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PREVALENCE OF MULTI- DRUG RESISTANCE AND HEALTH RISK POTENTIAL IN METAL TOLERANT ENTEROBACTER SPP. FROM POLLUTED RIVER WATER

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ABSTRACT

The aim of our study work is to determine the antibiotic resistance, resistance patterns and MAR indexing of the metal tolerant Enterobacter spp. isolated from the three different sampling sites of the Gomti river water of Lucknow city, India. Of the total 77 Isolates of metal tolerant Enterobacter spp. were found to be resistant to most of the 20 antibiotics tested by the disc diffusion method. All the isolates demonstrated 100% resistance against methicillin, penicillin G and ciprofloxacin in site-1, ampicillin, chloramphenicol, polymyxin B, methicillin, penicillin G and ofloxacin in site-2 and amoxicillin, methicillin and cefpodoxime in site-3 of the river water. Isolates from the sampling sites 1, 2 and 3 showed a diverse multi-drug resistance patterns against 10-18, 14-19 and 8-14 antibiotics at a time respectively. Isolates based MAR indexing profiling of the metal tolerant *Enterobacter spp.* from all three sites were also found very high ranged 0.08-0.7 indicating the high risk of environmental contamination and safety of the public health. The findings indicated that the aquatic pollution may enhance the survival as well as dissemination of the multi drug resistant *Enterobactor spp.* specially and other coliforms and pathogenic bacteria generally in the river water for the risk of human and animal health. **Abbreviations**: Multiple Antibiotic Resistance; M.A.R; antimicrobial-resistant; AMR; IMViC tests; Indole, Methyl Red, Voges Proskauer and Citrate Utilization Tests.

Keywords: Gomti River Water, Antibiotic sensitivity, Heavy metal tolerant, Multiple antibiotic resistance.

INTRODUCTION

Coliform resistance to antimicrobial drugs, especially in river water has emerged as a global concern [1-2]. Antibiotics are intensively used in human, veterinary and agriculture and considered as the most important factor promoting the emergence, selection, and dissemination of resistant organisms [3-4]. Rivers are being polluted by indiscriminate disposal of sewerage, industrial wastes and plethora of human activities, which affect their physico-chemical and biological quality [5].

Overuse and sometimes misuse of antibiotics in human and veterinary medicine are major promoters for the development and spread of multi-resistant bacteria in aquatic environment [6-7].

The occurrence of antibiotic resistant bacteria in the aquatic environment has been demonstrated in many studies [8-9], as a consequence of uncontrolled discharges urban and animal wastewater [10]. Antibiotics may be present at levels that could not only alter the ecology of the environment but also give rise to antibiotic resistance [11].

Several studies have reported that antibiotic resistance is a global problem [12]. Coliforms are the major microbial indicators of monitoring water quality [13]. Liquid manure of animals and human excretions has led to dissemination of resistant enteric bacteria in the environment [14]. Because of this and the potential for antibiotic resistance, there is a new level of risk associated with these bacteria. Recent studies have also identified antibiotics themselves in surface waters [15], and the role

of these antibiotics in the development, transfer, and maintenance of resistance is largely unknown.

Presence of heavy metals in the aquatic environment is also a key factor of microbial resistance against the antimicrobials. There are evidences for possible links between heavy metal and antibiotic resistance in bacteria because these traits are generally associated with transmissible plasmids and the genes are frequently found on the same plasmid [16-17].

The aim of our study was to establish the microbiological safety of water sources and to provide updated data on multiple antibiotic resistance (MAR), which may help in identifying the high risk contamination sites in the aquatic environment. The Enterobacter is indicative of general hygienic quality of the water and potential risk of infectious diseases from water.

MATERIAL AND METHODS

Sampling

The study was carried out on the Gomti River water of Lucknow City. Water samples were collected from three different sampling sites in sterile 250 ml polypropylene bottles, according to STAS 3001-91 [18]. Samples were taken at 4°C until their arrival in laboratory. This study was undertaken to determine the incidence and antibiotic resistant patterns of Enterobacter strains isolated from water samples. 77 Enterobacter isolates were isolated and tested against 20 commonly used antimicrobial agents.

Isolation and identification of metal tolerant Enterobacter isolates

Isolation of metal tolerant Enterobacter isolates from water samples were done on metal (Cr, Cd, Co, Cu, Zn, Ni and Hg) amended EMB agar plates at 100 µg/ml concentration. Serial dilutions of the water samples were plated by spreading 0.1 ml on EMB medium for metal tolerant Enterobacter spp. Plates were incubated at 37 °C for 24 h. pink and mucoid colonies were identified as Enterobacter spp, and further characterization was done by indole, methyl red, Voges Proskauer and citrate utilization tests (IMViC tests).

Determination of antibiotic resistance

The antibiotic resistance was determined by a standard disc diffusion technique using Mueller-Hinton agar (Difco) according to the recommendations of National Committee for Clinical Laboratory Standards (NCCLS 1997). The antimicrobial drugs tested and their sensidisk concentrations were: Amoxicillin (AMX) 25 µg, Nalidixic acid (NA) 30 µg, Neomycin (NEO) 30 µg, Kanamycin (KAN) 30 µg, Ampicillin (AMP) 10 µg, Cefradine (CEF) 25 µg, Gentamycin (GEN) 30 µg, Nitrofurozone (NR) 100 µg, Chloramphenicol (CHMP) 30 µg, Polymixin B (PB) 300 µg, Methicillin (MET) 5 µg, Streptomycin (STREPTO) 25 µg, Penicillin (PEN) 10 µg, Cefpodoxime (CPD) 10 µg, Rifampicin (RIF) 2 µg, Ciprofloxacin (CIP) 5 µg, Erythromycin (ERYTHRO) 15 µg, Ofloxacin (OF) 2 µg, Sulphadiazine (SZ) 300 µg and Tetracycline (TET) 10 µg. Within 15 min of the application of the discs, the plates were inverted and incubated at 37°C. After 24 h of incubation, the plates were examined, and the diameters of the zones of complete inhibition to the nearest whole millimetre were measured. The zone diameter for individual antimicrobial agents was then translated into sensitive and resistant categories. These antimicrobial agents were chosen based on their importance in treating human or animal enterobacter infections and their use as feed additives to promote growth in animals in agriculture, zootechny and aquaculture [19].

Multiple antibiotic resistances (MAR) indexing

The MAR index profile based on isolate and sampling site was performed to evaluate the health risk of the environment. MAR index for test isolates was calculated according to the formula: No. of antibiotics to which all isolates were resistant/No. of antibiotics tested x No. of isolates as recommended by [20]. Sampling site based MAR index was calculated by the same formula modified by the total number of isolates from a sampling site as described [21].

RESULTS

Total 77 Enterobacter isolates were isolated from three different sites of the Gomti river water. All the isolates were characterized on the basis of antibiotic susceptibility test. In the case of growth parameters study of Enterobacter isolates from site-1 against antibiotics, a variation in zone of inhibition of growth was observed among the isolates tested which is depicted in Figure 1.

Enterobacter isolates from site-1 showed a variable resistance against different antibiotics tested. All the isolates showed (96%) resistance against amoxicillin followed by nitrofurazone (89%), chloramphenical (77%) and minimum resistance was observed in kanamycin (11.11%), neomycin (7.4%) and ciprofloxacin among (3.7%) isolates of Enterobacter isolates.

In site-2, A high level of resistance was observed among the isolates, all the isolates demonstrated (100%) resistance against ampicillin, chloramphenicol, Polymixin b, methicilin, penicillin G, and ofloxacin respectively, 96% isolates showed resistance against sulphadiazine, erythromycin, rifampicin, kanamycin and nalidixic acid. 92% showed resistance against cefpodoxime, neomycin and amoxicillin. Least Drug resistance was observed by 24% isolates against Cefradine.

In site-3, all isolates (100%) demonstrated resistance against ampicillin, polymixin B and penicillin G, 92% isolates showed resistance against amoxicillin,

nitrofurazone, methicilin and erythromycin, 84% showed resistance against nitrofurazone. A very least number of isolates (16% and 4%) showed resistance against nalidixic acid and ciprofloxacin respectively.

Single and multiple antibiotic resistance patterns in 27 coliform isolates from site-I were also recorded. All isolates showed 7 patterns of antibiotic resistance among the antibiotics tested. 7.4% isolates showed resistance to 10, 12 and 17 antibiotics in two combinations. 11.1% isolates showed resistance to 18 antibiotics at a time in three different combinations. 14% of the isolates exhibited resistance to 13 and 16 antibiotics at a time in four combinations. 29.6% of the isolates showed resistance to 14 antibiotics at a time in eight different combinations respectively (Table 1).

Antibiotic resistance patterns among the 25 coliform isolates from site-2 were also recorded. All the isolates showed 6 different resistance patterns among the antibiotics tested. 8% isolates showed resistance to 14 and 15 antibiotics at a time in two different combinations at a time, 12%, 20%, 24% and 28% isolates exhibited resistance to 19, 17, 16 and 18 antibiotics at a time in 3, 5, 6 and 7 different combinations respectively (Table 2). Antibiotic resistance pattern in 25 Enterobacter isolates from site-3 was also recorded. All the isolates showed 7 different patterns of antibiotic resistance against the antibiotics tested. 4% and 8% of the isolates showed resistance to 14 and 8 antibiotics at a time in one and two different combinations respectively, 12% of the isolates exhibited resistance to 9 and 13 antibiotics at a time in three different combinations respectively. 16% and 24% of Enterobacter isolates exhibited resistance to 11 and 10, 12 antibiotics at a time in four and six different combinations respectively (Table 3).

MAR indexing based on isolates was also calculated. A varied trend of MAR Index was observed among the isolates from the three different sampling sites. 7.4% isolates from site-1 showed a MAR 0.25 - 0.4 range against different number of antibiotics. MAR 0.16, 0.087 and 0.3 were recorded by 14.8, 29.6 and 11.1% isolates respectively. In the case of sampling site-2, 8% isolates demonstrated 0.35- 0.37 MAR range, while, MAR 0.31 was recorded by 12% isolates against 19 antibiotics. In site-3 16% and 24% isolates showed MAR range 0.11–0.13 and 0.08 - 0.1 against different number of antibiotics, respectively (Table 1, 2 and 3).

Figure 1 MIC range of Enterobacter isolates from Gomti river water site 1 [ ], site 2 ( ) [ ] site 3 ( ) against antibiotic The values represent the mean ±SD, ***p< 0.001, **p<0.01, *p<0.05 and, (no star) not significant.

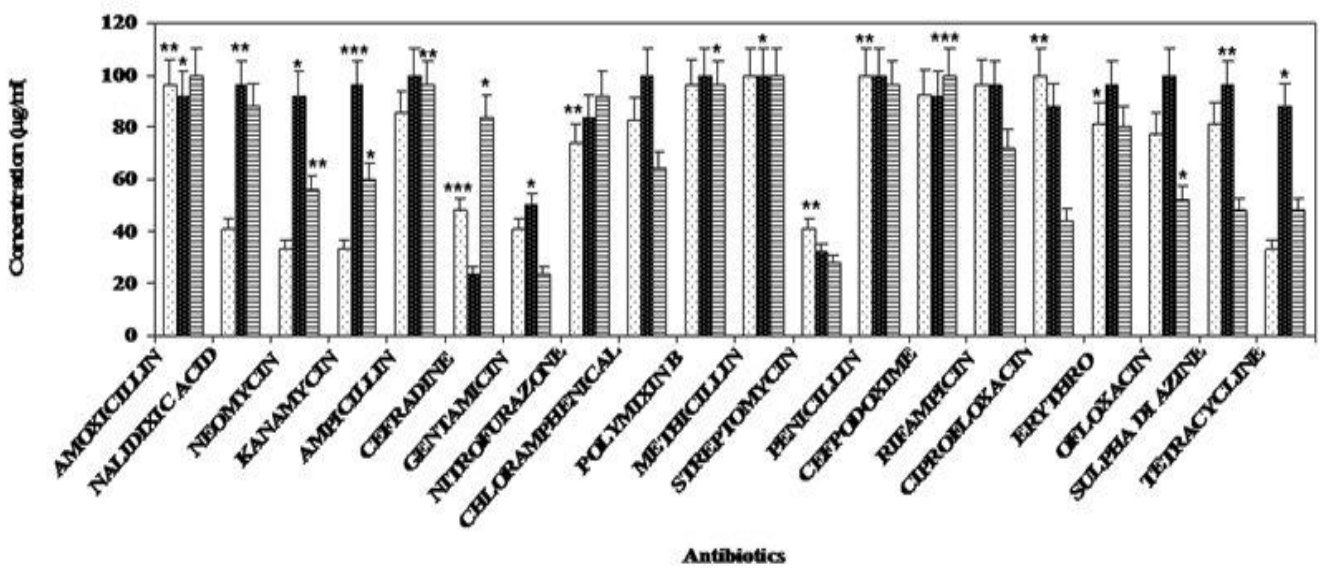

Table 1 Antibiotic Resistance Pattern in 27 Enterobacter Isolates From the Effluent of Gomti River (site-1)

No. of antibiotics	Resistance pattern	No. of resistance isolates	Percentage (%)	M.A.R.
10	NR,MET,STREPTO,PEN,CPD,RIF,CIP,AMX,PB,ERYTHRO. MET,STREPTO,PEN,CIP,CEF,CHMP,RIF,PB,OF,AMX.	1 1	7.4%	0.25
12	AMX,NA,KAN,AMP,CEF,GEN, CH,MET,PEN, RIF,CIP,0F. AMX,CEF,CPD,RIF,CIP,ERYTHRO,OF,CH, PB,NEO,PEN,MET.	1 1	7.4%	0.3
13	AMP,NR,PB,MET,STREPTO,PEN,CPD,RIF,CIP,ERYTHRO,CHMP, AMX,SZ. NR,MET,PEN,CPD,RIF,CIP,ER,PB,AMX,CHMP,AMP, OF, TET. NR,PB,MET,CPD,CIP,OF,SZ,RIF,CH,ERYTHRO, AMP, AMX, P. NR,PB,MET,PEN,CPD,RIF,CIP,SZ,CHMP,ERYTHRO, AMX,AMP, OF.	1 1 1 1	14.8%	0.16
14	PEN,CEF,AMP,CIP,CHMP,ERYTHRO,AMX,RIF,SZ,OF,TET, PB,MET,CPD. AMX,GEN,MET,STREPTO,PEN,CPD,RIF,CIP,AMP,CH,PB, NEO,OF. KAN,MET,PEN,CPD,ERYTHRO,OF,CIP,RIF,GEN,CHMP,PB, AMX,TET, AMP. MET,PEN,CPD,ERYTHRO,SZ,CEF,AMP,CHMP, RIF,CIP,AMX,NR,TET,PB. NR,MET,STREPTO,PEN,CPD,RIF,CIP,ERYTHRO,SZ,CH,PB,GEN,AMX,O F. NR,CH,MET,CPD,CIP,AMX,GEN,OF,CEF,PEN,PB,AMP, RIF,NEO. NA,NR,PB,MET,CPD,CIP,SZ,CEF,STREPTO,KAN,CHMP, PEN, AMX, RIF. CH,PB,MET,STREPTPO,PEN,CPD,RIF,CIP,SZ,GEN,OF,AMX, ERYTHRO,AMP.	1 1 1 1 1 1 1 1	29.6%	0.087
18	AMX,NA,KAN,AMP,CHMP,PB,PEN,MET,CPD,TET,CIP,ERYTHRO, GEN,NR ,RIF,OF,SZ,TET. NA,CEF,MET,PEN,CPD,RIF,CIP,SZ,KAN,GEN,CHMP,ERTH,AMP ,PB,AMX,NEO, OF,NR. AMX,NA,KAN,AMP,CEF,CHMP,MET,PEN,CPD,RIF,CIP, ERYTHRO,SZ,NR,GEN,PB,NEO,STREPTO.	1 1 1	11.1%	0.3
16	AMP,NR,CHMP,PB,MET,PEN,CPD,CIP,STREPTO,KAN,AMP ,TET,NA,OF,RIF,NEO. NA,KAN,AMP,PEN,CPD,RIF,CIP,OF,SZ,TET,CH,PB, MET,AMX,ERYTHRO,NEO. NA,AMP,PB,MET,STREPTO,PEN,CPD,RIF,CIP,SZ,CHMP,NR,GEN,NEO, ERYTHRO,OF. NR,CH,PB,MET,STREPTO,CPD,RIF,CIP,SZ,KAN,CEF,AMP,ERYTHRO, OF, AMX.	1 1 1 1	14.8%	0.08
17	NA,AMP,CEF,NR,CHMP,MET,STREPTO,PEN,CPD,RIF,CIP,ERYTHRO ,OF, SZ, STREPTO, AMX, PB. CH,MET,STREPTO,PEN,CPD,RIF,CIP,OF,NA,NR,ERYTHRO,SZ,AMX, AMP,PB, TET.	1 1	7.4%	0.4

Table 2 Antibiotic Resistance Pattern in 25 Enterobacter Isolates from the Effluent of Gomati River (site-2)

14.	SZ,CPD,MET,NR,NA,AMP,CHMP,OF,PEN,AMX,RIF,PB,KAN,TET. CHMP,NR,ERYTHRO,TET,OF,CPD,PB,CIP,NA,KAN,NEO,MET,AMP,STREPTO.	1 1	8	0.35
15.	OF,PEN,CHMP,NEO,NA,GEN,AMX,MET,PB,RIF,NR,CIP,KAN,CEF,SZ. OF,CHMP,NA,ERYTHRO,MET,PB,AMOX,AMP,RIF,TET,CIP,NEO,PEN,NR,KAN.	1 1	8	0.37
16.	AMX,SZ,CPD,MET,NA,ERYTHRO,TET,GEN,OF,PEN,NEO,PB,CHMP,CIP,KAN,RIF. RIF,AMX,PEN,SZ,CPD,MET,NR,NA,CIP,GEN,AMP,KAN,PB,CHMP,ERYTHRO,OF. AMX,PEN,SZ,MET,STREPTO,NA,RIF,CH,OF,CPD,NR,PB,NEO,KAN,TET,ERYTHRO. PEN,OF,SZ,CPD,NR,NA,ERYTHRO,MET,CHMP,AMX,PB,CIP,KAN,N,RIF,AMP. AMX,PEN,SZ,CPD,MET,AMP,NR,NA,ERYTHRO,CIP,RIF,OF,NEO,PB,CHMP,KAN. AMX,PEN,SZ,CPD,MET,NA,CIP,OF,GEN,AMP,CH,PB,KAN,NEO,TET,ERYTHRO.	1 1 1 1 1 1	2 4	0.13
17. .	NR,SZ,CPD,CHMP,NA,ERYTHRO,TET,MET,CIP,AMOX,OF,RIF,PB,NEO,PEN,KAN,AMP. AMX,PEN,SZ,MET,CHMP,NA,CPD,AMP,PB,OF,RIF,TET,NEO,ERYTHRO,GEN,CIP,KAN. AMX,SZ,CPD,MET,AMP,CHMP,NA,RIF,OF,TET,AMP,PEN,CEF,ERYTHRO,NEO,PB,GEN. AMX,PEN,SZ,MET,CHMP,NR,CIP,NA,KAN,RIF,OF,AMP,ERYTHRO,PB,CPD,NEO,TET. AMX,PEN,SZ,CPD,MET,AMP,CIP,RIF,STREPTO,OF,CHMP,PB,TET,ERYTHRO,NEO,KAN, GEN.	1 1 1 1 1	2 0	0.12
18.	OF,PEN,CPD,NR,NA,ERYTHRO,CHMP,RIF,PB,AMX,MET,CIP,NEO,SZ,AMP,KAN,TET,ST REPTO. OF,SZ,ERYTHRO,NEO,CEF,NR,RIF,AMOX,PEN,PB,MET,NA,CIP,CPD,AMP,KAN,CHMP,T ET. OF,PEN,SZ,MET,STREPTO,NR,CIP,NA,PB,CPD,ERYTHRO, OF,RIF,CHMP,KAN,TET,NEO,AMP. PEN,SZ,CPD,MET,AMP,CHMP,NR,CIP,NA,PB,OF,RIF,ERYTHRO,KAN,GEN,NEO,TET,CEF . PEN,CPD,MET,AMP,CH,NR,PB,RIF,KAN,STREPTO,AMOX,OF,CIP,ERYTHRO,TET,GEN,N EO,SZ. AMX,PEN,SZ,CPD,MET,CIP,NA,ERYTHRO,OF,PB,CHMP,AMP,RIF,KAN,TET,NEO,NR,GE N. AMX,PEN,SZ,CPD,MET,NA,ERYTHRO,NR,RIF,CH,OF,AMP,CIP,KAN,TET,NEO,PB,CEF.	1 1 1 1 1 1 1	2 8	0.12 8
19.	OF, AMX,PEN,SZ,CPD,MET,NR,NA,AMP,CHMP,ERYTHRO,CIP,PB,KAN,TET,RIF,NEO,GEN,N R. AMOX,PEN,CPD,MET,AMP,CHMP,NR,NA,ERYTHRO.RIF,STREPTO,CEF,SZ,KAN,CIP,PB, NEO,OF,TET. AMX,PEN,CEF,SZ,CPD,MET,AMP,CHMP,NA,TET,ERYTHRO,NEO,NR,CIP,RIF,PB,STREPT O,GEN,OFLOX.	1 1 1	1 2	0.31

Table 3 Antibiotic Resistance Pattern in 25 Enterobacter Isolates from the Effluent of Gomati River. (site-3).

No. of antibiotics	Resistance pattern	No of resistance isolates	Percentage (%)	M.A.R.
8.	AMP,CPD,AMX,GEN,CHMP,ERYTHRO,PB,PEN.	2	8	0.2
9.	MET, KAN, ERYTHRPO, AMP, PEN, AMX, STREPTO, PB, SZ NR, MET, SZ, ERYTHRO, AMP, PEN, CPD, AMX, PB. NR, MET, SZ, ERYTHRO, AMP, PEN, CPD, AMX, PB. MET,AMP,PEN,AMX,RIF,ERYTHRO,CPD,PB,CHMP.	4	16	0.11
10.	NR,MET,ERYTHRO,AMP,PEN,AMX,GEN,SZ,PB,CPD. MET,KAN,AMP,PEN,AMX,OF,GEN,PB,SZ,ERYTHRO. NR,MET,AMP,PEN,AMX,OF,GEN,ERYTHRO,SZ,PB. MET, PEN, AMX, OF, RIF, NR, AMP, ERYTHRO, PB, CPD. MET, PEN, PB, AMP, PEN, AMX, NR, NA, GEN, SZ. MET,AMP,PEN,ERYTHRO,STREPTO,PB,CPD,AMX,SZ,GEN.	6	24	0.08
11.	STREPTO,NR,MET,AMP,PEN,AMX,RIF,CPD,SZ,ERYHTRO,PB.	4	16	0.13

	NR,MET,ERYTHRO,AMP,PEN,CPD,AMX,PB,CEF,STREPTO,TET. MET,AMP,PEN,AMX,RIF,NR,ERYTHRO,SZ,PB,CPD,GEN. NR,SZ,KAN,AMP,PEN,RIF,PB,TET,CPD,NA,NEO.			
12.	NR,MET,SZ,CEF,ERYTHRO,PEN,CPD,AMX,OF,GEN,AMP,PB. NR,MET,CEF,PEN,CPD,AMX,OF,AMP,PB,ERYTHRO,SZ,KAN. STREPTO,NR,MET,PEN,CPD,AMX,AMP,ERYTHRO,RIF,PB,CH,NEO. NR,MET,SZ,ERYTHRO,AMP,PEN,TET,OF,NEO,PB,CEF,CPD. NR,MET,SZ,ERYTHRO,AMP,PEN,AMX,NR,TET,PB,CPD,GEN. NR,MET,AMP,PEN,CPD,NEO,RIF,ERYTHRO,AMOX,PB,CHMP,NA.	6	24	0.1
13.	NR,MET,CEF,ERYTHRO,PEN,AMX,OF,PB,AMP,SZ,RIF,GEN,KAN. NR,MET,SZ,CEF,ERYTHRO,AMP,PEN,CPD,TET,AMP,CIP,PB,GEN. MET,KAN,AMP,PEN,AMX,RIF,STREPTO,PB,NA,NR,GEN,CPD,OF.	3	12	0.21
14.	NR,MET,SZ,ERYTHRO,AMP,PEN,CPD,AMX,OF,TET,STREPTO,CH,PB,CEF.	1	4	0.7

DISCUSSION

Presence of antibiotic resistance Coliform bacteria in a given environment may be an indication that an area is contaminated with antibiotics [22]. Environmental antibiotic concentrations may exert selective pressure on environmental bacteria and may also foster the transfer of resistance genes, helping create the "resistome" mixing pot of genetic AMR traits [23].

Antibiotic resistance of fecal bacteria in surface waters has been studied by various researchers from different types of surface waters, rivers, estuaries, lakes and coastal waters. Several studies have used the antibiotic resistance pattern of fecal indicator bacteria to investigate the source of fecal pollution in the given marine environment [24]. Coliforms are normally present in human and animal intestines and are the most reliable indicator of fecal contamination in waters.

Many studies revealed that the co-selection took place in the various environmental bacteria with metal and antibiotic resistance [25]. Bacteria in metal-contaminated environments appeared to be easier to obtain antibiotic resistance phenotypes than in control areas [25] found that class 1 integrase gene was more abundant in the metal-exposed environments than in control, and the selective pressures shaped the structure of the gene cassette pool, indicating that relative gene transfer potential is higher in the microbial communities of the contaminated environments.

The rise in antibiotics resistance had been reported in the past two decade [26], and antibiotic resistance still remains a global problem today. High level of antibiotic resistance was observed in this study with twenty antibiotics. From the three sampling sites 77 isolates of Enterobacter spp. were isolated. All the isolates were tested for their resistance against particular as well as multiple antibiotic resistance. A varied trend of resistance among the isolates was recorded from the three different sampling sites (site-1, 2 and 3).

All isolates showed multiple resistance to antimicrobial agents tested. Of the 100% isolates from site- 1, site- 2 and site- 3 showed resistance against methicillin, penicillin, ciprofloxacin, ampicillin, chloramphenicol, polymixin B, penicillin G, ofloxacin, amoxicillin and cefpodoxime respectively. All the isolates from all sampling sites showed 7 resistance patterns for 20 antibiotics. In the case of site-1, 29.6% and 11.1% isolates showed resistance to 14 and 18 antibiotics at a time in 8 and 3 different combinations respectively while 28%, 24% and 20% isolates from site-2 showed resistance to 18, 16, and 17 antibiotics at a time in 7, 6 and 5 different combinations respectively. Of the 24%, 16% and 4% isolates from site-3 exhibited multiple resistance showed to 12, 11 and 14 antibiotics at a time in 6, 4 and 1 different combinations respectively. However, the high level of Enterobacter spp. resistance to tested antibiotic seems to correspond with the report of [27]. Most of the isolated strains of Enterobacter spp. high level of resistance more than other bacteria from the intestinal tract as reported by [28]. The coliform isolates showed high level of antibiotic resistance against all used antibiotics. The result was in agreement with [29] who reported that the abuse and misuse of antimicrobial agents for growth promotion and prevention of diseases has impressed a selective pressure that causes discovery of more resistant bacteria.

Kaspar and Burgess reported that there were larger multiple antibiotic resistance of coliforms isolated in urban areas than from rural areas (Kaspar and Burgess, 1990). Ramteke [27] studied on the antibiotic resistance of 448 coliforms isolated from drinking water and their tolerance to heavy metals [27]. More than 90% of metal tolerant isolates showed resistance to one or more antibiotics tested. Parveen *et al.*, [30] studied total 765 *Escherichia coli* isolates for their multiple-antibiotic resistance profiles with 10 antibiotics and stated antibiotics resistance pattern influenced by geographical condition [30].

MAR is considered as a good tool for risk assessment. This also gives an idea of the number of bacteria showing antibiotic resistance in the risk zone in the study's routine susceptibility testing. This MAR index also recommended that all isolates, somehow, originated from the environment where antibiotics were over used [31]. MAR index values higher than 0.2 were considered to have originated from high- risk sources where antibiotics are often used [32].

In our study we also determined the MAR index of Enterobacter isolates from all three sampling sites. Isolates showed a variation in their MAR index based on sampling sites. Low and high risk MAR were recorded among the Enterobacter spp. isolates from the water samples of the Gomti river. MAR range 0.08-0.4, 0.12-0.37 and 0.08-0.7 were recorded among the isolates from site-1 (polluted), Site-2 (polluted) and site-3 (less polluted receiving the treated water near the treatment plant) respectively. No significant difference among the isolates from polluted and less polluted sites was observed regarding their antibiotic [33].

All Enterobacter spp. isolated from river and polluted waters show a high incidence of multiple antibiotic resistance (MAR) phenotype. Many investigators have recognized that wastewater treatment plants are the principal recipients of enteric bacteria with multiple antibiotic resistance [34], and an important site for horizontal gene transfer, by containing nutrients and high concentrations of microorganisms [35]. MAR indexing is likely to provide a useful tool for better risk assessment by identifying contamination from high-risk environments. These investigations suggest that an unexpected increase in the MAR index of Enterobacter spp. isolates from food should prompt an immediate investigation even though the number of Enterobacter spp. organisms present is below the established guideline or standard. The disposal of treated sewage into rivers, lakes, or elsewhere may or may not influence environmental bacterial populations [35]. Some studies have found that wastewater treatment can raise or lower the proportions of antibiotic resistant bacteria which carry antibiotic resistance plasmids [36]. The observation of increased resistance frequency to ampicillin, tetracycline, streptomycin and chloramphenicol after wastewater treatment has previously been reported by Reinthaler *et al.* [37].

High MAR Enterobacter spp. are also the major reservoirs for enteric diseases which are transmitted to humans through food and water. It was also found that nitrofurazone-resistant Enterobacter organisms were frequently isolated from the poultry environment but seldom elsewhere. As mentioned earlier, nitrofurozone has very limited use but is allowed in animal feeds for the control of coccidiosis in poultry and bacterial enteritis (scours) in swine. Nitrofurozone may prove to be a useful marker, signaling fecal contamination from this source [38].

The aim of our study was to establish the microbiological safety of water sources and to provide updated data on resistance index, which may help in identifying the high risk contamination sites in the aquatic environment. The Enterobacter spp. is indicative of general hygienic quality of the water and potential risk of infectious diseases from water.

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