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Antibiotic Resistance of *Escherichia Coli* Isolated from Lake Nainital, Uttarakhand State, India

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Abstract: Researchers have encountered new challenges with the discovery of multiple drug resistance in microbes. Currently, multidrug resistant bacteria are considered a major public health concern and an emerging global epidemic. Presence of *Escherichia coli* in water is used as a faecal pollution measure. In this study *E. coli* isolates were collected from 20 sample collection sites at Lake Nainital. 20 *E. coli* isolates, 1 from each sample collection sites, were examined for their antibiotic response patterns against a panel of widely used 15 antibiotics. The result of this study showed 100% resistance to Penicillin G followed by Erythromycin (80%). All isolates (100%) were found susceptible for Gentamycin. The susceptibilities for Chloramphenicol and Co-trimoxazaole were found next to Gentamycin as 90 and 85% respectively. Multiple antibiotic resistance (MAR) index was also determined. 0.73 MAR index was observed as highest in 1 isolate. 13 out of 20 isolates had more than 0.2 MAR indices. The result reveals the origin of *E. coli* isolates from an area of high antibiotics use.

Keywords: *E. coli* • Antibiotic Resistance • Multi Drug Resistance • MAR Index

Introduction

Water-borne pathogen pollution of water bodies and related diseases is a major concern for water quality worldwide (Sahoo et al., 2012). Bacteria are well-known water contaminants, several of which can contribute to waterborne diseases. *Salmonella*, *Camphylobacter*, *Staphylococcus*, *Clostridium*, *Pseudomonas* and *Escherichia coli* are the chief human pathogens liable for water contamination and water borne diseases (Parveen et al., 1997; He et al., 2007).

In 1885 Theodor Escherich gave the first description of *E. coli* (Lim et al., 2010). *E. coli* is a Gram-negative, rod-shaped and facultative anaerobic bacterium. *E. coli* is one of the member of Enterobacteriaceae and considered as commensal of lower gasterointestinal tract of human and animal (Gordon and Cowling, 2003; Jafari et al., 2012; Ifeanyi et al., 2015).

Besides this the presence of *E. coli* in water bodies is generally considered as faecal contamination. Moreover, contamination of water bodies by faecal coliform bacteria carrying drug resistance genes has become an issue of water quality of national scope and significance (Panneerselvam and Arumugam, 2012). In natural environments *E. coli* is also attributed as an important vehicle for the dissemination of genes for antibiotic resistance (AR) in different

bacteria including human pathogens (Mohanta and Goel, 2014; Bisht et al., 2019). The main factor in resistance spreading is the ability of bacteria to acquire and transmit foreign genes by moving elements such as plasmids and transposons (Li et al., 2019). Over the past two decades, there has been a significant increase in the emergence and spread of multidrug resistant bacteria (Thenmozhi et al., 2014; Bala et al., 2020).

This study investigated the presence of AR and multiple antibiotic resistance (MAR) *E. coli* isolates in Nainital lake water samples. We believe our findings are of tremendous significance and our findings will shed some light on this important issue for the water quality of Nainital Lake.

Materials and Methods

Sampling sites and sample collection

Lake Nainital is a natural kidney shaped lake with a high altitude, situated at an average height of 1937 m above sea level, 29°24' N latitude and 79°28' E longitude (Singh and Gupta, 2014). The lake receives water from springs, rainwater and 22 inlet nullahs (Purushothaman et al., 2012). There are designated two parts of the lake at the extreme ends (northwest and southwest). There are water pump stations in each of these regions for the supply of water (through lakebank filtration technique) to the Nainital city. Within sterile 500 ml polypropylene bottles (Genaxy, India) water samples were obtained from 20 locations each (Table 1). Within 2 h of processing, the water samples were taken to the laboratory. Proper cold chain was maintained.

Table 1: Locations of sample collection sites at Nainital Lake

Sr. No.	Sample	Location	Sr. No.	Sample	Location
1	S1	Shri Maa Naina Devi Temple	11	S11	Library
2	S2	Pump House	12	S12	Hotel Prince
3	S3	Shani Maharaj Temple	13	S13	Alka Hotel
4	S4	Boat House Club	14	S14	Pasaan Devi Mata Temple
5	S5	Quality Boat Stand	15	S15	Hotel Elphinstone
6	S6	Shri Golu Devta Temple	16	S16	Darshan Park
7	S7	New Capitol Cinema	17	S17	PWD Guest House
8	S8	Hanuman Mandir	18	S18	Manu Maharani Regency
9	S9	Dilli Darbar DLX	19	S19	Jheel Oxygen Center
10	S10	St. Francis Catholic Church	20	S20	Army Rest and Recoup Centre

Isolation and identification of bacterial isolates

Membrane filtration and pour plate techniques were employed for analysing the water samples. 100 ml of water samples were filtered with sterile mixed cellulose esters (MCE) membranes (Millipore, USA) with a pore size of 0.45 µm

using a vacuum filtration system. Each MCE membrane filter was then transferred to Hi-Crome (HiMedia, India) *E. coli* agar (chromogenic selective agar for *E. coli*) plates. In addition, the water samples (1 ml; undiluted) were directly plated into Hi-Crome agar plates using pour plate technique. The isolates were also plated on Eosin Methylene Blue (EMB) agar culture medium (HiMedia, India) and incubated at 37°C for overnight. Luria-Bertani (LB) broth and agar (Difco, USA) were used for general culture of bacterial isolates. Further identification and confirmation of *E. coli* was done by IMViC (indole, methyl red, Voges-Proskauer and citrate utilization) test. *E. coli* DH5α strain was used in this study as reference strain (kindly provided by Prof. A.K. Johri, School of Life Sciences, J.N.U., New Delhi, India). All the recovered isolates and reference strain were preserved in LB broth with 25% glycerol at -80ºC in deep freezer for future use.

Antimicrobial susceptibility testing

Susceptibility to antimicrobial agents was evaluated by the Kirby-Bauer's disk diffusion method on Muller-Hinton agar (HiMedia, India). Selection of antibiotics was based on the antibiotic prescription patterns in local hospitals and veterinary dispensaries; and the Clinical and Laboratory Standards Institute (CLSI) guidelines (earlier National Committee for Clinical Laboratory Standards guidelines) (CLSI, 2010). All the dehydrated culture media and antibiotic discs were obtained from HiMedia, India. Following 15 antibiotics were tested in this examination: Amikacin (30 µg), Ampicillin (10 µg), Cefixime (5 µg), Cefotaxime (5 µg), Cefuroxime (30 µg), Chloramphenicol (30 µg), Co-Trimoxazaole (25 µg), Erythromycin (15 µg), Gentamicin (10 µg), Kanamycin (30 µg), Nalidixic Acid (30 µg), Norfloxacin (10 µg), Penicillin G (10 µg), Streptomycin (10 µg) and Tetracycline (30 µg). Antimicrobial sensitivity was determined by measuring the zone diameter. Zone diameters of susceptibility testing results were categorized as per CLSI guidelines (CLSI 2010). Co-resistance (resistance to 2 antibiotics) and multi-resistance (resistance to at least 3 antibiotics) were recorded. Percent resistance was determined (by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics tested X 100) for each isolate.

Calculation of MAR index

MAR indices were determined for each isolate by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics tested (i.e., a/b, where a is the number of antibiotics to which the isolate was resistant and b is the total number of antibiotics to which the isolate was exposed; i.e., b=15) (Krumperman, 1983; Odjadjare et al., 2012).

Result

Isolation and identification of bacterial isolates

Out of 20 sampling sites, 10 (S1 to S10) were located at northwest region (Mallital) and 10 (S11 to S20) at southwest region (Tallital). Typical bacterial colonies were appeared after isolation and overnight incubation on Hi-Crome *E. coli* agar media. Colonies with bluish green color were corresponding to *E. coli*. All 20 water samples were showing the presence of *E. coli* on Hi-Crome *E. coli* agar media. Bacterial enumeration was not performed in this study. Distinct colonies in triplicate from each sample with morphology consistent with *E. coli* were picked from Hi-Crome *E. coli* agar plates and inoculated onto EMB agar plates. All EMB plate showed colonies with characteristic metallic green sheen of *E. coli* (Fig. 1). From each water sample only one putative *E. coli* colony was picked and grown on LB broth. Thus, further study comprises 20 selected putative *E. coli* isolates only. These

isolates were labelled as EC1 through EC20 respectively for their sample collection sites, i.e., S1 to S20 (Table 2). IMViC test confirmed the selected putative isolates as confirmed *E. coli* isolates

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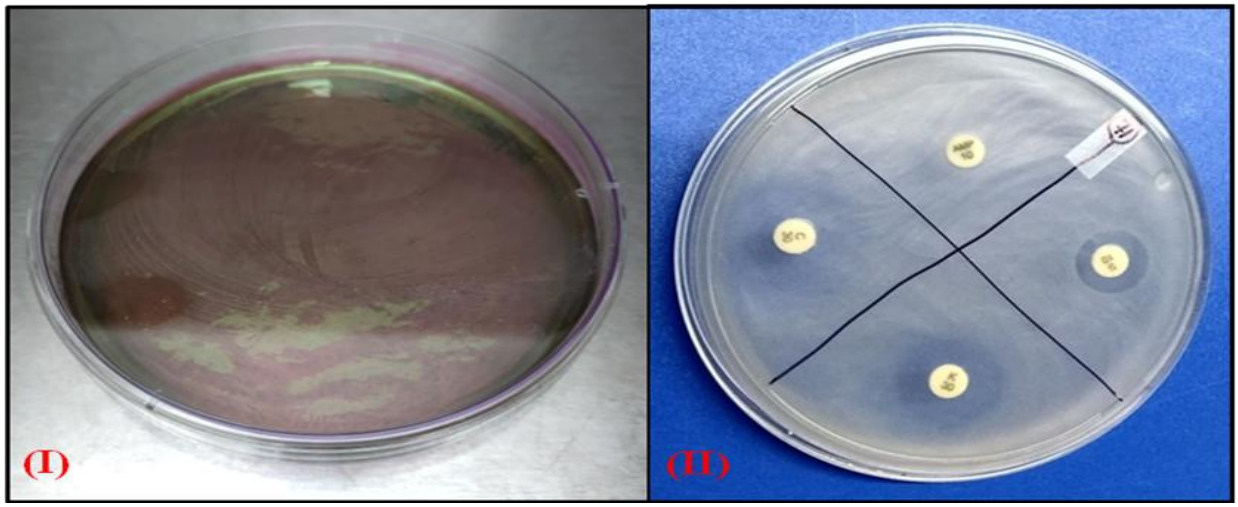

Fig. 1: *E. coli* isolates: (I). Sample 1 (S1/EC1) showing metallic green sheen on Eosin Methylene Blue (EMB) plate and (II). An isolate **(**EC8) showing resistance, susceptibility and intermediate response to antibiotics. The isolate is showing resistance against Ampicillin (AMP), susceptibility against Chloramphenicol (C); and intermediate response for Kanamycin (K) and Streptomycin (S).

Antimicrobial susceptibility testing and MAR index

E. coli isolates were categorized into 3 groups i.e. resistant (R), intermediate (I) and susceptible (S) on the basis of zone diameter measured as per CLSI standards (Fig. 1, Table 2). Of the 20 selected *E. coli* isolates (EC1 to EC20) EC1 showed 73.33% resistance against tested 15 antibiotics. 60% resistance was shown by EC5 and 53.33% by EC8. 2 isolates (EC9 and EC11) showed 46.66% resistance whereas 6 isolates (EC4, EC10, EC13, EC16, EC18 and EC20) showed 40% resistance. 2 isolates (EC15 and EC17) showed 33.33% resistance and 26.66% resistance was showed by EC2 isolate. 20% resistance was shown by EC7 and EC14, while 3 isolates (EC3, EC6 and EC19) showed 13.33% resistance. Least resistance was shown by EC12 (6.66%) against 15 antibiotics. Among the multidrug resistance (MDR) isolates, the resistance for Penicillin G was observed in all the isolates (n=20; 100%) followed by Erythromycin (80%), Ampicillin (60%), Cefotaxime (55%); Cefuroxime (45%); Cefixime, Norfloxacin and Tetracycline (40% each); Amikacin, Co-trimoxazole and Nalidixic Acid (15% each); Chloramphenicol (10%); Kanamycin and Streptomycin (5% each). All the isolates in this study showed resistance to one or more antibiotics except Gentamycin. Gentamycin showed 100% susceptibility in all tested *E. coli* isolates (n=20) (Table 3). Least MAR index (0.06) was estimated for EC12. Further, 0.10 to 0.33 MAR indices were observed in 8 isolates. Additional 8 isolates did show indices between 0.40 and 0.46. MAR indices were 0.53 and 0.60 in 1-1 isolates each. In this analysis we observed 1 isolate (EC1) with highest 0.73 MAR index (Table 2).

Table 2: Antibiotic$^{\Psi}$ sensitivity test$^{\varphi\omega}$ by Kirby-Bauer disk diffusion method on Muller-Hinton agar and MAR index

Sr. No.	*E. coli* Isolates	AK	AMP	CFM	CTX	CXM	C	COT	E	GEN	K	NA	NX	P	S	TE	Resistance (R) %	MAR Index
1	EC1	R	R	R	R	R	R	R	R	S	R	R	I	R	S	S	73.33	0.73
2	EC2	S	S	S	S	S	S	S	R	S	S	S	R	R	S	R	26.66	0.26
3	EC3	S	I	S	S	I	S	S	R	S	S	S	S	R	I	I	13.33	0.13
4	EC4	S	R	R	R	I	S	S	R	S	I	S	S	R	I	R	40.00	0.40
5	EC5	R	R	R	I	R	S	R	S	S	S	R	R	R	S	R	60.00	0.60
6	EC6	I	I	S	S	S	S	S	R	S	I	I	I	R	I	I	13.33	0.13
7	EC7	S	R	I	S	S	S	S	R	S	S	S	I	R	S	S	20.00	0.20
8	EC8	S	R	R	R	R	S	S	R	S	I	S	R	R	I	R	53.33	0.53
9	EC9	I	R	R	R	S	S	S	R	S	I	I	R	R	R	S	46.66	0.46
10	EC10	S	R	R	I	R	S	S	S	S	S	S	R	R	I	R	40.00	0.40
11	EC11	S	R	S	R	R	S	S	R	S	S	S	R	R	S	R	46.66	0.46
12	EC12	S	S	S	S	S	S	S	S	S	S	S	I	R	S	S	6.66	0.06
13	EC13	I	R	R	R	R	S	S	R	S	S	S	S	R	I	S	40.00	0.40
14	EC14	I	S	I	R	S	S	S	R	S	S	S	S	R	S	I	20.00	0.20
15	EC15	S	R	S	S	R	S	S	R	S	S	S	R	R	S	S	33.33	0.33
16	EC16	S	R	S	R	R	S	S	R	S	S	S	S	R	S	R	40.00	0.40
17	EC17	I	S	S	R	S	R	S	R	S	S	S	S	R	S	R	33.33	0.33
18	EC18	S	S	S	R	R	S	R	R	S	I	I	R	R	I	I	40.00	0.40
19	EC19	S	S	S	R	I	S	S	S	S	S	S	I	R	S	S	13.33	0.13
20	EC20	R	R	R	I	S	S	S	R	S	S	R	I	R	S	I	40.00	0.40

$^{\Psi}$Antibiotics: AK (Amikacin), AMP (AmpicilRlin), CFM (Cefixime), CTX (Cefotaxime), CXM (Cefuroxime), C (Chloramphenicol), CoT (Co-Trimoxazaole), E (Erythromycin), GEN (Gentamicin), K (Kanamycine), NA (Nalidixic Acid), NX (Norfloxacin), P (Penicillin G), S (Streptomycin) and TE (Tetracycline). $^{\omega}$R=Resistant, I=Intermediate and S=Susceptible $^{\varphi}$*E. coli* DH5α showed resistance to Nalidixic Acid only

Discussion

Nainital Lake is subtropical water body located in the Kumaun region of Uttarakhand. It serves to supply drinking water to the city and also is a big tourist attraction. The lake plays important role in socio-economic development of hill people. There has been a rapid deterioration in the water quality of the lake due to intense activities in the catchment area of the lake. The city has an over 60 year old sewage system which often chokes and bursts. The sewage overflows into rainwater channels which lead to the lake (Dash et al., 2008). This study was conducted to check the water quality and potential health risk assessment

of the Nainital Lake. Prevalence of *E. coli* in all lake water samples confirmed widespread faecal contamination in the waterbed. Unacceptable levels of coliform bacteria has already reported in the Nainital Lake and other water resources of the region (Dash et al., 2008; Jain et al., 2010; Rawat et al., 2012; Tyagi et al., 2015, Bisht et al., 2019). Most of the *E. coli* strains are harmless; some strains are pathogenic and cause death-causing diseases such as watery diarrhoea, bloody diarrhoea, urinary tract inflammation, meningitis and sepsis (Cho et al., 2018). Even though antibiotic sensitivity test was conducted on relatively less numbers of bacterial isolates, we got all isolates resistant to at least one of the tested antibiotics. The results showed high-level resistance against Penicillin G (100%) and Erythromycin (80%) whereas high level of susceptibility was recorded for Gentamicin (100%) followed by Chloramphenicol (90%) and Co-trimoxazole (85%). We already have found high Penicillin G resistance of *E. coli* isolates (recovered from drinking water sources) in our previous work (Bisht et al., 2019). It is may be due to inadequate and ineffective antimicrobial administration of antimicrobial agents in the area. Widespread AR in the environment is now at alarming situation for India and it has been reported every now and then (Walsh et al., 2011; Akiba et al., 2015; Gandra et al., 2016; DBT, 2017; Taneja and Sharma, 2019). Like ours, other studies also revealed hilly areas that have remained isolated for a long time are now reported to have potential AR threat (Rather et al., 2013; Ahammad et al., 2014; Poonia et al., 2014; DBT, 2017; Singh et al., 2020).

Table 3: Antibacterial resistance pattern of the *E. coli* isolates (n=20)

Sr. No.	Antibiotics	Resistance isolates		Intermediate isolates		Susceptible isolates	
		Numbers	Percentages	Numbers	Percentages	Numbers	Percentages
1	Amikacin	3	15	5	25	12	60
2	Ampicillin	12	60	2	10	6	30
3	Cefixime	8	40	2	10	10	50
4	Cefotaxime	11	55	3	15	6	30
5	Cefuroxime	9	45	3	15	8	40
6	Chloramphenicol	2	10	0	0	18	90
7	Co-trimoxazaole	3	15	0	0	17	85
8	Erythromycin	16	80	0	0	4	20
9	Gentamycin	0	0	0	0	20	100
10	Kanamycin	1	5	5	25	14	70
11	Nalidixic Acid	3	15	3	15	14	70
12	Norfloxacin	8	40	6	30	6	30
13	Penicillin G	20	100	0	0	0	0
14	Streptomycin	1	5	7	35	12	60
15	Tetracycline	8	40	5	25	7	35

MAR index or profile provides useful information regarding the overall health risk assessment. The 0.2 MAR indicate source bacteria with less antibiotic usage, MAR index >0.2 indicates high-

risk contamination sources while values 0.4 or higher MAR index is associated with human faecal contamination source (Kaneene et al., 2007; Kathleen et al., 2007; Mishra et al., 2013). High MAR indices need close monitoring and remedial action. In this analysis 13 out of 20 isolates had MAR index >0.2. A high MAR index for all samples suggests that these antibiotics have been used highly indiscriminately in the area. High MAR indices mandate vigilant surveillance and remedial measures. The Nainital Lake is a receptacle of water and waste water from adjoining areas and the catchments. Drains that enter the lake are one of the sources of pollution and contamination. The Nainital Lake has two basins Mallital and Tallital. Mallital (northwest basin) is a highly polluted site due to high population and intense tourist activities at this area. Also a major drain, Naina Devi Mandir drain, emptying into this area of lake. Tallital (southwest basin) on the other hand is relatively less polluted may be because of comparatively less human population and minor drains.

In developing countries such as India, AR has become a major problem, and the existence of resistant organisms in aquatic environment is a growing concern around the world. This is due to several reasons, including over-use and over-exposure of medicinal products, easy availability of antibiotics even without medical prescription, incomplete antibiotic treatments, poor hygiene, poor management of human waste and low literacy that cause poor water quality directly or indirectly (Watkinson et al., 2007; Ishii and Sadowsky, 2008). In the water bodies the AR character can easily passes from avirulent or less virulent bacteria to the other highly virulent bacteria and their virulent strains like diarrheagenic *E. coli*, *Clostridium*, *Campylobacter*, *Vibrio*, *Shigella*, *Salmonella*, *Pseudomonas*, *Legionella* through horizontal gene transfer. The expected transfer of AR might add to the burden of bacterial infectious diseases. Therefore, the issue of AR can be addressed by conducting antibiotic surveillance studies, raising public awareness and concerted efforts among physicians, research scientists, students, researchers, pharmaceutical industry and policy makers, involving government and non-governmental organizations. Moreover, this problem should be brought to the attention of all members of society so that we can maintain the effectiveness of antibiotics currently available.

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