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Public health implications of transferable antibiotic resistance among thermo-tolerant coliform species in rural drinking water

Suman Tewari[1], Pramod W Ramteke[2*] and Shashank Tiwari[1]

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[1]*Department of Environmental Microbiology, BB Ambedkar (Central) University, Lucknow 226025, Uttar Pradesh, India.*

[2]*Department of Biological Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad 211007, India.*

Corresponding Author:* Pramod W Ramteke, Department of Biological Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad 211007, India, E mail id- pwramteke@gmail.com

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Abstract

Antibiotic resistance in pathogenic bacteria is a serious public health issue. This study was carried out to determine the prevalence of multiple antibiotic resistance, heavy metal tolerance and transfer of resistance in 112 thermo-tolerant coliform species 30 E. coli, 32 Klebsiella and 50 other coliforms. Majority of the strains were found resistant to one or more antibiotics. All strains (100%) E. coli were resistant to bacitracin. Majority of E. coli, Klebsiella and other coliform species were found to be resistant to vancomycin. Multiple antibiotic resistance pattern (MAR) was observed in E. coli, Klebsiella and other coliforms. Large number of E. coli, Klebsiella and other coliforms were found to be tolerant to As, Cr, Cu and Cd respectively. Nine strains of thermo-tolerant E. coli representing different resistance pattern showed the presence of plasmid DNA. Transfer of antibiotic and heavy metal was observed in majority of E. coli, Klebsiella and other coliforms. In thirty three strains of therm-otolerant coliform species including E. coli and Klebsiella curing of resistance to antibiotics and heavy metals was detected. Since many water samples in this investigation exhibited the presence of E. coli, co-transfer of plasmid mediated resistance to antibiotics and metals was investigated.

Keywords: Antibiotic Resistance, Heavy Metal, Pathogenic, E. Coli, Bacitracin, Plasmid

Introduction

Water is essential for man and other life forms. It is required for various human daily activities such as drinking, cooking, tooth brushing, bathing, washing utensils and also for agricultural and industrial purposes [37], [10, 1-800-458-1158ext. 2-7650]. However, poor water quality continues to be a leading cause of health problems especially in developing countries where it is estimated that 80% of all illnesses are linked to water and sanitation and 15% of all child deaths under the age of 5 years result from diarrhoeal diseases. The presence of Escherichia coli (E. coli) directly relates to fecal contamination with its implied threat regarding incidence of enteric disease agents [48, 79: 121-127].

Faecal coliforms are a group of bacteria, which are natural inhabitants of the gut of humans and other warm-blooded animals. E. coli is a member of fecal coliforms that contaminate the drinking water from human and animal fecal waste. Piped supplies and dug wells are common sources containing pathogenic E. coli. Such isolates from drinking water were also found resistant to antibiotics and heavy metals, which were transferable in nature indicating the presence of R-plasmid [49, 130: 215-220]. During rainfalls these coliforms may be washed into creeks, rivers, streams, lakes, or ground water. Untreated drinking water coming from these sources contains coliforms including E. coli. E. coli is an opportunistic pathogen in neonatal and immuno-compromised patients. Bacteremia, wound infections, urinary tract infection, and gastrointestinal infections are the diseases associated with E. coli and are often fatal in newborns [43, 90: 172-175]. Food and water borne outbreaks of E. coli have been documented from a number of countries [41, 66: 111-117], [3, 2: 51-60]. The difficulties in the treatment of food and water associated gastrointestinal diseases due to E. coli have been reported. This problem is compounded by the continued emergence of antibiotic resistance to a growing number of antibiotics; i.e carbenicillin, tetracycline, streptomycin, [62, 39: 651-662] norfloxacin, amoxicillin, trimethoprim, nitrofurantoin, [21, 46: 223-228] nalidixic acid, gentamicin, cefuroxime, [52, 10: 322-328] etc. Increase in antibiotic resistance level is now a global problem. Infections with antibiotic resistant bacteria make the therapeutic options for infection treatment, extremely difficult or virtually impossible in some instances [18, 20: 365-370]. Microbial resistance to antibiotics and metal ions is a potential health hazard since these traits are generally associated with transmissible plasmids [30, 2: 379-383]. Antibiotic resistance, particularly MAR, is a major public health threat, and the presence of resistant organisms in environmental waters is an emerging concern around the world. This study has further emphasized the potential application of MAR indices for identifying the origin of fecal pollution. This application would provide a beneficial tool for administrators in managing waste discharge to the aquatic environment.

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Microbial resistance to antibiotics and metal ions is a potential health hazard since these traits are generally associated with transferable plasmids. Metal and antibiotic resistance genes are frequently found on the same plasmid [40, 2: 109-129]. Location of metal and drug resistance genes on a plasmid may be the result of independent insertion from mobile gene cassettes known as 'integrons'. Although, resistance to metal ions is of less clinical concern than resistance to antibiotics, such an association is significant as knowledge of resistance to metal ion which may provide useful information on mechanism of antibiotic resistance, plasmid genetics, physiology and ecology of the microorganism in polluted environments [57, 32: 39-54]. Microorganisms resistant to metal ions are useful for biological monitoring and may also be used as genetic marker [31].

This study was aimed at measuring the antibiotic and heavy metal sensitivity profile of thermotolerant coliform species E. coli, Klebsiella and other coliforms isolated from different sources of drinking water. Thermo-tolerant E. coli representing different resistance pattern was investigated for the presence of plasmid DNA.

Materials and Methods

Sampling and isolation procedure

Water samples were collected from villages of Lucknow and Kanpur districts, Uttar Pradesh, India. A total of 188 samples were analyzed for bacteriological quality of drinking water. The water samples were collected from 54 piped supplies, 51 hand pumps, 27 tube wells, 8 dug wells and 48 surface water sources (42 samples from rivers Gomti and Ganges, 2 ponds and 4 lakes). Water samples were collected in sterile glass bottles and transported to the laboratory on ice and processed within 6 hrs of collection. The most probable number (MPN) method (APHA, 1992), H2S strip test [34] and presence/absence (P-A) test [14, 14: 13-18] were employed to detect and isolate coliforms (2). Purified colonies were obtained by plating on MacConkey's agar (Hi-Media Pvt. Ltd, India). Microorganisms were identified as per the modified scheme of [12, 83: 152-154].

Antibiotic sensitivity and metal tolerance test

Sensitivity to antibiotics was determined on Muller-Hinton medium (Hi-media) containing antibiotics (µg ml-1): ampicillin (25), chloramphenicol (30), streptomycin (30), tetracycline (30), kanamycin (30), gentamycin (10), polymixin-B (50), cephaloridine (30), co-trimoxazole (25), nalidixic acid (30), carbenicillin (100), bacitracin (10), vancomycin (30), norfloxacin (20). Allantibiotics were supplied by Hi-Media Laboratory Pvt. Ltd, Mumbai. Multiple antibiotic resistance (MAR) index was calculated according to [28, 33: 679-687]. Tolerance to metal ions was determined by minimal inhibitory concentration technique [11, 55: 335-339]. The metal salts used were arsenic oxide, cobalt nitrate, cadmium chloride, potassium dichromate, nickel chloride, zinc chloride, copper sulfate and mercuric chloride. Concentrations of metals were: Zn, Ni, Cu (400 µg ml-1), Cr, Cd, Co, As (100 µg ml-1) and Hg (25 µg ml-1).

Resistance Transfer

A total of 24 thermotolerant coliforms representing different resistance patterns, were tested for their ability to transfer resistances to E. coli K12 J 62 (lac-, pro-, his-, trp-, Nal+) recipient strain [64, 50(4): 930-933]. Overnight grown cultures (0.1 ml each) were inoculated into 10 ml brain heart infusion (BHI) broth (HI-Media), incubated for 6 h, then 0.1 ml each of donor and recipient cultures were mixed and incubated for 18 h at 28°C for conjugation. Transconjugants were selected on MacConkey's agar containing nalidixic acid with appropriate antibiotic/heavy metal. To assess R-factor transfer in sterile water, the donors and recipient were grown to mid log phase, pelleted by centrifugation (4000 rpm for 30 min) and finally suspended to the original volume. Mating was performed in sterile water at 26°C with or without shaking for 6 h.

Plasmid curing

It was done using curing agent (µg ml^{-1}): acridine orange, 20; sodium dodecyl sulfate, 250-1000 or rifampicin, 0.5-1.0. Tubes containing 10 ml peptone water were supplemented with the curing agent, inoculated with 0.1 ml of overnight broth culture and incubated at 37°C for 24 h. Appropriate dilutions of the culture were plated on nutrient agar to obtain single colony isolates after 24 h incubation at 37°C. Resulting colonies were tested for loss of plasmid on nutrient agar plates incorporated with the appropriate antibiotic/metal ion.

Antibiotic resistance among thermo tolerant coliform species (E. coli, Klebsiella and other coliforms)

Of 112 thermotolerant coliforms, 30 (26.7%) and 32 (28.5%) isolates belonged to genus Escherichia coli and Klebsiella spp., respectively. Fifty (44.6%) thermotolerant coliforms belonged to other members of coliforms. Comparison of antibiotic resistance pattern among thermotolerant coliform species is depicted in Figure 1. All strains (100%) of E. coli were resistant to bacitracin. Majority of them were resistant to vancomycin (80.0%), low level of resistance was observed for polymixin B (16.6%), cephaloridine and co-trimazole (13.3%), ampicillin (10.0%), streptomycin and tetracycline (6.6%), carbencillin, nalidixic acid, kanamycin and chloramphenicol (3.3%).

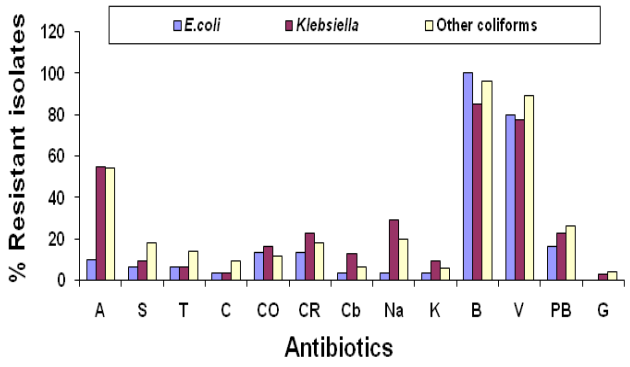


Figure 1 Antibiotic resistance among thermo-tolerant coliform species (E. coli, Klebsiella, other coliforms)
A- Ampicillin, S- Streptomycin, T- Tetracyclin, C- Chloramphenicol, CO- trimazole, CR- Cephaloridine, Cb- Carbencillin, Na- Nalidixic acid, B- Bacitracin, V- Vancomycin, PB- Polymixin-B, K- Kanamycin, G-Gentamycin.

Majority of thermotolerant Klebsiella spp.were resistant to bacitracin (85.1%), vancomycin (77.4%) followed by ampicillin (54.8%) (Fig. 4.3). Moderate resistance was observed for nalidixic acid (29.0%), polymixin B and cephaloridine (both 22.5%). Low level of resistance was observed for co-trimazole (16.6%), carbencillin (12.9%), kanamycin and streptomycin (both 9.6%), tetracycline (6.4%) chloramphenicol (3.3%) and gentamycin (3.2%).

Most of the coliform species other than E. coli and Klebsiella were found resistant to bacitracin (96.0%) and vancomycin (89.4%) (Fig.1). Moderate resistance was observed for ampicillin (54.3%). Comparatively low level of resistance was detected for polymixin B (26.3%), nalidixic acid (20.0%), cephaloridine and streptomycin (both 18.0%), tetracycline (14.2%), co-trimazole (11.7%), chloramphenicol (9.5%), carbencillin (6.3%), kanamycin (6.0%) and gentamycin (4.0%).

The results of the present investigation indicate the widespread occurrence of antibiotic resistance among thermotolerant coliforms in drinking water sources. Predominant resistance to bacitracin and vancomycin was found among the organisms included in the study. Most of the isolates were found resistant to ampicillin, the antibiotic very commonly used in the treatment. The results are somewhat similar to those reported by other workers [45, 91: 185-188] ; [44, 37:177-181]. The high value of ampicillin resistance may be due to production of β-lactamase enzymes by the bacterial strains nullifying the action of β-lactum family of antibiotics by opening up the β-lactum ring in their molecular structure [45, 91: 185-188].

Multiple antibiotic resistance (MAR) among thermotolerant coliform species (E. coli, Klebsiella and other coliforms)

Comparison of multiple antibiotic resistance among thermotolerant coliform species is depicted in Fig.2. Incidence of multiple antibiotic resistant (MAR) E. coli was observed in 33.3% strains studied (Figure 2). Resistance to one (1R) and two (2R) antibiotics was detected in 10% and 56.6% E.coli strains, respectively. Sixty seven per cent Klebsiella strains were multiple antibiotic resistant (MAR), 9.0% 1R and 19.3% 2R (Figure 2). Only one strain was found sensitive to all the antibiotics tested. Incidence of multiple antibiotic resistance (MAR) among coliform species other than E. coli and Klebsiella was observed in (62.0%) strains (Figure 2). Resistance to one (1R) and two (2R) antibiotics was observed in 26.0% and 12.0% organisms, respectively.

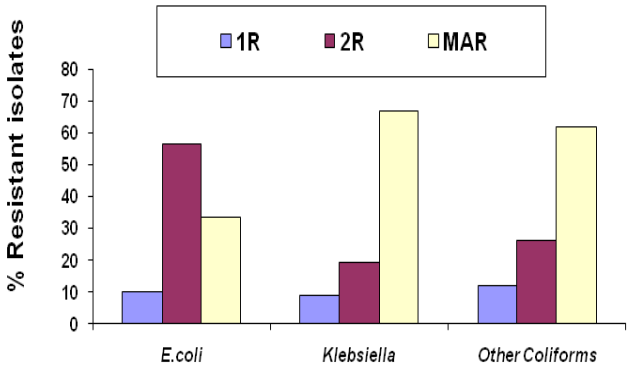


Figure 2 Comparison of multiple antibiotic resistance patterns among thermotolerant coliform species (E. coli, Klebsiella, other coliforms) 1R- Strains resistant to one antibiotic; 2R- Strains resistant to two antibiotics

Several workers have drawn attention to the antibiotic resistance among coliformsin treated and untreated drinking water [2, 42: 277-283]; [64, 50(4): 930-933] and concern has been expressed regarding the prevalence of multiple antibiotic resistance (MAR) strains.

Several workers have demonstrated prevalence of multiple antibiotic resistant (MAR) isolates in drinking water bodies in India [45, 91: 185-188]; [47, 32: 193-193]; [44, 37:177-181]. The extensive use and abuse of antibiotics in human therapy has resulted in co-existence of multiple antibiotic resistant and sensitive bacteria together in the natural reservoirs [56, 7(2): 130-136].

MAR- Multiple antibiotic resistant

Antibiotic resistance index (ARI) of thermotolerant coliform species (E. coli, Klebsiella and other coliforms) ARI of thermotolerant coliform species isolated from drinking water is presented in Table 1. Total 13 antibiotics were tested for resistance. Total resistance scored were 78, 84, 364 in case of E. coli, klebsiella and other coliforms. ARI of E. coli, Klebsiella and other coliform species were 0.20, 0.23 and 0.25, respectively.

Comparison of heavy metal tolerance among thermotolerant coliform species (E. coli, Klebsiella and other coliforms)

Comparison of heavy metal tolerance among thermotolerant coliform species is depicted in Fig.3. Large number of E. coli were found tolerant to As and Cr (both 80.0%), Cu and Cd (both 76.6%). Tolerance to Zn, Ni and Hg was observed among 70.0% isolates. Comparatively moderate tolerance was observed for Co (60.0%). Majority of Klebsiella strains were found tolerant to Cr (89.6%), As (86.2%), Cd (77.7%), Hg (72.4%) and Cu (68.9%). Moderate tolerance was observed for Co (48.2%), Ni and Zn (both 31.0%). Most of the thermotolerant coliforms other than E. coli and Klebsiella were found tolerant to Cd and Hg (both 88.0%), Cr (82.0%), Cu (76.0%) and As (72.0%). Moderate tolerance was observed for Co (52.0%), Ni (46.0%) and Zn (42.0%).

Heavy metal resistance may be possibly due to extrusion of metal species, bioaccumulation, transformation, production of low molecular weight binding proteins, etc. [53, 42: 717-743]; [56, 7(2): 130-136]. Bacterial resistance to antibiotics and heavy metals is an increasing problem in today's society [56, 7(2): 130-136]. The incidence of high level of metal tolerance among bacteria could be attributed to release of metal ions in water bodies due to industrial process [44, 37:177-181].

Plasmid characterization

Nine strains of thermotolerant E.coli representing different resistance pattern showed the presence of plasmid DNA. Of the nine strains studied, 6 strains possess only one plasmid of small to medium size (2.6-5.7 Kb). Strain one possesses two plasmids having molecular masses of 2.3 and 3.9 Kb. Strain no. 6 possesses two plasmids with molecular masses of 3.8 and 4.2 Kb. Strain no. 134 possesses two plasmids having molecular masses of 2.6 and 7.0 Kb (Plate 1).

The bacteria carrying R-plasmids have greater chances of survival and propagation in natural ecosystems than that of strains lacking plasmids [23, 8: 1-9]. Thus, there arises the necessity to limit the dissemination of R bacteria in drinking water sources in order to protect the human population against the hazard of infection with R bacteria. Microbial resistance to antibiotics and metal ions is a potential health hazard since these traits are generally associated with transmissible plasmids [7, 47: 1238-1242]; [60, 51(1): 61-69]. Transmission of R plasmid occurs in less than 1 min [65, 27: 87-115] and resistance may spread rapidly among bacteria.

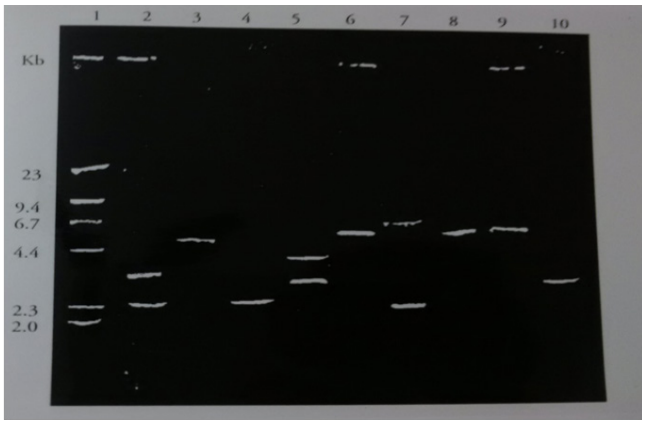


Plate 1 Plasmid DNA profile of thermotolerant E. coli observed after agarose gel electrophoresis
Lane 1: Marker- λ DNA / Hind III digest; Lane 2: Strain No. 1, (3.9 Kb and 2.3 Kb); Lane 3: Strain. No. 4, (5.0 Kb); Lane 4: Strain No. 5, (2.6 Kb); Lane 5: Strain No. 6, (4.2 Kb and 3.8 Kb); Lane 6: Strain No. 53, (5.6 Kb); Lane 7: Strain No. 134, (7.0 Kb and 2.6 Kb); Lane 8: Strain No. 139, (5.7 Kb); Lane 9: Strain No. 197, (5.7 Kb); Lane 10: Strain No. 207, (3.9 Kb)

Genera	Strains resistant to one or more antibiotics (%)	ARI
Escherichia coli	100	0.20
Klebsiella sp.	96.8	0.20
Other coliforms	100	0.25

Table 1 Antibiotic resistance index (ARI) of thermotolerant coliform species (E. coli, Klebsiella and other coliforms)

Transfer of resistance among thermoolerant E. coli and Klebsiella spp.

Transfer of antibiotic and heavy metal resistance among thermotolerant E. coli and Klebsiella spp.

Of 30 strains of thermotolerant E. coli, 19 were studied for ability to transfer their resistance. Among antibiotics, cent per cent transfer of resistance to bactracin was observed (Table 2). Ampicillin resistance was transferred in 5.2% strains of the conjugation experiments. The frequency of bacitracin transfer ranged between 3.3 x 10-6 to 5.0 x 10-1. Resistance to ampicillin was transferred in only one strain (no.146) with a transfer frequency of 3.8 x 10-4 (Table 2). Transfer of resistance to heavy metals among E. coli is presented in Table 3. Out of 19 E. coli strains studied for transfer of heavy metal resistance, in 17 (89.4%) strains transfer of one or more resistance to heavy metal were detected. Among heavy metals, high level transfer of resistance to As (63.1%) was detected followed by Ni (42.1%), Cu and Co (both 26.3%), Zn, Cr, and Cd (21.0%) (Table 3).

Five strains of Klebsiella spp. were studied by conjugation experiment for transfer of resistance to antibiotics (Table 5). In all the strains resistance to bacitracin was transferred. Additionally, in one strain (no. 63), alongwith bacitracin, resistance to ampicillin was also transferred with a frequency of 2.4 x 10-6. Transfer pattern of resistance to heavy metals among Klebsiella spp. is shown in Table 4.8. Of 5 strains of Klebsiella studied for transfer of heavy metal resistance, 4 (80.0%) strains exhibited transfer of heavy metal tolerance (Table 6). Resistance to arsenic was most frequently transferred. In all the 4 strains, arsenic resistance was transferred with a transfer frequency of 8.0 x 10-5-3.7 x 10-4. In one strain (no. 81), resistance to cadmium was co-transferred with a frequency of 1.2 x 10-4.Conjugation experiments were conducted in order to see the transfer of R-plasmid to sensitive population. The results of this study highlighted that almost all the organisms studied for transfer of R-plasmid demonstrated the transfer of at least one resistant trait. This finding is slightly different from earlier reports of [20, 190: 113-120] who observed that only 15.3% organisms were able to transfer their resistance to E. coli K12 recipient. The frequency of resistance trait transfer was found in as high as 10-1 in 16.6% strains studied which is higher than the frequency of 10-3 reported earlier in India [46, 28 (4): 859-870] and abroad [33,44: 1395-1403]; [64, 50(4): 930-933]. The present study also indicates that the incidence of resistance transfer is higher in MAR strains as well as strains with resistance to two or one antibiotic.

Strain No.	Resistance pattern of donor	Markers transferred	Frequency of transfer	
			B	A
104	S, V, B	B	2.3 x 10-4	—
146	A, B, CR	B,A	5.7x10-5	3.8x10-4
135	V, B	B	0.6x10-1	—
134	V, B	B	0.8x10-1	—
125	V, B	B	3.5x10-3	—
37	V, B	B	0.6x10-1	—
1	B	B	1.5x10-4	—
38	V, B, PB	B	1.8x10-4	—
138	V, B	B	5.0x10-4	—
137	V, B, CO	B	9.0x10-1	—
119	V, B	B	3.3x10-6	—
6	B, CO	B	2.0x10-5	—
124	V, B	B	1.5x10-2	—
108	B	B	2.0x10-1	—
131	V, B	B	8.0x10-3	—
176	V, B, CO	B	6.6x10-4	—
197	V, B	B	4.8x10-3	—
202	V, B	B	2.8x10-3	—
207	V, B	B	3.0x10-1	—

A- Ampicillin, B- Bacitracin, CR- Cephaloridine, CO-Co-trimazole, B-Polymixin-B, V- Vancomycin, S- Streptomycin

Table 2 Transfer of antibiotic resistance among thermo-tolerant E. coli

Conjugation occurs not only between closely related bacterial species but also between different genera and even between Gram-negative and Gram- positive organisms [59, 48: 289-294]; [35, 25: 147-71]. Conjugative DNAtranslocation is not restricted to bacteria. It has been shown that conjugative plasmids of E.coli can even mobilize DNA to the yeast Saccharomyces cerevisiae[27, 340: 205-209]. Thus conjugation has the broadest host range among the mechanisms for inter bacterial genetic exchange. The best studied model of a conjugative plasmid is the F factor of E.coli. The conjugation system of F-like plasmids has been reviewed [29, 20: 593-624]; [66,1110-1131]; [15]. Recently, initiation and termination of DNA transfer during conjugation was studied in E.coli.

Temperature is one of the important factors that significantly influences gene transfer by conjugation. The maximum transfer frequencies were reported in the temperature range of 20-30°C [44, 37:177-181]. The normal water temperature during the day time in tropical countries like India lies in the range of 20-30°C [20, 190: 113-120] which may be very favorable for gene transfer.

Strain No.	Heavy metal tolerance pattern of donor	Markers transferred	FREQUENCY OF TRANSFER						
			Zn	Ni	Cu	Cr	Cd	Co	As
104	Cr, Cd, As, Hg	-	-	-	-	-	-	-	-
146	Zn, Ni, Cu, Cr, Cd, As, Co, Hg	-	-	-	-	-	-	-	-
135	Zn, Ni, Cu, Cr, Cd, As, Co, Hg,	As, Ni, Co	-	1 . 3 x 10-6	-	-	-	1.4x 10-4	1.4x 10-2
134	Zn, Ni, Cu, Cr, Cd, As,	As, Co	-	-	-	-	-	5.4x 10-6	0.4x 10-1
125	Zn, Ni, Cu, Cd, As, Co	As, Co	-	-	-	-	-	1.1x 10-6	2.0x 10-2
37	Zn, Ni, Cu, Cr, As, Co	As, Ni, Co	-	1.0x 10-6	-	-	-	1.2x 10-6	1.7x 10-3
1	Zn, Ni, Cu, Cr, Cd, As, Co, Hg	As, Co	-	-	-	-	-	7.5x 10-5	1.1x 10-3
38	Zn, Ni, Cu, Cr, As, Co, Hg	As	-	-	-	-	-	-	9.0x 10-2
138	Ni, Cu, Cr, As, Co	Ni, As	-	1.0x 10-5	-	-	-	-	2.5x 10-5
137	Zn, Ni, Cu, Cr, As, Cd, Co, Hg	As, Co, Ni	-	1.0x 10-7	-	-	-	2.0x 10-6	7.5x 10-1
119	Zn, Ni, Cu, As, Cd, Hg	As	-	-	-	-	-	-	1.1x 10-6
6	Cu, Cr, Cd, As	As	-	-	-	-	-	-	3.8x 10-5
124	Zn, Ni, Cu, Cd, Co, As, Hg	As	-	-	-	-	-	-	5.0x 10-3
108	Cd, As	As	-	-	-	-	-	-	5.5x 10-1
131	Zn, Ni, Cu, Cd, Co, As, Cr, Hg,	Zn, Ni, Cu, Cr	15x 10-3	12x 10-4	11x 10-3	6x 10-4	-	-	-
176	Zn, Ni, Cu, Cd, Co, As	Zn, Ni, Cu, Cr, Cd	26x 10-4	6x 10-3	10x 10-4	15x 10-3	20x 10-4	-	-
197	Zn, Ni, Cu, Cr, Cd, Co, As	Zn, Ni, Cu, Cr, Cd	34x 10-3	14x 10-4	8x 10-	19x 10-4	27x 10-5	-	-
202	Zn, Ni, Cu, Co, Cd, As	Zn, Ni, Cu, Cd	28x 10-5	14x 10-4	18x 10-4	-	30x 10-5	-	-
207	Cu, Cr, Co, Hg	Cu, Cr	-	-	46x 10-4	46x 10-4	-	-	-

The frequency of resistance marker transfer was as follows.

Arsenic	1.1 x 10–6	–	7.5 x 10–2
Cobalt	1.1 x 10–5	–	1.4 x 10–4
Nickel	1.5 x 10–7	–	1.0 x 10–5
Cadmium	30 x 10–5	–	46 x 10–4
Zinc	28 x 10–5	–	34 x 10–3
Copper	8 x 10–5	–	46 x 10–4
Chromium	46 x 10–4	–	15 x 10–3

Table 3 Transfer of heavy metal resistance among thermotolerant E. coli.

Resistance pattern studied	No. of strains	No. of strains transferred (%)
MAR	6	6 (100)
2R	11	11 (100)
1R	2	2 (100)
Total	19	19 (100)

Table 4 Transfer of resistance among thermotolerant MAR E. coli

Linked transfer of resistance to antibiotics and heavy metals among thermotolerant coliform species (E. coli and Klebsiella)

Linked transfer of resistance among thermotolerant coliform species (E. coli and Klebsiella) is shown in Table 8. Linked transfer among thermotolerant E. coli showed that B-As-Ni-Co were transferred in 3 (15.7%) strains, whereas B-As were transferred in 6 (31.5%) strains.In case of Klebsiella, linked transfer was observed for B-As in 3 (60.0%) strains (Table 8).The study indicated the linked transfer of antibiotic(s) and metal (s) especially among MAR isolates. The linked transfer may have serious public health implications. The sensitive isolates present in the drinking water may acquire resistance (s) borne on conjugative plasmids rendering them resistant, which will have an added advantage to adapt and survive in metal containing water system [56,7(2): 130-136]. The study also clearly indicates the linked transfer of antibiotic(s) and metal(s) especially among MAR microorganisms. The combined linked transfer may be due to gene determinants present on the same plasmid adjacent to each other. This may have serious public health implications. The sensitive organisms present in the drinking water may acquire resistance(s) borne on conjugative plasmids rendering them resistant which will have an added advantage to adapt and survive in metal containing water systems. It also strengthens the argument of [23, 8: 1-9] that antibiotic resistant bacteria

Strain No.	Antibiotic resistance pattern of donor	Markers transferred	Frequency of transfer	
			B	A
57	CR, A, V, B	B	2.0 x 10–5	NT
46	A, V, B	B	1.3 x 10–6	NT
81	C, V, B	B	7.5 x 10–3	NT
79	C, V, B	B	1.0 x 10–5	NT
63	A, V, B	B, A	3.4 x 10–4	2.4 x 10–6

NT- Not Transferred

Table 5 Transfer of antibiotic resistance among thermotolerant Klebsiella spp.

Strain No.	Heavy metal tolerance pattern of donor	Marker(s) transferred	Frequency of transfer	
			As	Cd
57	Ni, Cr, Zn, Co, Cu	As	8.0x10-5	-
46	Ni, Cu, As	As	2.0x10-5	-
81	Ni, Cu, Cd, Hg, As	As, Cd	3.7x10-5	1.2x10-4
79	As, Co	As	1.0x10-5	-
63	Cu, Cr, Cd, Co, As, Hg	-	-	-

Table 6 Transfer of resistance to heavy metals amongthermo-tolerant Klebsiella spp.

Resistance pattern	No. of strains studied	No. of strains transferred (%)
MAR	3	3 (100)
2R	2	2 (100)
Total	5	5 (100)

Table 7 Transfer of antibiotic resistance among MAR Klebsiella spp.

Resistance pattern	No. of strains (%)	
	E. coli	Klebsiella
B-As-Ni-Co	3(15.7)	—
B-As	6 (31.5)	3 (60.0)

Table 8 Linked transfer of resistance to antibiotics and heavy metals among thermotolerant coliform species (E. coli and Klebsiella)

should not be considered harmless indicators and calls for revaluation of bacteriological quality criteria of water.

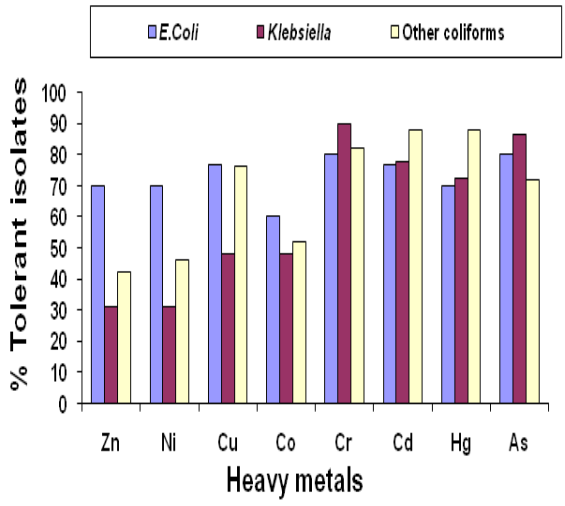


Fig.3 Comparison of the heavy metal resistance pattern among thermotolerant coliform Species
Zn- Zinc, Ni- Nickel, Cu- Copper, Co- Cobalt, Cr- Chromium, Cd- Cadmium, Hg- Mercury, As- Arsenic

Curing of resistance in E.coli. and Klebsiella

Twenty five E. coli strains were studied for curing of resistance. It was found that among antibiotics, curing was observed only for cephaloridine (66.6%). Curing was not detected in case of resistance to bacitracin, ampicillin, polymixin B, chloramphenicol and streptomycin (Table 9). Among heavy metals, in large number of organisms curing was detected for Ni (88.8%), Cr, (86.3%), Zn (83.3%), Co (82.3%) and Cu (78.9%). Moderate curing was observed for Cd (52.6%), Hg (50.0%) and As (31.3%). Eight strains of Klebsiella were studied for curing of resistance (Table 9). Among antibiotics, curing of resistance to only kanamycin (50.0%) was observed. Curing of resistance to ampicillin, bacitracin, cephaloridine and streptomycin was not detected. In case of heavy metals, cent per cent curing of resistance to Zn was observed. Curing was also observed for Cr (71.4%), Cu and Co (both 66.6%), Cd (60.0%) Hg and Ni (both 50.0%). Curing of resistance was detected in few organisms (33.3%).

The elimination of R-factor from bacteria conventionally is known as "Curing effect". Bacteria isolated from diverse habitats are known to contain plasmid DNA [57, 32: 39-54]. Some plasmids are stable and can be maintained through successive generation by being partitioned to each daughter cell during cell division. This allows each cell to receive at least one plasmid. Some plasmids undergo spontaneous segregation and deletion. However, the majority is extremely stable and requires the use of curing agents or other procedures (elevated growth temperature, thymine starvation) to increase the frequency of spontaneous segregation [9,97-122]. [8, 222–242] reported that the effective concentration of a particular curing agent can vary considerably, in the range of 100 to 1000 fold. This is dependent upon the species being treated, curing agent efficiency, and the mode of action of curing agent.

Relationship between plasmid transfer and curing among thermotolerant coliforms

The Relationship could not be established among thermotolerant coliforms in which transfer of ampicillin and bacitracin was observed, whereas these strains were not cured. On the other hand, resistance to streptomycin, cephaloridine and kanamycin was cured, however, their transfer was not possible (Figure 4A).

The relationship between resistance to heavy metal transferred and curing is shown in Figure 4 B.Resistance to Zn and Cd were transferred, whereas their curing was not detected. On the other hand, resistance to Hg was cured and its transfer did not occur.

The relationship between transfer and curing of resistance for Ni, Cu, Cr, Co and As was established but in very few organisms (Figure 4 B).

Relationship between plasmid transfer and curing among thermotolerant E. coli.

In E. coli, among antibiotics, relationship was not observed between plasmid-transferred and cured. Transfer of resistance to ampicillin and bacitracin was detected, whereas curing was observed only for cephaloridine (Figure 5A).

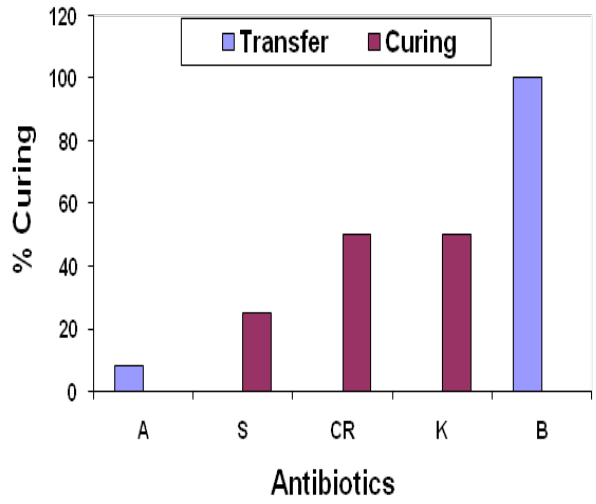
Organism	No. of strains studied											
		Antibiotics					Heavy Metals					
			S	Cr	K	Ni	Cu	Zn	Co	Cu	Hg	As
E.coli	25	1	2	-	16	19	15	15	14	10	11	6
		(40.0)	(8.0)		(88.8)	(8.0)	(78.9)	(83.3)	(82.3)	(52.6)	(50.0)	(31.5)
Klebsiella	8	-	-	1	2	5	4	3	2	3	3	1
				(50.0)	(50.0)	(71.4)	(66.6)	(100)	(66.6)	(60.0)	(50.0)	(33.3)
Total	33	1	2	1	18	24	19	18	16	13	14	7
		(3.0)	(6.0)	(3.0)	(54.5)	(72.7)	(57.5)	(54.5)	(48.4)	(39.3)	(42.4)	(21.2)

Table 9 Curing of R-plasmid among thermotolerant coliform species (E. coli and Klebsiella)

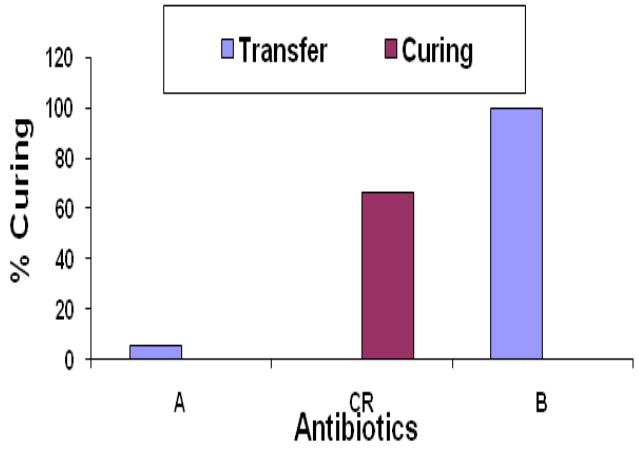
In case of heavy metals, relationship was observed between transfer and curing of resistance to Zn, Ni, Cu, Cr, Cd, Coand As, whereas Hg was only cured and not transferred. Relationship between transfer and curing for resistance to Ni was observed in 42.0% strains studied followed by resistance to arsenic (31.5%). Transfer and curing of resistance to Zn, Cr and Cd was detected in 21.0% strains only (Figure 5B).

Relationship between plasmid transfer and curing among thermotolerant Klebsiella species.

In Klebsiella spp., relationship was not observed between curing and plasmid transfer both for antibiotics as well as heavy metals. In case of antibiotics bacitracin was transferred and kanamycin was cured (Figgure 6A). Among heavy metals, Ni, Cu, Cr, Co, and Hg was cured and not



A-Ampicillin, S- Streptomycin, CR- Cephaloridine, K- Kanamycin, B- Bacitracin
Figure 4A- Antibiotics



A-Ampicillin, CR- Cephaloridine, B- Bacitracin
5A- Antibiotics
Figure

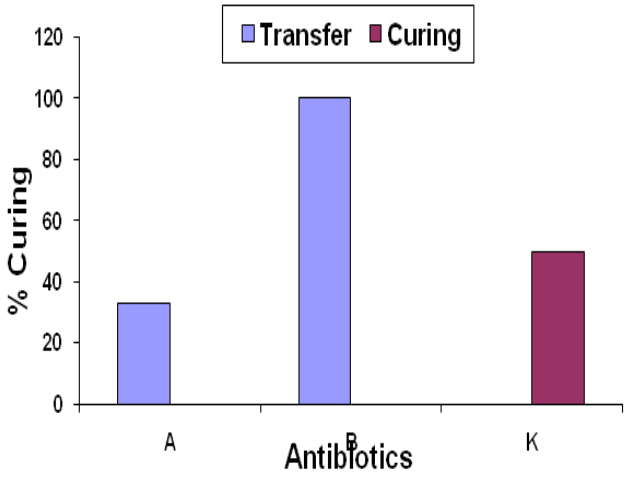
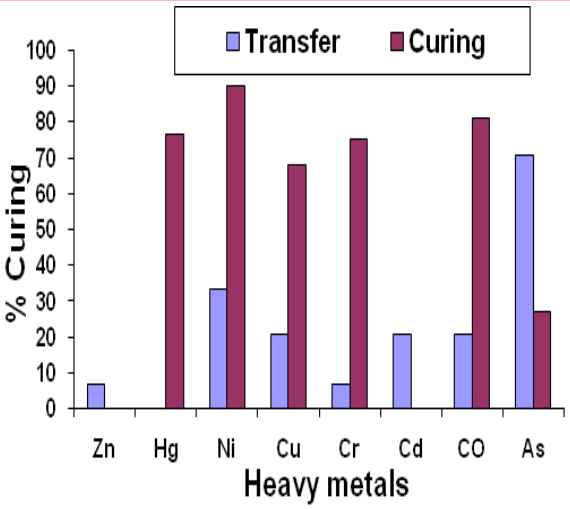
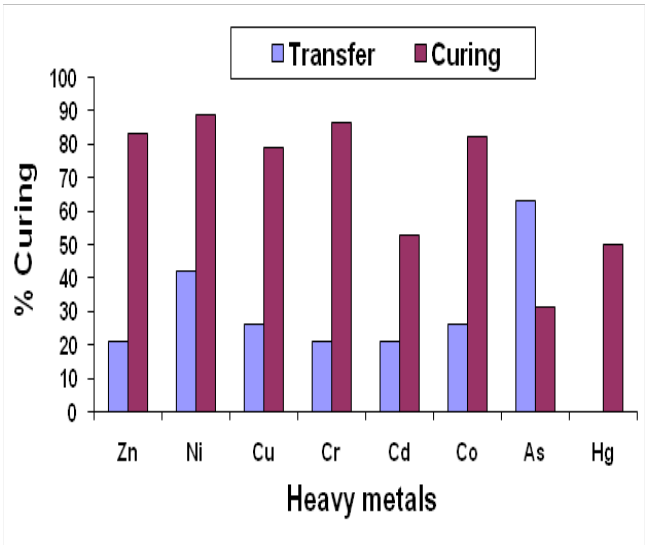


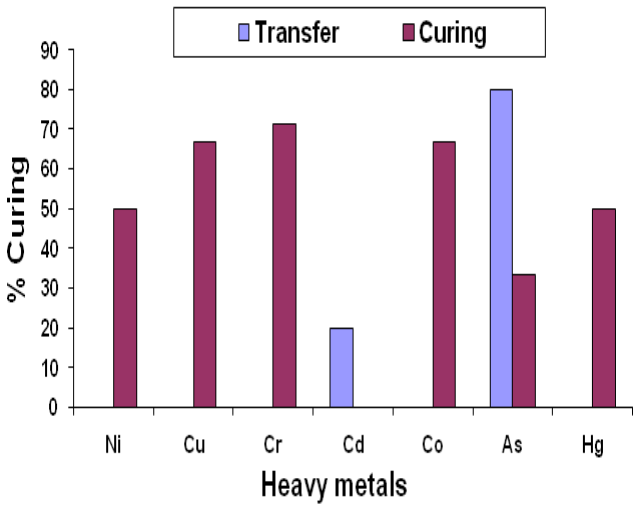
Figure- 6A
A- Ampicillin, B- Bacitracin, K- Kanamycin,



Zn- Zinc, Hg- Mercury, Ni- Nickel, Cu- Copper, Cr- Chromium, Cd- Cadmium, Co-Cobalt, As- Arsenic, Figure 4B Heavy Metal
Figure 4-Relationship between plasmid transfer and curing among thermotolerant coliform species



Zn- Zinc, Ni- Nickel, Cu- Copper, Cr- Chromium, Cd- Cadmium, Co-Cobalt, As- Arsenic, Hg- Mercury
Figure 5B Relationship between plasmid transfer and curing among thermotolerant E.coli



Ni- Nickel, Cu- Copper, Cr- Chromium, Cd- Cadmium, Co- Cobalt, As- Arsenic, Hg- Mercury
Figure 6B Relationship between plasmid transfer and curing among thermotolerant Klebsiella.

transferred, whereas Cd was transferred and not cured. Only As was transferred as well as cured. Relationship between transfer and curing for resistance to As was detected in 33.3% of Klebsiella spp. studied (Fig. 6B). Studies have suggested that antibiotic and metal resistance is frequently associated with plasmids (Foster, 1983).

Bacteria vary widely in their response to antibiotic induced stresses [26, 317(7159): 657-660]. There could be intrinsic resistance determined by chromosomal genes which are not transferable to other organisms [50, 2098-2107]. Bacterial resistance to antibiotics can also be acquired through mutation in the chromosomal genes or through acquisition of new genes responsible for antibiotic resistance. Genes responsible for acquired antibiotic resistance are often carried on genetic elements that can easily be transferred among bacteria. These could be plasmids, bacteriophages or transposons [63,285-295]; [32,10: 122-129]. In many cases, these genetic elements carry several antibiotic resistance genes, thus transferring multiple antibiotic resistances to other organisms.

Antibiotic resistance mechanisms utilized by bacteria include the production of enzymes that degrade the antibiotic [17,64, 375-382]; [36,13: 229-247]; [32,10: 122-129]. Some bacteria can rapidly pump the antibiotic out of the cell before it has chance to interact within the cell [13,29: 247-277]. Also some bacteria can produce enzymes that inactivate the antibiotic by adding additional chemical structures onto the antibiotic. Bacteria may also change their cell surface to reduce the affinity of the antibiotic to its target site. Bacteria may express more than one type of mechanisms to resist one antibiotic [32,10: 122-129]. As a result of the fact that many organisms typically found in surface waters have intrinsic antibiotic resistance (Farmer, 2003) or may not be pathogens, the identification of the resistant bacteria is required to interpret the health significance. Pathogenic bacteria with resistance to an antibiotic used to treat the infection caused by that organism are an obvious concern. Of equal importance but less obvious concern is the significance of the environmental (non pathogenic) bacteria with antibiotic resistances. This is because of the ability of environmental bacteria to transfer antibiotic resistance genes to human pathogens. The ability of environmental bacteria to transfer antibiotic resistance genes to human pathogens can have great consequences for human health.

The presence of antibiotic-resistant faecal indicator bacteria in streams and wells used as sources of water for human consumption may pose a threat to human health because of the potential for transfer of antibiotic resistance genes to pathogens and the environment in that they may act as reservoirs contributing to the maintenance and spread of antibiotic resistance genes [22,66(1): 125-132]; [61,14: 327-335]; [25,54: 321-332]. The increased incidence of MAR organisms has attracted the attention of many workers to the phenomenon of transferable drug resistance [16,21: 254-260]. Several workers reported that 50-75% of MAR strains are capable of transferring their resistance markers [55,18: 918-924]; [4,46: 227-232]; [64,50(4): 930-933]. Earlier studies in India reported the increasing trend in transfer of resistance among thermotolerant coliforms [45, 91: 185-188]; [20,190: 113-120]; [44, 37:177-181]. However, the results of present study revealed that all the thermotolerant coliforms were capable of transferring their resistance. The present study showed that acridine orange was able to eliminate resistance but the resistance pattern of the individual strains did not influence the percentage of cells cured. It was suggested by Singh and 54, 26(10): 668-670] that it is the characteristic of the plasmid which determines the curing frequencies. Although loss of antibiotic resistance on exposure to acridine was demonstrated, no relationship was observed between the strains exhibiting transfer of antibiotic resistance and those strains showing curing. In all the strains studied for transfer of resistance among antibiotics, bacitracin was transferred whereas it was not cured by acridine orange. However, plasmid borne nature of resistance is evident by their curing by curative agent. The high incidence of thermotolerant coliforms exhibiting multiple antibiotic resistance mediated by R-factors reinforces arguments previously put forth for a revalulation of the coliform standard and further emphasizes the fact that they should not be considered harmless indicators [23, 8: 1-9], [24, 9:777-782]. Further studies should be conducted on the treatment necessary for drinking water when antibiotic resistant bacteria are present in it.

Antibiotic resistance in bacteria is a serious problem facing society today and one of the reasons responsible for this problem is overuse of antibiotics in humans [42, 13(11): 1640-1646]. According to [51, 71(3): 1394-1404] the source of water contamination plays significant role in determining the extent of antimicrobial resistance as contaminating bacteria could come from domestic, wild animals or human sewage. Antibiotic resistance poses a threat to everyone most especially the children and the immunocompromised individuals that are more vulnerable to bacterial illnesses. For the general public as a whole antibiotic resistance limits the number of effective drugs available leading to fewer treatment options for the sick. There is therefore the need to control faecal pollution of water supply to avert the occurrence of waterborne diseases outbreak. Through effective public health education by relevant Government agencies, the people should be educated on the implications associated with the consumption of contaminated water for drinking and other domestic purposes. Public health education aimed at improving personal, household and community hygiene is imperative. For instance water needs to be adequately and appropriately treated or disinfected before consumption and well environments kept in hygienic conditions. To stem the tide of antibiotic resistance, infected persons should avoid self medication but seek proper medical attention so that appropriate antibiotic can be administered rather than using drugs indiscriminately which can lead to development of resistance by organisms. The importance of continuing with surveillance studies to follow the evolution of antimicrobial resistance in saprophytic bacteria of the intestinal tract recovered from water sources in order to detect possible reservoirs of antimicrobial resistance genes and antimicrobial-resistant bacteria should be emphasized.

Drinking water is heavily contaminated with potentially pathogenic multi drug resistant strains of E. coli. The source could possibly be the mixing of sewage lines with drinking water supply. Presence of multi drug resistant E. coli in drinking water can act as a vehicle to disseminate antibiotic resistance to other bacteria. This suggests a need to educate people regarding the rational use of antibiotics and safe

disposal of antibiotic containing waste. The water sources pose a threat to human health due to the danger of waterborne diseases and potential for the transfer of antibiotic resistance genes to pathogens. Effective public health education aimed at creating awareness of the implications of consumption of contaminated and untreated water is imperative. Antibiotics should only be administered based on physicians' prescription.

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