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Horizontal gene transfers of multiple antibiotic resistant plasmid among enteric bacteria isolated from the runoff of the Gangotri glacier, Western Himalaya, India

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Abstract

Gangotri glacier is situated in the Uttaranchal, India between 30º56' N and 79º15'E. The Gangotri glacier around 30 km in length covering an area of 143 km^2, flowing north-west, the largest valley type glacier. Glacier store in them enormous water wealth but with increasing anthropogenic activities around glacier area deterioration of glacier run off is expected. The incidence of antibiotic resistant bacteria in aquatic environments has increased world wide as a consequence of the widespread use of antibiotics. This increase has resulted from a variety of factors, perhaps the most important of which is the selection for resistant strains and the ability of such strains to exchange plasmids encoding resistance. Plasmid are frequently conjugative important agents of horizontal gene transfer and often encoding antibiotic resistance, pathogenesis, virulence determinants and ecologically important factors.

Sixty four coliforms and 64 faecal coliforms were isolated from different sites of Gangotri glacier. Out of them 13 coliforms and 11 faecal coliforms were selected for detailed study of isolation, characterization and transfer of antibiotic/heavy metal resistant plasmid. The selection was based on varying combination of resistance to different selection markers tested. In conclusion, this study contributes, towards knowledge of naturally occurring antibiotic resistance, confirms the presence of plasmids and their characterization. As enteric bacteria are an important bacterial group owing to the medical and ecophysiological significance of its many representatives, the aim of this work is to assess R-plasmid presence and their transfer in enterobacterial species of different sites and altitudes of runoff of the Gangotri glacier.

1. INTRODUCTION

Gangotri glacier around 30 km in length covering an area of 143 km^2, flowing north west, the largest valley type glacier in the Western Himalaya (Singh and Yadav, 2000). It originates from Chaukhamba group of peaks of an altitude of 7100 m. Glacier store in them enormous water wealth but with increasing anthropogenic activities around glacier area deterioration of glacier runoff is expected. Coliforms, faecal coliform and faecal streptococci are representative of enteric bacteria which is well recognized indicator of faecal contamination in aquatic system (Baghel et al 2005; Kistemann et al., 2002; McLellan et al., 2001; Harwood et al., 2001; Pathak and Gopal, 2001). The incidence of antibiotic resistant bacteria in aquatic environments have increased worldwide as a consequence of the widespread use of antibiotics (Davies and Davies 2010; Martinez et al 2009; Martinez J.L., 2009, Martinez and Baquero 2009; Baghel et al 2003; Iversen et al., 2002; Borgen et al., 2002). This increase has resulted from a variety of factors, perhaps the most important of which is the selection for resistant strains and the ability of such strains to exchange plasmids encoding resistance (Licht et al., 2002). Natural bacterial isolates frequently harbour plasmids of

various sizes (Boyd and Hartl, 1997). Plasmids are frequently conjugative important agents of bacterial horizontal gene transfer and often encode antibiotic resistance, pathogenecity, virulence determinants and ecologically important factors (Mulec et al., 2002). The issue of antibiotic resistancehas rrecieved considerable attention due to the problem of emergence and rapid expansion of antibiotic-resistant pathogenic bacteria (Aminov and Mackie 2007).

An important factor is horizontal conjugal transfer and the dissemination of plasmid encoded properties in plasmid host range, the ability to be transferred to and maintained in different bacterial species. As enteric bacteria are an important bacterial group owing to the medical and ecophysiological significance of its many representatives, the aim of this work to assess R-plasmid presence and their transfer in enterobacterial species of different sites and altitudes of runoff of the Gangotri glacier.

2. MATERIAL AND METHOD

2.1 Sampling

Water samples were collected from 21 sampling sites from the runoff of Gangotri glacier covering an stretch of 250 km from Gangotri to Haridwar (Fig. 1), in sterile glass bottles, transported on ice to the base laboratory and processed within 6-8 hour of collection. Samples were collected during winter (Feb. 2000), summer (June, 2000) and monsoon (Sept. 2000) seasons.

2.2 Microbiological analysis of the samples and bacterial isolation

Coliform, faecal coliform and faecal streptococci count was determined by the standard most probable number (MPN) method (APHA, 1998). Coliforms were isolated by inoculation of samples in MacConkey broth and incubated at 37±0.5ºC for 48 h. The positive tubes were subcultured into brilliant green bile broth (BGBB) and incubated at 44.5±0.5ºC. Gas production in BGBB at 44.5±0.5ºC were used for the detection of faecal coliform after 48 hours of incubation. Faecal streptococci were isolated by inoculation of water samples into KF streptococcal broth and incubation at 37±0.5ºC for 48 h. Coliform, faecal coliform and faecal streptococci representing every study site were isolated by positive MPN tubes. Purified colonies were obtained by repeated subculturing of the positive tubes of the total coliform, faecal coliform and faecal streptococci separately on nutrient agar plates. Total number of bacteria were determined from the colony forming units (CFU).

$$\text{CFU (number of cells/ml)} = \frac{\text{Number of colonies}}{\text{Amount plate x dilution}}$$

2.3 Antibiotic susceptibility test (WHO, 1961; Bauer et al., 1966)

Antibiotic susceptibility test was performed by following method Bauer et al., 1966.

A loopful of fresh culture was diluted in 5 ml sterile phosphate buffered saline (PBS) and seeded onto Muller Hinton Agar plates. The antibiotic impregnated discs (Himedia) were placed on freshly prepared lawns of each strain on agar plates, incubated at 35-37ºC for 24 h and examined for the inhibition zones. Discs containing the following antibiotics (µcg/disc) were tested: Polymixin-B (50), Nitrofuranjoin (30), Ampicillin (25), Colistin (25), Nalidixic acid (30), Streptomycin (25), Tetracycline (30), Chloramphenicol (30), Kanamycin (30), Gentamycin (10).

2.4 Resistance to heavy metals

Resistance to heavy metals was performed by MIC Method (Calomaris et al., 1984). A loopful of fresh broth culture of the isolates were diluted in 5 ml of sterile phosphate buffer saline (PBS) and were inoculated on Muller Hinton Agar plates supplemented individually with heavy metals. The metal salts used were $CuSO_4$ (E. Merck), $ZnSO_4$, $NiCl_2$, $CO(NO_3)_2$, $K_2Cr_2O_7$, $HgCl_2$, $CdCl_2$, As_2O_3 $(CH_3COO)_2Pb$. The metal ion concentration tested ranged from 25 to 3200 µg/ml. The concentration of heavy metals were selected for tolerance pattern on which more than 50% isolates were resistant. The isolates exhibiting growth after overnight incubation at 37±1ºC were considered resistant to the metal.

2.5 Isolation of plasmid DNA

Plasmid DNA was isolated by modified method of Birnboin and Doly (1979) and Ish-Horowicz and Burke (1981). The lysate was resolved on 0.8% agarose gel and was visualized under UV light after staining with ethedium bromide. The molecular weight of plasmid was determined using the standard plasmid molecular weight ladder (Bangalore Genie).

2.6 Curing of R-plasmids

Curing of antibiotic and metal resistance was conducted by using curing agents (µg/ml) acridine orange (20) and mitomycin-C (10-20). Tubes containing 10 ml peptone water was supplemented with the curing agent and inoculated with overnight broth culture (0.1 ml) and incubated at 37ºC for 24 h. Appropriate dilutions of the culture were plated on nutrient agar to obtain

individual colonies at 37°C for 24 h. Resulting colonies were tested for loss of resistance by spot inoculation method on Muller-Hinton Agar plates containing the appropriate antibiotic and metal salts. Strains were also spot inoculated on plain nutrient agar, without any selection marker as control. Plates were then incubated at 37°C for 36-48 h and curing effect was assessed (indicated) by absence of bacterial growth on selection plates. The percentage cure or elimination of R-factor was calculated by dividing the total number of completely cured strains by the total number of resistant strains under study the multiplied with 100.

2.7 Transfer of R-plasmids

2.7.1 Conjugation

Coliforms (13) and thermotolerent coliforms (11), resistant to one or more antibiotics and sensitive to nalidixic acid, were tested for their ability to transfer their resistance to recipient strain *E. coli* K-12 J-62.

Overnight grown exponential phase cultures (0.1 ml) were inoculated into 10 ml of peptone water, incubated for 6 h, then 0.1 ml each of donor and recipient cultures were mixed in fresh broth and incubated for 18 h at 30oC for conjugation. Transconjugants were selected on Mac Conkey agar plates containing nalidixic acid with appropriate antibiotic/heavy metal by spreading the dilution of the mixed culture. Dilution of the mixed cultures were also plated on Mac Conkey agar plates containing appropriate concentration of the respective antibiotic to enumerate the donors. The rates of plasmid transfer (transfer frequency) were expressed as the number of transconjugants formed per donor.

$$\text{CFU (number of cells/ml)} = \frac{\text{Number of transconjugants}}{\text{Number of donors}}$$

3. RESULTS

3.1 Coliforms

Based on varying combinations of resistance to different selection markers tested, out of 64, 13 isolates were selected for detailed study of isolation, characterization and transfer of antibiotic/heavy metal resistant plasmid.

3.2 Antibiotic and metal resistance

The antibiotic and heavy metal resistance pattern of the selected 13 isolates is shown in Table 1.

All coliforms were found resistant to Pb^{2+} (100%). These isolates also showed frequent resistance to other heavy metals tested. The order of resistance was as follows: Zn2+ (69.23%), Cd2+ (69.23%), Ni2+ (61.53%), Co2+ (53.84%), Cr6+ (53.84%), As3+ (46.15%) and Cu2+ (38.46%). However, resistance to Hg2+ was detected in only 7.69% isolates. All the isolates were resistant to multiple metals.

Among antibiotic resistance, majority of the isolates were found resistant to tetracycline (53.84%), followed by gentamycin (46.15%), polymixin-B (38.46%), ampicilin (38.46%), colistin (38.46%), nitrofuranjoin (30.76%), chloramphenicol (23.07%) and kanamycin (23.07%), seven (53.84%) isolates were resistant to multiple antibiotics (MAR) and 46.15% isolates were found resistant to two antibiotics (2R).

3.4 Plasmid characterization

The plasmid profile of 13 selected isolates in shown in Fig. 2 a,b. Out of 13 isolates examined, presence of plasmid was detected in 8 isolates (Fig.2a,b; Table 1). The molecular size of the plasmids were estimated by comparison with standard plasmid ladder of λDNA digested with *EcoRI* and *HindIII*. Moreover the stability and maintenance of the plasmid was monitored by periodic examination of plasmids on agarose gels after preservation and multiple sub-culturing of the organisms. The plasmid profile of strain no. 46 revealed the presence of a single plasmid of 2.0 kb which was of molecular weight in comparison to the plasmid DNA of other seven strains. Exceptionally, in Sl. No. 40 the plasmid band was detected in the agarose gel but its molecular weight was out of the marker range used. The molecular weight of the remaining 6 strains was found ranging between 3.8 to 4.9 kb.

3.4.1. Transfer of R-plasmid

All the eight strains under study (harbouring plasmid) were assayed for their ability to transfer resistance to heavy metal and antibiotics by mating with recipient strain of *E. col* K0-12 J-62. Result of the resistance pattern of 8 donor strains as well as the R-factors transferred, with the respective conjugal frequencies are listed in Table 1. Transmissibility of different heavy metal and antibiotic was detected in 7(87.50%) strains. Traits which transferred most frequently were among antibicrobial, polymixin-B (100%), ampicillin (100%) gentamycin (100%), chloramphenicol (100%), tetracycline (83.33%) and kanamycin (50%) and among metals, Co^{2+} (100%), Cd^{2+} (100%), Cu^{2+} (100%), Ni^{2+} (85.71%), Cr^{6+} (75%), As^{3+} (66.66%) and Zn^{2+} (50%). Frequency of transfer of resistance to colistin (33.33%) and Pb^{2+} (37.50%) was found very low. Among antibiotic, resistance to

nitrofuranjoin and among metals, mercury resistance was not found to be transferred under experimental conditions. The rate of transfer for resistance to polymixin-B, ampicillin, tetracycline, chloramphenicol and gentamycin ranged from $22x10^{-2}$ to $34x10^{-2}$, $26x10^{-2}$ to $33x10^{-2}$, $19x10^{-2}$ to $28x10^{-2}$, $25x10^{-2}$ to $30x10^{-2}$ and $29x10^{-2}$ to $34x10^{-2}$, respectively. Furthermore, the transfer rates of colistin and kanamycin are $24x10^{-2}$ and $32x10^{-2}$, respectively. Among metals, the transmission rates for Zn^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Cr^{6+}, Cd^{2+}, As^{2+}, As^{2+} and Pb^{2+}, ranged from $16x10^{-2}$ to $19x10^{-2}$, $18x10^{-2}$, to $25x10^{-2}$, $18x10^{-2}$ to $23x10^{-2}$, $9x10^{-2}$ to $16x10^{-2}$, $19x10^{-2}$ to $28x10^{-2}$, $20x10^{-2}$ to $31x10^{-2}$, $12x10^{-2}$ to $17x10^{-2}$ and $17x10^{-2}$ to $26x10^{-2}$, respectively. The presumptive transconjugants revealed the heavy metal and antibiotic resistance pattern identical to those selection markers, which were transferred during conjugation.

3.5.2. Curing of R-plasmid

All the strains studied for plasmid transfer were also studied for curing of resistance marker (Table 1). Elimination of one or more resistance marker was detected in all the strains when treated with acridine orange. Curing of resistance markers was observed both for transferable and non transferable marker. Curing of transferable marker revealed elimination of resistance to polymixin-B, ampicillin, chloramphericol, kanamycin, cobalt, copper, chromium and arsenic in all the strains tested. Resistance to tetracycline, colistin, gentamycin, nickel, zinc, cadmium and lead was observed in 88.33%, 66.66%, 50%, 85.71%, 75%, 60% and 50% isolates, respectively, Elimination of resistance to nitrofuranjoin and mercury was not observed by acridine orange treatment.

3.5. Faecal coliforms

Based on varying combination of resistance to different selection markers tested, out of 64, 11 strains were selected for the detailed study of isolation, characterization and transfer of antibiotic/heavy metal resistant plasmid.

3.6. Antibiotic and metal resistance

The antibiotic and heavy metal resistance pattern of the 11 selected faecal coliforms is shown in Table 2.

All faecal coliforms were resistant to Pb^{2+} (100%). The isolates also showed frequent resistance to other heavy metals tested. The order of resistance was as follows: Ni^{2+} (81.9%), Cd^{2+} (72.8%), Co^{2+} (72.8%), Cu^{2+} (63.7%), Cr^{6+} (54.5%) and Zn^{2+} (45.6%). All the isolates were resistant to multiple metals. None of the isolates were found resistant to Hg^{2+}.

Among antibiotic resistance, majority of the isolates were found resistant to tetracycline (72.8%), followed by polymixin B (54.5%), colistin (54.5%), kanamycin (36.3%), chloramphenicol (36.3%), ampicillin (27.2%), streptomycin (27.2%) and gentamycin (27.2%). About, 73% isolates were resistant to multiple antibiotics (MAR), whereas only 27% isolates were found resistant to two antibiotics (2R). None of the isolates was resistant to only one antibiotic tested (1R).

1.7 Plasmid characterization

All the eleven cultures of faecal coliforms representing different resistance pattern were screened for the presence of plasmids by agarose gel electrophoresis. Out of eleven strains, in three strains plasmid was not detected on agarose gel electrophoresis (Table 3). These are strain no 16, 33 and 56. Demonstration of plasmid on agarose gel electrophoresis is shown in Fig. 3. Of the eight plasmid bearing strains, one strains each of no. 21 and no. 25 exhibited the presence of two bands on agarose gel. The molecular size of the plasmid of no. 21 was 4.2 and 2.0 kb and that for no. 25 was 38 and 1.93 kb. The plasmid molecular weight of the remaining strains was found ranging between 1.8 to 5.1 kb.

3.8. Transfer of R-plasmid

All the eight strains under study were assayed for their ability to transfer resistance marker to *E. coli* K-12 J-62 by conjugation. The resistance pattern of the eight donor strains as well as the R-factors transferred, with the respective conjugal frequencies are shown in Table 2. Transmissibility of different heavy metal and antibiotic was detected in all the test strains. Traits which transferred most frequently were among antimicrobials, streptomycin (100%), tetracycline (100%), gentamycin (100%), chloramphenicol (100%), polymixin-B (75%), kanamycin (66.66%) and ampicillin (50%) and among metals, Ni^{2+} (100%), Cu^{2+} (100%), Zn^{2+} (100%), Cr^{6+} (100%), Cd^{2+} (80%), Co^{2+} (66.66%) and Pb^{2+} (50%). Frequency of transfer of resistance to colistin (40%) and As^{3+} (25%) was found very low. Among antibiotics, resistance to nitrofuranjoin was not found to be transferred under experimental conditions. Resistance to mercury was found to be transferred in strain no. 21 with a frequency of 23×10^{-2}. The rate of transfer of resistance to polymixin-B, colistin, streptomycin, tetracycline, chloramphenicol, kanamycin and gentamycin ranged from 21×10^{-2} to 35×10^{-2}, 25×10^{-2} to 32×10^{-2}, 24×10^{-2} to 29×10^{-2}, 19×10^{-2} to 34×10^{-2}, 25×10^{-2} to 36×10^{-2}, 18×10^{-2} to 23×10^{-2} and 18×10^{-2} to 29×10^{-2}, respectively. Furthermore, the transfer rate for ampicillin was 24×10^{-2}. Among metals, the transmission rates for Zn^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Cr^{6+}, Cd^{2+} and Pb^{2+} ranged from 9×10^{-2} to 15×10^{-2}, 18×10^{-2} to 29×10^{-2}, 18×10^{-2} to 31×10^{-2}, 13×10^{-2} to 24×10^{-2}, 19×10^{-2} to 32×10^{-2}, 8×10^{-2} to 19×10^{-2} and 23×10^{-2} to 31×10^{-2},

respectively. The transfer rate for arsenic was 11 $\times 10^{-2}$. Transconjugants recovered were resistant to the respective concentration of the heavy metal and antibiotic which were transferred during conjugation.

3.9. Curing of R-plasmid

All the strains studied for plasmid transfer were also studied for curing of resistance markers (Table 2). In all the strains tested, one or more resistance marker(s) was lost by treatment with acridine orange. Curing of resistance markers was observed both for transferable and non-transferable markers. Curing of transferable markers revealed elimination of resistance to streptomycin, tetracycline, gentamycin, chloramphenicol, nickel, copper, arsenic and chromium in all the strains tested. Resistance to colistin, polymixin-B, kanamycin, cobalt, cadmium, lead and zinc was observed in 80%, 75%, 33.33%, 83.33%, 80%, 62.5% and 33.33% isolates, respectively. Resistance to mercury was transferred during conjugation in strain no. 21 but was not cured by acridine orange. Elimination of resistance to nitrofuranjoin was not observed by acridine orange treatment.

4. DISCUSSION

The results clearly suggest that resistance to different antibiotics and heavy metals among coliforms and faecal coliforms is plasmid mediated. Resistance in bacteria to many antimicrobials and also toxic metals and metalloid ions has been known to be conferred by plasmids (Silver and Phung, 1996; Lawlor et al., 1999; Bruins et al., 2000; Richards et al., 2002; Nwosu and Ladapo, 1999; Oppeggard et al., 2001; Schroeder et al., 2002; Smallar et al., 2000). The results of the present study support the findings of earlier researchers. The development of antibiotic resistance in bacteria has been mostly attributed to the use of antimicrobials in human medicine and veterinary use (Teuber, 2001).The occurrence and spread of antibiotic–resistance bacteria(ARB) are pressing public health problems worldwide, and aquatic ecosystem are a recognized reservoir for ARB and antibiotic resistance genes (ARGs)(Xi et al 2009).The increased prevalence and dissemination of bacterial antimicrobial resistance is a natural expression of evolution and bacterial genetics (Martinez and Baquero 2009; Levy, 2000). The more a particular antimicrobial agent is used, the greater the chance of microorganisms developing resistance (Aminov and Mackie 2007; White and Dermott, 2001). However, bacterial resistances to metal ions seems to be directly related to the presence and adaptive response to these elements in the environment (Top et al., 1994). A cell may develop metal resistance system in an attempt to protect sensitive cellular components (Bruins et al., 2000). Association between resistance to antibiotics and heavy metals have been reported by several workers (Dhakephalkar and Chopade, 1994; Ramteke, 1997; Schwarz and Hobel, 1989, 2000, 2001). In all these cases, genes encoding resistance to antibiotics and metals were located on transmissible plasmids. Our study based on plasmid transfer and curing indicates that both antibiotic and metal resistance are plasmid mediated. The prevalence of such metal tolerant microorganisms is ecologically very important when they are antibiotic resistant (Timoney et al., 1978). Results of plasmid transfer studies indicates replication of plasmid from donor strains in recipient strains and possessed genetic information necessary for the expression of different selection markers. The study also indicated the linked transfer of antibiotic(s) and metal(s) especially among MAR organisms. The combined linked transfer may be due to gene determined present in the same plasmid (Ramteke, 1997; Lawlor et al., 1999). The sensitive organisms present in the water may thus acquire resistance's borne on transferable plasmids rendering then resistant which will have an added advantage to adopt and survive in polluted water system. Along with these strains, there were some other resistant strains which did not express their transfer potential in the present investigation and hence precludes the possibility that such bacteria might transfer under different conditions or with another recipient. Furthermore, plasmid borne nature of resistance was also evident by the curing of selection markers. Since curing of resistance marker in test strains was observed both for transferable and non-transferable traits, hence it suggest that the resistance marker may be possibly on a separate gene. The cured transconjugant lost the properties of transferable markers indicating that the plasmid of the isolates/strains encodes gene for resistance to selection marker, which is stable. Under environmental conditions of stress, such antibiotic and metal resistant population will adapt faster by the spread of R-factors than by mutation and natural selection thereby leading to a very rapid increase in their number (Bhattacherjee et al., 1988). Although the possession of antibiotic resistance may not have any survival importance under environmental conditions, these traits lead to a very serious public health hazard. The results of this study assume greater significance from the public health point of view because some of our test strain were found pathogenic, based on their serotypes. The genome of bacteria, especially of enterobacteriaceae family is of high plasticity allowing it to gain or lose genes at a relatively high frequency (Muhldorfer and Hacker, 1994). The conjugative plasmids contain more information than just the resistance markers. Apart from R-factors bacteria may also harbour other plasmids such as those which transmit enteropathogenecity among *E. coli* and other organisms (Kuhnert et al., 2000; Griffin, 1999; Schroeder et al., 2002, Bettelheim, 2001), so strain with new combination of virulence genes might emerge in the future. The presence of such pathogenic types of bacteria may post therapeutic problems and is a direct threat to human and animal health. This observation needs due consideration because infections caused by such antibiotic resistant pathogens will complicate the antibiotic therapy. The R-plasmid harbouring pathogens also have a greater chance of survival and propagation in natural ecosystems than that of strains lacking plasmid (Schubert et al., 1998). The development and spread of antibiotic resistance genes (Chadha, T 2012). The antibiotic resistance by horizontal gene transfer has

a major role on t Thus, this arises the necessity to limit the dissemination of R-plasmid bearing bacteria in drinking water bodies to protect the human population against the hazard of infection with R-bacteria.

It is also probable that some of our test isolates bear certain resistance traits, which have a chromosomal origin and probably genetically regulated to repress transfer. Some related metal resestance systems have been determined by chromosomal genes e.g. Hg and Cd resestance bacillus and As resestance in *E. coli* (Silver, 1996).A good programme for the prevention of resistance must include an active system for the surveillance of antimicrobial resistance and antibiotic usage (Foucault and Brouqui 2006)

As such waters are finally released in the environment such as in different water bodies for drinking or irrigation purpose, it ultimately enters the food chain (Barman et al., 2000). Therefore, cause of any newly emerging diseases may be the microbes present in water. The presence of such strains may post therapeutic problems and is a direct threat to human and animal health. This calls for a reevaluation of bacteriological water quality criteria, and stresses the importance of more abundant purification of water prior to its discharge for public use. The observation of this concern should be considered in dealing with water-borne public health problems.

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Table 1: Resistance pattern, curing pattern, trait transferred and conjugation frequency among coliforms

Strain No.	Resistance Pattern	Curing Pattern	TRAIT TRANSFERRED	Conjugation frequency	
1	PB, A, T, $Ni^{i2}+$, Pb^{2+}	PB, A, T, $Ni^{i2}+$, Pb^{2+}	PB, A, T Ni^{2+}	PB A T Ni^{2+}	32×10^{-2} 27×10^{-2} 19×10^{-2} 21×10^{-2}
15	NJ, T, Zn^{2+}, Ni^{2+}, Pb^{2+}	NJ Zn^{2+}	No Conjugation		-
20	NJ, A, T, C, Ni^{2+}, Co^{2+} Cd^{2+}, Pb^{2+}	A, T, C Ni^{2+}, Co^{2+}, Cd^{2+}	A, T, C Ni^{2+}, Co^{2+}, Cd^{2+}	A T C Ni^{2+} Co^{2+} Cd^{2+}	33×10^{-2} 24×10^{-2} 30×10^{-2} 24×10^{-2} 13×10^{-2} 25×10^{-2}
22	CO, K, Zn^{2+}, Cu^{2+}, Co^{2+}, Cr^{6+} Cd^{2+}, Pb^{2+}	CO, K, Zn^{2+}, Cu^{2+}, Co^{2+}, Cr^{6+} Cd^{2+}, Pb^{2+}	CO, K, Zn^{2+}, Cu^{2+}, Co^{2+}, Cr^{6+} Cd^{2+}, Pb^{2+}	CO K Zn^{2+} Cu^{2+} Co^{2+} Cd^{2+} Pb^{2+}	24×10^{-2} 32×10^{-2} 19×10^{-2} 21×10^{-2} 11×10^{-2} 27×10^{-2} 20×10^{-2}
25	A, CO, T, G, Zn^{2+}, Ni^{2+}, Cr^{6+}, Pb^{2+}	A, CO, T, Ni^{2+}, Cr^{6+}, As^{3+}, Pb^{2+}	A, T, G, Ni^{2+}, Cr^{6+}, As^{3+}, Pb^{2+}	A T G Ni^{2+} Cr^{6+} As^{3+} Pb^{2+}	20×10^{-2} 22×10^{-2} 29×10^{-2} 18×10^{-2} 28×10^{-2} 12×10^{-2} 17×10^{-2}
40	PB, C, Ni^{2+}, Co^{2+}, Cd^{2+}, $As3^{+,}$ Pb^{2+}	PB, C, Ni^{2+}, Co^{2+}, Cd^{2+}, $As3^{+,}$ Pb^{2+}	PB, C Ni^{2+}, Co^{2+}, Cd^{2+}, $As3^{+,}$ Pb^{2+}	PB C Ni^{2+} Co^{2+} Cd^{2+} As^{3+} Pb^{2+}	28×10^{-2} 25×10^{-2} 19×10^{-2} 16×10^{-2} 24×10^{-2} 17×10^{-2} 26×10^{-2}
46	A, CO, T, G Zn^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Cr^{6+}, Cd^{2+}, As^{3+}, Pb^{2+}	A, T, G Zn^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Cr^{6+}, Cd^{2+}, As^{3+}	A, T, G Zn^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Cr^{6+}, Cd^{2+}, As^{3+}	A T G Zn^{2+} Ni^{2+} Cu^{2+} Co^{2+} Cr^{6+} Cd^{2+}	32×10^{-2} 28×10^{-2} 34×10^{-2} 16×10^{-2} 22×10^{-2} 18×10^{-2} 13×10^{-2} 10×10^{-2} 31×10^{-2}
53	PB, A, T, K, Ni^{2+}, Cu^{2+}, Co^{2+}, Cr^{6+}, Hg^{2+} Cd^{2+}, Pb^{2+}	PB, A, T, K Ni^{2+}, Cu^{2+}, Co^{2+}, Cr^{6+}	PB, A, T, Ni^{2+}, Cu^{2+}, Co^{2+}, Cr^{6+}, Cd^{2+}	PB A T Ni^{2+}	34×10^{-2} 26×10^{-2} 21×10^{-2} 25×10^{-2}

Strain No.	Resistance Pattern	Curing Pattern	TRAIT TRANSFERRED	Conjugation frequency	
				Cu^{2+}	23×10^{-2}
				Co^{2+}	9×10^{-2}
				Cr^{6+}	26×10^{-2}
				Cd^{2+}	20×10^{-2}

Represents similar resistance marker for 1-14 isolates

Table 2: Resistance pattern, curing pattern, trait transferred and conjugation frequency among faecal coliforms

Strain No.	Resistance Pattern	CURING PATTERN	Trait transferred	Conjugation frequency	
2	A, CO, S, T, K $N^{i2}+$, Cu^{2+}, Cd^{2+}, Pb^{2+}	CO, S, T, $N^{i2}+$, Cu^{2+}, Cd^{2+}	CO,S,T,K Ni^{2+}, Cu^{2+}, Cd^{2+}	CO	32×10^{-2}
				S	29×10^{-2}
				T	22×10^{-2}
				K	18×10^{-2}
				Ni^{2+}	18×10^{-2}
				Cu^{2+}	31×10^{-2}
				Cd^{2+}	8×10^{-2}
7	PB, T, Zn^{2+}, Ni^{2+}, Pb^{2+}	PB, T Zn^{2+}, Ni^{2+}, Pb^{2+}	PB, T Zn^{2+}, Ni^{2+}	PB	35×10^{-2}
				T	19×10^{-2}
				Zn^{2+}	19×10^{-2}
				Ni^{2+}	23×10^{-2}
12	CO, G, Cu^{2+}, Co^{2+}, As^{3+}, Pb^{2+}	CO, G, Cu^{2+}, Co^{2+}, As^{3+}, Pb^{2+}	G Cu^{2+}, Co^{2+}, As^{3+}, Pb^{2+}	G	29×10^{-2}
				Cu^{6+}	18×10^{-2}
				Co^{2+}	21×10^{-2}
				As^{3+}	11×10^{-2}
				Pb^{2+}	23×10^{-2}
21	PB, A, T,C Ni^{2+}, Cu^{2+}, Co^{6+}, Cr^{6+}, Hg^{2+}, As^{3+}, Pb^{2+}	A, T,C Ni^{2+}, Cu^{6+}, Co^{2+}, $Cr^{6+,}$ Hg^{2+}, As^{3+}	A, T, C, Ni^{2+}, Cu^{6+}, Co^{2+}, $Cr^{6+,}$ Hg^{2+},	A	24×10^{-2}
				T	20×10^{-2}
				C	36×10^{-2}
				Ni^{2+}	29×10^{-2}
				Cu^{6+}	22×10^{-2}
				Co^{2+}	24×10^{-2}
				Cr^{6+}	23×10^{-2}
				Hg^{2+}	23×10^{-2}
25	CO, T, K, Ni^{2+}, Co^{2+}, Cr^{6+}, $Cd^{2+,}$ Pb^{2+}	CO,T,K Ni^{2+}, Co^{2+}, Cr^{6+}, $Cd^{2+,}$ Pb^{2+}	CO, T, K Ni^{2+}, Co^{2+}, Cr^{6+}, $Cd^{2+,}$ Pb^{2+}	CO	25×10^{-2}
				T	29×10^{-2}
				K	23×10^{-2}
				Ni^{2+}	24×10^{-2}
				Co^{2+}	13×10^{-2}
				Cr^{6+}	31×10^{-2}
				Cd^{3+}	19×10^{-2}
				Pb^{2+}	29×10^{-2}
40	PB, CO, T, G Zn^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Cr^{6+}, Cd^{2+}, Pb^{2+}	PB, T, G Zn^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Cr^{6+}, Cd^{2+}, Pb^{2+}	PB, T, G Zn^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Cr^{6+}, Cd^{2+}, Pb^{2+}	PB	21×10^{-2}
				T	28×10^{-2}
				G	18×10^{-2}
				Zn^{2+}	9×10^{-2}
				Ni^{2+}	29×10^{-2}
				Cu^{2+}	26×10^{-2}
				Co^{2+}	19×10^{-2}
				Cr^{6+}	32×10^{-2}
				Cd^{2+}	17×10^{-2}
				Pb^{2+}	25×10^{-2}
49	CO, S, C, K $Zn^{2+}Ni^{2+}$, Cu^{2+}, Co^{2+}, Cd^{2+}, As^{2+} Pb^{2+}	CO, S, C, $Zn^{2+}Ni^{2+}$, Cu^{2+}, Co^{2+}, Cd^{2+}, As^{2+}	S, C $Zn^{2+}Ni^{2+}$, Cu^{2+}, Co^{2+}, Cd^{2+},	S	24×10^{-2}
				C	33×10^{-2}
				Zn^{2+}	15×10^{-2}
				Ni^{2+}	26×10^{-2}
				Cu^{2+}	29×10^{-2}

Strain No.	Resistance Pattern	CURING PATTERN	Trait transferred	Conjugation frequency
				Co^{2+} 13×10^{-2} Cd^{2+} 13×10^{-2} Cd^{2+} 14×10^{-2}

Table contd.

Strain No.	Resistance Pattern	CURING PATTERN	Trait transferred	Conjugation frequency
61	PB, T, C, $Zn^{2+}Ni^{2+}$, Cu^{2+}, Co^{2+}, Cd^{2+}, As^{2+} Pb^{2+}	PB, T, C, Zn^{2+}, Cu^{2+}, As^{3+} Pb^{2+}	PB, T, C, Ni^{2+}, co^{2+}, Cr^{6+}, Pb^{2+}	PB 28×10^{-2} T 34×10^{-2} C 25×10^{-2} Cu^{2+} 25×10^{-2} Cr^{6+} 27×10^{-2} Pb^{2+} 31×10^{-2}

Table 3: Resistance pattern and presence/absence of plasmid among faecal coliforms

Strain No.	Resistance Pattern		Plasmid present/absent
2	A, CO, S, T, K	Ni^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}	Present
7	PB, T	Zn^{2+}, Ni^{2+}, Pb^{2+}	Present
12	CO, G	Cu^{2+}, Co^{2+}, As^{3+}, Pb^{2+}	Present
16	CO, S, C, G	Ni^{2+}, Co^{2+}, Cr^{6+}, Cd^{2+}, As^{3+}, Pb^{2+},	Absent
21.	PB, A, T, C	Ni^{2+}, Cu^{2+}, Co^{2+}, Cr^{6+}, Hg^{2+}, As^{3+}, Pb^{2+}	Present
25	CO, T, K	Ni^{2+}, Co^{2+}, Cr^{6+}, Cd^{2+}, Pb^{2+}	Present
33	PB, T	Zn^{2+}, Cu^{2+}, Cd^{2+}, As^{3+}, Pb^{2+}	Absent
40	PB, CO, T, G	Zn^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Cr^{6+}, Cd^{2+}, Pb^{2+}	Present
49	CO, S, C, K	Zn^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Cd^{2+}, As^{3+}, Pb^{2+}	Present
56	PB, NJ, A, T, K	Zn^{2+}, Ni^{2+}, Co^{2+}, Cr^{6+}, Hg^{2+}, Cd^{2+}, As^{3+}, Pb^{2+}	Absent
61	PB, T, C	Ni^{2+}, Cu^{2+}, Co^{2+}, Cr^{6+}, Cd^{2+}, As^{3+}, Pb^{2+}	Present