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RESEARCH ARTICLE

EVALUATION OF THE MICROBIAL POLLUTION OF WATER IN RAMASANDRA LAKE, BANGALORE, KARNATAKA AND ASSESSMENT OF MULTIPLE ANTIBIOTIC RESISTANCE AMONG ESCHERICHIA COLI

N. Latha* and M. Ramachandra Mohan
Lake Management and Water Quality Research Unit, Department of Zoology, Bangalore University, Bangalore-560 056.

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Corresponding Author

N. Latha

Abstract

An experiment was designed to examine microbiology of water samples of Ramasandra Lake and further assess the occurance of the multiple antibiotic resistance of *E coli*. Analysis of the water samples obtained from the lake indicates high microbial and fecal contamination with microbial load in order of 10^5 and MPN $\geq$1800/100ml. Collected water samples from four stations were screened for the *E coli* to assess their resistance to 10 different antibiotics. Of the 14 *E coli* isolates 0% were susceptible to all antibiotics used. The isolates were found resistance to penicillin and tetracycline (100%). Among the 10 antibiotics tested, four pattern of antibiotics resistance were obtained and all of them were multiple antibiotic resistance with the number of antibiotics ranging from 2 to 7. The result indicates the developing antibiotic resistant *E coli* may be a serious threat on public health, aquatic organisms and environment.

Introduction

Water sources are of much significance right from the history of natural world, it is our duty to preserve water resource as it is very essential for natural life (Lewandowska *et al*., 2000). The pollution of a particular waterbody can always be linked to an industry, sewage or agricultural runoff (Sathware *et al*., 2007). Numerous people suffer from different fatal diseases like typhoid, jaundice, etc., due to water pollution (Atlas and Bertha, 1997). Furthermore, unfavourable environment also cause trachoma, childhood and diarrhoea, etc., (Daunders and Warford, 1976). Generally, the surface water is not recommended for drinking purposes as it is contaminated mostly by organic, inorganic and biological pollutants (Kumar *et al.*, 1996). Among the biological pollutants, coliform, fecal coliform, *Escherichia coli* considered as main indicators. As *E coli* dwell in human intestine and other warm-blooded animals, these are most significant indicators of fecal contamination (Edberg, 2000). *E coli* routinely employed as an indicator of fecal contamination (APHA, 1985). Though *E coli* are considered as an ordinary commensal of intestinal microflora (commensal strains belong to several 'O' groups), they are potentially pathogenic elsewhere in the body, where they produce pathogenic and diarrhoeal diseases, especially in children, elderly people and those debilitated by other diseases. Enteropathogenic *E coli* (EPEC) and Enterotoxigenic *E coli* (ETEC) accounts for considerable proportions of infantile diarrhoea in the developing World (Mohammed hatha *et al.*, 1999).

MATERIAL AND METHODS:

Sample collection: The water samples from the lake were collected using sterile 250ml borosilicate glass bottles that were sterilized in hot air oven 160^0C for 1 hour and were covered tightly until used. The water samples were kept in ice box (4-10^0C) and transported to the laboratory < 4 hours of collection. All samples were analysed within 24 hours of collection.

Total colony count and isolation of organism- Pour plate method on nutrient agar were plated and the plates were incubated at 37^0C for 24-48hours. The colonies were screened and identified based on taxonomic schemes described by Bergeys manual.

Fecal coliform test and isolation and identification of *Ecoli:* Three tube procedure using Lactose broth was used to detect the coliform and Most Probable Number (MPN) of coliform bacilli.

Total coliform -Ferment lactose with the production of gas and acid at 44.5 ± 0.2^0C within the first 48hours of incubation in brilliant green bile broth. These tubes were plated on Eosin Methylene Blue Agar medium (EMB) and incubated. Colonies grown on EMB plates were selected and finally identified on the basis of morphological, cultural and biochemical characteristics for the isolation of *E coli* (APHA, 1985).

All the bacterial isolates were tested for their sensitivity to antibiotics by means of a disc diffusion method on Muller Hilton Agar medium (Bauer *et al.,* 1966).

Ten antibiotic disc viz., Norfloxacin, Oflaxacin, Ampicillin, Cotrimoxazole, Gentamycin, Furozolidone, Ciproplaxacin, Amoxycillin, Penicillin and Tetracylin.

Based on the occurrence of resistance to two and more than two antibiotics the isolates were categorised as multiple antibiotic resistant isolates.

Results and discussion:

It is evident from the results obtained in this study (table 1) that the water samples from the four stations from the lake Ramasandra are heavily contaminated with microorganisms. The index of the microbial load (10^5) is high and it indicates dense pollution of bacteria in the water samples. The comparative analysis of the microbial load of the station indicates that more bacteria were encountered at station 4, where dumping of waste were noticed. Further coliform bacilli with high MPN (>1800) may be a result of runoff from contaminated areas. *E coli* form a part of normal intestinal flora of man and animal and the commensal strains belong to several 'O' groups. The virulent strains of

E coli act as specific pathogens of the gut (enteritis) and of extra intestinal sites (urinary tract infection), wound infection. The clinical infections caused by *E coli* include urinary tract infection, septic infection of wound, diarrhoea, dysentry, septicemia, pneumonia, neonatal meningitis and abscess in variety of organs. The presence of abundant bacteria in the water samples from all the four stations indicate that the lake is exposed to nonpoint pollution particularly runoff from agricultural land and human wastes. The presence of fecal coliform and *E coli* clearly shows that the lake is not free of human fecal material. Thus, it can be concluded that the lake is exposed to both point and nonpoint pollution, which can alter the natural balance of population within the ecosystem, by selectively encouraging the development of those aerobic microorganisms which can degrade all or some of the added material. High levels of resistance were obtained among the bacterial isolates (21-42%) and the cumulative resistance of the bacterial isolates to the antibiotics ranged from 0-100% (as shown in table 2). The isolates showed very high rates of susceptible to all the evaluated antibiotics. Further, we detected all of the isolates resistant to penicillin and tetracycline. Based on the occurance of resistance to two and more than two antibiotics the isolates were categorized as multiple antibiotic resistant isolates (Lateef *et al.,* 2005). In this study Multiple antibiotic resistant to 3 to 6 antibiotic were observed. Incidence of antibiotic resistance in station 2 is 4. Among the bacterial isolates from the lake, four pattern of multiple drug resistance were encountered and the number of antibiotics ranged from 2 to 5 (table 3). *E coli* isolate showed 100% sensitivity to Norflox and Ofloxacin and 100% resistance to tetracycline and penicillin (Table 4). Recent studies have shown that antibiotic can accumulate in the environment and even persist for upto a year (Zuccato *et al.,* 2000). In 2000, Thurman and Hostetler found antibiotics in animal feedlots wastewater and ground water near lagoons. The number of antibiotics involved in the multiple antibiotic resistance as obtained in the study falls within the range obtained by earlier workers (Khan and Malik, 2001). The relatively high level of resistance to antimicrobial agents could be a reflection of misuse or abuse of these agents in the environment (Malik and Ahmed, 1994). Antibiotic prescription in hospitals are given without clear evidence of infection or adequate medical indication, toxic broad spectrum antibiotics are sometimes given in place of narrow spectrum drugs as substitute for culture sensitivity testing with the consequent risk of dangerous side effects, super infections and the selection of drug-resistant mutants (Prescott *et al.,* 1999). In developing countries, drugs are available to the public and thus people may practice self administration of antibiotic and further increase the prevalence of drug resistant strains. Many antibiotic are persistent in the environment (Zuccato *et al.,* 2000) and some of them have been isolated from waste water and ground water (Thurman and Hostetler, 2000). This could enhance the resistance of bacteria to antibiotics or drugs as shown in this study, and also spread bacterial resistance among the inhabitants who may get in contact with this water body.

Conclusion: Pollutants in the watershed mainly consist of pathogens and nutrients due to the densely populated areas, agricultural activities, and urban and storm-water runoff in the region. The microbiological data

obtained in the study gives the picture of the water quality, with *E coli*, matter of great concern. The continuous and increasing use of antibiotics has led to the emergence of *E coli* resistant to many antibiotics.

Table 1: Microbiologic results of the lake water samples

Sampling Station (No.)	Total aerobic bacteria (cfu/100mL)	Total coliform (cfu/100mL)	Fecal coliform (cfu/100mL)
1.	$2.8*10^5$	>1800	+
2.	$3.2*10^5$	>1800	+
3.	$3.0*10^5$	>1800	+
4.	$4.8*10^5$	>1800	+

Table 2: Showing multiple antibiotic resistance pattern

No. of antibiotics	Resistance pattern	No. of *E coli*	Resistance (%)
Sensitive		0	
2-Antibiotic	Tet, Pen	3	21.42
3-Antibiotic	Amp, Tet, Pen	1	35.72
	Fur, Tet, Pen	2	
	Gen, Tet, Pen	1	
	Cot, Tet, Pen	1	
4-Antibiotic	Amp, Cip, Tet, Pen	2	28.57
	Amp, Tet, Pen, Amo	1	
	Cot, Tet, Pen, Amo	1	
5-Antibiotic	Cip, Gen, Cot, Tet, Pen	1	14.28
	Cip, Cot, Tet, Pen, Amo	1	

Table 3: Sjowing antibiotic intermediate resistance pattern among *E coli* isolates

No. of antibiotics	Intermediate resistance pattern	No. of *E coli*	Resistance (%)
Sensitive		0	
Resistance			
1 Antibiotic	Amp	1	35.7
	Cip	1	
	Amo	1	
	Fur	1	
	Cot	1	
2 Antibiotic	Fur, Cot	2	35.7
	Cip, Gen	1	
	Gen, Amo	1	
	Cip, Amo	1	
3 Antibiotic	Gen, Cot, Amo	1	14.28
	Fur, Gen, Amo	1	
4 Antibiotic	Amp, Fur, Gen, Amo	1	7.14

Table 4: Showing the antibiotic resistance level of *E coli*

Station	Isolates	Amp	Fur	Cip	Gent	Cot	Tet	Nor	Ofl	Pen	Amox	blac	%S	% I	% R
I	1	S	I	S	S	I	I	S	S	R	S	+	60	20	20
	2	I	I	S	I	R	R	S	S	R	I	+	30	40	30
	3	S	S	S	I	I	R	S	S	R	I	-	50	30	20
	4	S	R	I	I	S	R	S	S	R	S	-	50	30	20
II	5	S	S	I	S	R	R	S	S	R	R	-	50	10	40
	6	R	S	R	I	S	R	S	S	R	I	-	40	20	40
	7	S	S	I	R	S	R	S	S	R	I	-	50	20	30
	8	S	I	S	I	S	R	S	S	R	I	-	50	30	20
III	9	S	I	R	S	R	R	S	S	R	R	-	40	10	50
	10	S	R	S	S	S	R	S	S	R	I	-	60	10	30
	11	R	S	S	S	S	R	S	S	R	I	-	70	00	30
IV	12	I	S	R	R	R	R	S	S	R	S	-	40	10	50
	13	R	I	S	S	I	R	S	S	R	R	-	40	20	40
	14	R	S	R	S	S	R	S	S	R	I	-	50	10	40
	% s	57.1	49.9	49.9	42.85	49.99	00	100	100	00	28.57				
	% i	28.57	35.7	21.4	35.71	21.42	00	00	00	00	49.99				
	% r	14.28	14.2	28.5	14.28	28.57	100	00	00	100	21.42				

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