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Research Article

INCIDENCE OF MULTI-DRUG RESISTANCE IN *Escherichia coli* STRAINS ISOLATED FROM THREE LAKES OF TOURIST ATTRACTION (MIRIK LAKE, JOREPOKHARI LAKE AND NAKHAPANI LAKE) OF DARJEELING HILLS, INDIA

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ABSTRACT
Eleven *Escherichia coli* (Enterobacteriaceae) strains previously isolated from the three lakes of Darjeeling hills were assessed for antibiotic sensitivity pattern. All the isolates were resistant to penicillin, eight isolates were resistant to Co-Trimoxazole, seven isolates were resistant to amoxicillin, six against cloxacillin, five against ampicillin, ceftazidime and erythromycin, two against cefadroxil and one isolate each was resistant to cefoperazone, ceftriaxone, nitrofurantoin and vancomycin. All isolates were sensitive to seven of the twenty antibiotics tested. The results indicate the prevalence of multidrug - resistant *Escherichia coli*.

***Key Words**: Lake Water, Darjeeling, antibiotic resistance, E. coli*

INTRODUCTION
The availability of safe drinking water is an indispensable feature for preventing epidemic diseases and improving the quality of life (Borchard *et al*., 2004). As water becomes scarce, people tend to use any locally available source without any form of pretreatment which however, leads to obvious problems of health and sanitation (Kritof, 1997). Water for human consumption and use are derived from different sources which can be natural or artificial. Natural sources include rainfall, well, bore-hole, spring, river, stream, sea, ocean and lake while artificial water sources include distilled water, purified or treated water (Nash, 1993). According to the World Health Organization (WHO, 1981), 80% of all diseases are attributed to unsafe water. Safe water should contain a maximum of one coliform bacterium per 100 ml of water (WHO, 1984). Lake waters which are used for drinking get polluted by faecal contaminanats which include several bacterial enteropathogens (Obi *et al.,* 2002). These enteric bacterial pathogens are variously incriminated in cases of diarrhea, which accounts for a substantial degree of morbidity and mortality in different age groups worldwide (Black, 1993; Nath *et. al*., 1993; El-Sheikh and El-Assouli, 2001). To further compound this problem, enteric bacterial pathogens have been widely reported to demontraate resistance to several antibiotics (Hoge *et al*., 1998; Obi *et al*., 1998; McArther and Tuckfield, 2000; Engberg *et al*., 2001; Ash *et al*., 2002). A remarkable increase in antibiotic resistance among the *E. coli* isolates has been observed during the last few years (Li *et al*, 2007).
More recently the importance of Gram-negative bacteria has increased since the advent of broad-spectrum antibiotics because these organisms often carry multiple antibiotics resistance (Mims *et al*., 1999). Sensitivity patterns and treatment must be guided by laboratory investigations (Gross, 1998).
Darjeeling hills are a favourable tourist destination and the man made as well as natural lakes in this region is crowded by tourists during peak seasons. The lakes get untreated water from several springs in its surrounding hilly catchment area.
Mirik is a small town in the Darjeeling Hills, located at 26°54’N, 88°10’E/26.9°N,88.17°E with average elevation of 1495 metres (4904 feet) above msl. It has a pleasant climate all the year round with temperatures of maximum 30°C in summer and a minimum of 2°C in winter. A man made lake in this small hilly town provides entertainment to tourists as well as water is pumped to different parts of the

town as municipal water supplies. Nakhapani is a natural lake surrounded by lustrous tea gardens and generally semi-rainfed. Jorepokhari is another man made lake of tourist attraction which is fed by untreated waters pumped up from nearby hill stream.

This study is designed to determine the antibiotic sensitivity pattern of *Escherichia coli* strains isolated from this lake during bacteriological monitoring in different seasons of the year. A remarkable increase in antibiotic resistance in bacteria and emerging multiple drug resistance strains, it seems necessary to conduct a regional research in the resistance patterns of the bacteria.

MATERIAL AND METHODS

Bacterial isolates

In total 11 *E. coli* strains were isolated previously (Sharma *et al.*, 2012) from three lakes *viz*. Mirik lake, Jorepokhari Lake and Nakhapani lake of Darjeeling hills following standard methods (APHA, 1985). They were identified by morphological, Gram Staining and biochemical characters based on Bergey's Manual of Determinative Bacteriology (1994).

Antibiotic sensitivity test

Overnight nutrient broth cultures of *E. coli* isolates were spreadplated on nutrient agar plates, antibiotic icosadiscs were placed on the plates and incubated at 37ºC for 24 hours. Inhibition zones thus obtained were measured and expressed as diameter of inhibition zone in mm. The antibiotic icosa discs was obtained from HiMedia Laboratories Ltd., Mumbai, India and antibiotic concentrations per disc included: amikacin (30 μg), ampicillin (10 μg), amoxicillin (10 μg), cefadroxil (30 μg), cefaperazone (75 μg), ceftazidime (30 μg), ceftriaxone (30 μg), chloramphenicol (30μg), ciprofloxacin (5 μg), cloxacillin (1 μg), Co-Trimoxazole (25 μg), erythromycin (15 μg), gentamicin (10 μg), nalidixic acid (10 μg), netillin (10 μg), nitrofurantoin (300 μg), norfloxacin (10 μg), penicillin (10 IU), tobramycin (10 μg) and vancomycin (30 μg).

RESULTS AND DISCUSSION

Out of the twenty antibiotics tested, the isolates showed resistance to thirteen antibiotics viz. ampicillin, amoxicillin, cefadroxil, cefaperazone, ceftazidime, ceftriaxone, cloxacillin, co-trimoxazole, erythromycin, nalidixic acid, nitrofurantoin, penicillin and vancomycin (Table 1). Of eleven isolates, ten (91%) showed resistance to penicillin, eight isolates (73%) were resistant to Co-Trimoxazole, seven (64%) were resistant to amoxicillin, six (55%) against cloxacillin and ampicillin, five (45%) against ceftazidime and erythromycin, two (18%) against cefadroxil and one isolate (9%) each was resistant to cefoperazone, ceftriaxone, nitrofurantoin and vancomycin (Table 2). The highest rate of resistance against ampicillin was also observed by Celebi *et. al*., (2007); Olowe *et al*., (2008) and Uma *et al*., (2009). This may be due to the indiscriminate use of inexpensive antibiotics in our country, India (Uma *et al.,* 2009) or may be due to production of beta-lactamase enzymes. The most common mechanism of resistance to cotrimoxazole is acquisition of plasmid mediated, variant diaminopyrimidine folate reductase enzymes (WHO, 2001).

All isolates were sensitive to seven of the twenty antibiotics tested. The highest sensitivity was shown against four antibiotics – cefoperazone, ceftriazone, nitrofurantoin and vancomycin. 91% of the isolates were sensitive to these antibiotics. 82% of the isolates were sensitive to cefadroxil, 55% to ceftazidime and erythromycin, 45% to cloxacillin and ampicillin, 36% to amoxicillin, 27% to co-trimoxazole and 9% to penicillin. Table 3 shows a detailed resistance pattern to antimicrobial agents. As strains susceptible to all drugs become less common, the proportion of isolates resistant to multiple antibiotics increase (Olowe *et al.,* 2008).

Table 1: Antibiotic Sensitivity Pattern Of Twenty Selected Strains Of *E. Coli* isolated From Lake Waters

Antibiotics	Diameter of inhibition zone in mm										
	*1	*2	*3	*4	*5	*6	*7	*8	*9	*10	*11
Amikacin(Ak 30mcg)	26	20	26	25	27	23	27	24	23	25	27
Ampicillin(AMP 10mcg)	24	0	0	24	0	15	21	0	0	0	38
Amoxycillin (AMX 10mcg)	18	0	0	21	0	0	22	0	0	0	25
Cefadroxil(CFR 30mcg)	20	37	38	0	32	15	22	0	26	35	23
Cefoperazone(CPZ 75mcg)	26	27	20	30	23	27	26	0	17	23	25
Ceftazidime(CAZ 30mcg)	30	18	22	27	0	19	0	0	0	0	30
Ceftriaxone (CTR 30mcg)	36	30	34	31	24	23	27	0	17	24	34
Chloramphenicol (C 30µg)	27	32	28	30	34	31	37	31	33	33	28
Ciprofloxacin (CIP 5mcg)	15	37	36	40	31	34	21	32	28	33	36
Cloxacillin(COX 1mcg)	15	16	0	21	0	18	18	0	0	0	0
Co-Trimoxazole(COT25mcg)	0	0	0	24	0	0	25	0	0	0	25
Erythromycin(E 15mcg)	24	26	24	0	28	0	0	0	28	24	0
Gentamycin(GEN 10mcg)	27	23	20	26	24	25	26	26	20	24	25
Nalidixic Acid (NA10mcg)	0	20	35	30	38	35	15	33	21	38	28
Netillin(NET 10mcg)	29	24	19	25	24	24	24	26	23	22	28
Nitrofurantoin(NIT300mcg)	22	20	20	20	19	19	19	25	14	0	29
Norfloxacin (NX 10mcg)	13	35	38	34	32	35	26	35	29	33	36
Penicillin (P 10units)	0	0	0	0	0	0	0	17	0	0	0
Tobramycin(TOB10mcg)	28	25	23	28	25	24	26	26	28	22	27
Vancomycin (VA 30mcg)	21	24	23	24	25	0	18	24	20	21	19

Table 2: Antibiotic sensitivity pattern of *E. coli* isolates from the lake water samples (N=11)

Antibiotics	Resistant isolates	Sensitive isolates
Ampicillin	6 (55%)	5 (45%)
Amoxycillin	7 (64%)	4 (36%)
Cefadroxil	2 (18%)	9 (82%)
Cefoperazone	1 (9%)	10 (91%)
Ceftazidime	5 (45%)	6 (55%)
Ceftriaxone	1 (9%)	10 (91%)
Cloxacillin	6 (55%)	5 (45%)
Co-Trimoxazole	8 (73%)	3 (27%)
Erythromycin	5 (45%)	6 (55%)
Nitrofurantoin	1 (9%)	10 (91%)
Penicillin	10 (91%)	1 (9%)
Vancomycin	1 (9%)	10 (91%)

Table 3: Antibiotic resistance profile of *E. coli isolates* from the lake water samples (N=11)

Number of antibiotics	**Number of isolates showing resistance**
Two antibiotics	1 (9%)
Three antibiotics	3 (27%)
Five antibiotics	3 (27%)
Six antibiotics	1 (9%)
Seven antibiotics	1 (9%)
Ten antibiotics	1 (9%)

The results show that 90% of the isolates are multiple drug resistant i.e. are resistant to two or more than two antibiotics. 81% show resistance to three or more antibiotics. Each of the isolates was resistant to at least one drug.

DISCUSSION

Pathogenic isolates of *E. coli* have relatively high potentials for developing resistance (Karlowsky *et al.*, 2004). High resistance of *E. coli* to antimicrobial agents tested was observed in this study. Our data shows that the prevalence of resistance to most drugs tested in *E. coli* isolates from the three lakes also coincides with the study of Aibinu *et al.*, (2004) who reported 100% resistance of their *E. coli* isolates to ampicillin and amoxicillin. The drugs for which *E. coli* isolates showed a considerable rise in resistance was seen for ampicillin, sulfonamides, co-trimoxazole, cefuroxine and tetracycline by Olowe *et al.*, (2008). They are extensively used in developing countries (Hart *et al.*, 1998; Okeke *et al.*, 1999). Low resistance rates to cefaperazone (9%), ceftriaxone (9%), cefadroxil (18%), nitrofurantoin (9%) and vancomycin ((9%) were observed in this study. Low resistance rates to cefotaxime, ceftriaxone , ceftazidime and Cefixime were observed by Alsara ,(2010) in his study, which is similar to other findings reported in Jordan (Younis *et al.*, 2009) and elsewhere (Hagei-Astanei *et al.*, 2007).
Such multi drug resistance has serious implications for the empiric therapy of infections caused by *E. coli* and related enteric gram negative pathogens and for the possible co-selection of antimicrobial resistance mediated by multi drug resistance plasmids (Sherley *et al.*, 2004). In India, the emergence of multidrug resistant strains and its variation over the years have been emerging (Taneja *et al.*, 2004). Obi *et al.*,

(2006) studied antibiotic resistance profiles and relatedness of enteric bacterial pathogens isolated from HIV/AIDS patients with and without diarrhea and their household drinking water in rural communities in Limpopo Province, South Africa and found that about 50% of *E. coli* isolated from the various study cohorts showed multiple antibiotic resistance to penicillin, amoxicillin, ampicillin, erythromycin, tetracycline, doxycycline and cotri-moxazole, whereas less than 10% resistance was consistently reported for ofloxacin, gentamicin, meropenem cefotaxime, cefuroxime and imipenem.

The inexpensive drugs are widely available without prescription from authorized health institutions and pharmacies, as well as from unauthorized patent medicine shops and other distributors (Hart *et al*., 1998; Okeke *et al.*, 1999). Ingestion of antibiotics is known to provide selective pressure ultimately leading to a higher prevalence of resistant bacteria (Levin *et al.*, 1997; Levy *et al*., 1997). Studies in other developing countries have shown that the trend in

Enteric pathogens are toward increasing antibiotic resistance (Hoge *et al*., 1998). Our study shows the need to monitor commensal organisms as well as pathogens by susceptibility testing to guide treatment. The data observed suggest that the antibiotics against which all the isolates were sensitive may be useful in treating infections caused by pathogenic *E. coli* and other related enteric gram negative pathogenic bacteria in the locality where the source of water supply largely depend on the lakes.

In addition, different pathologies may alter antibiotic sensitivity patterns, consequently, periodic evaluation of antibiotic susceptibility is recommended to guide management of patients requiring antibiotic treatment (Obi *et al*., 2006). It is speculated that the widespread use of antibiotics may create pressure that encourages the selection of multi-drug resistance among bacteria (Sack *et al*., 1997; Hoge *et. al*., 1998; Pratts *et al*., 2000). Such rising resistance is due to mechanisms of mutation and then resistance gene transfer by transport means. Since a plasmid or transposon can carry several resistance indexes, resistance to several antimicrobial agents may be acquired simultaneously and results in multiple drug resistant organisms (Hughes and Datta, 1983; Davies, 1994; Hall and Collis, 1995; Labee-lund and Sorum, 2001). It is known that majority of antibiotic resistance genes in *Escherichia coli* are palsmidborne and such plasmids are often transferable to same species or other enteric pathogenic species. Observation of incidence of multidrug resistant *E. coli* isolates in lake waters of Darjeeling Hills in our study may serve as database for formulation immediate and future sanitation and health related programmes in this area.

Conclusions

From the study it can be concluded that though several antibiotics are active against the isolates, periodic monitoring of antibiotic sensitivity is imperative to detect any changing patterns. The multiple resistance of isolates to some antibiotic classes are of great public health concern and calls for caution in the indiscriminate use of antibiotics on humans.

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