



Microbial source tracking through antibiotic resistance indexing of *Escherichia coli* to identify source of fecal contamination in drinking water

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Abstract

Fecal pollution of water leads to introduce variety of intestinal pathogens that causes water borne diseases. Microbial source tracking (MST) methods are increasingly being used to identify fecal contamination sources in drinking water. A total of 400 drinking water samples were collected from the houses (200), schools (100), hostels (50) and commercially available water cans (50), out of which 160 water samples were found nonpotable. A total of 154 E.coli were isolated and identified from these 160 nonpotable drinking water samples. The 123(79%) from human fecal origin and 31(21%) animal fecal origin E. coli were found after the microbial source tracking by multiple antibiotic resistances (MAR) analysis. In hostels and School's water identified a total of 19 and 18 human fecal originated E.coli. Out of 12 E.coli isolated from 15 Can's nonpotable water; 10(83%) were human and 2(17%) animal fecal origin. The 105 E.coli were isolated from the household drinking water out of these 76(72%) was human and 29(28%) was animal fecal origin. The study concluded that the water in urban area was mainly polluted due human fecal origin as compare to animal fecal origin.

Key words: Urban area, fecal pollution, Nonpotable drinking water, MST, MAR

I. INTRODUCTION

Microbial source tracking (MST) is a method to determine the sources of fecal contamination in and establish whether fecal bacteria are being introduced into water bodies through human, wildlife, agricultural or pet wastes [1]. There are different categories of methods currently being developed and used in MST such as biochemical (phenotype), molecular (genotype), chemical and immunological to identify the source of pollution [2]. The human beings, domestic animals and wildlife are exposed to various antibiotics and develop and express resistance to these different antibiotics. This distinctive resistance present in each contaminant makes the fecal bacteria reasonably unique to its source.

Water is a major way of dissemination of microorganism in nature and has been recognized as a significant reservoir of antibiotic resistance [3,4]. Poor infection control, inadequate sanitary conditions and inappropriate food-handling encourage the spread of antimicrobial resistance. Prevention of such diseases is highly essential, since they spread fast, affecting entire community [5]. Fecal contamination from animal and human sources is one of the major causes of water quality deterioration [6]. Fecal indicators may not be from one particular source, but rather variety of sources. *Escherichia coli* has long been used as an indicator of fecal pollution having good characteristics of a fecal indicator, such as not normally being pathogenic to humans and is present at much higher concentrations than the pathogens it predicts [7]. Antibiotic resistance profiling for the identification of sources of fecal contamination in water is promising and emerging procedure [8]. The multiple antibiotic resistance (MAR) indices of *E. coli* from wild animals was generally low, while human and poultry isolates had higher MAR indices [9]. There were larger multiple antibiotic resistance of *E. coli* isolated in urban areas than from rural areas [10]. Therefore antibiotic resistance

profiling and MAR indexing of *E.coli* was performed for microbial source tracking for source of fecal pollution in drinking water in Amravati city.

II. MATERIALS AND METHODS

A total of 400 drinking water samples were collected from houses, schools, hostels and commercially available water cans in Amravati city. The collected water samples were tested for its potability by rapid field Manja's test (H₂S test). The non potable (160) drinking water samples (H₂S positive) were further processed for presence of *E.coli* by inoculating on MacConky agar. *E.coli* produces pink coloured colonies at 37°C for 24h on MacConkey agar medium. These *E.coli* (154) isolates were subjected to antibiotic resistant pattern to track the source of fecal contamination by using disc diffusion technique with 8 different antibiotics supplied by Hi-media Pvt Ltd, Mumbai. The multiple antibiotic resistance indices (MARI) were calculated for these *E.coli* isolates.

Antibiotics	Quantity
Ceftriaxone (Ci)	30 mcg
Ceftazidime (Ca)	30 mcg
Cephotaxime (Ce)	30 mcg
Lincomycin (L)	2 mcg
Netilmicin (Nt)	30 mcg
Ofloxacin (Of)	2 mcg
Vancomycin (Va)	30 mcg
Amikacin (Am)	30 mcg

MAR Index:

$$\text{The MAR Index for a isolate} = \frac{\text{Number of antibiotics to which the isolates resistance}}{\text{No. of antibiotics tested}}$$

Multiple antibiotic resistance values for each isolate were calculated by summing the number of antibiotics to which the isolate was resistant and dividing by the total number of antibiotics assayed [10].

III. RESULTS AND DISCUSSION

The total 400 drinking water samples were collected from the house (200), school (100), hostel (50) and commercially available water cans (50), out of which 160 water samples were found nonpotable. A total of 154 *E.coli* were isolated and identified from these 160 nonpotable drinking water samples.

The 123(79%) from human fecal origin and 31(21%) animal fecal origin *E. coli* were found after the microbial source tracking by multiple antibiotic resistances (MAR) analysis as shown in table 1. The data of the study also revealed that total 19 and 18 *E.coli* were isolated from the nonpotable drinking water samples of hostels and Schools respectively, all the isolates were found human fecal origin. Out of 15 nonpotable water samples from commercial water cans, 12 *E.coli* were isolated and 10(83%) was human fecal origin and 2(17%) was animal fecal origin. The 105 *E.coli* were isolated from the household drinking water out of these 76(72%) *E.coli* was human fecal origin and 29(28%) was animal fecal origin (Fig.2).

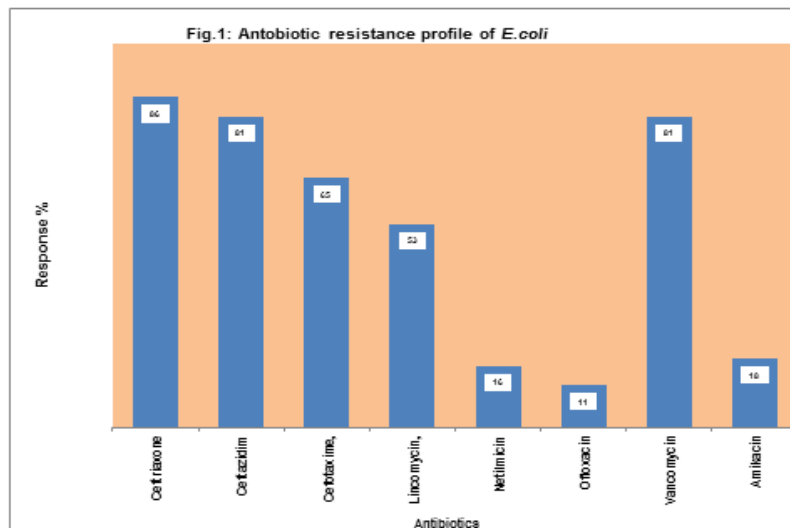
Site of collection	Total sample	Results of H ₂ S			Non potability	No. of <i>E.coli</i> Isolated	Source of contamination	
		24h	48h	72h			Human Fecal Origin	Animal Fecal Origin
Hostels	50	10	16	20	20	19	19	00
Schools	100	05	18	19	19	18	18	00
Cans	50	04	09	15	15	12	10	02
Houses	200	34	92	106	106	105	76	29
Total	400	53	135	160	160	154	123	31

The *E.coli* isolates showed variable antibiotic resistant patterns. The isolates were highly resistant to Ceftriaxone 86% followed by 81% resistant to Vancomycin and ciftazidime, 65% were resistant to Cefotaxime, 53% were resistant to Lincomycin, 18% were found resistant to Amikacin, 16% to Netilmicin and 11% to Ofloxacin. This results correlates with the Tambekar *et al.*, [11] where *E.coli* isolates were highly susceptible to Ofloxacin followed by Netilmicin, reported 58% resistant to ceftriaxone i.e., the antibiotic was moderately effective against the isolates (Fig.1).

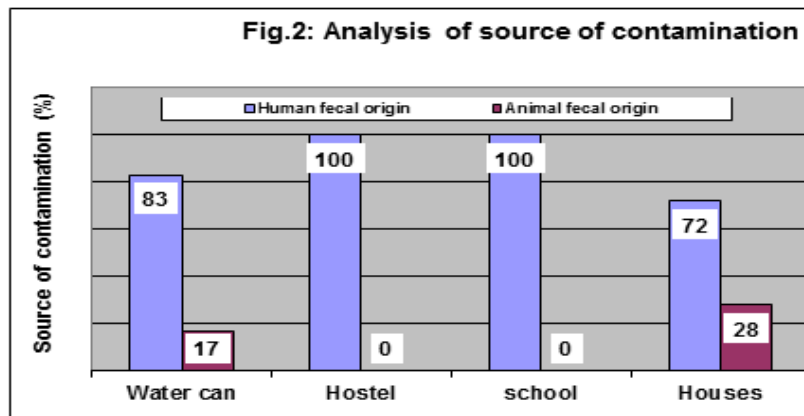
The data of the study showed that the isolates having MAR Indices 0.25, 0.125 were animal fecal origin and that of 0.375, 0.5, 0.625, 0.75, 0.875 and 1.0 were human fecal origin. 3% of the isolates have the MAR Index 0.125, 17% isolates have MAR index 0.25. The 15% *E.coli* isolates have MAR index 0.375, 20% have 0.5, 30% have 0.625, 10% have 0.75 MAR index. The 4% isolates have MAR index 0.875 and only 1% isolates have the MAR index 1.0 (Table.2).

Table. 2: MAR Index of *E.coli* obtain from different sites

Type of contamination	MAR Index	Site of collection of water sample				Total
		Hostel	School	Water can	House hold	
Animal fecal origin	0.125	0	0	0	4	4 (3%)
	0.25	0	0	2	25	27 (17%)
Human fecal origin	0.375	1	3	2	17	23 (15%)
	0.5	4	6	3	18	31 (20%)
	0.625	6	5	5	30	46 (30%)
	0.75	7	2	0	6	15 (10%)
	0.875	1	2	0	4	7 (4%)
	1.0	0	0	0	1	1 (1%)
Total <i>E.coli</i>		19	18	12	105	154



From the study it is cleared that in the urban area maximum fecal contamination occurs from the human fecal origin as compare to animal fecal origin. The contamination occurs in the urban area due to unhygienic water storage and handling practices, Microbial contamination of collected and stored household water is caused not only by the way of collection and use but unsanitary and inadequately protected (open, uncovered or poorly covered) water collection and storage containers. Unsanitary methods to dispense water from household storage vessels, including contaminated hands and dippers, and inadequate cleaning of vessels, which lead to accumulation of sediments and pathogens [12,13,14]. The easily available medical facilities and uncontrolled use of antibiotic in the urban area leading to cause increased in antibiotic resistance in bacteria which is alarming situation because the number of antibiotic are limited.



Similarly, Tambekar *et al.*, [15] reported that the thermotolerant *E.coli* isolated from the drinking water from salinity affected villages of Purna River basin of Vidarbha were 75% human fecal origin and 25% were non human fecal origin. The study done by Chitanand *et al.*, [16] showed that multiple antibiotic resistances was prominently seen in coliforms at downstream sites while it was low in coliforms at up streams sites along the bank of river and the differences in MAR indices provide a method for distinguishing high risk contamination sites in aquatic environment. The antibiotic resistance index for detected thermotolerant *E.coli* in ODNF (Open defecation not free) villages were found higher than in ODF (open defecation free) villages [17].

IV. CONCLUSION

From the above study it is cleared that, Microbial source tracking can be a useful tool for the detection of source of fecal contamination in drinking water. The study revealed that in urban area the pollution of drinking water was mainly human fecal origin as compare to animal fecal origin. The reason for contamination of drinking water was unhygienic water storage and handling practices. Thus study concluded that the water in urban area was mainly polluted due human fecal origin as compare to animal fecal origin.

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