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RESEARCH ARTICLE

Partial Characterization Of Various Bacteria Isolated From Indigenous Environments Of Karachi and Baluchistan

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Abstract

Bacteria were isolated from Soil samples, Air samples, Marine samples and Food Samples collected from different habitats. Bacterial isolates were tested for their antibiotic resistance for different antibiotics like Ampicillin, Kanamycin, and chloramphenicol, Tetracycline, Streptomycin and Neomycin. Resistance against heavy metals was also checked for copper, cobalt, Zinc, chromium, Nickel and Lead. Plasmid profiles were also carried out. This study will provide a brief idea of bacterial diversity in different environments.

INTRODUCTION

Bacteria are a diverse group of organisms found in every environment. Many species have adapted to a particular niche, allowing the species to exploit the resources necessary for survival. Bacteria are also metabolically diverse. Even among closely related species, nutritional needs may be significantly different. There is growing concern that metal contamination functions as a selective agent in the proliferation of antibiotic resistance. Documented associations between the types and levels of metal contamination and specific patterns of antibiotic resistance suggest that several mechanisms underlie this co-selection process. These co-selection mechanisms include co-resistance (different resistance determinants present on the same genetic element) and cross-resistance (the same genetic determinant responsible for resistance to antibiotics and metals). Metal and antibiotic contamination, therefore, represents a long-standing, widespread and recalcitrant selection pressure with both environmental and clinical importance that potentially contributes to the maintenance and spread of antibiotic resistance factors(Baker et al). This paper focuses on the characterization and interaction of microbes with environment by interacting with metal and antibiotics. Microbial interactions with metals may have several implications for the environment. These microbes may play a large role in the biogeochemical cycling of toxic heavy metals also in cleaning up or remediating metal-contaminated environments. There is also evidence of a correlation between tolerance to heavy metals and antibiotic resistance, a global problem currently threatening the treatment of infections in plants, animals, and humans (Spain et al).Bacteria are able to inherit antibiotic resistance genes to provide protection against most antibiotics. The dissemination of antibiotic resistance genes by horizontal gene transfer has led to the rapid emergence of antibiotic resistance among bacteria (Ploy et al). Extensive studies have shown that mobile genetic elements such as plasmids and transposons are able to facilitate the spread of genetic material between species or genera of bacteria. In the 1980s, genetic elements termed integrons were identified on these mobile elements (Stokes et al). Antibiotic use in clinical and nonclinical settings plays an important part in the development of antibiotic-resistant bacteria throughout the world (Cunha,B.A).

MATERIALS AND METHODS:

BACTERIAL ISOLATES:

A total of 511 strains of Bacteria were isolated from various environments. Soil Samples were collected from Petrol pumps, workshops, Shipyard and Garden soil. Marine Samples were collected from the coastal areas of Karachi and Baluchistan, Both Attached and free living bacteria were isolated. Air Samples were collected from different places in the University of Karachi. Samples of Industrial effluent were collected from Malir river water contaminated with industrial waste, effluent from Karachi shipyard, Metropolitan steel , Tannery and Pak steel mill. Lactobacilli were isolated from food samples like fruit juices, yogurt and Dairy products.

Samples	**Cotaminated Soil sample**	**Marine sample**	**Air sample**	**Industrial Effluent**	**Food Sample**
No of isolates	61	156	6	22	_
Pseudomonas sp	8	44	4	16	_
Bacillus sp	_	17	_	1	_
E.coli	_	7	_	3	_
Enterobacter sp	2	5	_	1	_
Vibrio	_	10	_	_	_
Staph sp	_	10	_	_	_
Lactobacilli sp	_	_	_	_	24
Micsillenious Organisms	1	58	2	1	_
Not Identified	50	12	_	_	_

MEDIA USED:

For the isolation and purification of bacteria Nutrient Agar, Luria Bertani Broth, Luria Agar, Tris Gluconate minimal media, ASW media (Lyman and Fleming, 1940) ,Brain Heart infusion broth, McConkey broth, Selenite broth, TCBS Agar, VRBDA Agar, SS AGAR, Blood agar and Baired Parker Agar of (Oxoid, Difco, Sigma) were used.

Antibiotic resistance testing:

The antibiotic resistance patterns of strains were routinely tested by Disk diffusion method and Pour plate method. Antibiotic resistance was checked against Ampicillin, Kanamycin, Chloramphenicol, Tetracycline, Streptomycin and Neomycin. Antibiotics resistance was checked from 20µl-----200 µl concentration.

Metal resistance testing:

All isolates were tested to determine the resistance against six metal salts by pour plate method. Metal resistance was checked for CoCl2, CuSO4, ZnCl2, Cr2O5, Nicl2, Pb(CHCOO)2 from 0.5 mM----2.0 mM concentration.

Plasmid Analysis:

For isolation of plasmids from understudied bacteria different methods were used. For Lactobacilli Anderson and McKay's method, Kado and Liu method (Kado and Liu, 1981), Flexiprep method (Amersham), Cesium Gradient method (, Birnboim and Doly method (Birnboim and Dolly, 1979)

Results:

511 strains of bacteria were isolated from different samples.

Table 1.1

Strain Code	Identification	Source of Isolation	Antibiotic Resistance	Metal Resistance
CMGKUKU57	Bacillus sp	Industrial effluent	Amp, Tc, Cm, Sm, Rif, Km	Ni, Cd, Cu, Co, Zn, Pb, Fe
CMGKU 603	Bacillus subtilis	Marine Sample	Amp	Co, Cr
CMGKU 615	Bacillus cereus	Marine Sample	Amp, Tc	Cu, Co
CMGKU 619	Bacillus subtilis	Marine Sample	Amp, Sm, Km	Co, Cu
CMGKU 621	Bacillus subtilis	Marine Sample	Amp, Sm, Km	Co, Cu
CMGKU 622	Bacillus subtilis	Marine Sample	Amp, Sm, Km	Cu, Cr
CMGKU 629	Bacillus polymyxa	Marine Sample	Amp, Sm	Co, Cd
CMGKU 630	Bacillus polymyxa	Marine Sample	Amp, Km	Co, Cr
CMGKU 370	Bacillus lichiniformis	Soil Sample	Cm, Ery, Km, Rif, Sm, Tc	Zn, Cu, Cr, Ni
CMGKU 1082	Bacillus sp	Marine Sample	Eryth	Cr, Cu, Zn, Pb, Ni, Co
CMGKU 1083	Bacillus sp	Marine Sample	Chlor	Cr, Cu, Zn, Pb, Ni
CMGKU 1084	Bacillus sp	Marine Sample	Chlor, Tet	Cr,Cu,Zn,Pb
CMGKU 1085	Bacillus sp	Marine Sample	Chlor, Tet	Cr,Cu,Zn,Pb,Ni
CMGKU 1086	Bacillus sp	Marine Sample	-	Cr,Cu,Zn,Pb,Ni
CMGKU 1087	Bacillus sp	Marine Sample	Eryth	Cr,Cu,Zn,Pb
CMGKU 1088	Bacillus sp	Marine Sample	-	Cr,Cu,Zn,Pb,Ni
CMGKU 1089	Bacillus sp	Marine Sample	Chlor, Tet, Eryth	Cr,Cu,Zn,Pb,Ni
CMGKU 1090	Bacillus sp	Marine Sample	Chlor,Tet	Cr,Cu,Zn,Pb,Ni
CMGKU 1091	Bacillus sp	Marine Sample	Chlor,Tet,Eryth	Cr,Cu,Zn,Pb

Table 1.2

Strain Code	**Identification**	**Source of Isolation**	**Antibiotic Resis**	**Metal Resis**
CMGKU 59	E.coli	Industrial effluent	Amp,Tc,Cm,Sm,Rif,Km	Ni,Cd,Cu,Co,Zn,Pb,Fe
CMGKU 60	E.coli	Industrial effluent	Amp,Tc,Cm,Sm,Rif,Km	Ni,Cd,Cu,Co,Zn,Pb,Fe
CMGKU 62	E.coli	Industrial effluent	Amp,Tc,Cm,Sm,Rif,Km	Ni,Cd,Cu,Co,Zn,Pb,Fe
CMGKU 604	E.coli	Marine Sample	Amp,Sm,Tc,Km	Co,Cu,Cr
CMGKU 606	E.coli	Marine Sample	Amp,Tc	Co,Cu,Cr
CMGKU 609	E.coli	Marine Sample	Sm,Tc	Co,Cu,Cr
CMGKU 610	E.coli	Marine Sample	Amp,Tc	Co,Cu,Cr
CMGKU 611	E.coli	Marine Sample	Amp,Tc,Km	Co,Cu,Cr
CMGKU 612	E.coli	Marine Sample	Amp,Tc	Co,Cu,Cr
CMGKU 1055	E.coli	Marine Sample	-	Cr,Cu,Zn,Pb

Table 1.3

Strain Code	**Identification**	**Source of Isolation**	**Metal Resistance**	**Antibiotic Resistance**
CMGKU 601	Enterobacter aerogenes	Marine Sample	Co, Cu, Cr	Amp, Tc
CMGKU 613	Enterobacter cloacae	Marine Sample	Co, Cr	Amp, Sm
CMGKU 371	Enterobacter sp	Soil Sample	Cu, Cr III, Ni, Zn, Cd, Co	Cm, Ery, Tc, Sm, Km, Rif,
CMGKU1065	Enterobacter sp	Marine Sample	Cr, Cu, Zn, Pb	Chlor
CMGKU1067	Enterobacter sp	Marine Sample	Cr, Cu, Zn, Pb	-

Table1.4

Strain Code	**Identification**	**Source of Isolation**	**Antibiotic Resistance**	**Metal Resistance**
CMGKU SA 120	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**
CMGKU SA 122	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**

CMGKU SA 135	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**
CMGKU SA 148	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**
CMGKU SA 154	**Lactobacillus Sp**	**Food Sample**	**Van, Chlor,Tet**	**N.D**
CMGKU SA 156	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**
CMGKU SA 158	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**
CMGKU SA 163	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**
CMGKU SA 164	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**
CMGKU SA 168	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**
CMGKU SA 179	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**
CMGKU SA 181	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**
CMGKU SA 182	**Lactobacillus Sp**	**Food Sample**	**Van, Kan, Neo**	**N.D**
CMGKU SA183	**Lactobacillus Sp**	**Food Sample**	**Van, Chlor, Tet, Neo**	**N.D**
CMGKU SA 228	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**
CMGKU SA 261	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**
CMGKU SA 264	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**
CMGKU SA 269	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**
CMGKU SA 186	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**
CMGKU SA 188	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**
CMGKU SA 161	**Lactobacillus Sp**	**Food Sample**	**Van, Tet**	**N.D**
CMGKU SA 1548	**Lactobacillus Sp**	**Food Sample**	**Van, Kan, Tet, Neo**	**N.D**
CMGKU SAE	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**
CMGKU SA	**Lactobacillus Sp**	**Food Sample**	**Van, Kan**	**N.D**

Table1.5

Strain Code	**Identification**	**Source of Isolation**	**Antibiotic Resistance**	**Metal Resistance**
CMGKU 617	Staphylococcus saprophyticus	Marine Sample	Amp, Tc ,Km	Co, Cr
CMGKU 1092	Staph sp	Marine Sample	Eryth, Tet, Chlor	Cr, Cu, Zn, Pb
CMGKU 1093	Staph sp	Marine Sample	-	Cr, Cu, Zn, Pb

CMGKU 1094	Staph sp	Marine Sample	Tet, Chlor	Cr,Cu,Zn
CMGKU 1095	Staph sp	Marine Sample	Tet, Chlor	Cr,Cu,Zn,Pb
CMGKU 1096	Staph sp	Marine Sample	Tet, Chlor	Cr,Cu,Zn,Pb
CMGKU 1097	Staph sp	Marine Sample	Tet,Chlor	Cr,Cu,Zn
CMGKU 1098	Staph sp	Marine Sample	Tet	Cr,Cu,Zn,Pb
CMGKU 1099	Staph sp	Marine Sample	Tet,Chlor	Cr,Cu,Zn
CMGKU 1100	Staph sp	Marine Sample	-	Cr,Cu,Zn,Pb,Ni

Table1.6

Strain Code	**Identification**	**Source of Isolation**	**Antibiotic Resistance**	**Metal Resistance**
CMGKU1027	**Vibrio sp**	**Marine Sample**	**-**	**Cr,Cu,Zn,**
CMGKU1028	**Vibrio sp**	**Marine Sample**	**Kan, Eryth, Tet, Chlor**	**Cr, Zn**
CMGKU1029	**Vibrio sp**	**Marine Sample**	**-**	**Cu,Zn,Ni**
CMGKU1045	**Vibrio sp**	**Marine Sample**	**Chlor**	**Cr,Cu,Zn**
CMGKU1046	**Vibrio sp**	**Marine Sample**	**-**	**Cr,Cu,Zn,Pb**
CMGKU1051	**Vibrio sp**	**Marine Sample**	**Kan, Eryth ,chlor,Strep**	**Cr,Cu,Zn**
CMGKU1052	**Vibrio sp**	**Marine Sample**	**Tet,Chlot**	**Cr,Zn**
CMGKU1074	**Vibrio sp**	**Marine Sample**	**-**	**Cu, Zn**
CMGKU1075	**Vibrio sp**	**Marine Sample**	**Tet, Chlor**	**Cr, Cu, Pb**

Table 1.7

Strain Code	**Identification**	**Source of Isolation**	**Metal Resistance**	**Antibiotic Resistance**

CMGKU2K1	**Not Identified**	**Soil sample**	**Cr, Cu**	**Sm, Neo**
CMGKU2K2	**Not Identified**	**Soil sample**	**Cr, Cu**	**Sm**
CMGKU2K3	**Not Identified**	**Soil sample**	**Ni**	**Sm**
CMGKU2K4	**Not Identified**	**Soil sample**	**Cu, Ni**	**Km, Tc, Amp, Cm, Sm, Neo**
CMGKU2K5	**Not Identified**	**Soil sample**	**Cu ,Ni**	**Tc, Amp, Sm ,Neo**
CMGKU2K6	**Not Identified**	**Soil sample**	**Ni, Cu**	**Km,Tc, Amp, Sm,Neo**
CMGKU2K7	**Not Identified**	**Soil sample**	**Ni, Cu**	**Km,Tc,Rif,Amp,Cm, Sm,Neo**
CMGKU2K8	**Not Identified**	**Soil sample**	**Ni, Cu**	**Km,Tc,Amp,Cm, Sm,Neo**
CMGKU2K9	**Not Identified**	**Soil sample**	**Cr**	**Sm**
CMGKU2K10	**Not Identified**	**Soil sample**	**Cr, Ni, Cu**	**Amp, Sm**
CMGKU2K11	**Not Identified**	**Soil sample**	**Cu**	**Tc,Amp**
CMGKU2K12	**Not Identified**	**Soil sample**	**Cr, Cu, Ni**	**Sm,Neo**
CMGKU2K13	**Not Identified**	**Soil sample**	**Ni, Cr, Cu**	**Sm,Neo**
CMGKU2K14	**Not Identified**	**Soil sample**	**Cr**	**Sm**
CMGKU2K15	**Not Identified**	**Soil sample**	**Ni, Cr**	**Sm**
CMGKU2K16	**Not Identified**	**Soil sample**	**Cr**	**Sm**
CMGKU2K17	**Not Identified**	**Soil sample**	**Cu**	**Km,Tc,Amp,Cm, Sm,Neo**
CMGKU2K18	**Not Identified**	**Soil sample**	**Cr, Cu**	**Sm,Neo**

CMGKU2K19	**Not Identified**	**Soil sample**	**Ni, Cr, Cu**	**Sm**
CMGKU2K20	**Not Identified**	**Soil sample**	**r, Ni, Cu**	**Sm**
CMGKU2K21	**Not Identified**	**Soil sample**	**Cu, Cr**	**Amp,Sm,Neo**
CMGKU2K22	**Not Identified**	**Soil sample**	**Cr**	**Sm**
CMGKU2K23	**Not Identified**	**Soil sample**	**Cr, Cu**	**Sm**
CMGKU2K24	**Not Identified**	**Soil sample**	**Cr, Ni**	**Sm**
CMGKU2K25	**Not Identified**	**Soil sample**	**Cu, Ni**	**Sm,Neo**
CMGKU2K26	**Not Identified**	**Soil sample**	**Cr, Cu**	**Sm,Nov,Neo**
CMGKU2K27	**Not Identified**	**Soil sample**	**_**	**Km,Tc,Amp,Cm, Sm**
CMGKU2K28	**Not Identified**	**Soil sample**	**Ni**	**Sm,Amp**
CMGKU2K29	**Not Identified**	**Soil sample**	**Cu**	**Sm,Tc**
CMGKU2K30	**Not Identified**	**Soil sample**	**Cr**	**Sm**
CMGKU2K31	**Not Identified**	**Soil sample**	**Cr, Ni,Cu**	**Nov,Sm,Neo**
CMGKU2K32	**Not Identified**	**Soil sample**	**-**	**Tc,Sm**
CMGKU2K33	**Not Identified**	**Soil sample**	**Cr**	**Tc**
CMGKU2K34	**Not Identified**	**Soil sample**	**_**	**Tc,Amp,Cm,Sm**
CMGKU2K35	**Not Identified**	**Soil sample**	**_**	**Tc,Sm**
CMGKU2K36	**Not Identified**	**Soil sample**	**Cr, Cu**	**Sm,Tc**
CMGKU2K37	**Not Identified**	**Soil sample**	**Cr, Cu,Ni**	**Sm,Nov,Neo**

CMGKU2K38	**Not Identified**	**Soil sample**	**Ni,Cu**	**Sm**
CMGKU2K39	**Not Identified**	**Soil sample**	**Cr, Cu**	**Sm,Neo**
CMGKU2K40	**Not Identified**	**Soil sample**	**Cr, Ni,Cu**	**Sm**
CMGKU2K41	**Not Identified**	**Soil sample**	**Cr**	**Sm**
CMGKU2K42	**Not Identified**	**Soil sample**	**Cr, Cu**	**Sm,Nov,Neo**
CMGKU2K43	**Not Identified**	**Soil sample**	**Cu**	**Tc,Amp,Cm,Sm,Neo**
CMGKU 1016	**Not Identified**	**Marine Sample**	**Cr,Cu,Pb**	**_**
CMGKU 1057	**Not Identified**	**Marine Sample**	**Cu,Pb**	**_**
CMGKU 1058	**Not Identified**	**Marine Sample**	**Cr,Cu,Pb,Zn**	**Tet**
CMGKU 1059	**Not Identified**	**Marine Sample**	**Cr,Cu,Pb**	**_**
CMGKU 1066	**Not Identified**	**Marine Sample**	**Cr,Cu,Ni**	**Eryth, Chlor**
CMGKU 1068	**Not Identified**	**Marine Sample**	**Cr, Cu,Zn**	**Eryth, Chlor**
CMGKU 1069	**Not Identified**	**Marine Sample**	**Cr ,Cu**	**_**
CMGKU 1070	**Not Identified**	**Marine Sample**	**Cr ,Zn**	**Eryth, Chlor**
CMGKU 1081	**Not Identified**	**Marine Sample**	**Cr Cu,Zn**	**Eryth, Chlor,Tet**

Table : 1.8

Strain Code	**Identification**	**Source of Isolation**	**Antibiotic Resistance**	**Metal Resistance**
CMGKU501	Pseudomonas cepacia	Marine sample	Ap,Km	Cr

CMGKU503	Pseudomonas cepacia	Marine sample	Ap,Km,Sm	Cr
CMGKU507	Pseudomonas mirabilis	Marine sample	Ap,Sm	Ni
CMGKU508	Pseudomonas vulgaris	Marine sample	Ap,Km,Sm,Cm,Rif,Tc	Zn,Ni,Cd,Co
CMGKU 510	P. fluorescene	Marine Sample	Ap,Cm, Rif	Zn
CMGKU 511	P.aeruginosa	Marine Sample	Ap,Cm,Km,Rif,Tc	Cd, Zn
CMGKU 512	P.stutzeri	Marine Sample	Ap, Km, Sm	Cu, Ni
CMGKU 522	Pseudomonas cepacia	Marine Sample	-	-
CMGKU 530	P. pseudomonellei	Marine Sample	-	Zn, Ni, Co
CMGKU 531	P. sp	Marine Sample	Km	-
CMGKU 532	P.aeruginosa	Marine Sample	Sm	-
CMGKU 554	Pseudomonas mallei	Marine Sample	Km, Rif	Cu
CMGKU 556	P.aeruginosa	Marine Sample	Ap,Cm,Km, Rif, Sm, Tc	Cd, Zn, Co, Ni
CMGKU 561	Pseudomonas aeruginosa	Marine Sample	Km	-
CMGKU 557	P.aeruginosa	Marine Sample	Km	-
CMGKU 558	Pseudomonas sp	Marine Sample	Km	-
CMGKU 562	Pseudomonas sp	Marine Sample	Ap,Cm,Km,Sm	Cu, Zn
CMGKU 545	P.aeruginosa	Marine Sample	Km	-
CMGKU 607w	P.aeruginosa	Marine Sample	Ap, Km,Tc	Co,Cr
CMGKU 625	P.Pseudomallei	Marine Sample	Tc, Sm	Co,Cu,Cd
CMGKU 627	Pseudomonas cepacia	Marine Sample	Ap,Sm	Cu
CMGKU 631	Pseudomonas cepacia	Marine Sample	Ap,Km	Cu,Cr
CMGKU 633	Pseudomonas sp	Marine Sample	Sm,Km	Co.Cu,Cr
CMGKU 636	Pseudomonas sp	Marine Sample	Ap, Km, Sm	Co.Cu,Cr
CMGKU 1024	Pseudomonas sp	Marine Sample	-	Cr,Cu,Zn,Pb
CMGKU 1022	Pseudomonas sp	Marine Sample	Kan,Eryth,Tet	Cr,Cu,Zn,Pb
CMGKU 1019	Pseudomonas sp	Marine Sample	_	Cr,Cu,Zn
CMGKU 1013	Pseudomonas sp	Marine Sample	_	Cr,Cu,Zn,Pb
CMGKU	Pseudomonas	Marine	Tet,Chlor	

1007	sp	Sample		Cr,Cu,Zn,Pb,Ni,Co
CMGKU I021	Pseudomonas sp	Marine Sample	Tet	Cr,Cu,Zn,Pb,Ni,Co
CMGKU 1006	Pseudomonas sp	Marine Sample	Tet	Cr,Cu,Zn,Pb,Ni,Co
CMGKU I023	Pseudomonas sp	Marine Sample	_	Cr,Cu,Zn,Pb
CMGKU l003	Pseudomonas sp	Marine Sample	_	Cr,Cu,Zn,Pb,Ni,Co
CMGKU1004	Pseudomonas sp	Marine Sample	_	Cr,Cu,Zn,Pb,Ni,Co
CMGKU1030	Pseudomonas sp	Marine Sample	_	Cr,Cu,Zn,Pb,Ni,
CMGKU1030	Pseudomonas sp	Marine Sample	_	Cr,Cu,Zn,Pb
CMGKU I034	Pseudomonas sp	Marine Sample	_	Cr,Cu,Zn,Pb
CMGKU I035	Pseudomonas sp	Marine Sample	Kan,Eryth	Cr,Cu,Zn,Pb,Ni,Co
CMGKU I039	Pseudomonas sp	Marine Sample	_	Cr,Cu,Zn,Pb,Ni
CMGKU I040	Pseudomonas sp	Marine Sample	Kan,Eryth,Chlor	Cr,Cu,Zn,Pb,Ni
CMGKU1044	Pseudomonas sp	Marine Sample	Chlor	Cr,Cu,Zn,Pb
CMGKU I047	Pseudomonas sp	Marine Sample	_	Cr,Cu,Zn,Pb
CMGKU I048	Pseudomonas sp	Marine Sample	Kan,Eryth,Chlor	Cr,Cu,Zn,Pb
CMGKU1050	Pseudomonas sp	Marine Sample	_	Cr,Cu,Zn,Pb
CMGKU1054	Pseudomonas sp	Marine Sample	_	Cr,Cu,Zn,Pb
CMGKU1053	Pseudomonas sp	Marine Sample	_	Cr,Cu,Zn,Pb
CMGKU l056	Pseudomonas sp	Marine Sample	Chlor	Cr,Cu,Zn,Pb
CMGKU1063	Pseudomonas sp	Marine Sample	Tet,Chlor	Cr,Cu,Zn,Pb,Ni
CMGKU1076	Pseudomonas sp	Marine Sample	_	
CMGKU1079	Pseudomonas sp	Marine Sample	Eryth,Tet	Cr,Cu,Zn,Pb,Ni
CMGKU 101	P.aeruginosa	Industrial Effluent	Sm,Amp, Km,Tc, Cm	Zn,Cd,Cu,Co,Ni,Te,Bi,Sn,As,Pb,Al
CMGKU102	P.aeruginosa	Industrial Effluent	Sm,Amp, Km,Tc, Cm	Zn,Cd,Cu,Co,Ni,Te,Bi,Sn,As,Pb,Al
CMGKU103	P.aeruginosa	Industrial Effluent	Sm,Amp, Km,Tc, Cm	Zn,Cd,Cu,Co,Ni,Te,Bi,Sn,As,Pb,Al
CMGKU104	P.aeruginosa	Industrial Effluent	Sm,Amp, Km,Tc, Cm	Zn,Cd,Cu,Co,Ni,Te,Bi,Sn,As,Pb,Al
CMGKU105	P.aeruginosa	Industrial Effluent	Sm,Amp, Km,Tc, Cm	Zn,Cd,Cu,Co,Ni,Te,Bi,Sn,As,Pb,Al
CMGKU106	P.aeruginosa	Industrial Effluent	Sm,Amp, Km,Tc, Cm	Zn,Cd,Cu,Co,Ni,Te,Bi,Sn,As,Pb,Al

CMGKU51	P.aeruginosa	Industrial Effluent	Sm,Amp, Km,Tc, Cm,Rif	Ni,Cd,Co,Cu,Zn,Pb,Fe
CMGKU52	P.aeruginosa	Industrial Effluent	Sm,Amp,Km,Tc,Cm,,Rif	Ni,Cd,Co,Cu,Zn,Pb,Fe
CMGKU53	P.aeruginosa	Industrial Effluent	Sm,Amp, Km,Tc, Cm,Rif	Ni,Cd,Co,Cu,Zn,Pb,Fe
CMGKU54	P.aeruginosa	Industrial Effluent	Sm,Amp, Km,Tc, Cm,Rif	Ni,Cd,Co,Cu,Zn,Pb,Fe
CMGKU56	P.aeruginosa	Industrial Effluent	Sm,Amp, Km,Tc, Cm,Rif	Ni,Cd,Co,Cu,Zn,Pb,Fe
CMGKU58	P.aeruginosa	Industrial Effluent	Sm,Amp, Km,Tc, Cm,Rif	Ni,Cd,Co,Cu,Zn,Pb,Fe
CMGKU64	P.aeruginosa	Industrial Effluent	Sm,Amp, Km,Tc, Cm	Ni,Cd,Co,Cu,Zn,Pb,Fe
CMGKU107	P.aeruginosa	Industrial Effluent	Sm,Amp, Km,Tc, Cm	Zn,Cd,Cu,Co,Ni,Te,Bi,Sn,As,Pb,Al
CMGKU108	P.aeruginosa	Industrial Effluent	Sm,Amp, Km,Tc, Cm	Zn,Cd,Cu,Co,Ni,Te,Bi,Sn,As,Pb,Al
CMGKU 818	Pseudomonas sp	Air Sample	Sm, Tet	Cr(VI),Cu,Co,Ni
CMGKU 821	Pseudomonas sp	Air Sample	Sm,Tet	Cd,Cu,Ni
CMGKU 823	P.aeruginosa	Air Sample	Amp,Sm	Cu,Ni
CMGKU 826	P.aeruginosa	Air Sample	Sm,Tet	Cu,Co.Ni
CMGKU 01	Pseudomonas sp	Soil Sample	N.D	Cd,Co,Cu,Cr,Pb
CMGKU 04	Pseudomonas sp	Soil Sample	N.D	Cd,Co,Cr,Pb
CMGKU 05	Pseudomonas sp	Soil Sample	N.D	Cd,Co,Cr,Pb
CMGKU 16	Pseudomonas sp	Soil Sample	N.D	Cd,Co,Cr,Pb
CMGKU 106	Pseudomonas sp	Soil Sample	N.D	Cd,Co,Cr,Pb
CMGKU 457	Pseudomonas sp	Soil Sample	Sm,Amp,Tet,	Cd,Co,Cr,Pb
CMGKU 462	P.stutzeri	Soil Sample	Sm,Amp,	Cd,Co,Cu,Cr,Pb
CMGKU 463	P.stutzeri	Soil Sample	Sm,Amp,	Ni,Co,Cr,Pb
CMGKU 581	Pseudomonas	Soil	Sm,Amp,	Zn, Ni, Cd, Co,Cr,Cu

	sp	Sample	Neo,Cm,Km,Tet	
CMGKU 585	Pseudomonas sp	Soil Sample	Sm,Amp, Neo,Cm,Km,Tet	Zn, Cd, Co,Cr,Cu
CMGKU 586	Pseudomonas sp	Soil Sample	Sm,Amp, Neo,Cm,Km	Zn, Cd, Co,Cr,Cu
CMGKU 587	Pseudomonas sp	Soil Sample	Sm,Amp, Neo,Cm,Km,Tet	Zn, Cd, Co,Cr,Cu
CMGKU 589	Pseudomonas sp	Soil Sample	Sm,Amp, Cm,Km	Co,Cr,Cu
CMGKU 590	Pseudomonas sp	Soil Sample	Sm,Amp, Km,Tet	Zn,Ni

Table:1.9

CULTURE CODE	IDENTIFICATION	SOURCE OF ISOLATION	METAL RESISTANCE	ANTIBIOTIC RESISTANCE
CMGKU 61	**Morexella**	**Industrial Effluent**	**Ni,Cd,Co,Cu,Zn,Pb,Fe**	**Ap, Km,Tc,Cm,Sm, Rif**
CMGKU 55	**Enterococcus sp**	**Industrial Effluent**	**,Ni,Cd,Co,Cu,Zn,Pb,Fe**	**Ap, Tc, Sm,Cm,Rif**
CMGKU 602	**Klebsiella oxytoca**	**Marine Sample**	**Co,Cu,Cr**	**Amp,Tc**
CMGKU 605	**Serretia liquifacies**	**Marine Sample**	**Co,Cu,Cr**	**Tc**
CMGKU 608	**Aeromona Salmonicida**	**Marine Sample**	**Co,Cr**	**Amp,Tc**
CMGKU 613	**Klebsiella ozanae**	**Marine Sample**	**Co,Cu**	**Tc**
CMGKU 614	**Enterobacter cloacae**	**Marine Sample**	**Co,Cr**	**Amp,Sm**
CMGKU 620	**Actinobacillus lignieresii**	**Marine Sample**	**Co,Cu,Cr**	**Amp,Tc,Km, Sm**
CMGKU 623	**Actinobacillus lignieresii**	**Marine Sample**	**Co,Cu**	**Amp, Sm**
CMGKU 624	**Stomatococcus mucilaginosa**	**Marine Sample**	**Co,Cu**	**Amp,Tc, Sm**
CMGKU 626	**Haemophilus arphrophilus**	**Marine Sample**	**Co,Cu,Cd**	**Amp,Sm**
CMGKU 632	**Serratia**	**Marine Sample**	**Co,Cu**	**Amp,Km**
CMGKU 634	**Alcalagenes sp**	**Marine Sample**	**Co,Cu**	**Amp,Km, Sm**
CMGKU 480	**Curcubit yellow vine disease Bacterium**	**Air Sample**	**Cr,Cu,Cd,Ni,**	**Amp,Km,Sm**
CMGKU 814	**Klebsiella sp**	**Air Sample**	**Cd,Cu,Co,Ni**	**Amp,Km,Sm**
CMGKU 1020	**Citrobacter sp.**	**Marine Sample**	**Cr,Cu,Zn,Pb,Ni,Co**	**Tet**
CMGKU 1018	**Shigella sp.**	**Marine Sample**	**Cr,Cu,Zn,Pb**	**_**
CMGKU 1017	**Shigella sp.**	**Marine Sample**	**Cr,Cu,Zn,Co**	**Chlor**
CMGKU	**Klebsiella sp.**	**Marine**	**Cr,Cu,**	**_**

1015		**Sample**		
CMGKU 1014	**Proteus sp.**	**Marine Sample**	**Cr,Cu,Zn**	**Chlor**
CMGKU 1011	**Shigella sp.**	**Marine Sample**	**Cr,Cu,Zn**	**_**
CMGKU 1008	**Serratia sp.**	**Marine Sample**	**Cr,Cu,Zn,Co**	**Kan,Eryth,Chlor**
CMGKU 1005	**Proteus sp.**	**Marine Sample**	**Cr,Cu,Zn**	**Chlor**
CMGKU 1001	**Klebsiella sp.**	**Marine Sample**	**Cr,Cu,Zn**	**Tet**
CMGKU I002	**Proteus sp.**	**Marine Sample**	**Cr,Cu**	**Tet**
CMGKU I009	**Klebsiella sp.**	**Marine Sample**	**Cr,Co**	**Kan,Eryth**

Antibiotic Resistance:

Most of the bacterial strains showed resistance to multiple antibiotics.Antibiotic resistance against selected antibiotics is compared by calculating the percentage of a certain bacteria resistant to the given antibiotic (Table:2.1).E.coli show high percentage of resistance against Tetracycline and Ampicillin.Lactobacilli sp were highly resistant to Kanamycine and Neomycine. Vibrio sp were highly resistant to Chloramphenicol.

Table:2.1

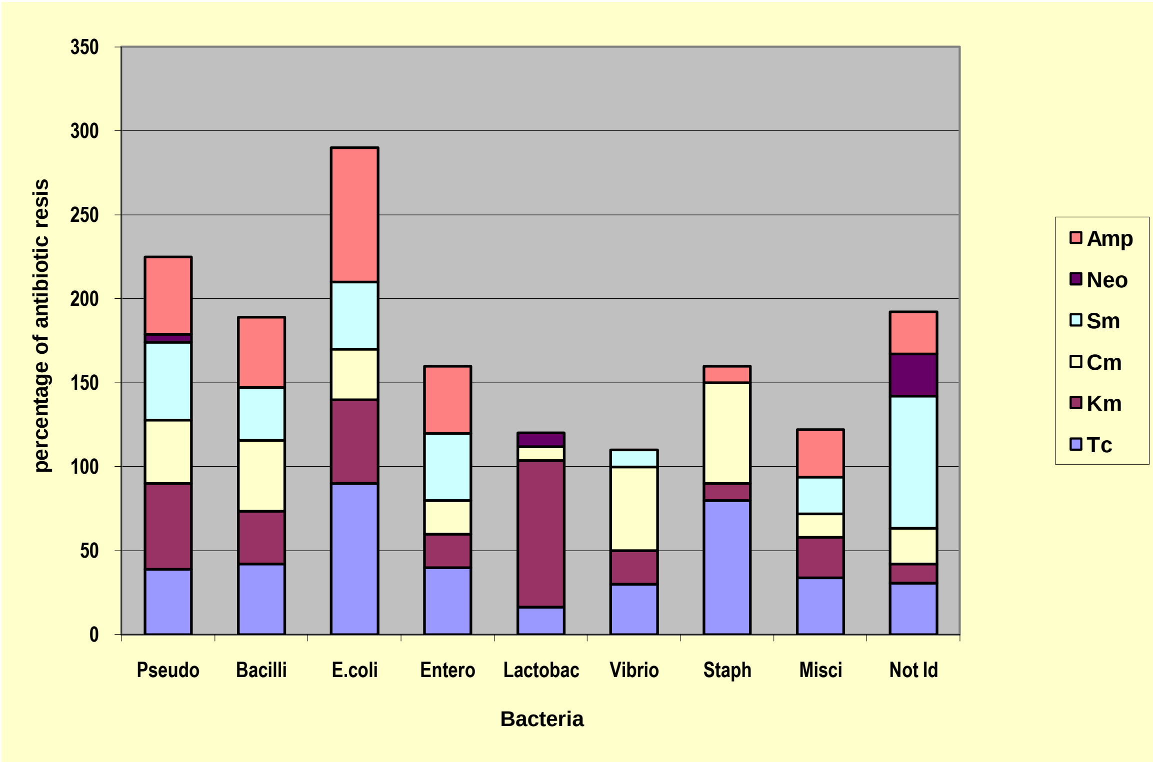
Antibiotic Resistance Pattern of Various bacteria

Antibiotics	**Percentage of Antibiotic Resistance**								
	Pseudomonas	Bacilli	E.coli	Enterobacter	Lactobaccilli	Vibrio sp	Staphylococcus	Micsillenious Organisms	Not Identified
Tc	**39%**	**42.1%**	**90%**	**40%**	**16.3%**	**30%**	**80%**	**34%**	**30.7%**
Km	**51.2%**	**31.5%**	**50%**	**20%**	**87.4%**	**20%**	**10%**	**24%**	**11.5%**
Cm	**37.8%**	**42.1%**	**30%**	**20%**	**8.3%**	**50%**	**60%**	**14%**	**21.1%**
Sm	**46.3%**	**31.5%**	**40%**	**40%**	**-**	**10%**	**N.D**	**22%**	**78.8%**
Neo	**4.8%**	**N.D**	**N.D**	**-**	**8.3%**	**N.D**	**-**	**-**	**25%**
Amp	**46%**	**42%**	**80%**	**40%**	**-**	**N.D**	**10%**	**28%**	**25%**

KEY

Antibiotic resistance is checked up to 20---200 µl.
Antibotics;Tc :Tetracycline,Km :Kanamycin,Cm:Chloramphenicol,Sm :Streptomycin,Neo: Neomycin, Amp: Ampicillin,N.D :Not Done

Figure :1



Abbreviations
Bacteria: Pseudo: Pseudomonas sp,Entero: Enterobacter sp, Lactobac: Lactobacilli sp, Staph: Staphylococcus sp, Misci: Miscillenious organisms.
Antibiotics: Tc :Tetracycline, Km: Kanamycin, Cm:Chloramphenicol, Sm:Streptomycin, Neo:Neomycin, Amp: Ampicillin, Not Id: Not Identified.

Heavy Metal Resistance:

Most of the bacterieal isolates showed resistance to multiple heavy metal salts.Resistance against selected metal salts is compared by calculating the percentage of certain bacteria resistant to the given metal salt (Table 2.2).E.coli showed higher percentage of resistance against Cobalt and Copper .

Table: 2.2

Heavy Metal Resistance Pattern of Various bacteria

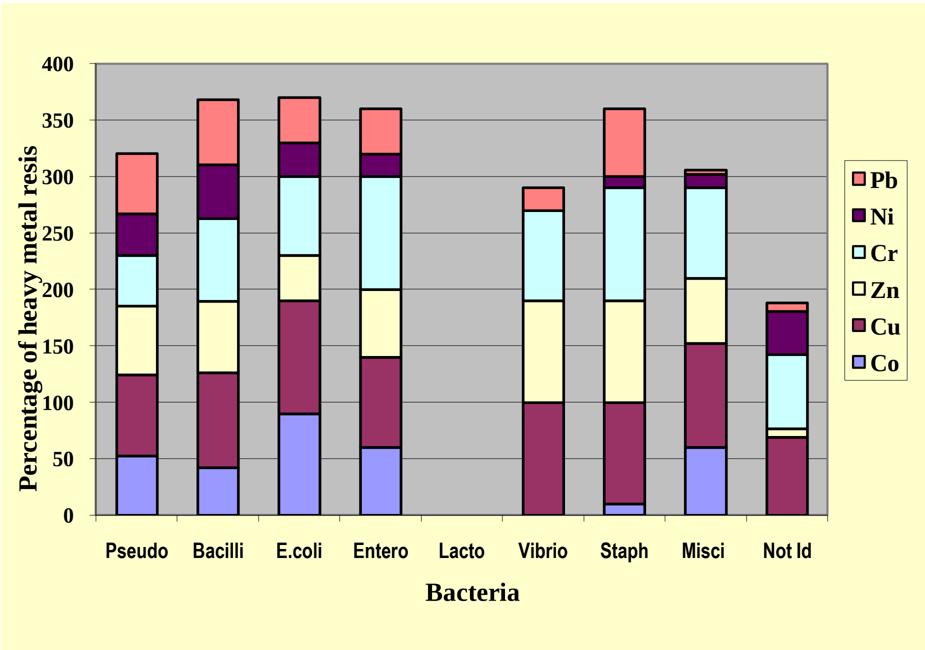
	Percentage of Heavy Metal Resistance

Heavy Metals	Pseudomonas	Bacilli	E.coli	Enterobacter	Lactobaccili	Vibrio sp	Staphylococcus	Micsillenious Organisms	Not Identified
Co	**52.4%**	**42.1%**	**90%**	**60%**	**N.D**	**-**	**10%**	**60%**	**-**
Cu	**71.9%**	**84.2%**	**100%**	**80%**	**N.D**	**100%**	**90%**	**92%**	**69.2%**
Zn	**60.9%**	**63.1%**	**40%**	**60%**	**N.D**	**90%**	**90%**	**58%**	**7.6%**
Cr	**45.1%**	**73.6%**	**70%**	**100%**	**N.D**	**80%**	**100%**	**80%**	**65.3%**
Ni	**36.5%**	**47.3%**	**30%**	**20%**	**N.D**	**-**	**10%**	**12%**	**38.4%**
Pb	**53.6%**	**57.8%**	**40%**	**40%**	**N.D**	**20%**	**60%**	**4%**	**7.6%**

KEY:

Heavy Metal resistance is checked upto 0.5mM----2.0mM concentration
Heavy Metals;Co: CoCl2, Cu:CuSo4,Zn:ZnCl2,Cr:Cr2O5,Ni:Nicl2,Pb:Pb(CHCOO)2 ,N.D :Not Done

Figure:2



Abbreviations :
Bacteria: Pseudo: Pseudomonas sp,Entero: Enterobacter sp, Lactobac: Lactobacilli sp,
Staph: Staphylococcus sp, Misci: Miscillenious organisms.
Heavy Metals;Co: CoCl2, Cu:CuSo4,Zn:ZnCl2,Cr:Cr2O5,Ni:Nicl2,Pb:Pb(CHCOO)2 ,N.D :Not Done

Plasmid profile:

Out of 511 strains in 37 strains plasmids of different molecular sizes are found:

Table: 2.3

Culture code	Plasmid
CMGKU 57	No
CMGKU 603	No
CMGKU 615	No
CMGKU 619	No
CMGKU 621	No
CMGKU 622	No
CMGKU 629	No
CMGKU 630	No
CMGKU 370	No
CMGKU 1082	No
CMGKU 1083	No
CMGKU 1084	No
CMGKU 1085	No
CMGKU 1086	No
CMGKU 1087	No
CMGKU 1088	No
CMGKU 1089	No
CMGKU 1090	No
CMGKU 1091	No
CMGKU 59	3.5 kb
CMGKU 60	No
CMGKU 62	No
CMGKU 604	Yes
CMGKU 606	Yes
CMGKU 609	No
CMGKU 610	No
CMGKU 611	No
CMGKU 612	No
CMGKU 1055	1 kb
CMGKU 59	No
CMGKU 60	No
CMGKU 601	No
CMGKU 613	No
CMGKU 371	No
CMGKU1065	1 kb
CMGKU1067	No
CMGKU 601	No
CMGKU 613	No
CMGKU2K1	No
CMGKU2K2	No
CMGKU2K3	No

CMGKU2K4	No
CMGKU2K5	No
CMGKU2K6	No
CMGKU2K7	No
CMGKU2K8	Yes
CMGKU2K9	No
CMGKU2K10	No
CMGKU2K11	No
CMGKU2K12	No
CMGKU2K13	No
CMGKU2K14	Yes
CMGKU2K15	No
CMGKU2K16	No
CMGKU2K17	Yes
CMGKU2K18	Yes
CMGKU2K19	No
CMGKU2K20	No
CMGKU2K21	No
CMGKU2K22	Yes
CMGKU2K23	No
CMGKU2K24	Yes
CMGKU2K25	Yes
CMGKU2K26	Yes
CMGKU2K27	Yes
CMGKU2K28	Yes
CMGKU2K29	No
CMGKU2K30	No
CMGKU2K31	Yes
CMGKU2K32	No
CMGKU2K33	Yes
CMGKU2K34	Yes
CMGKU2K35	Yes
CMGKU2K36	Yes
CMGKU2K37	No
CMGKU2K38	No
CMGKU2K39	No
CMGKU2K40	Yes
CMGKU2K41	No
CMGKU2K42	No
CMGKU2K43	Yes
CMGKU 1016	No
CMGKU 1057	No
CMGKU 1058	No
CMGKU 1059	No
CMGKU 1066	No
CMGKU 1068	No
CMGKU 1069	No
CMGKU 1070	No
CMGKU 1081	No
CMGKU SA 120	Yes
CMGKU SA 122	No
CMGKU SA 135	Yes
CMGKU SA 148	No
CMGKU SA 154	No

CMGKU SA 156	Yes
CMGKU SA 158	Yes
CMGKU SA 163	No
CMGKU SA 164	Yes
CMGKU SA 168	No
CMGKU SA 179	No
CMGKU SA 181	No
CMGKU SA 182	Yes
CMGKU SA183	Yes
CMGKU SA 228	No
CMGKU SA 261	No
CMGKU SA 264	No
CMGKU SA 269	No
CMGKU SA 186	No
CMGKU SA 188	No
CMGKU SA 161	No
CMGKUSA 1548	No
CMGKUSAE	No
CMGKUSA	No
CMGKU 61	3.93 Kb
CMGKU55	No
CMGKU 602	No
CMGKU 605	No
CMGKU 607	No
CMGKU608	No
CMGKU613	No
CMGKU624	No
CMGKU 614	No
CMGKU620	No
CMGKU623	No
CMGKU626	No
CMGKU632	No
CMGKU I080	No
CMGKU634	No
CMGKU 480	No
CMGKU 814	9.9kb/7.5kb
CMGKU 1020	No
CMGKU 1018	No
CMGKU 1017	No
CMGKU1015	No
CMGKU 1014	No
CMGKU 1011	No
CMGKU 1008	No
CMGKU 1005	No
CMGKU 1001	No
CMGKU I002	No
CMGKU I009	No
CMGKU I012	No
CMGKU I025	No
CMGKU1010	No
CMGKU I026	No
CMGKU l031	No
CMGKU 1032	No
CMGKU 1033	No

CMGKU 1036	No
CMGKU l037	No
CMGKUI038	No
CMGKUI041	No
CMGKU I042	No
CMGKU 1043	No
CMGKU 1049	No
CMGKU l060	No
CMGKU 1061	No
CMGKU1062	No
CMGKU 1071	No
CMGKU l072	No
CMGKU 1073	No
CMGKU1077	No
CMGKU1078	No
CMGKU I080	No
CMGKU 501	No
CMGKU503	No
CMGKU 507	No
CMGKU 508	No
CMGKU 510	No
CMGKU 511	No
CMGKU 512	No
CMGKU 522	No
CMGKU 530	No
CMGKU 531	No
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CMGKU 554	No
CMGKU 556	No
CMGKU 561	No
CMGKU 557	No
CMGKU 558	No
CMGKU 562	No
CMGKU 545	No
CMGKU 607w	No
CMGKU 625	No
CMGKU 627	No
CMGKU 631	No
CMGKU 633	No
CMGKU 636	No
CMGKU 1024	No
CMGKU 1022	No
CMGKU 1019	No
CMGKU 1013	No
CMGKU 1007	No
CMGKU I021	No
CMGKU 1006	No
CMGKU I023	No
CMGKU l003	No
CMGKU1004	No
CMGKU1030	No
CMGKU1030	No
CMGKU I034	No
CMGKU I035	No

CMGKU I039	No
CMGKU I040	No
CMGKU1044	No
CMGKU I047	No
CMGKU I048	No
CMGKU1050	No
CMGKU1054	No
CMGKU1053	No
CMGKU l056	No
CMGKU1063	No
	No
CMGKU1076	No
CMGKU1079	No
CMGKU 101	No
CMGKU102	No
CMGKU103	No
CMGKU104	No
CMGKU105	No
CMGKU106	No
CMGKU51	No
CMGKU52	No
CMGKU53	No
CMGKU54	No
CMGKU56	No
CMGKU58	10.02kb
CMGKU64	10.68kb
CMGKU107	Yes
CMGKU108	No
CMGKU 818	No
CMGKU 821	No
CMGKU 823	No
CMGKU 826	No
CMGKU 01	No
CMGKU 04	No
CMGKU 05	No
CMGKU 16	No
CMGKU 106	No
CMGKU 457	Yes
CMGKU 462	Yes
CMGKU 463	No
CMGKU 581	No
CMGKU 585	No
CMGKU 586	No
CMGKU 587	No
CMGKU 589	No
CMGKU 590	No
CMGKU 617	5.19kb
CMGKU 1092	No
CMGKU 1093	No
CMGKU 1094	No
CMGKU 1095	No
CMGKU 1096	No
CMGKU 1097	No
CMGKU 1098	No

CMGKU 1099	No
CMGKU 1100	No
CMGKU1027	No
CMGKU1028	No
CMGKU1029	No
CMGKU1045	No
CMGKU1046	No
CMGKU1051	No
CMGKU1052	No
CMGKU1052	No
CMGKU1074	No
CMGKU1075	No

Discussion

Bacteria isolated from different habitats showed resistance to multiple antibiotics and heavy metals. E.coli is highly resistant bacteria either isolated from Marine samples or industrial effluent. Infections caused by E.coli can be difficult to treat as it has acquired

Resistance to many antibiotics. Bacteria isolated from marine samples showed high percentage of resistance to multiple antibiotics (Table2.1) and heavy metals (Table 2.2) because in the sea bacterial flora is diverse .Bacteria isolated from soil samples contaminated with metal waste have developed resistance mechanisms to many heavy metals. In spite of the plasmids present in the bacterial isolates (Table2.3), there was no consistent correlation between plasmid profiles and antibiotic and heavy metal resistance pattern.

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