

Antibiotic Resistance Pattern and Plasmid Profiling of Thermotolerant *Escherichia coli* Isolates in Drinking Water

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ABSTRACT

Background: Antibiotic resistant *Escherichia coli* is potential source of transmission of resistance to other water borne pathogens where plasmid borne resistance is most significant.

Methods: Drinking water samples were collected from different water sources: that is to say- tap, well and spring from different places of Kathmandu where *E. coli* and thermotolerant *E. coli* were isolated using membrane filtration technique. Antibiotic susceptibility was determined using a modified Kirby Bauer disc diffusion method and thermotolerant *E. coli* isolates from tap water were subjected for plasmid profiling.

Results: Type of water sources were not associated with the presence of coliform ($P=0.155$) and thermotolerant coliform ($P=0.235$) but the significant association was observed in thermotolerant coliform and thermotolerant *E. coli* for all sources tap ($P=0.029$), well ($P=0.028$), spring ($P=0.05$) but total coliform and *E. coli* association was found for well ($P=0.01$). All *E. coli* and thermotolerant *E. coli* isolates were susceptible to Ofloxacin, Chloramphenicol and Cotrimixazole. Resistance to Cefexime, Amikacin, Nalidixic acid, Amoxicillin, Tetracycline were 17 (54.8%), 9 (29%), 11 (35.5%), 25 (80.6%), 29 (93.5%) and 19 (57.6%), 12 (36.4%), 13 (39.4%), 31 (94%), 33 (100%) was observed in *E. coli* and thermotolerant *E. coli* respectively where 25 (75.8%) thermotolerant *E. coli* and 22 (70.9%) *E. coli* were observed with multiple drug resistance patterns. Single band of plasmid were observed in three MDRs and one non-MDR isolates and size varied from 2kb to >10kb. All Nalidixic acid resistant thermotolerant *E. coli* were found to harbor a plasmid.

Conclusions: Presence of plasmid in Nalidixic acid resistant thermotolerant *E. coli* heightens public health issue and the need of monitoring Quinolone resistance bacteria in environment.

Keywords: Nalidixic acid; plasmid; thermotolerant.

INTRODUCTION

Occurrence of *Escherichia coli* in drinking water is indicative of recent faecal contamination and possible incidence of water-borne diseases that is most serious threat to health.¹ However, recovery of indicator bacteria may depend upon level of contamination in particular water source.²

Being commensal in the human and animal gut, antibiotic resistant *E. coli* may be a sensitive indicator of distinct

therapeutic and non-therapeutic uses of antimicrobial drugs.³ *E. coli* has been reported to transfer the antibiotic resistant genes to enteric pathogenic and normal flora bacteria.^{4,5} The problem in increasing infectious disease is the acquisition and transfer of antibiotic resistance and virulence factor genes by the bacterium through horizontal transfer of the resistance (R) plasmids, transposons and integrons.^{6,7}

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We investigated the antibiotic susceptibility pattern of both *E. coli* and thermotolerant *E. coli* with the commonly used antibiotics and antibiotic resistance in relation to their plasmid profile of thermotolerant *E. coli*.

METHODS

A cross-sectional study was carried out using random sampling technique from January to August, 2011. Total of 66 drinking water samples comprising 28 from tap water; 24 from well water and 14 from spring water sources were collected randomly from different places of Kathmandu and membrane filtration technique was followed for microbial examination according to standard guideline,⁸ where growth on higher temperature (44.5°C) distinguished thermotolerant coliform. Pure cultures of both *E. coli* and thermotolerant *E. coli* were obtained using EMB (Eosin Methylene Blue) agar medium and further identified by colony morphology, gram staining and biochemical test,⁹ including MUG (4-methyl umbelliferyl- β -glucuronide) hydrolysis test. Antibiotic susceptibility of isolated enteric bacteria was assayed using a modified Kirby Bauer disc diffusion method with eight different antibiotics (Hi-Media Pvt. Ltd, India) Ofloxacin (OF5 μ g), Chloramphenicol (C30 μ g), Cotrimoxazole (Co25 μ g), Amoxicillin (Am10 μ g), Cefixime (Cfx5 μ g), Tetracycline (T30 μ g), Amikacin (Ak30 μ g) and Nalidixic acid (NA30 μ g) and interpretation was done following CLSI guideline.¹⁰

The selected thermotolerant *E. coli* strain (single colony) isolated from tap water was grown overnight in Luria-Bertani (LB) broth at 37°C with aeration using an

orbital shaker and plasmid DNA was extracted through mini alkaline lysis by SDS.¹¹ Electrophoresis was carried out in a horizontal gel apparatus. Electrophoresis was conducted in agarose (0.8%) gel containing Ethidium bromide (EtBr). Supermix 1kb DNA marker (GeNei Pvt. Ltd., Bangalore, India) was used as a reference marker and *E. coli* ATCC 25922 was used as control. The plasmid size was estimated comparing with the DNA marker using semi log graph.

RESULTS

All water sources were contaminated with coliform and thermotolerant coliform. Average count of *E. coli* (cfu/100ml) was \log_{10} 1.55, \log_{10} 1.19 and \log_{10} 2.13 and thermotolerant *E. coli* was \log_{10} 1.99, \log_{10} 2.05 and \log_{10} 2.17 in tap, well and spring water sources respectively. Significant association was observed on presence of *E. coli* (P=0.002) and thermotolerant *E. coli* (P=0.011) among different drinking water sources. However no significant relation occurred between water sources with presence of coliform (P=0.155) and thermotolerant coliform (P=0.235). In case of particular sources, the significant association in thermotolerant coliform and thermotolerant *E. coli* was found for all sources tap (P=0.029), well (P=0.028), spring (P=0.05) but total coliform and *E. coli* association was found for well (P=0.01) only. Resistance to Cefixime, Amikacin and Nalidixic acid, Amoxicillin, Tetracycline were 17 (54.8%), 9 (29%), 11 (35.5%), 25 (80.6%), 29 (93.5%) and 19 (57.6%), 12 (36.4%), 13 (39.4%), 31 (94%), 33 (100%) observed in *E. coli* and thermotolerant *E. coli* respectively while all isolates were susceptible to Ofloxacin, Chloramphenicol and Cotrimoxazole (Table 1).

Table 1. Antibiotic resistance pattern of *E. coli* and thermotolerant *E. coli*.

Antibiotics	<i>E. coli</i> (n=31)			Thermotolerant <i>E. coli</i> (n=33)		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
OF	31 (100)	0 (0)	0 (0)	33 (100)	0 (0)	0 (0)
C	31(100)	0 (0)	0 (0)	33 (100)	0 (0)	0 (0)
Am	0 (0)	6 (19.4)	25 (80.6)	1 (3)	1 (3)	31 (94)
Ak	11 (35.5)	11 (35.5)	9 (29)	12 (36.4)	9 (27.2)	12 (36.4)
Cfx	1 (3.2)	13 (42)	17 (54.8)	0 (0)	14 (42.4)	19 (57.6)
NA	8 (25.8)	12 (38.7)	11 (35.4)	6 (18.2)	14 (42.4)	13 (39.4)
Co	31 (100)	0 (0)	0 (0)	33 (100)	0 (0)	0 (0)
T	0 (0)	2 (6.5)	29 (93.5)	0 (0)	0 (0)	33 (100)

Note: OF = Ofloxacin, C= Chloramphenicol, Am = Amoxicillin, Ak= Amikacin, Cfx= Cefixime, NA= Nalidixic acid, Co= Cotrimoxazole, T= Tetracycline, S= Sensitive, I= Intermediate, R= Resistance

Table 2. Comparative antibiotic susceptibility pattern for *E. coli* and thermotolerant *E. coli* from different sources.

		OF	C	Am		Ak		Cfx		NA		Co		T	
		S	S	S	I	R	S	I	R	S	I	R	S	I	R
Tap	<i>E. coli</i> (n=6)	6	6	1	5	2	3	1	3	3	2	3	1	6	6
	Thermotolerant <i>E. coli</i> (n=8)	8	8	1	7	3	3	2	5	3	2	3	3	8	8
Well	<i>E. coli</i> (n=16)	16	16	3	13	6	5	5	1	6	9	4	6	16	14
	Thermotolerant <i>E. coli</i> (n=16)	16	16	1	15	6	4	6	5	11	2	8	6	16	16
Spring	<i>E. coli</i> (n=9)	9	9	1	8	3	3	3	4	5	2	3	4	9	9
	Thermotolerant <i>E. coli</i> (n=9)	9	9	9	3	2	4	4	4	5	2	3	4	9	9

OF = Ofloxacin, C= Chloramphenicol, Am = Amoxicillin, Ak= Amikacin, Cfx= Cefexime, NA= Nalidixic acid, Co= Cotimoxazole, T= Tetracycline, S= Sensitive, I= Intermediate, R= Resistance

Comparatively, thermotolerant *E. coli* isolates were found more resistant than *E. coli* isolates to Amoxicillin, Amikacin, Cefexime and Nalidixic acid though Ofloxacin, Chloramphenicol and Cotimoxazole were equally inhibitory to both *E. coli*. Tetracycline resistance was found in all thermotolerant *E. coli* isolates and two *E. coli* from well water did not show resistivity to Tetracycline. Higher proportion of thermotolerant *E. coli* 25 (75.8%) isolates expressed multiple drug resistance whereas, 22 (70.9%) *E. coli* isolates expressed multiple drug resistance (Table 2).

All thermotolerant *E. coli* isolates from tap water were subjected for plasmid profiling (Figure 1).

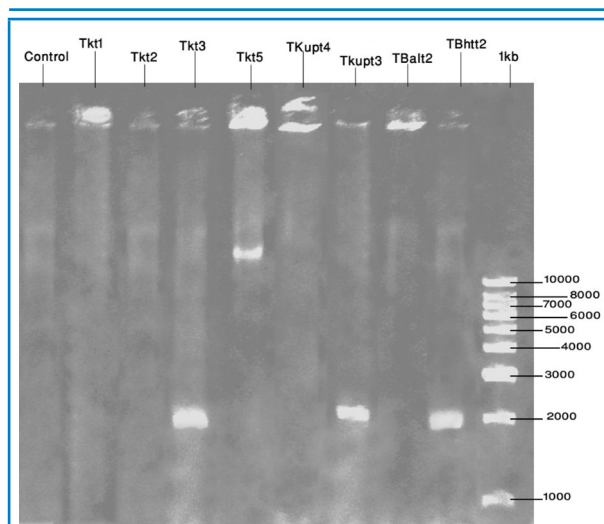


Figure 1. Agarose gel photograph of plasmid profiling of thermotolerant *E. coli* isolates from tap water, Lane 1, 1 kb ladder; Lane 2-9, Thermotolerant *E. coli* isolates; Lane 10, Control strain *E. coli* ATCC25922.

Single band of plasmid were observed in three Multiple drug resistant (MDR) isolates and one non-MDR isolate and size varied from 2 kb to >10 kb were obtained. All Nalidixic acid resistant (NAR) thermotolerant *E. coli* were found to harbour plasmid (Table 3).

Table 3. Plasmid profiling of thermotolerant *E. coli* isolated from tap water.

Organism code	Antibiotic Resistance pattern	No. of Plasmid band	Plasmid size
Tkt1	Am, Cfx, T	-	-
Tkt2	Am, Ak, Cfx, T	-	-
Tkt3	Am, Na, T	1	2 kb
Tkt5	Na, T	1	>10 kb
TKupt3	Am, Ak, T	1	2.1 kb
TKupt4	Am, T	-	-
TBatt2	Am, Cfx, T	-	-
TBhtt2	Am, Na, T	1	2 kb

Key: Am = Amoxicillin, Ak= Amikacin, Cfx= Cefexime, NA= Nalidixic acid, T= Tetracycline

DISCUSSION

Faecal contamination in water is an important water quality issue and public health concern because water serves as genetic pool for the antibiotic resistance and its dissemination. Faecal coliform contamination with *E. coli* is comparative to previous results^{2,12} ranging from 70.6% to 72% contamination with *E. coli* in water sources.

Lack of significant association between water sources with presence of coliform ($P=0.155$) and thermotolerant coliform ($P=0.235$) in our study is in agreement

with results from Turkey.¹³ Besides the degree of contamination, the presence of coliforms could also be a result of direct contamination caused by human activities and indirect effect caused by ecological disturbances. The significant association of thermotolerant coliform and thermotolerant *E. coli* was found for all sources that is tap ($P=0.029$), well ($P=0.028$), spring ($P=0.05$) but total coliform and *E. coli* association was found only for well ($P=0.01$). The result strengthened the fact that thermotolerant *E. coli* (alternatively thermotolerant coliform) provides the best indication for faecal pollution and must be detected for all water sources.

Multiple drug resistant isolates, *E. coli* (70.9%) and thermotolerant *E. coli* (75.8%) were prevalent in drinking waters. The frequency of MDR in aquatic isolates of *E. coli* ranges from 61.2% to 97.1% elsewhere in the world.¹⁴⁻¹⁷ High level of resistance to Tetracycline is in accordance to result from Niagra¹⁴ in water isolates. Previous result from Iran¹⁸ showed the high level of Tetracycline, Amoxicillin, and Nalidixic acid resistance in *E. coli* from buffalo faeces while Bayat¹⁹ reported that 100% resistance to Amoxicillin, Tetracycline, Cefexime in the hospital isolates. Similar sensitivity of *E. coli* against Chloramphenicol and Ofloxacin were documented by Tambekar²⁰ which may be attributed to restricted use of Chloramphenicol. Previous studies^{15,21,22} showed an increasing resistance of *E. coli* against Nalidixic acid. We observed 35.4% and 39.4% Nalidixic acid resistance among *E. coli* and thermotolerant *E. coli* respectively. Resistance to Nalidixic acid is an important considering that the Fluoroquinolones are used to treat a range of *E. coli* infections in humans.

Increased level of resistance of *E. coli* to Tetracycline, Amoxicillin with Nalidixic acid and Cefexime can create the public health problem and spread of drug resistance among enteric organism.

In this study, only thermotolerant *E. coli* isolates from tap water were subjected for plasmid profiling because the Kathmandu valley water supply system treats and distributes the water and most of the communities in Kathmandu drink tap water directly.

Plasmid profiling of thermotolerant *E. coli* showed single band of plasmid in three multiple drug resistant isolates and one non-multiple drug resistant isolate where size varied from 2kb to >10kb. Notably, all Nalidixic acid resistant *E. coli* had found to harbour single plasmid. Our result showed that some carry single plasmid and some carrying no plasmids was comparable with the results of Ozakabir²³ and Rahman.²⁴ Alam,²⁵ reported plasmid of 0.5 to 40kb in size having one or more bands in *E. coli* isolates from different sources and among the 8 isolates of water, 4 isolates showed plasmid bands.

Similarly, Miles²⁶ reported one or more plasmid bands between 2 kb and ≥ 12 kb. Correspondingly, Nandy²⁷ reported that smaller plasmids (<20 kb) were distributed in Fluoroquinolone (FQ)-resistant *S. dysenteriae* which are frequently isolated yearly (2000-2007) in Kolkata, India.

In this study, the two different organisms from different places, Bhaktapur (TBht2) and Kritipur (Tkt3) which have same antibiotic resistance pattern also found to contain similar size of single plasmid and this may indicate that there was a common source of contamination.

In present study, the presence of plasmid in all Nalidixic acid resistant thermotolerant *E. coli* isolates is against the theory of Quinolone resistance involves chromosomal mutations. This suggests that plasmid encoded resistance to Nalidixic acid is emerging. Therefore, we underline the need to monitor Quinolone resistant bacteria as emergence is important public health concern and resistance dissemination.

CONCLUSIONS

Water quality of Kathmandu is polluted due to faecal contamination with the high level of resistance towards antibiotics and presence of plasmid in Nalidixic acid resistance isolates have emerged creating alarming concern to public health and environment.

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