academicJournals

Vol. 8(7), pp. 619-627, 12 February, 2014
DOI: 10.5897/AJMR2013.6417
ISSN 1996-0808 ©2014 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Determination of multiple antibiotic resistance patterns and indexing among metal tolerant β-lactamase-producing *Escherichia coli*

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Accepted 30 January, 2014

The antibiotic resistance profiles of *Escherichia coli* isolated from three different sampling sites of the Gomti River at Lucknow city was evaluated. Water samples were collected and then analyzed for the presence of *E. coli*, using standard methods. Antibiotic susceptibility testing was performed by the disc diffusion method. Of the 77 *E. coli* isolates tested, marked antibiotic resistances (over 70%) were observed for amoxicillin, nitrofurozone, chloramphenicol, polymixin B, methicilin, ampicillin, nalidixic acid, cefpodoxime, erythromycin, penicillin, rifampicin and ofloxacin depending upon the sampling sites. All *E. coli* isolates also showed multiple resistance patterns in different combination of antibiotics. The MAR index ranges were found very high indicating the high risk of environmental contamination. The findings indicated that pollution of aquatic environments from different sources of the city may have a potential impact on the dissemination and survival of *E. coli*, as well as other pathogenic bacteria in the Gomti River water for public and animal health. This may result to a negative effect on antibiotic therapy for infectious diseases.

Key words: Gomti River, coliforms, antibiotic susceptibility, multiple antibiotic resistance (M.A.R).

INTRODUCTION

Many antibiotics have been used in the last several years in medical, veterinary, agriculture and aquaculture practices (Alpay-Karaoglu et al., 2007). Recently, there has been a growing interest in the presence of different pharmaceutical substances, mainly antibiotics in the aquatic environment (Vulliet and Cren-Olive, 2011). The wide application of antibiotics by human has led to large-scale dissemination of bacteria resistant to antibiotics in water basins (Dang et al., 2006).

Bacterial resistance to antibiotics in the aquatic environment has received comparatively little attention. Bacterial contamination of surface water, particularly contaminated with faecally derived bacteria, has long been a water quality issue due to the potential for disease transmission. 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 (Batt et al., 2006) and the role of these antibiotics in the development, transfer and maintenance of resistance is largely unknown. The number of antimicrobial-resistant (AMR) bacteria in the environment increases exponentially with the use of

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Abbreviations: MAR, Multiple antibiotic resistance; **AMR,** antimicrobial-resistant; **IMViC tests,** indole, methyl red, Voges Proskauer and citrate utilization tests.

antimicrobials, as a result of increasing selective pressure on bacterial populations (Tsiodras et al., 2008). Furthermore, AMR is increasing, and its spread between different bacterial strains in different habitats has been demonstrated (Drewnowska and Swiecicka, 2013; SVARM, 2006).

Untreated drinking water coming from different sources contains coliforms including *Esherichia coli*. In developing countries, drinking water supply lines and open sewage drains are laid side by side resulting in frequent contamination of water (Patoli et al., 2010). *E. coli* is an opportunistic pathogen. The disposal of treated sewage into rivers, lakes, or elsewhere may or may not influence environmental bacterial populations (Ahmed et al., 2010). Food and water borne outbreaks of *E. coli* have been documented from a number of countries (Soderstrom et al., 2008). Increase in antibiotic resistance level is now a global problem. Since water is one of the four components of environment, and a usual habitat for *E. coli*, therefore, the availability of antibiotic resistant *E. coli* strains in water cannot be denied.

They represent one of the major contaminants in surface and ground water in developing countries. In recent decades, the increased usage of antibiotics has led to antibiotic resistance among enteric bacteria. River water is the main receptacle reservoir of antibiotics and antibiotic resistant bacteria in the environment. They are directly introduced into surface water through animal farms and agricultural practices. The antibiotic resistance bacteria in drinking water are a prime concern to public health (Igbinosa and Okoh, 2008). The wide use and abuse of antibiotics in human therapy has produced MAR *E. coli* in the faeces of human as well (Fischbach and Walsh, 2009). These practices have resulted in the coexistence of MAR *E.coli* within these major reservoirs of enteric disease for human. The aim of this study was to investigate the multiple antibiotic resistance and their patterns among the beta lactamase producing *E. coli* strains from the Gomti river water in the vicinity of Lucknow city.

MATERIALS 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. Samples were taken at 4°C until their arrival in laboratory. This study was undertaken to determine the incidence and antibiotic resistant patterns of *E. coli* strains isolated from water samples. 77 *E. coli* isolates were isolated and tested against 20 commonly used antimicrobial agents.

Isolation and identification of metal tolerant *E. coli* isolates

Isolation of metal tolerant *E. coli* 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 *E. coli*. Plates were incubated at 37°C for 24 h. Greenish with metallic sheen colonies were identified as *E. coli* and further characterization was done by indole, methyl red, Voges Proskauer and citrate utilization tests (IMViC tests).

Beta lactamase production

For detection of beta lactamase producing bacteria, a loopfull of grown culture was transferred into small tube containing 1 ml of penicillin G solution and incubated at 37°C for 30 min. 0.5 ml of iodine solution was added and mixed for 2-3 min. Change in colour to colourless, indicates positive result (Dierikx et al., 2010)

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 2008) including *Escherichia coli* ATCC 25922 as a control strain. 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 *E. coli* infections and their use as feed additives to promote growth in animals in agriculture, zootechny and aquaculture (Florea, 2011)

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 Downing et al. (2011). Sampling site based MAR index was calculated by the same formula modified by the total number of isolates from a sampling site as described (Riaz et al., 2011).

RESULTS

A total of 77 *E. coli* isolates were isolated from three different sites, 27 from site I and 25 each from sites II and III of the Gomti River Water. All the isolates were found beta lactamase positive and characterized on the basis of antibiotic susceptibility test. *E. coli* isolates from site I, II and III showed a variable resistance against 20 different antibiotics tested showed in Figure 1. 96% of the isolates showed resistance against amoxicillin followed by 89, 85 and 77% against Nitrofurozone, Penicillin, Chloramphenicol, respectively. Lower number of isolates showed resistance against Rifampicin (14.8%) followed by Gentamicin (11.11%), (Neo 7.40%) and ciprofloxacin (3.7%).

In case of site II: A high level of resistance was also observed among the isolates; all the isolates demonstrated resistance against Amoxicillin, Polymixin B and Methicilin.

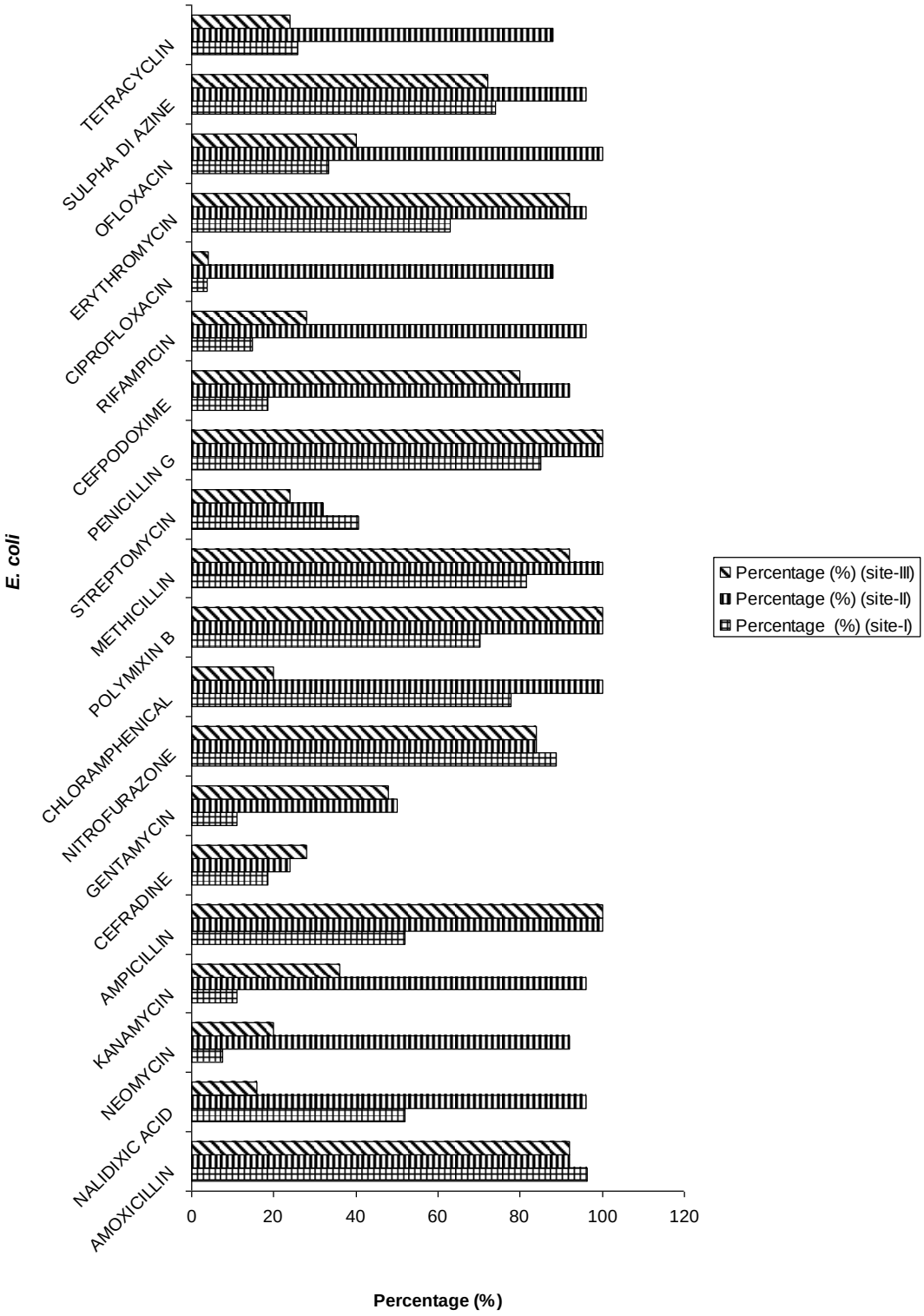

Figure 1. Percentage of resistance against antibiotics in *E. coli* isolates from Gomti River water (sites I, II and III).

96% of the isolates showed resistance against cefpodoxime, rifampicin and ciprofloxacin. Minimum drug resistance was observed by 40, 36, 32% isolates against Kanamycin, Tetracyclin and Gentamicin, respectively.

Similar observations were recorded in the case of site III: All isolates demonstrated resistance against Ampicillin, Polymixin B and Penicillin, while 92 and 84% isolates showed resistance against Amoxicillin and Nitrofurozone,

Table 1. Antibiotic resistance pattern in 27 *E. coli* isolates from Gomti River water (sample 1).

Number of antibiotics	Resistance pattern	Number of resistance isolates	Percentage (%)	MAR
2	AMX, MET.	1	3.7	0.1
4	AMX, AMP, ERYTHRO, PB.	1	3.7	0.2
5	PEN, ERYTHRO, NEO, PB, AMX	1	3.7	0.25
6	NR, NA, AMX, OF, RIF, PB. NR, MET, AMOX, PB, RIF, SZ.	2	7.4	0.3
8	AMX, NA, NR, CHMP, PEN, ERYTHRO, SZ, OF. AMX, NR, MET, PEN, ERYTHRO, SZ, KAN, CHMP. NR, CHMP, MET, PEN, ERYTHRO, SZ, AMP, TET.	3	11.1%	0.4
9	AMX, NR, CHMP, MET, STREPTO, PEN, SZ, AMP, PB. AMX, NR, CHMP, MET, STREPTO, SZ, PEN, PB, AMP. AMX, NR, MET, STREPTO, PEN, SZ, CHMP, TET, OF.	4	14.8%	0.45
10	AMX, NA, NEO, GEN, NR, ERYTHRO, SZ, CHMP, PB. AMX, NA, NR, MET, PEN, CPD, ERYTHRO, SZ, PB, CHMP. MET, CHMP, NR, NA, AMOX, PEN, ERYTHRO, TET, CPD, PB. NA, NR, MET, PEN, CPD, ERYTHRO, CHMP, AMOX, AMP, PB. AMX, NR, CH, MET, STREPTO, PEN, SZ, AMP, PB, RIF. AMX, NA, NR , MET, STREPTO, PEN, SZ, CEF, CHMP, NEO. AMX, NA, AMP, NR, CHMP, MET, PEN, ERYTHRO, SZ, PB. AMX, NR, CHMP, PB, MET, PEN, ERYTHRO, SZ, TET, KAN. AMX, AMP, GEN, NR, CHMP, MET, PEN, ERYTHRO, OF, SZ.	8	25.6%	0.5
11	AMX, NA, NR, MET, STREPTO, PEN, CPD, SZ, ERYTHRO, OF, PB. AMOX, NA, CEF, NR, CHMP, MET, PEN, CPD, ERYTHRO, OF, AMP. AMX, NA, NR, CHMP, MET, STREPTO, PEN, SZ, AMP, PB, OF. AMX, NR, MET, STREPTO, PEN, SZ, CHMP, TET, PB, AMP, CEF. AMX, NA, NR, MET, STREPTO, PEN, SZ, CH, PB, AMP, OF.	5	18.5%	0.55
12	AMX, NA, NR, CHMP, MET, STREPTO, PEN, SZ, AMP, PB, OF, ERYTHRO.	1	3.7	0.6
14	AMX, NA, CEF, GEN, NR, CHMP, PB, MET, PEN, RIF, CIP, ERYTHRO, SZ, AMP.	1	3.7	0.7

Sensitive strains- 2.

respectively. There in site III, the least number of isolates by 16 and 4% showed resistance against Nalidixic acid and Ciprofloxacin, respectively.

Single and multiple antibiotic resistance patterns in 27 *E. coli* isolates were also recorded in the sites of the Gomti River Water, as depicted in Tables 1, 2 and 3. In site I, all the isolates showed 11 patterns of antibiotic resistance against the antibiotics tested. 3.7% isolates showed resistance to 2, 4, 5, 12 and 14 antibiotics in one and two combinations, respectively. 7.4% isolates showed resistance to 6 antibiotics at a time in 2 different combinations. 11.1% isolates showed resistance to 8 antibiotics at a time in three different combinations. 14% of the isolates exhibited resistance to 9 antibiotics at a time in four combinations. 18.5 and 29.6% of the isolates showed resistance to 11 and 10 antibiotics at a time in five and eight different combinations, respectively.

Antibiotic resistance patterns among the 25 *E. coli* isolates from site II were also recorded. All the isolates showed 12 different resistance patterns among the antibiotics tested. 4% isolates showed resistance to 6, 8, 9, 10 and 14 antibiotics at a time in one combination and 8% isolates exhibited resistance to 12, 13, 16 and 18 antibiotics at a time in two different combinations and 12, 16 and 20% isolates showed resistance to 17, 11and 15 antibiotics at a time in three, four and five different combinations, respectively.

In the case of site III (25 *E. coli* isolates), all the isolates showed 12 different patterns of antibiotic resistance against the antibiotics tested. 4% of the isolates showed resistance to 7, 8, 11, 18 and 20 antibiotics at a time in one combination, respectively, while 8% of the isolates exhibited resistance to 10, 12, 14, 15 and 17 antibiotics at a time in two different combinations respectively. 16 and 24% of *E. coli* isolates exhibited resistance to 16 and 13 antibiotics at a time in four and six different combinations, respectively.

M.A.R. indexing based on isolates was also calculated. A varied trend of MAR Index was observed among the isolates from the three different sampling sites. 3.7%

Table 2. Antibiotic resistance pattern in 25 *E. coli* isolates from the effluent of Gomati River water (sample 2).

Number of antibiotics	Resistance pattern	Number of resistance isolates	Percentage (%)	MAR
6.	AMX, PEN, SZ, CPD, MET, PB.	1	4	0.3
8.	CPD, MET, NA, PB, GEN, RIF, AMX, NR.	1	4	0.4
9.	PB, CPD, OF, GEN, RIF, AMX, PEN, STREPTO, MET.	1	4	0.45
10.	AMX, PEN, MET, PB, AMP, OF, KAN, CHMP, RIF, ERYTHRO.	1	4	0.5
11.	AMX, PEN, MET, NA, PB, AMP, CHMP, ERYTHRO, RIF, NEO, OF, PB, AMX, CPD, MET, NR, NA, NEO, PEN, OF, RIF, ERYTHRO. CPD, MET, CHMP, NR, CEF, SZ, PB, PEN, ERYTHRO, AMOX, RIF. AMX, PEN, CPD, MET, CH, NA, PB, AMP, STREPTO, OF, RIF.	4	16	0.55
12.	AMX, PEN, CPD, MET, CHMP, NA, KAN, STREPTO, RIF, OF, NEO, PB, PB, OF, AMX, PEN, MET, NA, GEN, STREPTO, KAN, AMP, RIF, CPD.	2	8	0.6
13.	AMX, PEN, SZ, CPD, MET, AMP, NA, STREPTO, CEF, NR, PB, RIF, ERYTHRO, SZ, CPD, MET, PB, AMX, PEN, NR, NA, ERYTHRO, NEO, RIF, OF, AMP.	2	8	0.05
14.	AMX, PEN, SZ, CPD, MET, AMP, CHMP, NA, STREPTO, ERYTHRO, RIF, PB, OF, CEF.	1	4	0.7
15.	AMX, PEN, MET, AMP, CH, NA, TET, RIF, KAN, CPD, PB, NEO, OF, SZ, ERYTHRO. AMX, PEN, SZ, CPD, MET, NR, NA, AMP, CIP, CEF, OF, CH, ERYTHRO, RIF, PB. OF, AMX, PEN, SZ, CPD, MET, AMP, CHMP, NA, ERYTHRO, KAN, RIF, PB, CIP, TET.	5	20	0.75
16.	AMX, PEN, CEF, SZ, MET, CHMP, NR, ERYTHRO, PB, OF, KAN, RIF, AMP, CPD, TET. CEF, SZ, CPD, MET, RIF, AMX, PEN, CHMP, CIP, PEN, NA, OF, AMP, TET, ERYTHRO. PEN, MET, AMP, CHMP, NA, RIF, AMX, OF, KAN, PB, CPD, TET, ERYTHRO, NEO, STREPTO, NR. AMP, PEN, CPD, MET, AMP, CHMP, NA, ERYTHRO, PB, GEN, KAN, RIF, SZ, OF, NR, NEO.	2	8	0.8
17.	PB, AMX, PEN, CEF, SZ, CPD, MET, AMP, CHMP, NR, NA, RIF, ERYTHRO, GEN, OF, NEO, CIP. RIF, AMX, OF, PEN, SZ, CPD, MET, AMP, CHMP, CIP, NA, TET, PB, GEN, KAN, NEO, NR.	3	12	0.85
18.	MET, CHMP, ERYTHRO, PB, OF, KAN, RIF, AMP, TET, NA, ERYTHRO, STREPTO, CHMP, PB, TET, NEO, OF. PB, PEN, SZ, CPD, MET, NR, CHMP, AMX, RIF, GEN, CEF, AMP, TET, NEO, CIP, NA, OF, KAN. AMX, PEN, CPD, MET, CIP, NA, ERYTHRO, PB, GEN, CEF, SZ, CHMP, STREPTO, NR, RIF, OF, NEO, AMP.	2	8	0.9

isolates from site I showed a MAR 0.1 - 0.7 range against different number of antibiotics. M.A.R. 0.45, 0.5 and 0.55 were recorded by 14.8, 29.6 and 18.5% isolates respectively. In the case of sampling site II, 4% isolates demonstrated 0.3 - 0.7 MAR. range, while, MAR 0.9 was recorded by 8% isolates against 18 antibiotics. Maximum MAR was recorded among the isolates from site III. 4 and 8% isolates showed MAR range 0.35 - 1.0 and 0.6 - 0.9 against different number of antibiotics, respectively.

DISCUSSION

Many studies revealed that the co-selection took place in antibiotic resistance (Berg et al., 2005; Stepanauskas et al., 2005; Wright et al., 2006). Bacteria in metal-contaminated environments appeared to be easier to obtain antibiotic resistance phenotypes than in control areas (Baker-Austin et al., 2006). Wright et al. (2008) found that class 1 integrase gene was more abundant in the metal-exposed

Table 3. Antibiotic resistance pattern in 25 *E. coli* isolates from the effluent of Gomati River water (sample 3).

Number of antibiotics	Resistance pattern	Number of resistance isolates	Percentage (%)	MAR
7.	CPD, AMX, PEN, CEF, AMP, PB, MET.	1	4	0.35
8.	CPD, AMX, MET, ERYTHRO, NR, PEN, PB, RIF	1	4	0.4
10.	CPD, PEN, MET, ERYTHRO, PB, AMX, KAN, CHMP, NR, AMP. CPD, SZ, OF, PEN, ERYTHRO, NR, CHMP, MET, PB, AMX.	2	8	0.5
11.	CPD, CEF, MET, KAN, AMX, PB, ERYTHRO, NEO, NA, AMP, OF.	1	4	0.55
12.	CPD, AMX, MET, NR, RIF, STREPTO, AMP, CHMP, PB, NA, CEF, PEN. CPD, PEN, CEF, CIP, AMP, MET, AMX, PB, SZ, OF, NA, NR	2	8	0.6
13.	CPD, SZ, AMX, PEN, CEF, NA, MET, ERYTHRO, NR, PB, RIF, CHMP, AMP. CPD, AMX, PEN, CHMP, MET, ERYTHRO, RIF, STREPTO, AMP, NR, OF, NA, NEO. CPD, AMX, PEN, CEF, AMP, MET, RIF, PB, CHMP, NA, ERYTHRO, NR, KAN. CPD, AMX, PEN, AMP, MET, PB, TET, NA, ERYTHRO, NR, KAN, GEN. CPD, AMX, PEN, CEF, MET, NR, ERYTHRO, AMP, OF, CIP, NA, PB, SZ. CPD, AMX, PEN, CEF, TET, OF, ERYTHRO, PB, KAN, MET, AMP, NR, NA.	6	24	0.65
14.	CPD, PEN, MET, ERYTHRO, RIF, CHMP, TET, CIP, AMX, PB, KAN, NR, NEO, AMP. CPD, SZ, AMX, CEF, NA, AMP, MET, NR, RIF, TET, OF, PEN, PB, KAN.	2	8	0.7
15.	CPD, SZ, AMX, PEN, CEF, TET, CHMP, MET, RIF, OF, NA, PB, AMP, GEN, NR. CPD, AMX, PEN, CEF, MET, NEO, PB, SZ, ERYTHRO, NR, OF, NA, AMP, TET, KAN.	2	8	0.75
16.	CPD, AMX, PEN, NA, CHMP, MET, NEO, ERYTHRO, NR, RIF, STREPTO, CIP, AMP, OF, PB, CEF. CPD, AMX, SZ, PEN, CEF, NA, AMP, CHMP, MET, ERYTHRO, NR, RIF, TET, STREPTO, KAN, NEO. CPD, SZ, AMX, PEN, CHMP, MET, ERYTHRO, CEF, AMP, CIP, PB, RIF, NEO, NR, NA, OF, TET. PEN, CEF, MET, RIF, CIP, PB, AMX, CPD, NR, CHMP, NA, ERYTHRO, KAN, NEO, AMP, OF.	4	16	0.8
17.	CPD, AMX, PEN, AMP, CHMP, MET, ERYTHRO, NR, RIF, SZ, CEF, CIP, KAN, NA, OF, TET, NEO. CPD, AMX, PEN, AMP, CHMP, MET, ERYTHRO, NR, RIF, STREPTO, CIP, KAN, NA, TET, PB, SZ, OF.	2	8	0.85
18.	CPD, PEN, MET, ERYTHRO, NR, RIF, STREPTO, CHMP, CIP, AMX, NA, PB, CEF, NEO, TET, AMP, KAN, GEN.	1	4	0.9
20.	CPD, AMX, PEN, CEF, CHMP, PB, MET, ERYTHRO, RIF, STREPTO, AMP, CIP, GEN, NR, TET, KAN, SZ, NA, NEO, OF.	1	4	1

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.

A variety of *bla* genes identified in bacteria derived from different environmental sources such as water or sediments of aquaculture areas (Srinivasan et al., 2005; Jacobs and Chenia, 2007), STPs (Szczepanowski et al., 2004; Leski et al., 2013; Antunes et al., 2006; Taviani et al., 2008), and surface water (Poppe et al., 2006). The environmen-

tal compartments may further serve as reservoirs for β-lactam resistance genes. The *bla* genes are often detected in environmental pathogens including *Aeromonas* (Jacobs and Chenia 2007), *Enterobacter* (Leski et al., 2013), *Salmonella* (Antunes et al., 2006; Moura et al., 2007), *Staphylococcus* (Volkmann et al., 2004), and *Vibrio* spp. (Taviani et al., 2008). AmpC gene encoding β-lactamases has been detected in the microbial isolates from wastewater, surface water and even from drinking water films (Blaak et al., 2010). *bla* genes often coexist with other antimicrobial resistance determinants and can also be associated with mobile genetic elements, increasing the possibility of multidrug resistance and environmental dissemination (Schlüter et al., 2007).

A high antibiotic resistance had been reported in the past two decade (Michael, 2009) 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 *E. coli* 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 (I, II and III). All isolates showed multiple resistance to antimicrobial agents tested. Of the 100% isolates from sites II and III there was resistance against Amoxicillin, Polymixin B, Methicillin and Ampicillin, Polymixin B, Penicillin, respectively. In the case of site I, 100% resistance was not observed against any antibiotic. Of the >50% isolates were found resistant against most of the antibiotics tested from site I while it was observed against 7 and 6 antibiotics among the isolates from sites II and III, respectively. In the case of multiple resistance, most of the isolates showed multiple antibiotic resistance. All the isolates from all sampling sites showed 11-12 resistance patterns for 20 antibiotics. In the case of site I, 18.5 and 25.6% isolates showed resistance to 11 and 10 antibiotics at a time in 5 and 7 different combinations, respectively, while 12, 16% and 20% isolates from site II showed resistance to 17, 11, and 15 antibiotics at a time in 3, 4 and 5 different combinations, respectively. Of the 4%, isolates from site-III exhibited multiple resistance to 7, 8, 11, 18 and 20 antibiotics at a time in one combination, respectively. However, the high level of *E. coli* resistance to tested antibiotic seems to correspond to the report of Adegunloye (2006). Most of the isolated strains of *E. coli* showed high level of resistance more than other bacteria from the intestinal tract as reported by Esposito and Leone (2007). The bacterial isolates showed high level of antibiotic resistance against all used antibiotics. The result was in agreement with that of Muhammad et al. (2010) 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.

In another study conducted in Pakistan, the susceptibility pattern of the urinary tract infection causing *E. coli* was studied. The susceptibility pattern of imepenem was 98%, while meropenem was 97%. Gentamicin had a sensitivity of 48%, while Ciprofloxacin was 35% and Cotrimoxazole was 17%. They also concluded that multidrug resistant and ESBL producing *E. coli* was in large proportion in this region (Ullah et al., 2009). The pattern of sensitivity was also affected with the type of infection, as ESBL producers had high rate of resistance to cephalosporin and penicillin groups as compared to non ESBL producers.

CM Jardine (2012) reported that there were larger multiple antibiotic resistance of *E. coli* isolated in urban areas than from rural areas. Ramteke (1997) studied the antibiotic resistance of 448 coliforms isolated from drinking water and their tolerance to heavy metals. More than 90% of metal tolerant isolates showed resistance to one or more antibiotics tested. Parveen et al. (1997) studied total 765 *E. coli* isolates for their multiple-antibiotic resistance profiles with 10 antibiotics and stated antibiotics resistance pattern influenced by geographical condition.

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 (Moon, 2013). MAR index values higher than 0.2 were considered to have originated from high-risk sources where antibiotics are often used (Hemen et al., 2012).

In our study we also determined the MAR index of *E. coli* 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 *E. coli* isolates from the water samples of the Gomti River. MAR range 0.1-0.7, 0.3-0.9 and 0.35-1.0 were recorded among the isolates from site I (polluted), site II (polluted) and site III (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 (Chitanand et al., 2010)

All *E. coli* strains isolated from river and polluted waters show a high incidence of MAR phenotype. Many investigators have recognized that wastewater treatment plants are the principal recipients of enteric bacteria with multiple antibiotic resistance (Selvaratnum and Kuberger, 2004) and an important site for horizontal gene transfer, by containing nutrients and high concentrations of microorganisms (Sloan et al., 2014).

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 *E. coli* isolates from food should prompt an immediate investigation even though the number of *E. coli* 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 (Sloan et al., 2014). Some studies have found that wastewater treatment can raise or lower the proportions of antibiotic resistant bacteria which carry antibiotic resistance plasmids (Silva et al., 2006). The observation of increased resistance frequency to ampicillin, tetracycline, streptomycin and chloramphenicol after wastewater treatment has previously been reported by Reinthaler et al. (2013).

High-MAR *E. coli* are also the major reservoirs for enteric diseases which are transmitted to human through food and water. It was also found that nitrofurazone-resistant *E. coli* 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, signalling fecal contamination from this source (Bendall, 2009).

The aim of this 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 *E. coli* is indicative of general hygienic quality of the water and potential risk of infectious diseases from water.

ACKNOWLEDGEMENTS

We are thankful to Prof. S.W. Akhtar, Vice Chancellor, Integral University, for providing the necessary facility to conduct this research. The authors also thank the HOD, Department of Bio-Sciences and Bio-Engineering, Integral University, Lucknow for guidance and their cooperation with regard the research work.

REFERENCES

Adegunloye DV (2006). Microorganisms associated with poultry faeces. J. Food Agric. Environ. 4:41-42.

Ahmed W, Goonetilleke A, Gardner T (2010). Implications of faecal indicator bacteria for the microbiological assessment of roof-harvested rainwater quality in southeast Queensland, Australia. Can. J. Microbiol. 56(6): 471-479.

Alpay-Karaoglu S, Ozgumus OB, Sevim E, Kolayli F, Sevim A, Yesilgil P (2007). Investigation of antibiotic resistance profile and TEM-type β-lactamase gene carriage of ampicillin- resistant Escherichia coli strains isolated from drinking water. Annal. Microbiol. 57: 281-288.

Batt AL, Bruce IB, Aga DS (2006). Evaluating the vulnerability of surface waters to antibiotic contamination from varying wastewater treatment plant discharges. Environ. Pollut.142: 295-302.

Bendall JG (2009) Comment on New Reagent for Trace Determination of Protein-Bound Metabolites of Nitrofurans in Shrimp Using Liquid Chromatography with Diode Array Detector. J. Agric. Food Chem. 57(23):11446–11447.

Blaak H, Schets FM, Italiaander R, Schmitt H, de Roda Husman AM (2010) Antibioticaresistente bacteriën in Nederlands oppervlaktewater in veeteeltrijk gebied. RIVM rapport 70371903, 9-10.

Chitanand MP, Kadam TA, Gyananath G, Totewad ND, Balhal DK (2010). Multiple antibiotic resistance indexing of coliforms to identify high risk contamination sites in aquatic environment. Indian J. Microbiol. 50: 216-220.

Dang H, Zhang X, Song L, Chang Y, Yang G (2006). Molecular characterizations of oxytetracycline resistant bacteria and their genes from mariculture waters of China. Mar. Poll. Bull. 52: 1494-1503.

Dierikx C, Essen-Zandbergen AV, Veldman K, Smith H, Mevius D. (2010). Increased detection of extended spectrum beta-lactamase producing *Salmonella enterica* and *Escherichia coli* isolates from poultry. Vet. Microbiol. 145(3-4) 273–278.

Downing T, Imamura H, Decuypere S, Clark TG, Coombs GH, Cotton JA, et al (2011). Whole genome sequencing of multiple*Leishmania donovani* clinical isolates provides insights into population structure and mechanisms of drug resistance. Genome Res. 21: 2143-2156

Drewnowska JM, Swiecicka I (2013). Eco-Genetic Structure of Bacillus cereus sensu lato Populations from Different Environments in Northeastern Poland. PLoS ONE 8(12): e80175. doi:10.1371/journal.pone.0080175.

Esposito S, Leone S (2007). Antimicrobial treatment for Intensive Care Unit (ICU) infections including the role of the infectious diseases specialist. Int. J. Antimicrob. Agents 29: 494-500.

Fischbach MA, Walsh CT (2009) Antibiotics for Emerging Pathogens. Science 325: 1089-1093.

Florea AB (2011). Antimicrobial susceptibility of Eschericia coli isolated from aries river (Romania). Analele Universităţii din Oradea - Fascicula Biologie 18(1): 34-38.

Hemen JT, Johnson JT, Ambo EE, Ekam VS, Odey MO, Fila WA (2012). Multi-Antibiotic Resistance of Some Gram Negative Bacterial Isolates from Poultry Litters of Selected Farms in Benue State. IJST 2(8): 543-547.

Igbinosa EO, Okoh AI (2008). Emerging *Vibrio* species: an unending threat to public health in developing countries. Res. Microbiol. 159(7–8): 495–506.

Jacobs L, Chenia HY (2007) Characterization of integrons and tetracycline resistance determinants in Aeromonas spp. Isolated from South African aquaculture systems. Int. J. Food Microbiol. 114: 295–306.

Jardine CM, Janecko N, Allan M, Boerlin P, Chalmers G, Kozak G, McEwen SA, Reid-Smith RJ (2012) Antimicrobial Resistance in Escherichia coli Isolates from Raccoons (Procyon lotor) in Southern Ontario, Canada. Appl. Environ. Microbiol. 78(11):3873.

Leski TA, Bangura U, Jimmy DH, Ansumana R, Lizewski SE, Li RW, Stenger DA, Taitt CR, Vora GJ (2013) Identification of blaOXA-$_{51}$-like, blaOXA-$_{58}$, blaDIM-$_{1}$, and blaVIM carbapenemase genes in hospital Enterobacteriaceae isolates from Sierra Leone. J. Clin. Microbiol. 51(7): 2435-2438.

Moon H (2013) Efficacy of essential oils as an anti-bacterial agent for the therapeutic management of Clinical MAR Index E. coli. Asiatic J. Biotechnol. Resour. 04 (01) 44-48.

Moura A, Henriques I, Ribeiro R, Correia A (2007). Prevalence and characterization of integrons from bacteria isolated from a slaughter house wastewater treatment plant. J. Antimicrob. Chemother. 60: 1243–1250.

Muhammad M, Muhammad LU, Ambali AG, Mani AU (2010). A survey of early chick mortality on small-scale poultry farms in Jos, Central Nigeria. Int. J. Poult. Sci., 9: 446-449.

Parveen S, Murphree RL, Edmiston L, Kaspar CW, Portier KM, Tamplin ML (1997). Association of multiple antibiotic resistance profiles with point and nonpoint sources of Escherichia coli: in Apalachicola bay. Appl. Environ. Microbiol. 63(7): 2607-2612.

Patoli AA, Patoli BB, Mehraj V (2010) High Prevalence of drug resistant Escherichia coli in drinking water samples from Hyderabad. Gomal. J. Med. Sci. 8 (1):23-26

Poppe C, Martin L, Muckle A, Archambault M, McEwen S, Weir E (2006). Characterization of antimicrobial resistance of Salmonella Newport isolated from animals, the environment, and animal food products in Canada. Can. J. Vet. Res. 70: 105–114.

Ramteke PW (1997). Plasmid mediated cotransfer of antibiotic resistance and heavy metal tolerance in coliforms. Indian J. Microbiol. 37: 177-181.

Riaz S, Faisal M, Hasnain S (2011). Antibiotic susceptibility pattern and multiple antibiotic resistances (MAR) calculation of extended spectrum β- lactamase (ESBL) producing Escherichia coli and Klebsiella species in Pakistan. Afr. J. Biotechnol. 10(33): 6325-6331.

Schlüter A, Szczepanowski R, Pühler A, Top EM (2007). Genomics of IncP-1 antibiotic resistance plasmids isolated from wastewater treatment plants provides evidence for a widely accessible drug resistance gene pool. FEMS Microbiol. Rev. 31: 449–477.

Silva J, Castillo G, Callejas L, López H, Olmos J (2006). Frequency of transferable multiple antibiotic resistance amongst coliform bacteria isolated from a treated sewage effluent in Antofagasta, Chile. Electron. J. Biotechnol. 9(5): 533-540.
Sloan DB, Nakabachi A, Richards S, Qu J, Murali SC, Gibbs RA, Moran NA (2014) Parallel Histories of Horizontal Gene Transfer Facilitated Extreme Reduction of Endosymbiont Genomes in Sap-Feeding Insects. Mol. Biol. Evol. doi:10.1093/molbev/msu004.
Soderstrom A, Osterberg P, Lindqvist A, Jönsson B, Lindberg A, Blide S et al., (2008). A Large Escherichia coli O157 Outbreak in Sweden Associated with Locally Produced Lettuce. Foodborne Pathog. Dis. 5(3): 339-349.
STAS 3001-91 – Water, bacteriological analysis.(Please provide Year)
Taviani E, Ceccarelli D, Lazaro N, Bani S, Cappuccinelli P, Colwell RR, Colombo MM (2008). Environmental Vibrio spp., isolated in Mozambique, contain a polymorphic group of integrative conjugative elements and class 1 integrons. FEMS Microbiol. Ecol. 64: 45–54
Tsiodras S, Kelesidis T, Kelesidis I, Bauchinger U, Falagas ME (2008). Human infections associated with wild birds. J. Infect. 56: 83-98.
Ullah F, Malik SA, Ahmed J (2009). Antibiotic susceptibility pattern and ESBL prevalence in nosocomial Escherichia coli from urinary tract infections in Pakistan. Afr. J. Biotechnol. 8: 3921-3926.
Vulliet E, Cren-Olive C (2011) Screening of pharmaceuticals and hormones at the regional scale, in surface and groundwaters intended to human consumption. Environ. Pol. 159 (10):2929–2934.
Wright MS, Baker-Austin C, Lindell AH, Stepanauskas R, Stikes HW, McArthur JV (2008). Influence of industrial contamination on mobile genetic elements: class 1 integron abundance and gene cassette structure in aquatic bacterial communities. ISME J. 2: 417–428
Wright MS, Peltier GL, Stepanauskas R, McArthur JV (2006). Bacterial tolerances to metals and antibiotics in metal-contaminated and reference streams. FEMS Microbiol. Ecol. 58: 293–302.