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Antimicrobial resistant coliform bacteria in the Gomti river water and determination of their tolerance level

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Abstract:
The distribution of resistance to ampicillin, chloramphenicol, sulfonamides, tetracycline, and streptomycin among coliform in the Gomti river water samples was investigated. The coliform populations were isolated on Mac Conky and eosin methylene blue (EMB) agar plates supplemented with antibiotics. The incidence of resistance among the coliform population varied considerably in different drug and water sampling sites. Coliform bacteria showed lower drug resistant viable count in sampling site-III (receiving treated wastewater) as compared to more polluted site-I and site-II. Viable count of coliform population obtained on both medium was recorded higher against erythromycin from sampling site-III. Lower viable count of coliforms was recorded against tetracycline in site-II and III. Similar resistance pattern was obtained in the frequency of *E.coli* and Enterobacter species from all the three sampling sites. Percentage of antibiotic resistant *E. coli* was observed higher than Enterobacter spp among the total coliforms against all antibiotics tested without Erythromycin and penicillin in site-I and II respectively. Isolates of E.*coli* and Enterobacter spp. showed their tolerance level (MIC) in the range of 2-100 against the antibiotics tested. Maximum number of isolates of both genus exhibited their MICs at lower concentration range 2-5µg/ml against ciprofloxacin, tetracyclin and amoxycillin.

Key Words: River Water, Viable Count, Antibiotics, Coliform Bacteria, Multidrug Resistance

Abbreviations: Eosin methylene blue, EMB; IMViC tests, Indole, Methyl Red, Voges Proskauer and Citrate Utilization Tests; Minimum inhibitory concentration, MIC.

Background:
The occurrence of antibiotic resistant bacteria in the aquatic environment has been demonstrated in many studies as a consequence of uncontrolled discharges urban and animal wastewater **[1-2].** Antibiotics may be present at levels that could not only alter the ecology of the environment but also give rise to antibiotic resistance **[3].** Several studies have reported that antibiotic resistance patterns are becoming a global problem **[4-5].** Antibiotic resistance genes commonly transfer via conjugation or transformation. Conjugative gene transfer mediated by plasmids with a broad host range is generally believed to be a common and widespread mechanism for the transfer of genes across a broad phylogenetic range of bacteria. Horizontal transfer of antibiotic resistance genes in the environment is one of the main reason contributing to the evolution and emergence of antibiotic resistant bacteria and has been demonstrated in many studies **[6].** As consequence of uncontrolled discharges of various waste products containing antibiotics and heavy metals, lakes and sewages are principle recipients of enteric bacteria encoding multiple antibiotic and

metal resistance genes **[7].** The significance of this finding is that, gram negative bacteria and related organisms harbor different plasmids which confer them multiple antibiotic resistances to many unrelated antibiotics and give the ability to survive in these hostile environments especially in the sewage, where many toxic compounds are routinely discharged from factories in India and do not have any proper disposal facilities. Since, the coliforms are dominant bacteria in the sewage and lake, horizontal transfer of antibiotic resistance genes through conjugative plasmids do often occur among them and therefore, make these important bacteria multiple resistant to several antibiotics (sometimes 10 antibiotics). The incidence of resistance among bacteria has been noted mainly among clinical isolates and little is known about the antibiotic resistance of bacteria that occur in the environment. Monitoring the density of coliform bacteria in surface water is critical in order to protect public health **[8].** Coliforms are Gram-negative, rod shaped bacteria that are members of the family Enterobacteriaceae. They are normal flora of the gastrointestinal tracts of all warm-blooded and some cold-blooded animals. *Escherichia coli,* a well-known resident of animal digestive tracts, is a coliform that can be shed in faeces that is used as an indicator of fecal contamination in water **[8].** Several workers have drawn attention to the incidence of antibiotic resistance among coliforms in treated and untreated drinking water **[9].** The aim of our study was to investigate the drug resistant coliforms contamination in the Gomti river water at Lucknow city to mitigate the public health risks.

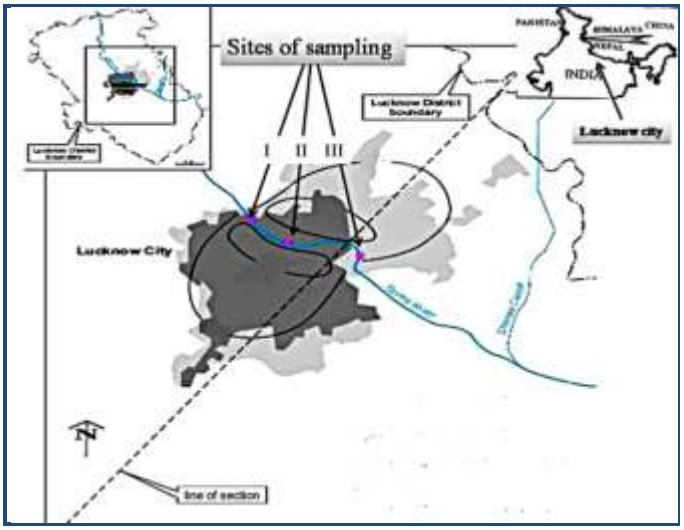

Figure 1: Map showing sampling sites I, II and III**.**

Methodology:

Sample collection

Water samples were collected from three sites of Gomati River Water along with Nadwa Bridge (I), Gomati Nagar Bridge (II), and Nishatganj Bridge (III) at Lucknow city as shown in **Figure 1.** Samples were collected in sterile 250-ml polypropylene bottles, according to internationally recommended methodology **[10].** Samples were kept at 4°C until their arrival to laboratory.

Isolation and identification of coliform bacteria

Isolation of antibiotic resistant coliform bacteria from water samples were done on antibiotic amended Mac conky and EMB agar plates at varying concentration (5-100µg/ml). Serial dilutions of the water samples were plated by spreading 0.1 ml on both medium for total antibiotic resistant coliforms. Plates incubated at 37°C for 24 hours and coliform counts were expressed as CFU/ml. On Mac conky agar medium, pink colored colonies were identified as coliform bacteria. On EMB agar, only two types of bacterial colonies were observed greenish with metallic sheen and pink mucoid which were identified as E.*coli* and Enterobacter spp. respectively and further identified on the basis of IMViC tests (Indole, Methyl Red, Voges Proskauer and Citrate Utilization Tests).

Determination of minimum inhibitory concentration (MIC) of antibiotics among the coliforms

The MIC of five different drugs (Ciprofloxacin, Erythromycin, Tetracyclin, Amoxicillin, and Penicillin) was determined by the plate dilution method as adopted by Rennie *et al.,* 2012 **[11].** The antibiotics were used in varying concentrations ranging from 2 to 100 µg/ml and supplemented individually in nutrient agar, which were then spot inoculated with approximately $3x10^6$ microbial cells with the help of platinum loop of 5mm diameter. The plates were incubated at 37° C for 24 hr. lowest concentration of the metal which inhibits the growth of the microorganisms was considered as MIC.

Results:

In this study, antibiotic tolerant population of coliforms from the river water samples was observed against five antibiotics (Erythromycin, Amoxicillin, Ciprofloxacin, Tetracyclin, Penicillin) at their varying concentrations (5 to 100µg/ml). Viable count of coliforms was higher in non antibiotic supplemented control plate than antibiotic supplemented plates in site I, II and III respectively. A decrease in viable count was recorded with the increase of antibiotic concentrations tested in all I, II and III sampling sites. The prevalence of drug resistant coliforms indicates the faecal pollution in the Gomti river water at Lucknow city. Higher population of antibiotic resistant coliforms was observed on Mac conky medium as compared on Emb medium. On Eosin Methylene blue (EMB), only two types of bacterial colonies were observed and identified as *E.coli* and Enterobacter spp, on the basis of their morphological and biochemical tests.

On Mac conky medium, **Table 1 (see supplementary material)** the viable count of coliforms in different concentrations (5-100 µg/ml) of antibiotics ranged from ($3.54x10^4$-$1.7x10^3$), ($9.3x10^3$-$4.0x10^2$), ($4.5x10^3$-$5.0x10^2$) cfu/ml of water in site I, II and III respectively. In case of site-I maximum viable count was recorded against erythromycin ($3.54x10^4$), followed by ciprofloxacin ($1.16x10^4$), amoxicillin ($3.2x10^4$), tetracycline ($3.2x10^3$), and penicillin ($1.8x10^3$) at 5 µg/ml respectively. All antibiotics showed no viable count at 100 µg/ml rather than amoxicillin. In case of site II, maximum viable coliforms count was recorded against erythromycin ($9.3x10^3$) followed by amoxicillin ($6.4x10^3$), tetracycline ($4.1x10^3$), ciprofloxacin ($2.4x10^3$) and penicillin ($1.4x10^3$) at 5µg/ml concentration respectively. Similar trend of coliform count was recorded at 10, 20 and 40 µg/ml concentration of the antibiotics tested. No viable count was found at 50, 75 and 100 µg/ml concentration of ciprofloxacin, tetracycline and penicillin respectively. In site III: A different trend of antibiotic toxicity was observed as compared to site-A and B. Maximum viable count of coliform ranged from ($4.5x10^3$-$1.6x10^3$) against erythromycin followed by

(3.9 x10^3- 5.0 x10^2), (3.2x10^3-4.0 x10^2), (2.5 x10^3- 5.0 x10^2) and (8.0 x10^2-2.0 x10^2) against amoxicillin, ciprofloxacin, tetracycline and penicillin at 5-75 µg/ml concentration range respectively. No viable coliform count was observed against tetracycline and penicillin at above 50 µg/ml concentration as compared to other antibiotics tested. Almost same trend was observed in Erythromycin and Amoxicillin no growth was detected at 75 and 100 µg/ml concentration. Similar trend of antibiotic resistant coliforms (cfu/ml) recorded on EMB medium. Antibiotics and sampling site based variations were also recorded regarding the occurrence of E.*coli* and Enterobacter spp **Table 1.**

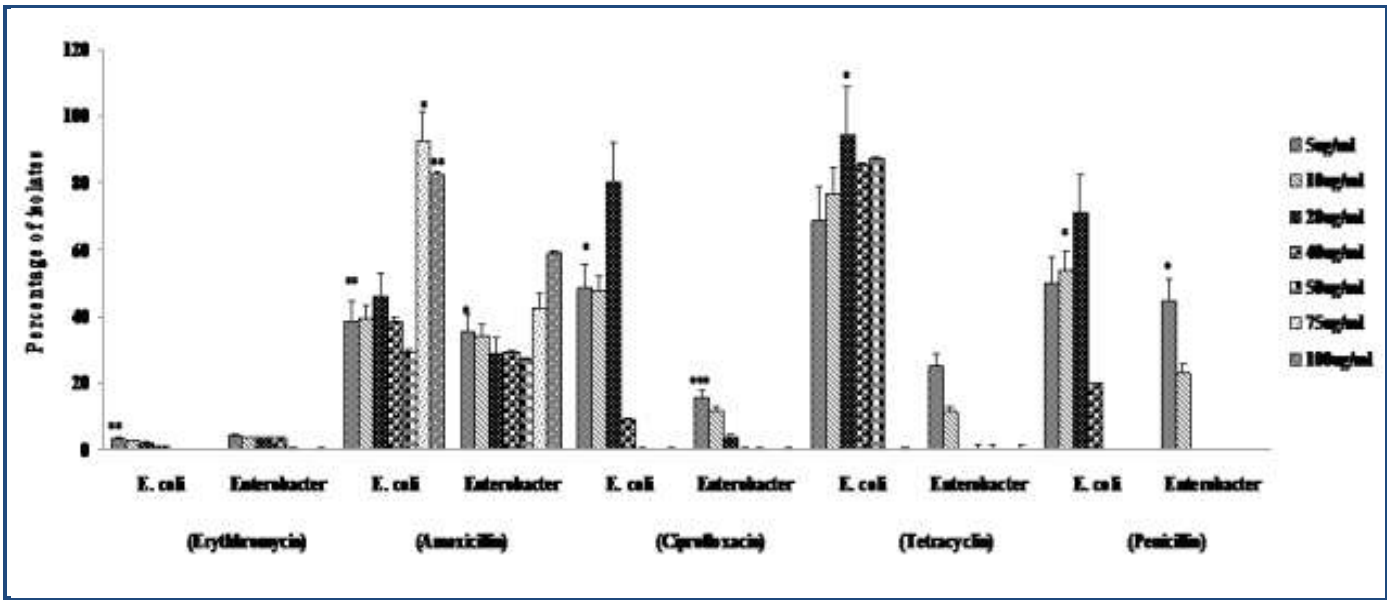

Figure 2: Percentage of Antimicrobial resistant *E.coli* and Enterobacter isolates among Coliforms (site-I)

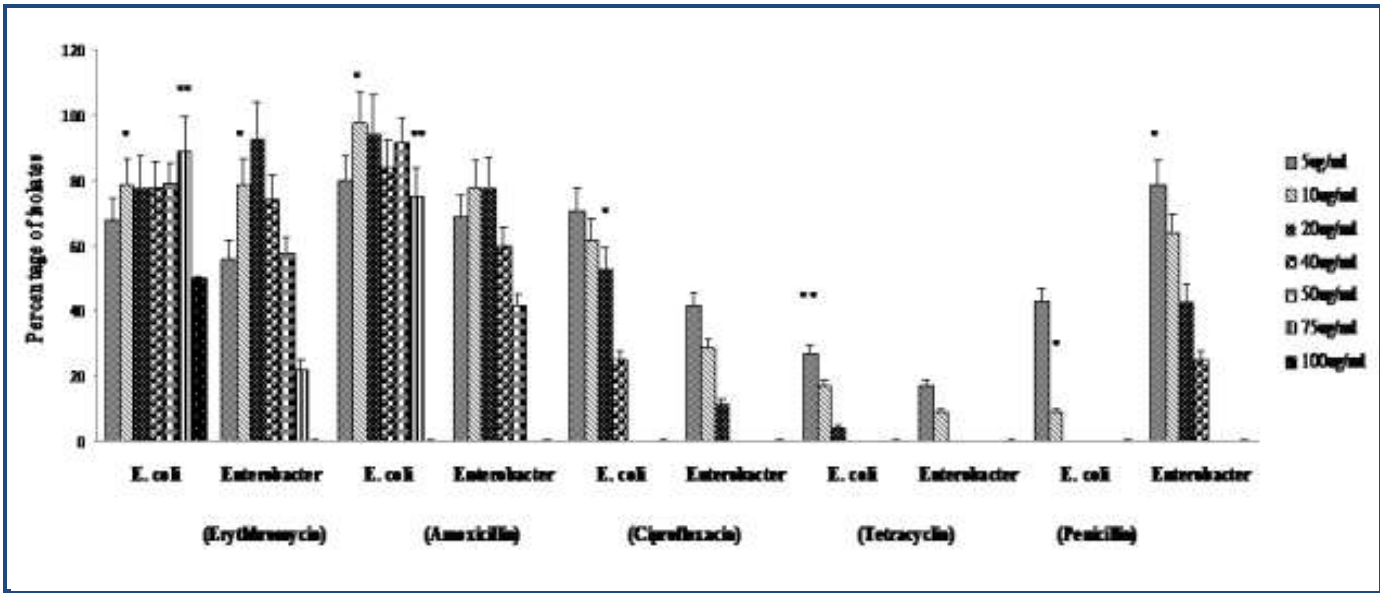

Figure 3: Percentage of Antimicrobial resistant *E.coli* and Enterobacter isolates among Coliforms (site-II)

Percentage of antibiotic resistant E. *coli* isolates was observed higher among the total coliforms against Erythromycin, Amoxicillin, Ciprofloxacin, and Tetracycline as compared to Enterobacter spp. Percentage of Enterobacter isolates was obtained higher only against erythromycin and penicillin in the sampling site I and II respectively. A varied trend of resistance order Penicillin >Ciprofloxacin >Tetracycline >Amoxicillin > Erythromycin, Penicillin >Amoxicillin > Ciprofloxacin > Erythromycin > Tetracycline and Penicillin > Erythromycin > Amoxicillin > Ciprofloxacin > Tetracycline was recorded among the coliforms from site I, II and III respectively. Percentage of Drug resistant *E.coli* and Enterobacter isolates among the total coliforms against individual antibiotic at its different concentration has been shown in **Figure 2, Figure 3 & Figure 4.**

Drug tolerance was also determined among the isolates of E.*coli* and Enterobacter species in terms of their MIC level. Isolates showed similar trend of resistance (MIC level) against antibiotics tested. Maximum number of E.*coli* and Enterobacter isolates exhibited their MICs at lower range (2-5µg/ml) against ciprofloxacin, tetracycline and amoxicillin. No MIC level was

found in the range of 2- 5, 5- 10 and 40- 50µg/ml among the *E.coli* isolates against Penicillin as compared to Enterobacter spp. Maximum number of isolates of E.*coli* and Enterobacter demonstrated their MIC range 50- 80 and 20- 40µg/ml of Penicillin respectively. Isolates of E.*coli* showed no MIC level in the range of 50- 80 and 80- 100 µg/ml against ciprofloxacin and tetracycline as compared to Enterobacter spp. higher number of E.*coli* isolates exhibited their MIC in the range of 80-100 µg/ml against Amoxicillin and Erythromycin than Enterobacter isolates shown in **Table 2 &Table 3 (see supplementary material).**

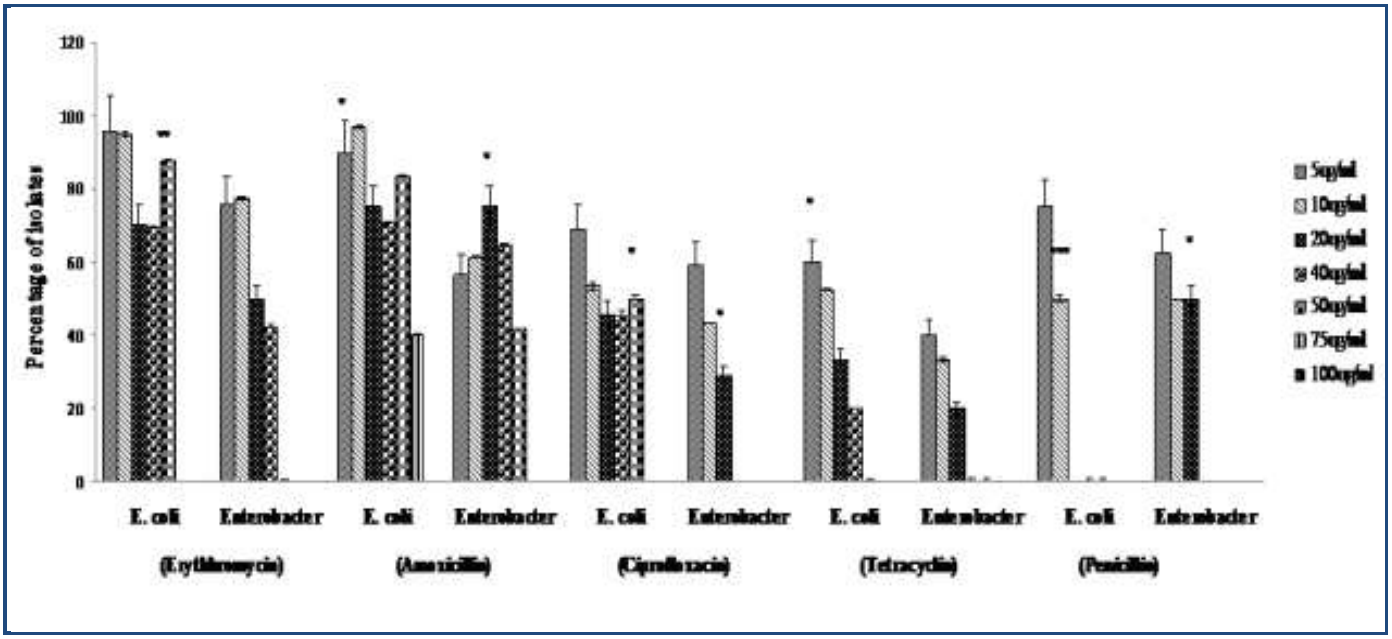

Figure 4: Percentage of Antimicrobial resistant *E.coli* and Enterobacter isolates among Coliforms (site-III)

Discussion:

During recent years, the distribution of antibiotic-resistant strains of Enterobacteriaceae in the aquatic environment like river and sewage waters, surface waters, sea water, and shellfish has been studied in different parts of the world. River and sewage waters, surface waters, sea water, and shellfish have been investigated for the presence of antibiotic-resistant Enterobacteriaceae. Drug concentrations sufficiently high to represent an ecological threat have already been reported in different rivers worldwide **[12-14].** Antimicrobial resistance (AR) determinants may be considered as a form of pollution **[15]** when they are introduced into the environment by the release of faecal bacteria that have been exposed to high levels of antimicrobials in the human or animal digestive track **[16].** An extensive literature describes and analyses the resistance pattern of faecal bacteria, mainly Escherichia coli and enterococci, in aquatic environments **[17].**

In our observation, a varying trend of antibiotic resistant coliform population in the Gomti river water receiving long term domestic, municipal small industries and hospital wastewater was observed. We recorded a varying viable count of multidrug resistant coliform bacteria in three different sampling sites of the Gomti River. Viable count of coliforms was higher in non antibiotic supplemented control plate than antibiotic supplemented plates in site I, II and III respectively. A decrease in viable count was recorded with the increase of antibiotic concentrations tested in all I, II and III sampling sites. The viable count of coliforms in different concentrations (5-100 µg/ml) of antibiotics ranged from ($3.54x10^4$-$1.7x10^3$), ($9.3x10^3$-$4.0x10^2$), ($4.5x10^3$-$5.0x10^2$) cfu/ml of water in site I, II and III respectively. The viable count from sampling site-c receiving water from treatment plant showed lower count as compared to site-I and II our findings are in agreement of many people reports, **[18-20]** also reported E. coli strains from sewage treatment plants were less resistant against quinolones, while Namboodiri *et al*. **[21]** have reported quinolones-resistant E. coli from the faecal flora of Accra residents, Ghana. **[22]** Hsu *et al*. pointed out that the differences in the extent of bacterial resistance to various antibiotics might reflect the history of antibiotic applications and allow bacterial drug resistance to be used as an indicator of antibiotic application. β-Lactamase is the major defense systems of Enterobacter species **[23].** However, efflux pump mediated resistance to β-lactam antibiotics, quinolones, tetracycline, and chloramphenicol has been reported **[24-25]** observed higher numbers of antibiotic resistant bacteria from upland tarns (receiving no sewage/effluent). They noticed variation in bacterial species in different aquatic habitats and growth in oligotrophic environments contributes to the incidence of antibiotic resistance.

Most of the authors consider that faecal bacteria released by wastewaters (treated or not) could play a key role in AR determinants dissemination. Indeed, in sewage contaminated rivers **[13].** Although the mortality of pathogenic bacteria or their indicators is very high in extraenteral environments, their great abundance and certain environmental conditions might keep these populations viable for quite some time. Several authors have found a high correlation between bacteria density in the water of different environments (beaches and freshwater shores) **[26].** In general, treatment plants reduce the abundance of inflow water bacteria by between 1 and 3 log units **[20].** This reduction, however, is not necessarily accompanied by a

reduction in the number of resistant bacteria; quite the contrary, the number of resistant bacteria increases **[27].**

In this study, Antibiotics and sampling site based viable count showed variations in the ccurrence of E. *coli* and Enterobacter spp. Percentage of antibiotic resistant E. *coli* isolates was observed higher among the total coliforms against Erythromycin, Amoxicillin, Ciprofloxacin, and Tetracycline as compared to Enterobacter from the sampling site- I, site-II and site III. Percentage of Enterobacter isolates was obtained higher only against erythromycin and penicillin from the sampling sites I and II respectively. Similar results have also been obtained in other studies **[28-29].** Variations in the occurrence of E.*coli* and Enterobacter spp. Was due to the survival of the fittest depending upon the antibiotics and sampling site. The resistance percentages obtained in this study tally with the resistance ranges found by other authors, Manji and Antai in 2012 **[30];** Kumar and Joseph in 2011 **[31].** Thus, the *Enterococci* show very low amoxicillin resistance rates, coinciding with the findings of Fernandes and Watanabe **[26].** The resistance levels of *E. coli* and Enterobacter spp. populations are also comparable to those found by other authors **[32-34].** Resistance patterns in the population of coliforms were recorded as erythromycin> amoxicillin > tetracycline> ciprofloxacin> penicillin, tetracycline >erythromycin >ciprofloxacin >amoxicillin >penicillin and tetracycline >ciprofloxacin >amoxicillin >erythromycin >penicillin from site I, II and site III respectively. This resistance order has similarity with the antibiogram among the members of Enterobacteriaceae by other reporters. Reinthaler *et al*. chiming in with the findings of this study. Multiresistance is another constantly studied factor **[35].** Chelosi *et al*. found that more than 56% of the Gram negative bacteria from cultivated marine sediment were resistant to 5 or more antibiotics. Lefkowitz and Durán **[34]** measured the multi-resistance of *E. coli* in wastewater treatment plants, obtaining outflow readings of 60% of bacteria multi-resistant to 2 or more antibiotics and 25% to 4 or more. Other authors have studied the same factor **[36],** and the findings of this study fall within the same ranges found therein.

We also determined tolerance level among the coliform isolates against tetracycline, penicillin, amoxicillin, erythromycin and ciprofloxacin. All the isolates exhibited their MIC in between the 2-100µg/ml against antibiotics tested. Maximum number of isolates of E. coli and Enterobacter spp. showed their MIC at lower range 2-5 µg/ml against ciprofloxacin and tetracycline while no MIC level was recorded in the range of 5-10 µg/ml against penicillin. Higher number of E. *coli* isolates showed their MIC in the range of 80-100 µg/ml against erythromycin and amoxicillin than Enterobacter isolates. Similar findings regarding the drug MIC levels in the members of Enterobacteriaceae have been reported by many workers **[37].** Significant rise in bacterial contamination exhibited by pollution indicator organisms is a risk to public health, particularly due to the emergence of resistance and microbial diversity in the Gomti river water. This study may be relevant and useful in conservation of riverine systems for the safety of the aquatic environment and human health. In this study, the distribution of resistance to antimicrobial drugs among coliforms in surface water was investigated without differentiating transferable and nontransferable resistance, but paying attention to the effect of the species composition of the sample on the incidence of resistance and resistance orders.

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Conflict of Interests:
The authors declare that they have no conflict of interests.

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Supplementary material:

Table 1: Viable count of antimicrobial resistant coliform bacteria from different water sampling sites

Antibiotic	Conc.	Eosin Methylene blue (EMB)						Mac conkey		
		Site1		Site2		Site3		Site1	Site2	Site3
		E.*coli*	*Enterobacter spp*	E.*coli*	*Enterobacter spp*	E.*coli*	*Enterobacter spp*			
Control	No Antibiotic	1.40 x10^4±0.5	1.23 x10^4±0.8**	2.38x10^4 ±0.2	2.12x10^4± 2.0	5.6x10^3±1 .8	4.3x10^3±3 .0	3.90 x10^4±0.5	2.59x10^4± 1.2*	5.8x10^3±1.1
Erythromycin	5	0.5x10^3±1. 0	0.5x10^3±1 .5**	0.3x10^3± 6.3	0.2x10^3±5 .2	0.07x10^3± 4.3	0.3x10^3±3 .4*	0.02x10^4± 3.54	0.09x10^3± 9.3	0.2x10^3±4. 5
	10	0.5x10^3±0. 8*	0.1x10^3±1 .2	0.1x10^3± 4.1*	0.310^3±4. 1*	0.2x10^3±3 .8	0.1x10^3±3 .1	0.04x10^4± 3.28	0.4x10^3±5 .2	0.08x10^3± 4.0**
	20	0.3x10^3±0. 5	0.4x10^3±0 .9*	0.3x10^3± 3.2*	0.3x10^3±3 .8	0.2x10^3±2 .1	0.06x10^3± 1.5	0.03x10^4± 2.78	0.4x10^3±4 .1	0.2x10^3±3. 0
	40	0.1x10^3±0. 2	0.4x10^3±0 .8	0.4x10^3± 2.1**	0.5x10^3±2 .0	0.1x10^3±1 .8	0.1x10^3±1 .1	0.04x10^4± 2.34	0.2x10^3±2 .7	0.2x10^3±2. 6
	50	0	0	0.4x10^3± 1.5	0.3x10^3±1 .1	0.3x10^3±1 .4	0	0	0.3x10^3±1 .9**	0.09x10^3± 1.6*
	75	0	0	0.2x10^3± 0.8	0.1x10^3±0 .2**	0	0	0	0.5x10^3±0 .9*	0
	100	0	0	0.1x10^3± 0.2	0	0	0	0	0.2x10^3±0 .4	0
Amoxicillin	5	0.04x10^4± 1.24*	0.04x10^4± 1.13	0.1x10^3± 5.1	0.4x10^3±4 .4	0.1x10^3±3 .5*	0.1x10^3±2 .2	0.5x10^4±3 .2	0.4x10^3±6 .4	0.09x10^3± 3.9
	10	0.05x10^4± 1.10*	0.2x10^4±0 .95	0.2x10^3± 4.0**	0.1x10^3±3 .2	0.1 x10^3±3.0	0.3x10^3±1 .9	0.09x10^4± 2.8	0.1x10^3±4 .1	0.1x10^3± 3.1*
	20	0.1x10^4±0. 92*	0.5x10^4±0 .58	0.3x10^3± 3.4	0.5x10^3±2 .8	0.05x10^3± 1.8*	0.09x10^3± 1.8	0.2x10^4±2 .0	0.3x10^3±3 .6	0.1 x10^3±2.4
	40	0.7x10^4±0. 58	0.4x10^4±0 .44*	0.2x10^3± 2.1	0.3x10^3±1 .5	0.1x10^3±1 .2 *	0.3x10^3±1 .1**	0.2x10^4±1 .5	0.5x10^3±2 .5	0.08x10^3± 1.7
	50	0.2x10^4±0. 32	0.3x10^4±0 .30*	0.6x10^3± 1.1*	0.2x10^3±0 .5	0.2x10^3±1 .0*	0.3x10^3±0 .5	0.1x10^4±1 .1	0.4x10^3±1 .2	0.06x10^3± 1.2
	75	0.6x10^4±0. 24	0.3x10^4±0 .11	0.1x10^3± 0.3	0	0.2x10^3±0 .2	0	0.1x10^4±0 .26	0.2x10^3±0 .4	0.06x10^3± 0.5*
	100	0.6x10^4±0. 14	0.2x10^3±0 .1	0	0	0	0	0.3x10^4±0 .17	0	0
Ciproflox acin	5	0.2x10^3±5. 6*	0.3x10^3±1 .8	0.2x10^3± 1.7	0.3x10^3±1 .0**	0.1x10^3±2 .2	0.1x10^3±1 .9*	0.2x10^4±1 .16	0.3x10^3±2 .4	0.09x10^3± 3.2*
	10	0.4x10^3±4. 5	0.2x10^3±1 .1	0.1x10±1 .3^3	0.2x10^3±0 .6	0.2x10^3±1 .6	0.1x10^3±1 .3 **	0.4x10^3±9 .5	0.2x10^3±2 .1	0.1x10^3±3. 0*
	20	0.2x10^3±4. 0*	0.1x10^3±0 .2*	0.2x10^3± 0.9	0.1x10^3±0 .2	0.1x10^3±1 .1	0.2x10^3±0 .7	0.2x10^3±5 .0	0.06x10^3± 1.7	0.3x10^3±2. 4
	40	0.1x10^3±0. 2	0	0.2x10^3± 0.3	0	0.2x10^{3}0. 5±	0	0.1x10^3±2 .3	0.06x10^3± 1.2	0.08x10^3± 1.1
	50	0	0	0	0	0.2x10^3±0 .2	0	0	0	0.08x10^3± 0.4*
	75	0	0	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0	0	0
Tetracycli n	5	0.1 x10^3±2.2	0.3x10^3±0 .8	0.4x10^3± 1.1	0.2x10^3±0 .7*	0.3x10^3±1 .5	0.3x10^3±1 .0	0.1x10^3±3 .2	0.08x10^3± 4.1	0.3x10^3±2. 5*
	10	0.3 x10^3±2.0*	0.1x10^3±0 .3**	0.1x10^3± 0.6	0.1x10^3±0 .2	0.1x10^3±1 .1	0.3x10^3±0 .7	0.1x10^3±2 .6	0.3x10^3±3 .5	0.1x10^3±2. 1
	20	0.3 x10^3±1.8	0	0.1x10^3± 0.1	0	0.2x10^3±0 .5	0.2x10^3±0 .3***	0.1x10^3±1 .9	0.1x10^3±2 .2*	0.2x10^3±1. 5
	40	0.4 x10^3±1.2	0	0	0	0.3 x10^3±0.1	0	0.1x10^3±1 .4	0	0.3 x10^3±0.5*
	50	0.2 x10^3±0.7*	0	0	0	0	0	0.4x10^3±0 .8	0	0
	75	0	0	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0	0	0

		0						0		
	5	0.4x10³±0.	0.4x10³±0	0.2x10³±	0.5x10³±1	0.08x10³±	0.2x10³±0	0.1x10³±1	0.3x10³±1	0.08x10³±
Penicillin	10	9*	.8	0.6*	.1	0.6*	.5	.8	.4*	0.8
	20	0.2x10³±0.	0.2x10³±0	0.2x10³±	0.3x10³±0	0.3x10³±0	0.07x10³±	0.4x10³±1	0.2x10³±1	0.3x10³±0.
	40	7	.3	0.1*	.7	.2	0.2*	.3*	.1	4*
	50	0.3x10³±0.	0	0	0.1x10³±0	0	0.2x10³±0	0.2x10³±0	0.3x10³±0	0.3x10³±0.
	75	5	0	0	.3	0	.1	.7	.7*	2*
	100	0.1x10³±0.	0	0	0.3x10³±0	0	0	0.3x10³±0	0. ±.4	0
		1**	0	0	.1	0	0	.5	0	0
		0	0	0	0	0	0	0	0	0
		0			0		0	0	0	0
		0			0			0		

The data are expressed in mean ± SEM). The comparisons were made by ANOVA followed by Dunnett's test.
*P < 0.05 significant, **P < 0.01 very significant, ***P < 0.001 extremely significant, ns-non-significant.

Table 2: MIC of antibiotics among *E.coli.*

Antibiotics	2-5 µg/ml	5-10 µg/ml	10-20 µg/ml	20-40 µg/ml	40-50 µg/ml	50-80 µg/ml	80-100 µg/ml
Ciprofloxacin	49(63.63%)	11(14.28%)	3(3.89%)	13(16.88%)	1(1.29%)	NIL	NIL
Erythromycin	2(2.59%)	NIL	23(29.8%)	29(37.66%)	2(2.59%)	20(25.9%)	1(1.29%)
Tetracyclin	54(70.12%)	10(12.9%)	9(11.6%)	1(1.29%)	3(3.89%)	NIL	NIL
Amoxicillin	21(27.2%)	1(1.29%)	2(2.59%)	1(1.29%)	1(1.29%)	8(10.3%)	43(55.8%)
Penicillin	NIL	NIL	18(23.3%)	11(14.2%)	NIL	48(62.3%)	NIL

Table 3: MIC of antibiotics among Enterobacter spp.

Antibiotics	2-5 µg/ml	5-10 µg/ml	10-20 µg/ml	20-40 µg/ml	40-50 µg/ml	50-80 µg/ml	80-100 µg/ml
Ciprofloxacin	51(70.1%)	4(5.19%)	4(5.19%)	7(9.09%)	3(3.89%)	2(2.59%)	6(7.7%)
Erythromycin	13(16.8%)	4(5.19%)	16(20.7%)	36(46.7%)	5(6.4%)	2(2.59%)	1(1.29%)
Tetracyclin	42(54.5%)	6(7.7%)	10(12.9%)	8(10.3%)	4(5.19%)	4(5.19%)	3(3.89%)
Amoxicillin	24(31.1%)	14(18.1%)	4(5.19%)	19(24.6%)	10(12.9%)	6(7.7%)	NIL
Penicillin	5(6.4%)	NIL	15(19.4%)	30(38.9%)	7(9.09%)	19(24.6%)	19(24.6%)