidal foodborne infections resulting in outbreaks due to consumption of contaminated food items (Foley & Lynne, 2008). In this study *Salmonella* Typhimurium was found as a major pathogen in water of river Yamuna. *S. Typhimurium* is found in both human and animals though it can cause diseases in a large range of host species, (Tsolis *et al.*, 1999) as it exhibit significant variation in its host preference. *Salmonella* Typhimurium is invasive in nature but not host adapted and may cause dreadful systemic infections in a wide range of host especially more invasive infection in immunocompromised host. (P.A. & T.J., 1993) In present study Ampicillin, Cefotaxime, Clindamycin and Nitrofurantoin were found to be 100% resistant where as Aztreonam, Chloramphenicol, Chloramphenicol, COT, Levofloxacin Tetracycline in showed no resistance (Table 2). *Salmonella* is reported in many Indian studies as invariably sensitive to Chloramphenicol, (Shekhar & Singh, 2014) like the result obtained in present study. Streptomycin exhibited higher resistance 81.81% resistance and the combination of Amoxicillin and Clavulanic acid as 63.63%. Gentamicin as 45.45%, Nalidixic acid as

36.36% whereas Azithromycin and Ceftazidime both had 27.27% resistance and Ceftriaxone, Ciprofloxacin and Piperacillin and Tazobactam showed less resistance as 9.09%. In our study the isolate S2 of Group C2 showed resistance to five antibiotics comprising Cefotaxime, Nitrofurantoin, Streptomycin, Piperacillin and Tazobactam and was susceptible to rest of the antibiotics. The other isolate S14 was untypable considering it may be a new or uncommon or rare serovar. Though the phenomenon of emergence of new serovar is there in *Salmonella* spp. but the untypable/new serovar is needed to be studied in details regarding its virulence factor and potential to study its potential in contributing in emergence and spread of AMR. Though in our study this untypable serovar was found to be resistant for six antibiotics only out of 23 antibiotics tested and was susceptible for the rest of the antibiotics so its MARI (Table 4) is also not very high considering it not to have been originated from high risk of contamination. The other isolate S12 and S15 obtained from Yamuna water showed resistant for eight and ten antibiotics respectively which comprised Nalidixic acid but they showed susceptibility for Ciprofloxacin and Levofloxacin, still fluoroquinolones may not completely eradicate the infection caused by the isolate as it was resistant to NA. Most of the isolates were intermediate sensitive for Nalidixic acid (*S. et al.*, 2016) with few isolates as sensitive and rest as resistant with 36.36% resistance but Ciprofloxacin exhibited higher sensitivity like that of Levofloxacin thus revealing that second and fourth generation fluoroquinolones are still effective and resistance is not higher for them in *Salmonella* isolates obtained from river Yamuna whereas three Ciprofloxacin resistant strains were reported to have been isolated from river Ganga in North India (Kalaiyarasu *et al.*, 2013). The isolates of *Salmonella* spp.

tested with the combination of Amoxicillin and clavulanic acid, (Wong, Epstein, & Westropp, 2015) showed resistance with exception for three isolates showing intermediate sensitivity. The overall resistant percentage observed was 63.63%. Ampicillin was 100% resistant. Azithromycin, (Day *et al.*, 2017) showed more sensitivity than resistant that was recorded as 27.27% only. The isolates were all resistant for Cefotaxime with 100% resistance. (T., 2015) Isolates were either resistant of Ceftazidime with 27.27% resistance or showed intermediate sensitivity (63.63%) only one isolate exhibited sensitivity for Ceftazidime, whereas when ceftazidime was used in combination with clavulanic acid there was absolute sensitivity in all the isolates with no or zero percent resistance that showed that clavulanic acid inhibited the bacterial enzymes and allowed the ceftazidime to produce its effect. Ceftriaxone mainly produced intermediate sensitivity or few sensitive results with only one isolate as resistant and recorded 9.09% resistance. Chloramphenicol, (Ghatak *et al.*, 2013) was all sensitive with zero resistance. Clindamycin was as usual resistant as it is reported to have no effect on gram negative bacteria and it showed presence of no mutant that gave any sensitive reaction for Clindamycin. Colistin, (Poirel, Jayol, & Nordmanna, 2017) produced larger ZI in reference to the ZI described for ATCC 25922 as it is not defined for *Enterobacteriaceae* so Colistin was considered sensitive. Cotrimoxazole, (Nhung, Chansiripornchai, & Carrique-Mas, 2017) produced absolute sensitive results with zero percent resistance in all the isolates. Gentamicin, (Poonia, Singh, & Tsering, 2014) was sensitive as well as resistant in many isolate the overall resistance recorded was 45.45%. Meropenem was sensitive or intermediate sensitive with no resistance. But all the isolates were 100% resistant for Nitrofurantoin. The isolates were mostly intermediate sensitive for combination

of Piperacilin and Tazobactam (Quan *et al.*, 2017) with 9.09% resistance only. Polmyxin B produced larger ZI like in case of Colistin (Giamarellou & Poulakou, 2009). The streptomycin was all resistant with 81.81% resistance and exception of few being intermediate sensitive (Davis, 1987). Tetracycline was sensitive or intermediate sensitive with no resistant isolate (Winokur *et al.*, 2000). Tigecycline, (Quan *et al.*, 2017) also produced the similar results as Tetracycline with higher sensitivity. The MARI obtained from all the MDR isolates was found to be varying from 0.208 to 0.417 with average MARI as 0.297. The MARI above 0.2 is considered to have originated from source of high risk of contamination. The MARI in present study is very high compare to other studies reporting maximum MARI as 0.67 in *Salmonella* Typhimurium (Shekhar & Singh, 2014). The isolates were tested against 26 FDA approved drugs and on examining the drug resistance pattern it was noticed that all the isolates were MDR and were resistant to maximum of 10 drugs and minimum of 5 drugs (Table 3).

In conclusion the study revealed the presence of multidrug resistant *Salmonella* spp. in water of the river Yamuna in Delhi region though the prevalence was not high and most of the isolates were obtained from Yamuna Bank region in Delhi. *Salmonella* Typhimurium was the major serovar detected in serotyping that causes invasive and systemic infection in wide range of hosts. *Salmonella* isolates exhibited high level of resistance and MARI that raise the public health concern of dissemination of AMR genes to the other pathogenic and non-pathogenic bacteria in the river water via horizontal gene transmission thereby contributing in the increase of the AMR, so effective measure and guidelines needs to be formulated to contain the spread of AMR in *Salmonella* spp. in the water of river Yamuna

to further protect the vulnerable population of human and animals that coming in contact with river water.

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