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RESEARCH PAPER

Incidence of resistance plasmid in thermotolerant bacterial indicators isolated from various sites of Ganges and Yamuna rivers, Allahabad, U.P.

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In this study the thermotolerant bacterial indicators *E.coli* and *Klebsiella* were isolated from the water samples of rivers Ganges and Yamuna from various sampling stations. Antibiotic susceptibility revealed the MDR among most of the thermotolerant bacterial species. In case of metal tolerance the thermotolerant species were found to be highly tolerant towards most of the metals tested. The curing pattern for the resistance traits were tested and found that the curing effect was observed in thermotolerant species. The resistant traits were found to be plasmid borne and were able to transfer their resistance to the sensitive ones. Presence of plasmid DNA was also observed in resistant strains of thermotolerant species which were found to be cured. The plasmids were of small to medium size. All the strains possess only one plasmid. The study demonstrates that the resistance to antibiotic and metal ions were found to be generally associated with transmissible R-plasmid.

Key words : Thermotolerant, Resistant plasmid, DNA, Transfer of resistance

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INTRODUCTION

Potable water supports public health and ensures economic growth. Water of poor quality can cause social and economic damages through water-related epidemics such as cholera which in turn increases medical treatment costs (Pritchard *et al.*, 2007). Total coliform (TC) and thermotolerant (fecal) coliform (FC) indicator tests are common public health tests of the safety of water and wastewater which might be contaminated with sewage of fecal material (APHA, 1995). Historically, water has played a significant role in the transmission of human disease. Typhoid fever, cholera, infectious hepatitis, bacillary and amoebic dysenteries and many varieties of gastrointestinal disease can all be transmitted by water (Rompre *et al.*, 2002). The EPA has determined that *E. coli* are one of the best indicators for the presence of potentially pathogenic bacteria (EPA, 1999).

Plasmids are known to carry resistance to antibiotics and metals (Sobecky, 1999; Rasmussen and Sorensen, 1998 and Smith *et al.*, 1993). Hence, the possibility of bacteria to harbor antibiotic/metal resistance traits via horizontal transformation can be anticipated. Bacteria adsorb, accumulate and transform heavy metals (Chan and Dean, 1988) in most food chains.

Due to the increasing phenomenon of incidence of R-plasmids and their transmissibility in bacteria which harbour the genes for multi drug resistance and heavy metal tolerance among bacterial population. Therefore, keeping this view in mind, the investigation was undertaken to study the ecological parameters of the rivers with special emphasis on thermotolerant bacterial indicators.

RESEARCH METHODOLOGY

Study area :

The water samples were collected from 7 sampling stations Baluaghat, Gaughat, Saraswati, (Yamuna ghats) Dashaswamedh ghat, Ramghat, Someshwar (Ganga ghats) and Sangam, (confluence). The samples were collected on the basis of monthly frequency.

Sample collection:

Water samples were collected from the rivers Ganga and Yamuna and also their confluence point, Sangam .The sample were collected from the upstream and downstream of flowing water of both the rivers in sterile containers and transported to the laboratory on ice.

Isolation, identification and characterization:

Thermotolerant bacterial indicators. Thermotolerant *E.coli* and *Klebsiella* spp. were isolated from positive coliform test using most probable number method (Aneja, 2003). Organisms were identified according to (Aneja, 2003).Tests employed to identify bacteria indole, methyl red, bouges-proskuaer test, citrate oxidase and oxidation fermentation test.

Bacterial strains :

A total of 156 thermotolerant coliforms were isolated and employed in the study: Strains of *E.coli* (36), *Klebsiella* spp. (30) and other coliforms (90).

Antibiotic susceptibility test : Susceptibility to antibiotics were determined by Curvy Bauer's method (Bauer *et al.*, 1966).

Metal tolerance test :

Tolerance to metal ion was determined by minimal inhibitory concentration technique. The metal salts used were cobalt chloride, cadmium chloride, potassium dichromate, nickel chloride, copper sulphate and mercuric chloride.

Screening of transferable resistance factors :

The screening of resistance factors was done as discussed by Walter and Vennes (1985)

Plasmid curing :

Plasmid curing was done according to plasmid curing method (Ramteke, 1993).

$$\text{Per cent cured} = \frac{\text{No. of organisms cured x 100}}{120}$$

RESEARCH FINDINGS AND ANALYSIS

Table 1 shows the percentage of *E.coli* and *Klebsiella* and other coliforms at different sampling stations. Among all the sampling stations percentage of *E.coli* were found to be highest in Someshwar ghat. Klebsiella per cent were found to be highest in Dashaswamedh ghat. Other coliforms were found to be highest in Saraswati ghat.

Antibiotic resistance among thermotolerant coliform species *E.coli*, *Klebsiella* and other coliforms :

E.coli :

Out of 156 thermotolerant coliforms 36 (23.07%) belonged to genus *E.coli* and 30 (19.2%) belonged to genus *Klebsiella* and and other coliforms 90 (57.69%). All strains of *E.coli* (100%) were resistant to ampicillin, vancomycin, bacitracin, cephalexin, trimethoprim, co-

Table 1 : Identification of organisms isolated from coliform tests

Sources	No. of isolates	*E.coli* (%)	*Klebsiella* (%)	Other coliforms (%)
Baluaghat ghat	24	4 (16.7%)	5 (20.83%)	15 (62.50)
Gaughat ghat	29	4 (13.79%)	5 (17.24%)	20 (68.96%)
Saraswati ghat	20	3 (15%)	2 (10%)	15 (75%)
Dashaswamedh ghat	18	3 (16.7%)	5 (27.8%)	10 (55.5%)
Ram ghat	24	9 (37.5%)	5 (20.8%)	10 (41.7%)
Someshwar ghat	23	9 (39.13%)	4 (17.39%)	10 (43.4%)
Sangam ghat	18	4 (22.2%)	4 (22.2%)	10 (55.6%)
Total	156	36 (23.1%)	30 (19.23%)	90 (57.69%)

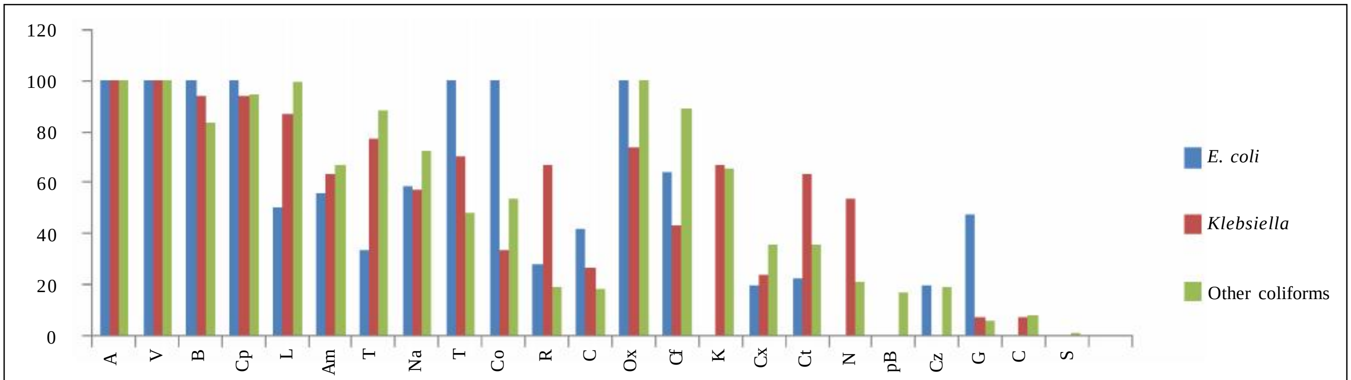
trimazole. The resistance observed in different antibiotics were cefadroxil (63.88%), nalidixicacid (58.33%), amoxicillin trihydrate (55.5%), lincomycin (50%), gentamycin (47.22%), carbenicillin (41.66%), tetracycline (33.3%) rifampicin (27.77%), cephotaxime (22.2%), ceftazidime (19.44%), cefixime (19.44%), kanamycin, neomycin, polymixin-B, chloramphenicol, streptomycin were all found to be sensitive to the strains of *E.coli* (Fig 1).

Klebsiella :

Majority of the thermotolerant *Klebsiella* spp. were resistant to ampicillin, vancomycin (100%), bacitracin, cephalexin (93.33%) followed by lincomycin (86.66%), tetracycline (76.66%), oxacillin sodium (73.33%), trimethoprim (70%), rifampicin and kanamycin (66.66%), cephotaxime (63.33%), nalidixicacid (56.66%), neomycin (53.33%).The resistance observed for cefadroxil (43.33%), co-trimazole (33.33%) and carbenicillin (26.66%) (Fig 1.) Low level of resistance was observed for gentamycin (6.6%), chloramphenicol (6.6%). polmixin-B, ceftazidime and streptomycin were found to be sensitive to all the *Klebsiella* spp. (Fig 1.)

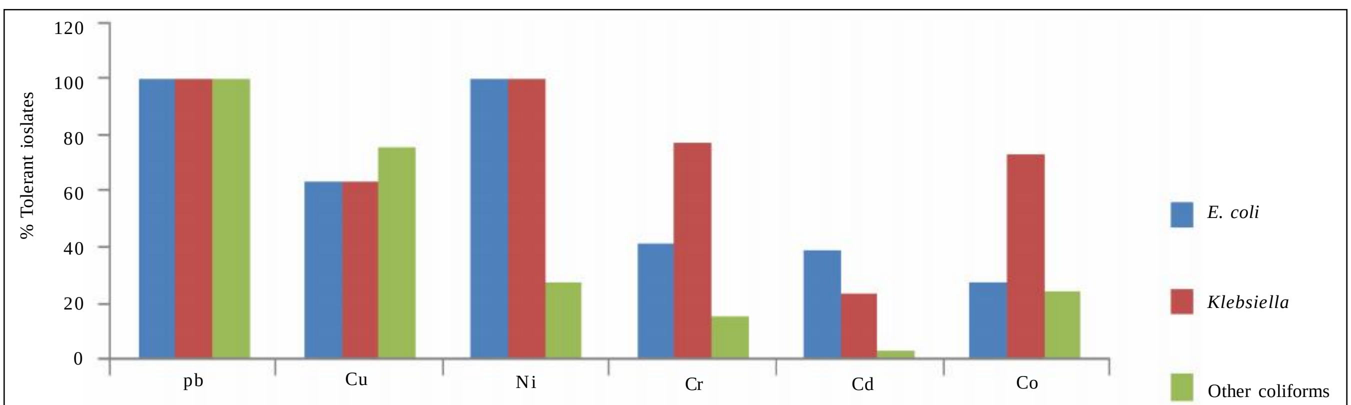
Other coliforms :

Most of the other coliforms species other than *E.coli* and *Klebsiella* were found resistant to, oxacillin sodium (100%) ampicillin (100%), vancomycin (100%), lincomycin (98.88%), cephalexin (94.4%), tetracycline (87.7%) and cefadroxil (88.8%), nalidixic acid (72.22%). The resistance was observed for kanamycin (65.55%), bacitracin (83.33%) and amoxicilin trihydrate (66.6%), trimethoprim (47.7%), cotrimazole (53.33%), rifampicin (18.88%),



A-Ampicilin,V-Vancomycin,B-Bcitracin,Cp-Cephalexin,L-lincomycin,Am-Amoxicillin,T-tetracycline,Co-Co-trimazole,R-Rifampicin, C-Carbenicillin,Ox-Oxacillin,Cf-Cefadroxil,K-Kanamycin,Cx-Cefixime,Ct-Cephotaxime,N-Naladixicacid, PB-Polymixin–B, Cz-Ceftazidime,G-Gentamycin,C-Chloramphenicol,S-Streptomycin

Fig. 1: Antibiotic resistances among thermotolerant coliform species (*E.coli*, *Klebsiella*, other coliforms)



Pb- lead, Ni-Nickel, Cr-Chromium, Cd- Cadmium, Co-Cobalt, Cu-Copper

Heavy metals

Fig. 2: Comparison of heavy metal tolerance pattern among thermotolerant coliform species

carbenicillin (17.77%), cefixime (35.55%), cephotaxime (35.55%), neomycin (21.1%), polymixinB (16.66%), ceftazidime (18.88%), gentamycin (5.55%), chloramphenicol (7.77%) and streptomycin (1%)

Metal tolerance among thermotolerant coliforms :

E.coli :

E.coli were found tolerant to Ni 100 per cent, Pb 100 per cent. The tolerance observed for Cu 63.3 per cent, Cr 41.66. Low level of tolerance were observed for Cd 38.8 per cent and Co 27.7 per cent (Fig. 2).

Klebsiella :

Ni and Pb were found to be 100 per cent and Cr 76.6 per cent tolerant towards *Klebsiella* sp. Cu showed tolerance towards 63.3 per cent isolates. *Klebsiella* sp. were found to be 73.3 per cent resistant to Co. Low level of tolerance was observed for Cd 23.3 per cent (Fig. 2).

Other coliforms:

Most of the thermotolerant coliforms other than *E.coli* and *Klebsiella* were found tolerant to Pb 100 per cent, Cu 75.5 per cent. Low levels of tolerance were observed in Ni 27.7 per cent, Co 24.4 per cent, Cr 15.55 per cent and Cd 3.33 per cent. (Fig. 2).

Curing of plasmid in *E.coli* :

Thiry six strains of *E.coli* were studied in for curing of resistance which were found to be resistance towards different antibiotics and metals. It was found that among antibiotics, curing was observed for ampicillin 61.1 per cent, tetracycline 63.88 per cent, cephalexin 55.85 per cent. Low level of curing was observed for vancomycin 16.66 per cent, trimethoprim 5.55 per cent and amoxicillin 2.7 per cent (Table 2).

Table 2 : Curing of resistance among *E.coli*

Resistance markers(µg/ml)	No. of strains cured (%)
Antibiotics	
Ampicillin	22 (61.1)
Tetracycline	23 (63.88)
Cephalexin	20 (55.5)
Co-Trimazole	1 (2.7)
Amoxicillin	1 (2.7)
Vancomycin	6 (16.66)
Trimethoprim	2 (5.55)

Among heavy metals curing was detected only in Pb (75%), Cr (72.2%) and Cu (22.2%). Curing was not detected in Ni, Co and Cd. (Table 3).

Table 3: Shows the percentage of *E.coli* strains cured in heavy metals

Heavy metals	No. of strains cured (%)
Ni(100µg)	-
Pb(200µg)	27 (75)
Cu(200µg)	8 (22.2)
Cr(50µg)	26 (72.2)
Co(25µg)	-
Cd(25µg)	-

Ni-Nickel, Pb-lead, Cu-Copper, Cr-Chromium, Co-Cobalt, Cd-Cadmium

Curing of resistance among *Klebsiella* :

Thirty strains of *Klebsiella* sp. which were found to be resistant to different antibiotics and metals were studied for curing of resistance (Table 4).

Table 4 : Curing of resistance in *Klebsiella* sp. (Antibiotics)

Resistance markers antibiotics	No. of strains cured (%)
Ampicillin	20 (66.6)
Kanamycin	15 (50)
Tetracycline	4 (13.33)
Cephalexin	16 (53.3)
Cefadroxil	12 (40)
Amoxicillin	10 (33.3)
Oxacillin	4 (13.33)
Vancomycin	4 (13.33)
Trimethoprim	6 (20)
Cotrimazole	14 (46.6)
Chloramphenicol	14 (46.6)

(Figures in parantheses indicate percentage)

Table 5: Curing of resistance in *E.coli* sp. (metals)

Resistance marker metals	No. of strains cured (%)
Pb (400µg)	28 (93.3)
Cu (200µg)	4 (13.33)
Cr (50µg)	24 (80)
Ni (200µg)	-
Cd(25µg)	-
Co(25µg)	-

(Figures in parantheses indicate the percentage of strains cured)

Transfer of antibiotic resistance among thermotolerant *E.coli.* :

Out of 36 strains of thermotolerant *E.coli*, 26 strains were studied for ability to transfer their resistance (Table 5). Among antibiotics, higher number of resistance was transferred in cephalexin (38.46%) of the strains, vancomycin (34.6%), oxacillin (30.76%), co trimazole (26.9%) followed by lincomycin (15.38%), neomycin (15.38%) and streptomycin (15.38%) of the conjugation experiments. The transfer frequency of cephalexin was $0.4x10^{-9}$-$8.9x10^{-9}$, vancomycin ($3.6x10^{-9}$-$8.9x10^{-9}$), oxacillin ($3.7x10^{-9}$-$7.3x10^{-9}$), cotrimazole ($3.2x10^{-9}$-$9.2x10^{-9}$), lincomycin ($5.3x10^{-9}$-$7.2x10^{-9}$), neomycin ($2.02x10^{-9}$-$7.2x10^{-9}$), streptomycin ($2.5x10^{-9}$-$6.4x10^{-9}$). The results of the present investigation indicate the wide spread occurance of transfer of resistance to antibiotics among thermotolerant coliforms in surface water sources (Alcaide and Garay (1984); Bell (1980 and 1981), Corliss (1985) and McPherson and Gealt (1986) demonstrated the same results.

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