



Potential of Surface Water Gram-negative Bacterial Flora as a Reservoir of Heterogeneous Plasmid and Multi-drug Resistance Phenomenon

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Authors' contributions

This work was carried out in collaboration between all authors. Author MHR designed the study. Authors FA, MIR and SB performed the statistical analysis. Author MHR wrote the protocol and the first draft of the manuscript. Authors FA, MIR managed the data acquisition of the study and literature searches. Authors NJ and ZUA did critical revision. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Present study highlights the diversity of Gram-negative bacterial community at surface water and their potential as a reservoir of antibiotic resistance genes. We also explore the potential of this heterogeneous community to host plasmids of diverse origin.

Place and Duration of Study: The study was carried out at the Department of Microbiology, Jahangirnagar University, Bangladesh during a period from July 2016 to June 2017.

Methodology: Water samples were cultured onto MacConkey plates for selective isolation of Gram-negative bacteria and identified according to Bergey's Manual of Systematic Bacteriology.

Antimicrobial susceptibility was done by disc-diffusion method followed by determination of MIC assay by agar dilution method. Plasmid extraction was done according to the hot alkaline method.

Results: A total of 197 representative isolates from 310 water sample culture, 110 were identified as *E. coli* and 87 as non-*E. coli* belonged to 14 different species of the genera *Escherichia*, *Enterobacter*, *Citrobacter*, *Klebsiella*, *Serratia*, *Proteus*, *Kluyvera*, *Obesumbacterium* and *Yersinia*. Susceptibility test showed highest incidence of resistance phenomenon against amoxicillin (66.5%), followed by cefixime (56.8%), sulfamethoxazole-trimethoprim (33.5%), ciprofloxacin (20.3%), tetracycline (19.79%) and gentamicin (18.78%). A notable proportion of the isolates (33.9%) showed multi-antibiotic resistance (MAR) phenomenon. The occurrence of MAR phenotype was almost twice (42%) as much as in *E. coli* population in compared to non-*E. coli* (22%) population. Plasmid extraction revealed that majority (57%) of the isolates contained plasmids with 59 different profile, incidence and diversity being higher in *E. coli* population.

Conclusion: The study indicates that surface-water Gram-negative bacterial flora is a reservoir of plasmids and multidrug resistance gene. *E. coli* population seems to be more potential in compared to non-*E. coli* population as a reservoir of both multi-antibiotic resistance genes and heterogeneous plasmids.

Keywords: Plasmid; multidrug resistance; gram-negative bacteria.

1. INTRODUCTION

Non-clinical environment such as surface water, wastewater, sediments and soils have been increasingly appreciated as a reservoir and source of widespread occurrence of resistance genes [1,2]. Discharge of wastewater from clinical sources and use of antibiotic in animal agriculture are the predominant source of surface water pollution with antibiotics escalating antibiotic resistance problem. Plasmids are directly implicated in carrying resistance in many enteric bacteria [3,4,5,6]. In addition to drug resistance genes, plasmids have been reported to encode virulence genes among diverse pathogenic bacteria including enteropathogens [7,8]. Bangladesh is a country of highest population density. Several recent studies show heterogeneous plasmids population and accumulation of resistance phenomenon against major clinically important antibiotic groups in intestinal normal Gram-negative flora [9,10,11]. As antibiotic resistance in the aquatic environment is thought to be related to proximity of anthropogenic activity [2], it was worth speculation that surface water isolates will exhibit similar trends of antibiotic resistance and would host diverse plasmid population. This might have serious public health implications as household usage of surface water still exist in the rural community. Limited information is available on the occurrence of resistance phenomenon against clinically related antibiotic in environmental Gram-negative microbiota of Bangladesh. Even less information can be found on the occurrence of plasmid in this heterogeneous population. Several studies have

been focused on *E. coli*- associated antibiotic resistance of clinical or environmental origin [12,13,14]. While Gram-negative bacteria other than *E. coli*, that are frequently found in environment and intestine is generally remained unexplored as a reservoir of resistance and/or pathogenic genes.

Therefore, the present study aimed to explore the species diversity of Gram-negative microbiota of surface water and describe their potential as a reservoir of antibiotic resistance genes. Additionally, we also wanted to explore the potential of this heterogeneous community to host plasmids of diverse origin and explore the possible association between the resistance phenomenon with their plasmid content.

2. MATERIALS AND METHODS

2.1 Isolation of Gram-negative Bacteria from Surface Water

A total of 310 surface water samples were collected from 50 different ponds at 25 sampling site from Jahangirnagar University (JU) and villages at the vicinity of JU campus. The water samples were used for the isolation of Gram-negative bacteria during a period from July 2015 to June 2016. After plating diluted water samples onto MacConkey plate, a total of 197 representative colonies were picked up for purification, storage and further characterisation. These isolates were identified by biochemical characterisation according to Bergey's Manual of Systematic Bacteriology [15] and Cappuccino & Sherman [16].

2.2 Antimicrobial Assay

Bacterial susceptibility test to antibiotics was performed by disc-diffusion method [17] followed by determination of MIC by agar dilution method and interpreted according to CLSI standards and guidelines [18]. Each isolate was checked for their possession of resistance phenotype against commonly used antibiotics belonging to six different groups. The groups of antibiotics included β -lactam (Amoxicillin), aromatic acetogenin (Tetracycline), aminoglycoside (Gentamicin), sulfonamide (Trimethoprim-sulphamethoxazole), fluoroquinolones (Ciprofloxacin), and cephalosporin.

2.3 Plasmid DNA Isolation

Plasmids were extracted according to modified hot alkaline by Kado & Liu [19] and electrophoresis in a 0.7% agarose gel.

3. RESULTS

3.1 Multiplicity of Gram-negative Bacterial Flora in Surface Water

A total of 310 surface water samples from 50 different ponds at 25 sites were collected during a period from July 2015 to June 2016. The water samples were cultured on--into MacConkey plates for selective isolation of Gram-negative environmental bacteria. A total of 197 representative colonies were selected and preserved parallel at 4°C and -80°C. Among 197

biochemically characterised isolates 110 were identified as *E. coli* while other 87 were non- *E. coli* belonging to *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Enterobacter cancerogenus*, *Enterobacter asburiae*, *Yersinia intermedia*, *Citrobacter rodentium*, *Serratia marcescens*, *Serratia fonticola*, *Escherichia fergusonii*, *Kluyvera ascorbata*, *Obesumbacterium proteus* and *Proteus spp.*(Table 1).

3.2 Major Proportion of Environmental Gram-negative Bacteria Carries Resistance Phenomenon against Clinically Important Antibiotics

All 197 isolates were tested for susceptibility against commonly used antibiotics by disc-diffusion [17] and MIC assay [18]. Majority of the Gram-negative isolates (87%; 171 of 197) showed resistance to one or more antibiotic in susceptibility test. A notable proportion of the isolates (33.9%; 65 of 197) showed multi-drug resistance phenotype. In general, the highest incidence of resistance phenomenon in a diverse community of Gram-negative bacteria was recorded against amoxicillin (66.5%), followed by cefixime (56.8%), sulfamethoxazole-trimethoprim (33.5%), ciprofloxacin (20.3%), tetracycline (19.79%), and gentamicin (18.78%). Table 2 shows the resistance percentage against individual antibiotics and a range of MIC displayed by the diverse community of environmental Gram-negative bacteria.

Table 1. Multiplicity of environmental gram-negative bacteria in surface water

Name of the isolates	Number of isolates (n)	% of occurrence	Total number of isolates
<i>Escherichia coli</i>	110	56	197
<i>Klebsiella pneumoniae</i>	15	7.6	
<i>Serratia marcescens</i>	12	6	
<i>Klebsiella oxytoca</i>	10	5	
<i>Enterobacter cancerogenus</i>	9	4.6	
<i>Enterobacter asburiae</i>	7	3.5	
<i>Enterobacter aerogenes</i>	6	3	
<i>Enterobacter cloacae</i>	5	2.5	
<i>Obesumbacterium proteus</i>	4	2	
<i>Yersinia intermedia</i>	4	2	
<i>Citrobacter rodentium</i>	4	2	
<i>Serratia fonticola</i>	4	2	
<i>Escherichia fergusonii</i>	3	1.5	
<i>Kluyvera ascorbata</i>	2	1	
<i>Proteus sp.</i>	2	1	

Table 2. Resistance frequency and MIC range against individual antibiotics in environmental Gram-negative bacteria (n=197)

Bacteria	Percent of resistance against different antibiotics					
	AMX	CFM	CIP	CN	SXT	TE
Total Gram-negative isolates (n=197)	65	57	20	19	33	20
<i>E. coli</i> isolates (n=110)	55	43	34	26	43	28
Non- <i>E. coli</i> isolates (n=87)	80	75	3	9	22	9
MIC range of Resistant isolates ($\mu\text{g/ml}$)	≥ 25 to ≥ 200	≥ 10 to ≤ 40	≥ 10 to ≥ 40	≥ 10 to ≥ 20	$\geq 41.5/ 8.5$ to $>166/34$	≥ 20 to ≥ 200

AMX, Amoxicillin; CFM, Cefixime; CIP, Ciprofloxacin; CN, Gentamicin; SXT, Sulphamethoxazole-Trimethoprim; TE, Tetracycline

3.3 Resistance Phenomenon in *E. coli* Isolates is Higher than Non-*E. coli* Community against Most of the Antibiotics

Among 197 isolates (110 *E. coli* and 87 non-*E. coli*) prevalence of resistance against individual antibiotics were analysed for comparison between *E. coli* and non-*E. coli* isolates. Among 110 isolates, 37 isolates (34%) of *E. coli* were resistant to ciprofloxacin while only about 3% (3 of 87) non-*E. coli* isolates showed resistance phenotype to ciprofloxacin. Similarly, *E. coli* isolates showed higher percentage of resistance against sulphamethoxazole-trimethoprim (43%; 47 of 110), tetracycline (28%; 31 of 110) and gentamicin (26%; 29 of 110) in compared to non-*E. coli* community that showed only 22%, 9% and 9% resistance respectively. In contrast, *E. coli* showed 55% (61 of 110) and 43% (47 of 110) resistance for amoxicillin and cefixime respectively, which is much lower in compared to non-*E. coli* isolates that showed 80% (70 of 87) and 75% (65 of 87) resistance phenotype respectively. Table 2 indicates the relative resistance percentage of *E. coli* and non-*E. coli* population against six clinically important antibiotics belonging to six different groups.

3.4 Multi-antibiotic Resistance Phenomenon is More Frequent in *E. coli* (n=110) than that of Non-*E. coli* (n=87) Isolates of Intestinal Bacteria

A notable proportion of the isolates (34%; 65 of 197) showed resistance to three or more groups of antibiotics. The genotypes of the multidrug resistance phenomenon observed in *E. coli* and non-*E. coli* population was shown separately in Tables 3 and 4 respectively. % among 110

isolates, 46 isolates of *E. coli* (42%) was found to be multi-antibiotic resistant. The predominant MAR phenotypes for *E. coli* isolates were AML-CFM-CIP-CN-SXT-TE and AML-CFM-CIP observed in 5.5% (6 of 110) of the isolates. MDR phenotypes AML-CIP-CN-SXT-TE and AML-CFM-CN were observed in 3.64% (4 of 110) isolates. Each of the AML-CFM-CIP-SXT-TE, AML-CFM-CIP-CN-SXT and AML-CIP-SXT-TE were detected in 2.73% (3 of 110) of the isolates. CFM-CIP-SXT-TE, AML-CIP-CN-SXT, AML-CIP-TE and AML-CFM-CIP phenotypes were observed in 1.82 % isolates. Besides these, 9 unique phenotypes of multi-antibiotic resistance against various combinations of 3, 4 and 5 group of antibiotics were detected in 9 individual isolates (Table 3).

The occurrence of multi-antibiotic resistance is only 22% (19 of 87) in non-*E. coli* community, much lower than that of *E. coli* isolates with 42% MAR phenomenon. The predominant MAR phenotype for the non-*E. coli* isolates was AML-CFM-SXT reported in 12.64 % (11 of 87) isolates. Each of AML-CFM-SXT-TE and AML-CFM-CN MAR phenotypes was observed in two isolates. MAR phenotype AML-CFM-CIP-CN-SXT-TE, with resistance against six antibiotics, was observed in single isolates. Similarly unique phenotypes AML-CFM-CIP-SXT, AML-CFM-CIP-CN and CFM-SXT-TE were exhibited by three individual isolates.

3.5 Gram-negative Bacteria in Surface Water Host Heterogeneous Plasmid Profile with Higher Incidence in *E. coli* than Non-*E. coli* Population

Plasmid extraction of 197 gram-negative isolates revealed that 57% (113 of 197) were found to

contain plasmids with 57 different profiles, number of bands varied from 1 to 6. Reference strain PDK-9 and V-517 that were included in each extraction- consistently produced a fixed number of bands. So, multiple bands in the gel were regarded as different plasmids. Plasmid profile of 197 (110 *E. coli* and 87 non-*E. coli*) Gram-negative bacteria were analysed for comparison between *E. coli* and non-*E. coli* isolates. Among 110, 85 isolates of *E. coli* (77%) were found to contain plasmids with 43 different profiles (Fig. 1). Table 5 shows plasmid patterns and plasmid contents of each pattern, along with their approximate molecular weight as shown in Fig. 1. Among 85 plasmid containing isolates, 25 exhibited unique profiles while the rest 60 isolates shared 18 plasmid profiles. In non-*E. coli* population prevalence of plasmid is 32 % (28

of 87) with 16 different profiles. Of 28 plasmid positive non-*E. coli* isolates, 9 showed unique profiles while the rest 6 profiles were shared by 19 isolates (Table 6; Fig. 2). The occurrence of plasmid is about 2.5 fold higher in *E. coli* (77%) in compared to non-*E. coli* population (32%).

Lane 1, 32 represent plasmid DNA markers PDK-9 and Lane 16, 48 represent V517, the two *E. coli* K-12 strain derivatives. Lane 18 represents 25 plasmidless isolates shared by both all sensitive and resistant *E. coli*. Lane 25 displays seven plasmids shared by seven isolates susceptible to all six antibiotics. Lane 3, 8, 15, 44, 46 shared by phenotypically resistant isolates to six antibiotics carried a variable number of small and large plasmids. Each of the lanes 2, 17, 22 shared by single isolate sensitive

Table 3. Multiple antibiotic resistant phenotypes detected in *E. coli* (n=110) from surface water

Phenotype	No. of isolates (n)	Percentage
AML-CFM-CIP	2	1.82
AML-CFM-CN	4	3.64
AML-CFM-SXT	1	0.91
AML-CFM-TE	1	0.91
AML-CIP-CN	1	0.91
AML-CIP-TE	2	1.82
CIP-SXT-TE	1	0.91
CN-SXT-TE	1	0.91
AML-CFM-CIP-SXT	6	5.45
AML-CFM-SXT-TE	1	0.91
AML-CIP-CN-SXT	2	1.82
AML-CIP-SXT-TE	3	2.73
AML-CN-SXT-TE	1	0.91
CFM-CIP-SXT-TE	2	1.82
AML-CFM-CIP-CN-SXT	3	2.73
AML-CFM-CIP-CN-TE	1	0.91
AML-CFM-CIP-SXT-TE	3	2.73
AML-CIP-CN-SXT-TE	4	3.64
CFM-CIP-CN-SXT-TE	1	0.91
AML-CFM-CIP-CN-SXT-TE	6	5.45
Total = 46		

Table 4. Multiple antibiotic resistant phenotypes detected in non-*E. coli* bacteria (n=87) from surface water

Phenotype	No. of isolates (n)	Percentage
AML-CFM-CN	2	2.29
AML-CFM-SXT	11	12.64
CFM-SXT-TE	1	1.14
AML-CFM-CIP-CN	1	1.14
AML-CFM-CIP-SXT	1	1.14
AML-CFM-SXT-TE	2	2.29
AML-CFM-CIP-CN-SXT-TE	1	1.14
Total= 19		

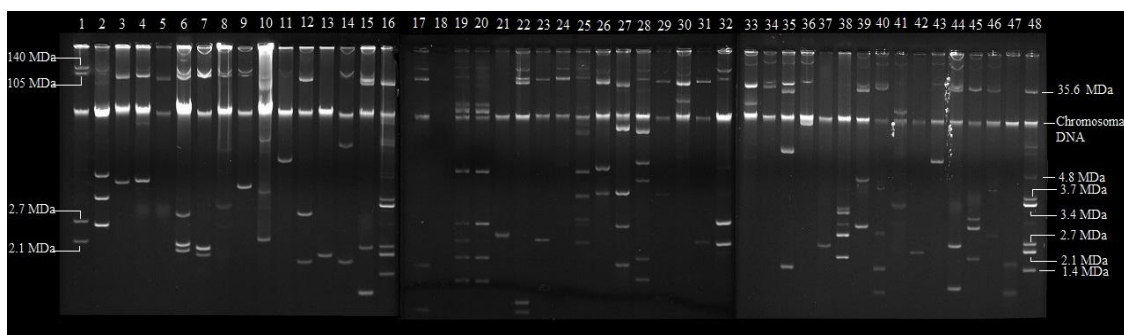


Fig. 1. Plasmid profile of *E. coli* isolates from surface water

to all antibiotics, carried 4 to 6 plasmids. Each of lane 19 and 28 shared by 1 and 4 isolates respectively with both all sensitive and multidrug resistant phenotypes carried six plasmids ranges from ~1.2 to ~140 MDal.

Lane 1 represents plasmid DNA markers obtained from *E. coli* K-12 strains PDK-9 and 10 and 19 for V-517. Lane 2-9 and lane 11-18 represent 16 different plasmid profiles. Lane 2

represents 59 plasmidless non-*E. coli* shared by both sensitive and multidrug resistant isolates. Lane 7 and 8 shared by both sensitive and multi drug resistant isolates shows two large plasmids. Lane 12 belongs to a multi-antibiotic resistant single isolate carries multiple plasmids. Each of the lanes 13, 14, 17 and 18 also represented unique profile phenotypically resistant to either one of the six antibiotics.

Table 5. Plasmid pattern and distribution in *E. coli* isolates of surface water origin

Plasmid pattern	Lane in Fig. 1	Plasmid content (MDal)	Number of isolates	Total isolates
1	2	140, 3.65, 3.4, 2.7	1	110
2	3	120, 3.6	4	
3	4	140, 3.5	5	
4	5	35	3	
5	6	130, 3.0, 2.0, 1.9	2	
6	7	130, 2.0, 1.8	2	
7	8	130, 3.1, 2.5	2	
8	9	3.3	3	
9	10	4.5, 3.5, 2.2	2	
10	11	3.6	3	
11	12	130, 2.9, 1.5	1	
12	13	1.8	1	
13	14	4.5, 1.6	1	
14	15	130, 35.8, 2.0, 1.2	1	
15	17	120, 4.8, 3.6, 3.5, 2.7, 2.0	1	
-	18	No plasmid	25	
16	19	140, 3.7, 3.0, 2.4, 1.8, 1.4	1	
17	20	140, 3.7, 3.0, 1.8, 1.4	1	
18	21	2.4	1	
19	22	140, 35.8, 1.3, 1.2	1	
20	23	140, 2.4	1	
21	24	140	3	
22	25	140, 6, 3.7, 3.4, 2.7, 2.6, 2.1	7	
23	26	120, 35, 4, 3.5	1	
24	27	30, 6, 3.5, 2.7, 1.6	1	
25	28	140, 6, 4.1, 3.7, 1.9, 1.5	3	
26	29	90, 2.0, 1.2	1	

Plasmid pattern	Lane in Fig. 1	Plasmid content (MDal)	Number of isolates	Total isolates
27	30	120	6	
28	31	90, 2.4	4	
29	33	90	3	
30	34	120, 90	1	
31	35	120, 35, 4.0, 1.4	1	
32	36	140, 10	1	
33	37	2.1	2	
34	38	2.9, 2.7, 2.4, 2.1, 1.7	1	
35	39	90, 30, 3.5, 2.4	1	
36	40	140, 90, 3.4, 1.8, 1.2	1	
37	41	4.5, 3.0	4	
38	42	1.3	1	
39	43	35.8, 4.8, 4	1	
40	44	35.8, 2.0, 1.2	1	
41	45	90, 2.8, 2.5, 1.6	2	
42	46	30, 3.1	1	
43	47	1.6, 1.2	1	

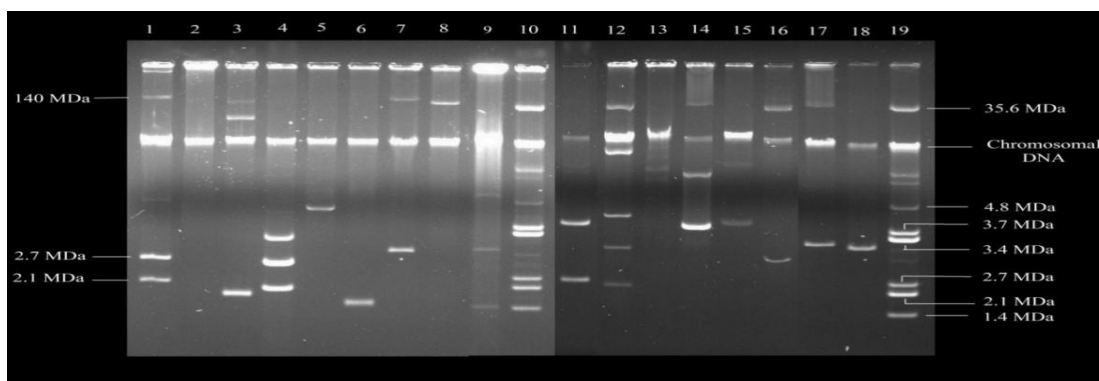


Fig. 2. Plasmid profiles of non- *E. coli* isolates from surface water

Table 6. Plasmid pattern and distribution in non-*E.coli* isolates of surface water origin

Pattern	Lane in Fig 2	Plasmid content (MDal)	Number of isolates hosting the pattern	Total isolates
-	2	No plasmid	59	87
1	3	25, 1.7	2	
2	4	3.3, 2.6, 1.8	6	
3	5	3.9	1	
4	6	1.6	2	
5	7	140, 2.9	4	
6	8	120	3	
7	9	6, 2.8, 1.6	1	
8	11	12, 10, 3.8, 2.1	1	
9	12	35.8, 16, 4.2, 2.9, 1.8	1	
10	13	14, 7	1	
11	14	6.8, 3.8	1	
12	15	15, 3.9	2	
13	16	35.8, 2.6	1	
14	17	40, 3.5	1	
15	18	3.2	1	

3.6 Occurrence of Large Plasmids is Several Folds Higher in *E. coli* Population than Non- *E. coli* Community

Among 110 *E. coli* isolates, 65 isolates (59%) contained large plasmid (≥ 20 MDal) while occurrence of large plasmid is only about 14% (12 of 87) in non-*E. coli* population. Among 110, 47 isolates of *E. coli* (43%) were found to contain plasmids with approximate molecular weight of 140 Mdal that corresponds with the large plasmid of the control strain PDK-9. On the other hand, only 8% (7 of 87) non- *E. coli* bacteria carried 140 Mdal plasmid, a size equal to virulence plasmid usually found in *Shigella sp* and entero invasive *E. coli*. Five-fold higher occurrence of large plasmid in *E. coli* along with the widespread occurrence of 140 Mdal plasmid indicates that surface water *E. coli* is a potential reservoir of the possible virulence related plasmid.

4. DISCUSSION

Surface water is known to be a reservoir of a diverse population of the gram-negative bacteria. Our objective was to study the multiplicity of Gram-negative microbiota of surface water and their potential as a reservoir of plasmids and antibiotic resistance genes. Accordingly, we have studied 197 environmental Gram-negative isolates for their resistance phenomenon against clinically important antibiotic. We also screened the isolates for the possession of plasmids and checked possible association of the resistance phenomenon with the plasmid content. A total of 197 representative Gram-negative colonies were obtained from overnight selective culture of 310 surface water samples onto MacConkey plates. Among 197 representative colonies, 110 were identified as *E. coli* by biochemical tests while the other 87 isolates belonged to 14 different species of the genera *Escherichia*, *Enterobacter*, *Citrobacter*, *Klebsiella*, *Serratia*, *Proteus*, *Kluyvera*, *Obesumbacterium* and *Yersinia*. The most dominant genera observed in this study include *Escherichia*, *Enterobacter*, *Citrobacter*, *Klebsiella* and *Serratia*. Several previous ecological studies gives testimony to the universal presence of these genera [20,21,22] in surface water. Disc-diffusion and MIC assay based susceptibility tests of this heterogeneous population were carried out against clinically relevant antibiotics. Highest incidence of resistance phenomenon was observed against

amoxicillin followed by cefixime, sulfamethoxazole-trimethoprim, ciprofloxacin, tetracycline and gentamicin in *E. coli* population. However, in case of non- *E. coli* population the order of incidence was amoxicillin, cefixime, sulfamethoxazole-trimethoprim, tetracycline, gentamicin and ciprofloxacin. Higher incidence of amoxicillin and cefixime resistance is also reported from Bangladesh in a previous study involving Gram-negative intestinal microbiota [11]. Ceftriaxone, cefixime and amoxicillin being the top listing among the frequently prescribed antibiotics in Bangladesh probably explain the reason behind higher incidence of resistance phenomenon against cefixime and amoxicillin in intestinal and environmental bacterial flora [9,11]. Ten-fold lower incidence of ciprofloxacin resistance phenomenon in non-*E. coli* community was reported in compared to *E. coli* population is in agreement with our previous studies with Gram-negative bacteria of intestinal origin [9,11]. It appears that, in general, there is no direct association between plasmid content and level of antibiotic tolerance. Among *E. coli*, there are nine isolates that were found resistant to all 6 antibiotics and harbouring 2 to 5 plasmids. However, we had other nine isolates carrying multiple plasmids but phenotypically sensitive to all six antibiotics under investigation. More interestingly, 10 isolates carried no detectable plasmids showed high MIC to several antibiotics, one of which was resistant to all six antibiotics under investigation. Similar trends of lacking association of plasmid content with resistance phenomenon were also observed in non-*E. coli* population. This finding is in agreement with our previous reports on *E. coli* and non-*E. coli* population of intestinal non-*E. coli* population [9,10].

Plasmid extraction revealed that a notable proportion of Gram-negative bacteria (57%; 120 of 197) contained plasmids with 59 different profiles. Prevalence of plasmid in majority of Gram-negative population and diversity of profile suggested that environmental Gram-negative bacteria might be serve as a reservoir of heterogeneous plasmid population. Data analysis of 197 gram-negative bacteria for comparison between *E. coli* and non- *E. coli* isolates revealed that occurrence of plasmid is more than twice as much as in *E. coli* (77% ; 85 of 110) isolates than non- *E. coli* (32 % ; 28 of 87) population. Similar trends were observed in our previous studies with intestinal bacteria from healthy human volunteers inhabiting the same locality of surface

water sampling for the present study [9,11]. However, the incidence of plasmid in non- *E. coli* community of previous study was higher (57%) than non-*E. coli* community of surface water (32%), as observed in this study. Incidence of large plasmids was about four fold higher in surface water *E. coli* (59%; 65 Of 110) isolates than non-*E. coli* (14%; 12 of 87) community. These findings deviate from our previous findings with intestinal bacteria that shows only 1.5 fold higher incidence of large plasmid in *E. coli* (60%) than non-*E. coli* (36%) community of intestinal habitat [9,11]. Together this findings indicate that, although as a reservoir of plasmid, surface water *E. coli* is comparable to *E. coli* of intestinal origin, non-*E. coli* community of the later seems more valuable than surface water community. The widespread occurrence of plasmids along with ~ 120 to 140 MDal size is indicative of the possible presence of plasmid mediated pathogenic genes (e.g., *eltA*, *eltB*, *est*, *ial*, *bfpA*) that occur in enteric bacteria [23,24,25,26]. Large plasmids carry invasive genes [27,28,29] and also known to code for mobilisable antibiotic resistance determinants that occur in *Shigellae* and enteroinvasive *E. coli* [30,31,32,33]. Frequent possession of the large plasmids with a size equivalent to the invasive plasmid indicates the possible pathogenic potential of surface water gram-negative microbiota. However, screening of this large plasmid for invasive gene is required to bring testimony for this assumption.

5. CONCLUSION

The present study suggests that surface water Gram-negative environmental bacterial flora is a treasure of heterogeneous plasmid and multidrug resistance phenomenon. In general, *E. coli* population seems to be more potential than non-*E. coli* population as a reservoir of both multi-drug resistance phenomenon and heterogeneous plasmids. Although no direct association between plasmid content and level of antibiotic tolerance was observed, screening the isolates for the presence virulence genes may clarify the role(s) of these plasmids as well as the pathogenic potential of these environmental isolates.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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