Plant Archives Vol. 19 No. 2, 2019 pp. 2759-2774 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

PREVALENCE OF POTENTIAL HAZARDOUS MICROORGANISMS AT FREQUENTLY PUBLIC USED SITES

A.S. Shishodia[1]*, Mukta Sharma[2] and Neha Wal[3]

[1]*Department of Microbiology, Mewar University, Chittorgarh–312901 (Raj.), India.
[2]Department of Microbiology, S.B.B. Dental College & Research Centre, Ghaziabad–201302 (U.P.), India.
[3]Department of Life Sciences, Mewar University, Chittorgarh–312901 (Raj.), India.

Abstract

The study was conducted in the five districts of Uttrakhand and Uttar Pradesh state of North India. Present study was carried out among 150 samples including 50 water samples and 100 swab samples (50 swab samples of bus handles and 50 swab samples of grocery shop counters from districts namely Haridwar, Muzaffarnagar, Meerut, Ghaziabad and Bulandshahr) collected from Uttarakhand and Western U.P. Frequently publically used sites were preferred for sampling purpose. To determine the bacterial load on these sites, 10 fold dilutions were prepared from the test sample. Colonies were examined and sub-cultured on different media for identification of the isolates. Further identification was made by morphological, microscopic, biochemical and RAPD based molecular characterization. Our study investigated the level of contamination and prevalence of potential hazardous microorganisms at individual sampling sites. 100% samples (50 water samples) of water bodies were found positive for viable count; and ranges between 21×10^4 to 16.6×10^5 total viable counts per ml of water sample. While, 100 swab samples (50 from bus handles and 50 from shop counters) were found 100% positive for the isolation of bacteria ranges from $18x10^4$ to 17.5×10^5 total viable count/4cm^2 surface area of bus handles and 21×10^4 to 11.3×10^5 total viable count/4cm^2 area of shop counters, respectively. These findings from our study are also distinguishing the need to take urgent measures to improve the sanitary conditions of potable water, public transport buses and shop counters. Therefore, removing soil, dust and dirt from them are necessary steps on the reduction of surface contamination by microbes.

Key words: Sanitary condition, Hazardous microorganisms, Water & swab samples, RAPD

Introduction

Microorganisms are found ubiquitously in nature as in water, soil, air and rock etc; being their presence in environment there is usual contact between microbes and humans. In fact, the regular relationship among microbes and human beings is delicate and complex. Due to the presence of microorganism in all activities of human life; high microorganism load (that are potentially hazardous to human health) has been found in environment is expected which may potentially transmitted person to person via common items/sites of frequent public use. Our study investigated the prevalence of potential hazardous microorganisms and the level of contamination at individual sites of river/canal bank water of frequent public use, bus handles of commercial transport buses and shop counters, particularly in Uttarakhand and Western U.P. of India. Although collectively water samples of river/canal bank water and moistened swab samples of bus handles and shop counters were processed by using microbiological techniques like enumeration, identification of bacteria using colony characteristics, growth on different culture media, microscopy, different biochemical tests and molecular methods. High level of heterotrophic plate count (HPC) of bacteria were found. Our study focused on the prevalence of five common genera including *Streptococcus, Staphylococcus, Pseudomonas, Escherichia* and *Klebsiella.* Further study was focused on the bacterial species *Streptococcus anginosus*, *Streptococcus pneumonia*, *Streptococcus bovis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Pseudomonas*

Author for correspondence :

aeruginosa, *Pseudomonas oryzihabitans*, *Pseudomonas fluorescens*, *Escherichia coli*, *Klebsiella pneumonia* and *Klebsiella oxytoca.* Our study suggests that the water of study area harbor highly pathogenic bacteria; and the inanimate surfaces of bus handles and shop counters are identified as the priority surfaces for the risk of infection by bacterial pathogens.

Materials and Methods

To study the prevalence of potential hazardous microorganisms and to check the level of contamination at individual sites of river/canal bank water of frequent public use, bus handles of commercial transport buses and shop counters following qualitative and quantitative methods were used.

Microscopic and Biochemical characterization of bacteria in collected samples

The samples were processed using microbiological techniques *i.e.* enumeration by 10 fold serial dilution method, identification of bacteria using colony characteristics, growth on different culture media, microscopy, different biochemical tests including Catalase test, Cytochrome oxidase test, Coagulase test, IMViC tests, Sugar fermentation test, Bile esculin test, Urease test, Optochin sensitivity test, Novobiocin test, Gelatin hydrolysis test, Nitrate reduction test, DNA hydrolysis test or DNase test, Mannitol salt agar (MSA) test, Lancefield grouping antigen test, King's medium B base test and Phenylalanine deaminase test.

DNA isolation of characterized bacterial isolates:

The genomic DNA isolation of characterized bacteria was done by C-TAB method. 1 ml bacterial broth culture was processed in 1.5 ml eppendorf tube. Then the tubes were subjected to centrifugation at 5000 rpm for 5 minutes to collect cells. The pellet was taken and supernatant was discarded. Warm 700 µl C-TAB buffer was added in each eppendorf tube and 15 µl β-mercaptoethanol was also added in the eppendorf tubes. After vortexing for few seconds the eppendorf tubes were placed at room temperature for 30 minutes. After incubation period, 500 µl Chloroform isoamyl alcohol (CIA) was added into eppendorf tube. After centrifugation at 12,000 rpm for 12 minutes the top layer was collected precisely in fresh eppendorf tube and chilled iso-propanol was added in equal volume and then centrifuged at 13,000 rpm for 15 minutes. After that top layer was discarded and pellet was collected; and 70% ethyl alcohol was added then tubes were further centrifuged at 5000 rpm for 5 minutes. Then pellet was stored in 50 µl nuclease free water. The quality of isolated DNA was checked on 0.8% agarose gel electrophorasis in 1X TAE buffer. The purity of isolated DNA was also calculated by spectrophotometer. OD was calculated at 260 nm and 280 nm by spectrophotometer. The ratio between the OD at 260 nm and 280 nm provided an estimation of purity and impurities in the nucleic acid. A pure preparation of DNA has the OD values between 1.8 to 2.0.

RAPD based molecular characterization by Polymerase Chain Reaction

All the DNA samples were subjected to molecular identification. Forty randomly selected RAPD primers (RAPD 10mer kits from Eurofins Genomics, Bangalore) including OPA-01, OPA-02, OPA-03, OPA-04, OPA-05, OPA-06, OPA-07, OPA-08,OPA-09, OPA-10, OPA-11, OPA-12, OPA-13, OPA-14, OPA-15, OPB-01, OPB-02, OPB-03, OPB-04, OPB-05, OPB-06, OPB-07, OPB-08, OPC-01, OPC-02, OPC-03, OPC-04, OPC-05, OPC-06, OPC-07, OPD-11, OPD-12, OPD-13, OPD-14, OPD-15, OPD-16, OPD-17, OPD-18, OPD-19 and OPD-20 were used for bacterial identification.

All the PCR tube was placed in the well of thermal cycler (MWG-Biotech). Thermal cycling reactions were performed in conditions as: initial denaturation at 94^0C for 4 minutes after that denaturation at 95^0C for 20 seconds for 35 cycles, annealing at 34^0C for 20 seconds and extension at 72^0C for 10 seconds followed by final extension at 72^0C for 3 minutes. A total 36 cycles of PCR were performed for complete amplification reaction. The total time in 36 cycles were 2 hour 37 minutes and 40 sec. Each isolate was twice tested under the same conditions with the selected oligonucleotides. For Documentation/visualization of RAPD-PCR products, 1.5% agarose gel (prepared in 1X TBE buffer containing 0.5 mg/ml of ethidium bromide used as a fluorescent tag) electrophoresis at 5 volt/cm for 45 min to 1 hour along with gene ruler Optilad DNA ladder 100 bp (GCC, Biotech.) was used. PCR amplicons were visualized under UV light and read by Gel document system (Fig. 1). The captured images were subjected to Total Lab software Reporting Tool version 13.2 for analyzing the number/ size of base pairs of all amplicons. Genetic relationships were established by making the scoring sheet as the presence of band was marked as 1 or absence of the band as 0 of each RAPD polymorphic band. The dendrograms were generated by unweighted pair group method with arithmetic averages (UPGMA) method (Fig. 2, 3, 4, 5 and 6) .

Results and Discussion

During our study total 150 samples (50 river/canal bank water samples, 50 swab samples of door handles of public transport buses and 50 swab samples of shop

Table 1: Collection of samples from different sites (river/canal bank water, bus handles and shop counters) and their viable counts

S. No.	Accession number	Total viable count	Source of Samples	Place	Lati-tude	Longi-tude
1	MUM-1	42×10^4	Water (Ganga River)	Ramjhoola, Rishikesh	30.1240	78.3148
2	MUM-2	33×10^4	Water (Ganga River)	LakshmanJhoola, Rishikesh	30.1263	78.3306
3	MUM-3	28×10^4	Water (Ganga River)	Shiv Puri, Rishikesh	30.1361	78.3928
4	MUM-4	34×10^4	Water (Ganga River)	Triveni Ghat, Rishikesh	30.1030	78.2997
5	MUM-5	21×10^4	Water (Ganga River)	VashishtaGuha Temple, Rishikesh	30.1142	78.4320
6	MUM-6	33×10^4	Water (Tons River)	Guchhupani, Malsi, Dehradun	30.3771	78.0606
7	MUM-7	36×10^4	Water (Sahastradhara)	Sahastradhara, Timilimansingh, Dehradun	30.3885	78.1300
8	MUM-8	23×10^4	Water (Asan River)	Tapkeshwar, Deharadun	30.3572	78.0166
9	MUM-9	31×10^4	Water (Ganga River)	Sarvananda Ghat, Haridwar	29.9709	78.1804
10	MUM-10	55×10^4	Water (Ganga River)	HarkiPauri, Haridwar	29.9574	78.1750
11	MUM-11	64×10^4	Water (Ganga River)	Chandi Ghat, Haridwar	29.5644	78.0951
12	MUM-12	37×10^4	Water (Ganga River)	Khanna Nagar Ghat, Haridwar	29.9243	78.1255
13	MUM-13	51×10^4	Water (Ganga River)	Kankhal (Sati Ghat) Haridwar	29.9249	78.1496
14	MUM-14	29×10^4	Water (Upper Ganga Canal)	Alaknanda Hotel, Haridwar	29.5645	78.9042
15	MUM-15	43×10^4	Water (Upper Ganga Canal)	Manglaur, Haridwar	29.7898	77.8639
16	MUM-16	71×10^4	Water (Yamuna Canal)	Kalsiya, Saharanpur	30.1350	77.6167
17	MUM-17	12×10^5	Water (Ganga River)	Brijghat, Hapur	28.7587	78.1443
18	MUM-18	13×10^5	Water (Ganga River)	Brijghat 2, Hapur	28.7569	78.1447
19	MUM-19	12.4×10^5	Water (Ramganga River)	Ramganga, Moradabad	28.8254	78.7980
20	MUM-20	79×10^4	Water (Ganga River)	Tigri, Hapur	28.8246	78.1549
21	MUM-21	74×10^4	Water (Ganga River)	Bijnor	29.2833	78.1013
22	MUM-22	79×10^4	Water (Ganga River)	Shukratal, Muzaffarnagar	29.4876	77.9824
23	MUM-23	23×10^4	Water (Upper Ganga Canal)	BholaJhal, Meerut	29.0010	77.5681
24	MUM-24	24×10^4	Water (Upper Ganga Canal)	Khatoli, Muzaffarnagar	29.2897	77.7308
25	MUM-25	68×10^4	Water (Upper Ganga Canal)	Sardhana, Meerut	29.0945	77.6061
26	MUM-26	11.7×10^5	Water (Upper Ganga Canal)	Muradnagar, Ghaziabad	28.7784	77.5185
27	MUM-27	41×10^4	Water (Upper Ganga Canal)	Masuri, Ghazaiabad	28.7095	77.5483
28	MUM-28	25×10^4	Water (Upper Ganga Canal)	Dehra, Ghaziabad	28.6494	77.5928
29	MUM-29	65×10^4	Water (Upper Ganga Canal)	Pyawli, NTPC, G.B Nagar	28.6155	77.5855
30	MUM-30	16.6×10^5	Water (Hindon River)	Hindon, Vaishali Ghaziabad	28.6705	77.4011
31	MUM-31	51×10^4	Water (Upper Ganga Canal)	Sanota, Bulandshahr	28.5204	77.7314
32	MUM-32	59×10^4	Water (Upper Ganga Canal)	Akbarpur, Bulandshahr	28.3861	77.8300
33	MUM-33	10.6×10^5	Water (Ganga River)	Karnwas, Bulandshahr	28.2695	78.3291
34	MUM-34	43×10^4	Water (Ganga River)	Rajghat, Bulandshahr	28.2362	78.3542
35	MUM-35	10.2×10^5	Water (Ganga River)	Narwar Ghat, Bulandshahr	28.1236	78.2340
36	MUM-36	12×10^5	Water (Ganga River)	Anoopshahr	28.3557	78.2715
37	MUM-37	96×10^4	Water (Ganga River)	Anupshahr bypass, Anupshahr	28.3645	78.2699
38	MUM-38	11.2×10^5	Water (Ganga River)	Kachhla Ghat, Badaun	27.9339	78.8577
39	MUM-39	12.8×10^5	Water (Ganga River)	SoronJi Ghat, Kasganj	27.8839	78.7493
40	MUM-40	10.8×10^5	Water (Yamuna River)	Anwara, Tundla, Firozabad	27.1733	78.2173
41	MUM-41	12.1×10^5	Water (Yamuna River)	Cremation Ghat, Etawah	26.7555	79.0182
42	MUM-42	14×10^5	Water (Ganga River)	Sarsaiya Ghat, Kanpur	26.4791	80.3584
43	MUM-43	14.1×10^5	Water (Ganga River)	Massacre Ghat, Kanpur	26.4585	80.3804
44	MUM-44	13.5×10^5	Water (Ganga River)	Anandeshwar, Kanpur	26.4875	80.3426
45	MUM-45	15.7×10^5	Water (Ganga River)	Brahmavart Ghat, Bithoor, Kanpur	26.6138	80.2747
46	MUM-46	12.4×10^5	Water (Yamuna River)	Parwatighat, Agra	27.2201	78.0317

continued table 1 .....

continued table 1 .....

S. No.	Accession number	Total viable count	Source of Samples	Place	Lati-tude	Longi-tude
47	MUM-47	13×10^5	Water (Yamuna River)	Dusehra Ghat, Agra	27.1761	78.0436
48	MUM-48	16.2×10^5	Water (Yamuna River)	Vishram Ghat, Mathura	27.5043	77.6870
49	MUM-49	15.5×10^5	Water (Yamuna River)	ShriGoverdhanNath Temple, Gokul, Mathura	27.4391	77.7181
50	MUM-50	13.3×10^5	Water (Yamuna River)	Keshi Ghat, Vrindavan, Mathura	27.5864	77.7005
51	MUM-51	73×10^4	Bus handle (UP11-T-4894)	Saharanpur Depot, Haridwar	29.9392	78.1422
52	MUM-52	14.4×10^5	Bus handle (UP25-B-1705)	Bareilly Depot, Haridwar	29.9392	78.1422
53	MUM-53	11×10^5	Bus handle (UK07-PA-0221)	Haridwar Depot, Haridwar	29.9392	78.1422
54	MUM-54	17.5×10^5	Bus handle (UK12-PA-5847)	Haridwar Depot, Haridwar	29.9392	78.1422
55	MUM-55	12.8×10^5	Bus handle (HR69-5073)	Mathura Depot, Haridwar	29.9392	78.1422
56	MUM-56	64×10^4	Bus handle (UP85-AT-3948)	Foundry Nagar Depot, Roorkee	29.8639	77.8887
57	MUM-57	75×10^4	Bus handle (UP85-AF-9894)	Taj Depot, Roorkee	29.8639	77.8887
58	MUM-58	43×10^4	Bus handle (UP85-Z-9463)	Foundry Nagar Depot, Roorkee	29.8639	77.8887
59	MUM-59	42×10^4	Bus handle (UP-85-AJ-9016)	Idgah Depot, Roorkee	29.8639	77.8887
60	MUM-60	38×10^4	Bus handle (HR46-D-2147)	Rohtak Depot, Roorkee	29.8639	77.8887
61	MUM-61	18×10^4	Bus handle (RJ05-PA-1878)	Bharatpur Depot, Muzaffarnagar	29.4679	77.7047
62	MUM-62	72×10^4	Bus handle (UP11-T-7500)	Chhutmulpur Depot, Muzaffarnagar	29.4679	77.7047
63	MUM-63	49×10^4	Bus handle (UP12-T-5114)	Ambala Depot, Muzaffarnagar	29.4679	77.7047
64	MUM-64	21×10^4	Bus handle (UP17-T-4761)	Muzaffarnagar Depot, Muzaffarnagar	29.4679	77.7047
65	MUM-65	34×10^4	Bus handle (UP11-T-7284)	Bhaisali Depot, Muzaffarnagar	29.4679	77.7047
66	MUM-66	10.7×10^5	Bus handle (UP15-AT-0931)	Muzaffarnagar Depot, Khatoli	29.2704	77.7296
67	MUM-67	78×10^4	Bus handle (UP12-T-3709)	Khatoli Depot, Khatoli	29.2704	77.7296
68	MUM-68	73×10^4	Bus handle (UP15-AT-8719)	Muzaffarnagar Depot, Khatoli	29.2704	77.7296
69	MUM-69	60×10^4	Bus handle (UP15-AT-0449)	Khatoli Depot, Khatoli	29.2704	77.7296
70	MUM-70	84×10^4	Bus handle (UP12-T-8210)	Muzaffarnagar Depot, Khatoli	29.2704	77.7296
71	MUM-71	32×10^4	Bus handle (UP12-AN-1755)	Bijnor Depot, Bhaisali, Meerut	28.9895	77.7013
72	MUM-72	10.4×10^5	Bus handle (UP16-P-9562)	Noida Depot, Bhaisali, Meerut	28.9895	77.7013
73	MUM-73	74×10^4	Bus handle (UP12-T-0644)	Muzaffarnagar Depot, Bhaisali, Meerut	28.9895	77.7013
74	MUM-74	48×10^4	Bus handle (UP85-Z-9190)	Foundry Nagar Depot, Bhaisali, Meerut	28.9895	77.7013
75	MUM-75	38×10^4	Bus handle (UP42-A-0673)	Agra Depot, Bhaisali, Meerut	28.9895	77.7013
76	MUM-76	63×10^4	Bus handle (UP12-T-1193)	Sohrabgate Depot, Sohrabgate, Meerut	28.9703	77.7220
77	MUM-77	52×10^4	Bus handle (UP17-D-4917)	Bulandshahr Depot, Sohrabgate, Meerut	28.9703	77.7220
78	MUM-78	38×10^4	Bus handle (UP13-P-9833)	Sohrabgate Depot, Sohrabgate, Meerut	28.9703	77.7220
79	MUM-79	36×10^4	Bus handle (UP15-AT-6283)	Sohrabgate Depot, Sohrabgate, Meerut	28.9703	77.7220
80	MUM-80	55×10^4	Bus handle (UP81-AF-5691)	Sohrabgate Depot, Sohrabgate, Meerut	28.9703	77.7220
81	MUM-81	46×10^4	Bus handle (UP15-AT-8447)	Bhaisali Depot, Ghaziabad	28.6705	77.4318
82	MUM-82	28×10^4	Bus handle (UP15-AT-7657)	Bhaisali Depot, Ghaziabad	28.6705	77.4318
83	MUM-83	49×10^4	Bus handle (DL1P-B-5591)	Bhajanpura Depot, Ghaziabad	28.6705	77.4318
84	MUM-84	40×10^4	Bus handle (UP17-T-3035)	Bulandshahr Depot, Ghaziabad	28.6705	77.4318

continued table 1 .....

continued table 1 .....

S. No.	Accession number	Total viable count	Source of Samples	Place	Lati-tude	Longi-tude
85	MUM-85	55×10^4	Bus handle (UP15-AT-7202)	Bhaisali Depot, Ghaziabad	28.6705	77.4318
86	MUM-86	58×10^4	Bus handle (UP21-AN-2053)	Moradabad Depot, Kaushambi Ghaziabad	28.6458	77.3184
87	MUM-87	48×10^4	Bus handle (UP17-C-7326)	Bhaisali Depot, Kaushambi Ghaziabad	28.6458	77.3184
88	MUM-88	53×10^4	Bus handle (UP21-AN-2678)	Moradabad Depot, Kaushambi Ghaziabad	28.6458	77.3184
89	MUM-89	71×10^4	Bus handle (UP17-C-7310)	Bhaisali Depot, Kaushambi Ghaziabad	28.6458	77.3184
90	MUM-90	57×10^4	Bus handle (UP14-AT-6389)	Hapur Depot, Kaushambi Ghaziabad	28.6458	77.3184
91	MUM-91	55×10^4	Bus handle (UP14-DT-3346)	Bulandshahr Depot, Bulandshahr	28.3980	77.8491
92	MUM-92	37×10^4	Bus handle (UP15-AT-1072)	Bulandshahr Depot, Bulandshahr	28.3980	77.8491
93	MUM-93	21×10^4	Bus handle (UP14-AT-6390)	Khurja Depot, Bulandshahr	28.3980	77.8491
94	MUM-94	79×10^4	Bus handle (UP15-Z-1590)	Atroli Depot, Bulandshahr	28.3980	77.8491
95	MUM-95	64×10^4	Bus handle (UP23-BT-0223)	Hathras Depot, Bulandshahr	28.3980	77.8491
96	MUM-96	60×10^4	Bus handle (UP81-AA-9037)	Etah Depot, New Bus Stand, Bulandshahr	28.3904	77.8313
97	MUM-97	73×10^4	Bus handle (UP81-AF-9289)	Aligarh Depot, New Bus Stand, Bulandshahr	28.3904	77.8313
98	MUM-98	52×10^4	Bus handle (UP86-E-9745)	Hathras Depot, New Bus Stand, Bulandshahr	28.3904	77.8313
99	MUM-99	35×10^4	Bus handle (UP86-T-4919)	Kasganj Depot, New Bus Stand, Bulandshahr	28.3904	77.8313
100	MUM-100	27×10^4	Bus handle (UP84-T-5213)	Mainpuri Depot, New Bus Stand, Bulandshahr	28.3904	77.8313
101	MUM-101	78×10^4	Shop counter (Rajul General Store)	Delhi Road Roorkee, Haridwar	29.5229	77.5323
102	MUM-102	10.6×10^5	Shop counter (Chaurasia Provision Store)	Bahadrabad, Haridwar	29.5736	79.9036
103	MUM-103	32×10^4	Shop counter (Monu Provision Store)	Bhoopatwala, Haridwar	29.9747	78.1778
104	MUM-104	53×10^4	Shop counter (Ashish Confectioners)	Jwalapur, Haridwar	29.9271	78.1080
105	MUM-105	38×10^4	Shop counter (PanditJi Provision Store)	HaripurKalan, Haridwar	29.9948	78.2020
106	MUM-106	76×10^4	Shop counter (Vishal Megamart)	Rishikul, Haridwar	29.9374	78.1436
107	MUM-107	60×10^4	Shop counter (HariKhanna Provision Store)	Rajghat, Haridwar	29.9245	78.1483
108	MUM-108	62×10^4	Shop counter (Harinandan Gift Emporium)	Har Ki Pauri, Haridwar	29.9580	78.1710
109	MUM-109	65×10^4	Shop counter (Sanju Provision Store)	Jagjeetpur, Haridwar	29.9168	78.1301
110	MUM-110	50×10^4	Shop counter (Jai Balaji Provision Store)	Rosanpuri, Haridwar	29.9380	78.0602
111	MUM-111	36×10^4	Shop counter (Brijmohan Kiryana Store)	Near Hanuman Chowk, Muzaffarnagar	29.4725	77.6906

continued table 1 .....

continued table 1 .....

S. No.	Accession number	Total viable count	Source of Samples	Place	Lati-tude	Longi-tude
112	MUM-112	40×10^4	Shop counter (Malik General Store)	Civil Lines North, Muzaffarnagar	29.4802	77.7036
113	MUM-113	36×10^4	Shop counter (Vimal General Store)	Rampuri, Muzaffarnagar	29.4901	77.6978
114	MUM-114	11.3×10^5	Shop counter (King Confectioners)	Roorkee Road, Muzaffarnagar	29.4743	77.6965
115	MUM-115	68×10^4	Shop counter (Durgadas General Store)	Shiv Chowk, Muzaffarnagar	29.4716	77.6966
116	MUM-116	35×10^4	Shop counter (Haldiram)	Mansoorpur, Muzaffarnagar	29.3441	77.7155
117	MUM-117	42×10^4	Shop counter (Goyal Provision Store)	G.T. Road Khatoli, Muzaffarnagar	29.2812	77.7312
118	MUM-118	61×10^4	Shop counter (Annpurna Confectioners)	GhantagharKhatoli, Muzaffarnagar	29.2802	77.7323
119	MUM-119	26×10^4	Shop counter (Moolchand Resort)	KhalaparMansoorpur, Muzaffarnagar	29.4660	77.6960
120	MUM-120	48×10^4	Shop counter (Cheetal Grand)	Khatoli Bypass, Muzaffarnagar	29.2439	77.7248
121	MUM-121	22×10^4	Shop counter (ManojKiranaStore)	Kharkhoda, Meerut	28.8395	77.7426
122	MUM-122	70×10^4	Shop counter (UjjawalKirana Store)	Near 44, PAC, Meerut	28.9363	77.7269
123	MUM-123	28×10^4	Shop counter (Gupta Traders)	Shastrinagar, Meerut	28.9525	77.7296
124	MUM-124	76×10^4	Shop counter (Aadi Shree Kirana Store)	Near Tejgarhi, Meerut	28.9627	77.7404
125	MUM-125	32×10^4	Shop counter (Ram Kirana Store)	NaiSadak, Meerut	28.9664	77.7300
126	MUM-126	60×10^4	Shop counter (Praveen Kirana Store)	Near GarhAdda, Meerut	28.9702	77.7202
127	MUM-127	43×10^4	Shop counter (Garg Provision Store)	Arya Nagar, Meerut	28.9778	77.7170
128	MUM-128	90×10^4	Shop counter (BansalKirana Store)	LalKurti, Meerut	28.9978	77.7059
129	MUM-129	68×10^4	Shop counter (AgrawalKirana Store)	Begumbridge, Meerut	28.9948	77.7055
130	MUM-130	52×10^4	Shop counter (SandeepKirana Store)	Kesarganj, Meerut	28.9857	77.6962
131	MUM-131	21×10^4	Shop counter (Yadav Provision Store)	Ghukna,Ghaziabad	28.6865	77.4195
132	MUM-132	34×10^4	Shop counter (Prince Sinha General Store)	Nandgram,Ghaziabad	28.6925	77.4283
133	MUM-133	62×10^4	Shop counter (Luthra Provision Store)	Patel Nagar-II,Ghaziabad	28.6760	77.4207
134	MUM-134	28×10^4	Shop counter (ChaddhaKirana Store)	KiranaMandi,Ghaziabad	28.6577	77.4330
135	MUM-135	42×10^4	Shop counter (MangalamKirana Store)	KiranaMandi,Ghaziabad	28.6577	77.4330

continued table 1 .....

continued table 1 .....

S. No.	Accession number	Total viable count	Source of Samples	Place	Lati-tude	Longi-tude
136	MUM-136	42×10^4	Shop counter (Pooja General Store)	RakeshMarg,Ghaziabad	28.6511	77.4421
137	MUM-137	34×10^4	Shop counter (Easyday)	Govindpuram,Ghaziabad	28.6898	77.4912
138	MUM-138	66×10^4	Shop counter (Vishal Provision Store)	Indergarhi,Ghaziabad	28.6816	77.4970
139	MUM-139	45×10^4	Shop counter (Toofan Grocery)	Shastrinagar,Ghaziabad	28.6632	77.4636
140	MUM-140	60×10^4	Shop counter (Goel Stores)	Kavinagar,Ghaziabad	28.6711	77.4491
141	MUM-141	48×10^4	Shop counter (New Classic Provision Store)	Near Malka Park, Bulandshahr	28.4064	77.8515
142	MUM-142	42×10^4	Shop counter (Kedia Provision Store)	D.M Road, Bulandshahr	28.4092	88.8501
143	MUM-143	46×10^4	Shop counter (Bansal General Store)	Kali Nadi Road, Bulandshahr	28.4081	77.8588
144	MUM-144	26×10^4	Shop counter (PriyankaKirana Store)	Near Bus Stand, Bulandshahr	28.3947	77.8445
145	MUM-145	35×10^4	Shop counter (KajiKirana Store)	Gulaothi, Bulandshahr	28.5930	77.7883
146	MUM-146	10.2×10^5	Shop counter (RamashishKirana Store)	Delhi Road Khurja, Bulandshahr	28.2590	77.8606
147	MUM-147	70×10^4	Shop counter (Rahul General Store)	JewarAdda, Bulandshahr	28.2486	77.8552
148	MUM-148	62×10^4	Shop counter (Ma Durga Provision Store)	Near Nav-Durga Temple, Bulandshahr	28.2384	77.8630
149	MUM-149	58×10^4	Shop counter (Akki General Store)	Dankaur Road Sikandrabad, Bulandshahr	28.4513	77.6954
150	MUM-150	38×10^4	Shop counter (Sharma Confectioners)	Lajja Ram Market Gulaothi, Bulandshahr	28.5884	77.7923

counters) were collected from Western Uttar Pradesh and Uttarakhand of North India (Table 1). 100% samples (50 water samples) of water bodies were positive for viable count; and ranges between 21×10^4 to 16.6×10^5 total viable counts per ml of water sample (Table 2). Water sample from the Vashishta Guha Temple, Rishikesh showed the minimum viable count (21.0×10^4 cfu/ml), whereas water sample from Hindon River, Vaishali, Ghaziabad showed the maximum viable count (16.6×10^5 cfu/ml). It is manifestated that all the sampling sites, the counts were significantly higher than the Central Pollution Control Board (CPCB) of India the permissible count in potable water source without conventional treatment but after disinfection should be <50 MPN/100 ml, for outdoor bathing (Organized) should be <500 MPN/100 ml and in potable water source after conventional treatment and disinfection should be <5000 MPN/100 ml. According to the WHO (2011) the microbial load in drinking water is 500 cfu/ml. Our study shows the high microorganism load in all water bodies of the studied area so it is concluded that these water bodies are not fit for drinking, swimming and other domestic purpose use.

In the present study 100 swab samples (50 from bus handles and 50 from shop counters) were also processed for the total viable count; it was found that 100% of the swab samples were positive for the isolation of bacteria ranges from $18x10^4$ to 17.5×10^5 total viable count/4cm^2 surface area of bus handles and 21×10^4 to 11.3×10^5 total viable count/4cm^2 area of shop counters, respectively (Table 1). Total viable count range with a median value of almost 60.72×10^4/4cm^2 which means that more than third part of the samples contain bacterial load higher than the median value. According to Surface Hygiene Guideline, BC Centre for Disease Control, Vancouver, British Columbia (2010) the count <5 CFU/cm^2 will be clean, ~ 5 to 10 CFU/cm^2 will be contaminated and >10 CFU/cm^2 will be very contaminated. The results of our study are showing that the high microorganism load and poor sanitation and hygiene conditions exist among the tested swab samples of selected bus handles and shop

Table 2: Prevalence of bacteria in collected samples.

Isolated Strains														
S. No.	Accession Number	*Streptococcus* spp.			*Staphylococcus* spp.			*Pseudomonas* spp.			*E. coli*	*Klebsiella*spp.		Total
		S. angi-nosus	*S. pneu-monia*	*S. bovis*	*S aureus*	*S.epider-midis*	*S.sapro-phyticus*	*P. aeru-ginosa*	*P. oryzi-habitans*	*P. fluo-rescens*		*K. pneumonia*	*K. oxytoca*	
1	MUM-1				+									01
2	MUM-2		+	+		+					+			04
3	MUM-3						+							01
4	MUM-4				+									01
5	MUM-5	+		+		+				+	+		+	06
6	MUM-6	+		+			+		+					04
7	MUM-7						+							01
8	MUM-8		+				+			+	+			04
9	MUM-9													00
10	MUM-10				+									01
11	MUM-11		+	+	+				+	+		+		06
12	MUM-12						+							01
13	MUM-13						+							01
14	MUM-14					+								01
15	MUM-15	+			+			+	+				+	05
16	MUM-16				+							+		02
17	MUM-17				+									01
18	MUM-18													00
19	MUM-19													00
20	MUM-20		+	+	+			+			+			05
21	MUM-21													00
22	MUM-22	+			+			+			+			04
23	MUM-23													00
24	MUM-24													00
25	MUM-25		+					+						02
26	MUM-26			+	+			+			+			04
27	MUM-27													00
28	MUM-28							+						01
29	MUM-29						+							01
30	MUM-30					+		+		+		+		04
31	MUM-31													00
32	MUM-32													00

continued table 2 .....

continued table 2 .....

S. No.	Accession Number	Isolated Strains												
		Streptococcus spp.			*Staphylococcus* spp.			*Pseudomonas* spp.			*E. coli*	*Klebsiella*spp.		Total
		S. angi- nosus	*S. pneu-* monia	*S.* bovis	*S* aureus	*S.epider-* midis	*S.sapro-* phyticus	*P. aeru-* ginosa	*P. oryzi-* habitans	*P.fluo-* rescens		*K.* pneumonia	*K.* oxytoca	
33	MUM-33						+							01
34	MUM-34													00
35	MUM-35					+								01
36	MUM-36	+		+		+		+					+	05
37	MUM-37													00
38	MUM-38													00
39	MUM-39	+			+			+	+		+		+	06
40	MUM-40				+									01
41	MUM-41													00
42	MUM-42	+						+	+		+			04
43	MUM-43													00
44	MUM-44													00
45	MUM-45		+		+			+	+		+	+		06
46	MUM-46											+		01
47	MUM-47													00
48	MUM-48													00
49	MUM-49											+		01
50	MUM-50													00
51	MUM-51													00
52	MUM-52						+							01
53	MUM-53		+				+	+			+			04
54	MUM-54						+							01
55	MUM-55	+		+	+				+		+			05
56	MUM-56													00
57	MUM-57						+							01
58	MUM-58				+						+			02
59	MUM-59						+							01
60	MUM-60				+									01
61	MUM-61				+									01
62	MUM-62						+							01
63	MUM-63					+								01
64	MUM-64	+					+				+			03

continued table 2 .....

continued table 2 .....

S. No.	Accession Number	Isolated Strains												
		Streptococcus spp.			*Staphylococcus* spp.			*Pseudomonas* spp.			*E. coli*	*Klebsiella* spp.		Total
		S. angi-nosus	*S. pneu-monia*	*S. bovis*	*S aureus*	*S.epider-midis*	*S.sapro-phyticus*	*P. aeru-ginosa*	*P. oryzi-habitans*	*P. fluo-rescens*		*K. pneumonia*	*K. oxytoca*	
65	MUM-65			+			+		+	+	+			05
66	MUM-66		+									+		02
67	MUM-67						+							01
68	MUM-68	+	+											02
69	MUM-69						+							01
70	MUM-70													00
71	MUM-71						+	+	+		+			04
72	MUM-72			+			+	+		+	+			05
73	MUM-73													00
74	MUM-74	+	+				+							03
75	MUM-75						+		+				+	03
76	MUM-76					+								01
77	MUM-77				+									01
78	MUM-78				+							+	+	03
79	MUM-79				+									01
80	MUM-80				+									01
81	MUM-81						+							01
82	MUM-82						+							01
83	MUM-83				+									01
84	MUM-84					+								01
85	MUM-85					+				+	+			03
86	MUM-86	+					+		+					03
87	MUM-87		+	+			+			+				04
88	MUM-88						+					+		02
89	MUM-89				+									01
90	MUM-90				+							+		02
91	MUM-91				+									01
92	MUM-92	+	+		+					+	+			05
93	MUM-93													00
94	MUM-94				+									01
95	MUM-95					+								01
96	MUM-96													00

continued table 2 .....

continued table 2 .....

S. No.	Accession Number	Isolated Strains												
		Streptococcus spp.			*Staphylococcus* spp.			*Pseudomonas* spp.			*E. coli*	*Klebsiella*spp.		Total
		S. angi- nosus	*S. pneu-* monia	*S.* bovis	*S* aureus	*S.epider-* midis	*S.sapro-* phyticus	*P. aeru-* ginosa	*P. oryzi-* habitans	*P. fluo-* rescens		*K.* pneumonia	*K.* oxytoca	
97	MUM-97													00
98	MUM-98		+	+								+		03
99	MUM-99						+							01
100	MUM-100	+					+							02
101	MUM-101				+									01
102	MUM-102						+					+	+	03
103	MUM-103						+							01
104	MUM-104		+				+	+		+	+			05
105	MUM-105						+						+	02
106	MUM-106						+							01
107	MUM-107				+									01
108	MUM-108				+			+			+			03
109	MUM-109				+									01
110	MUM-110	+	+	+	+			+	+					06
111	MUM-111						+	+						02
112	MUM-112						+							01
113	MUM-113						+							01
114	MUM-114		+				+	+		+				04
115	MUM-115	+			+									02
116	MUM-116	+					+							02
117	MUM-117					+								01
118	MUM-118			+		+			+					03
119	MUM-119						+							01
120	MUM-120						+		+	+	+			04
121	MUM-121	+	+				+							03
122	MUM-122						+				+			02
123	MUM-123	+	+	+			+		+					05
124	MUM-124		+	+			+				+			04
125	MUM-125						+					+	+	03
126	MUM-126						+							01
127	MUM-127						+			+	+			03
128	MUM-128						+							01

continued table 2 .....

continued table 2 .....

Isolated Strains														
S. No.	**Accession Number**	***Streptococcus* spp.**			***Staphylococcus* spp.**			***Pseudomonas* spp.**			***E. coli***	***Klebsiella*spp.**		**Total**
		***S. angi-*nosus**	***S. pneu-*monia**	***S.* bovis**	***S* aureus**	***S.epider-*midis**	***S.sapro-*phyticus**	***P. aeru-*ginosa**	***P. oryzi-*habitans**	***P.fluo-*rescens**		***K.* pneumonia**	***K.* oxytoca**	
129	MUM-129	+					+			+	+			04
130	MUM-130					+				+				02
131	MUM-131	+					+							02
132	MUM-132						+							01
133	MUM-133	+					+		+					03
134	MUM-134						+							01
135	MUM-135						+							01
136	MUM-136				+									01
137	MUM-137			+		+			+		+			04
138	MUM-138	+					+		+			+		04
139	MUM-139					+								01
140	MUM-140											+	+	02
141	MUM-141						+							01
142	MUM-142						+							01
143	MUM-143					+								01
144	MUM-144		+		+						+			03
145	MUM-145				+									01
146	MUM-146				+									01
147	MUM-147						+							01
148	MUM-148						+							01
149	MUM-149						+							01
150	MUM-150				+									01
Total isolates		23	20	17	38	17	61	19	18	15	27	15	10	280

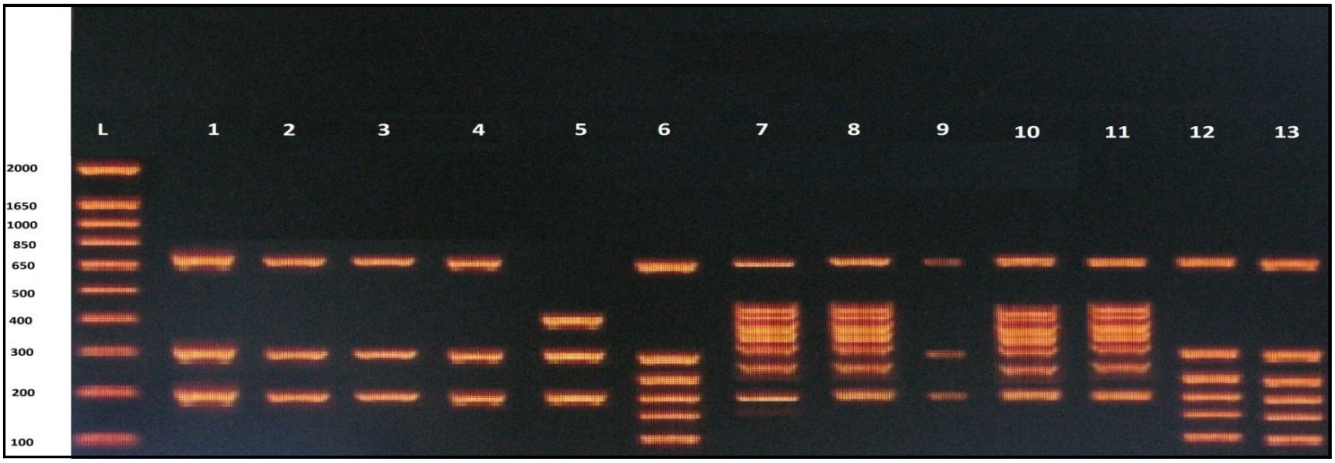

Fig. 1: RAPD profiles of bacterial isolates subjected to PCR amplification by using 10-mer RAPD primer. Lane 1–13 represents bacterial isolates whereas L represents 100 bp DNA ladder.

counters.

In the present study river/canal bank water samples were contaminated by 05 different bacterial genera *Streptococcus* sp., (23%) *Staphyloccocus* sp., (31.04%) *Pseudomonas* sp., (24.13%) *Escherichia coli* (10.34%) and *Klebsiella* sp. (11.49%) identified on the basis of morphology, cultural, biochemical and molecular characterization. Further, total 87 isolates of 12 different bacterial species were identified including *S. anginosus* (8.05%), *S. pneumonia* (6.90%), *S. bovis* (8.05%), *S. aureus* (14.94%), *S. epidermidis* (6.90%), *S. saprophyticus* (9.20%), *P. aeruginosa* (12.64%), *P. oryzihabitans* (6.90%), *P. fluorescens* (4.59%), *Escherichia coli* (10.34%), *K. pneumonia* (6.90%) and *K. oxytoca* (4.59%). Our study estimates that *Staphylococcus* sp. (31.04%) as most frequently distributed isolate followed by *Pseudomonas* sp. (24.13%) as well as the highest prevalence of *S. aureus* (14.94%) followed by *P. aeruginosa* (12.64%) and the lowest was of *K. oxytoca* (4.59%). Our study indicates huge hazardous bacterial load on river /canal bank water in the sampling area. Previous studies have also been suggested that a little number of potential pathogens are sufficient to cause infectious disease.

In our study swab samples from surfaces of bus handles were contaminated by 05 different bacterial genera including *Streptococcus* sp., (21.58%) *Staphyloccocus* sp., (45.45%) *Pseudomonas* sp., (14.77%) *Escherichia coli* (10.25%) and *Klebsiella* sp. (7.95%) identified on the basis of morphology, cultural, biochemical and molecular characterization. Total 88 isolates of 12 different bacterial species were identified including *S. anginosus* (7.95%), *S. pneumonia* (7.95%), *S. bovis* (5.68%), *S. aureus* (15.91%), *S. epidermidis* (5.68%), *S. saprophyticus* (23.86%), *P. aeruginosa* (3.41%), *P. oryzihabitans* (5.68%), *P. fluorescens* (5.68%), *Escherichia coli* (10.25%), *K. pneumonia* (5.68%) and *K. oxytoca* (2.27%). In our study we have found a maximum count of *S. saprophyticus* (23.86%) followed by *S. aureus* (15.91%) and a least count of *P. aeruginosa* (3.41%) and *K. oxytoca* (2.27%). Our study also suggests that the existence of hazardous bacteria on these common sites represents that they might act as environmental vehicles for the transmission of potentially pathogenic bacteria.

Furthermore, in our study; swab samples from shop counters were also contaminated by 05 different bacterial genera including *Streptococcus* sp., (20%) *Staphyloccocus* sp., (46.67%) *Pseudomonas* sp., (17.14%) *Escherichia coli* (8.57%) and *Klebsiella* sp. (7.62%) identified on the basis of morphology, cultural, biochemical and molecular characterization. From these 50 swab samples total 105 isolates of 12 different bacterial species were identified including *S. anginosus* (8.57%), *S. pneumonia* (6.67%), *S. bovis* (4.76%), *S. aureus* (10.48%), *S. epidermidis* (5.71%), *S. saprophyticus* (30.48%), *P. aeruginosa* (4.76%), *P. oryzihabitans* (6.67%), *P. fluorescens* (5.71%), *Escherichia coli* (8.57%), *K. pneumonia* (3.81%) and *K. oxytoca* (3.81%). Our results also indicate that the shop counters were highly contaminated with *S. saprophyticus* (30.48%) comparatively to other objects in the study. The presence of pathogenic microorganisms on shop counters indicate that the goods or any other daily basic need provisions we are purchasing from these sites; may be contaminated by hazardous bacteria as well as they might act as environmental vehicles/vectors for the transmitting of potentially pathogenic bacteria. At the time of weighing of provisions which are often radiate on the shop counter. Due to the availability of nutrients environmental

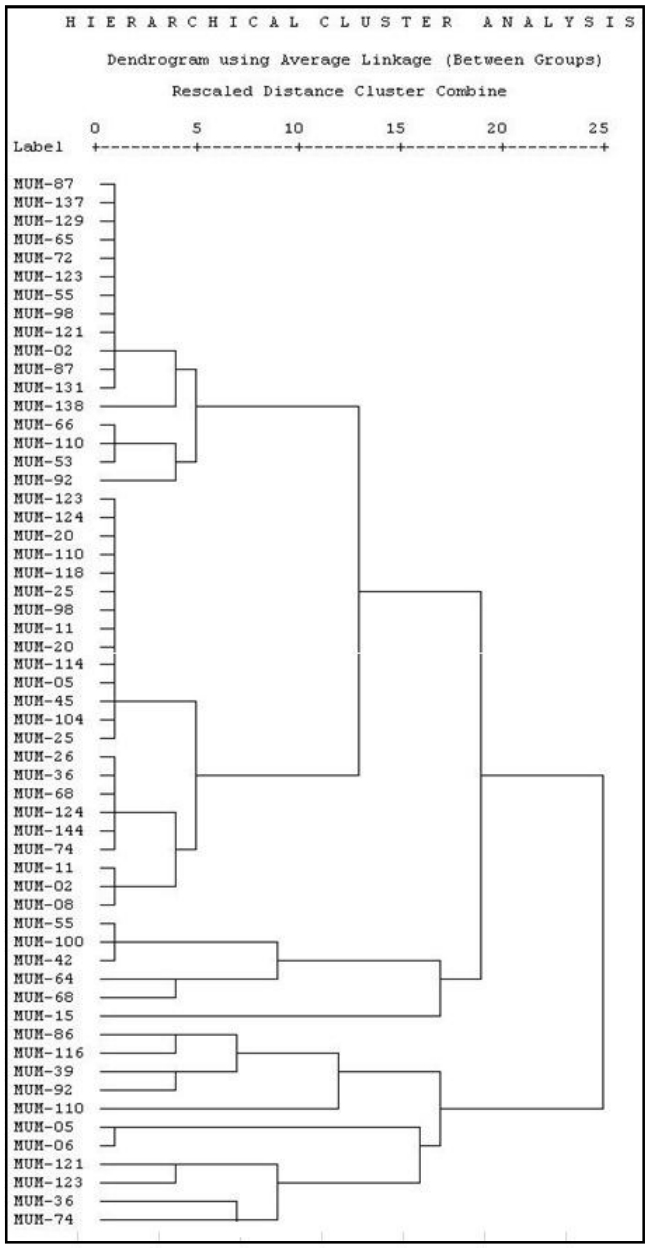

Fig. 2: Dendrogram of *Streptococcus* species on the basis of RAPD similarity matrix data by Hierarchical cluster analysis.

microorganisms along with contaminated finger microbes of customers are grown at that site.

In our study 40 primers were examined, out of these 18 primers were used for Random Amplified Polymorphic DNA (RAPD) screening for detection of polymorphism in bacterial isolates and used to know the genetic diversity or variability analysis among 280 bacterial isolates. A total 1034 bands were scored, out of which *Streptococcus* genera (233) including *S. anginosus* (108), *S. pneumonia* (64) and *S. bovis* (61), *Staphylococcus* genera (405) including *S. aureus* (144), *S. epidermidis* (58) and *S. saprophyticus* (203), *Pseudomonas* genera (216) including *P. aeruginosa* (82), *P. oryzihabitans*

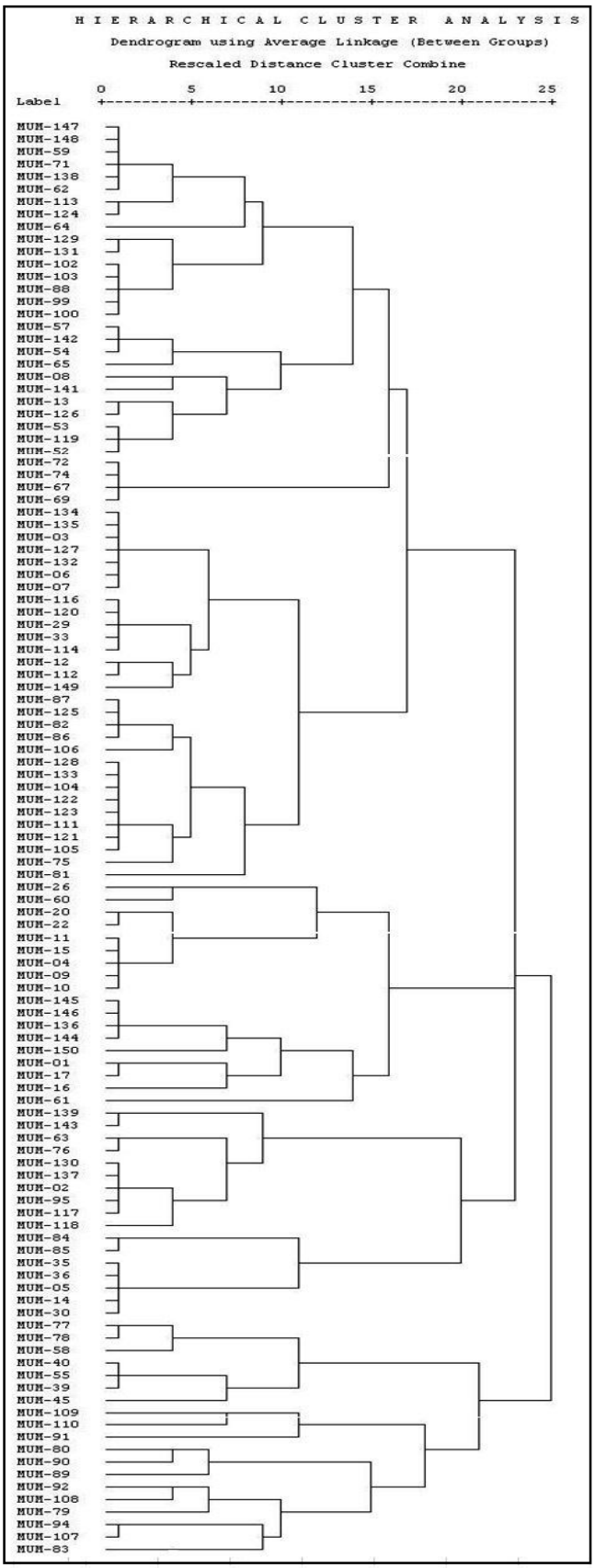

Fig. 3: Dendrogram of *Staphylococcus* species on the basis of RAPD similarity matrix data by Hierarchical cluster analysis.

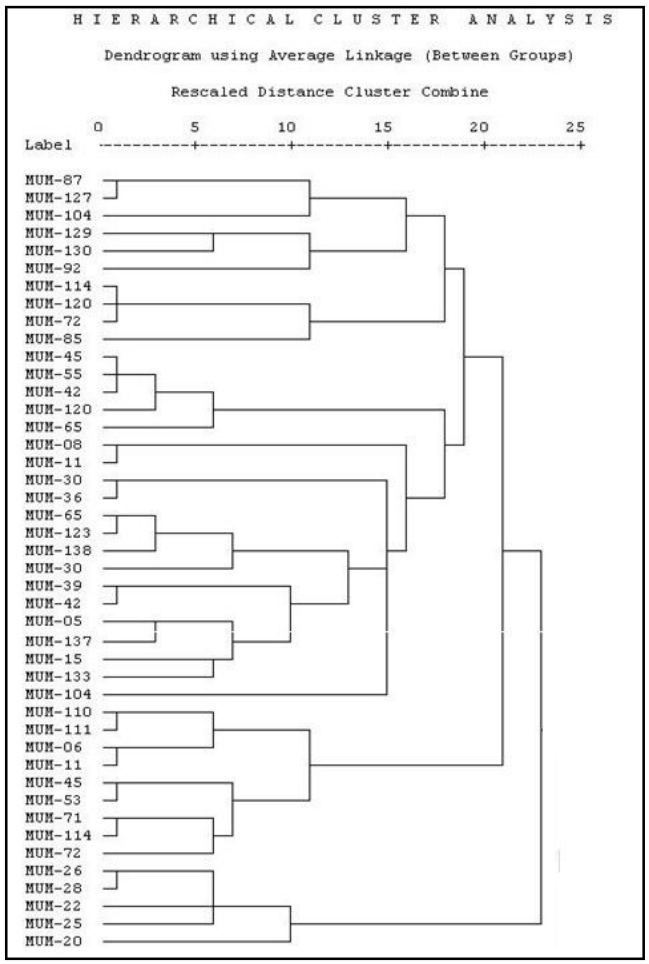

Fig. 4 : Dendrogram of *Pseudomonas* species on the basis of RAPD similarity matrix data by Hierarchical cluster analysis.

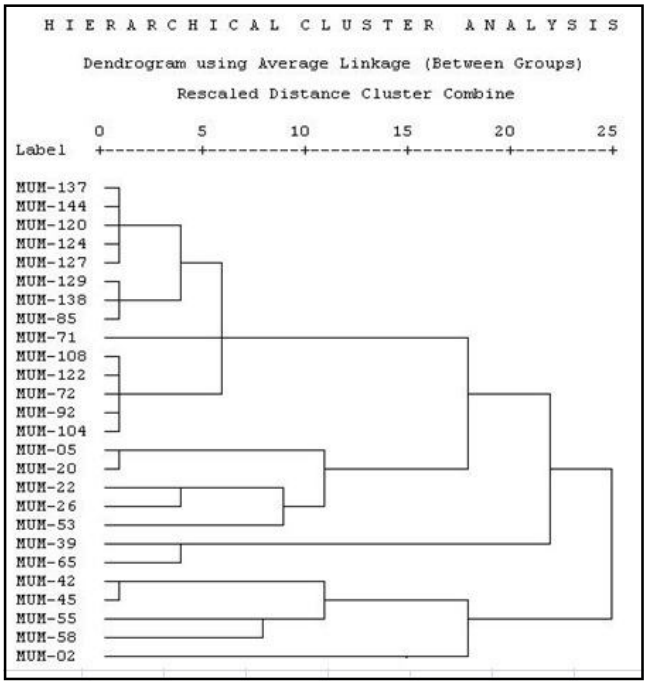

Fig. 5: Dendrogram of *Escherichia coli* on the basis of RAPD similarity matrix data by Hierarchical cluster analysis.

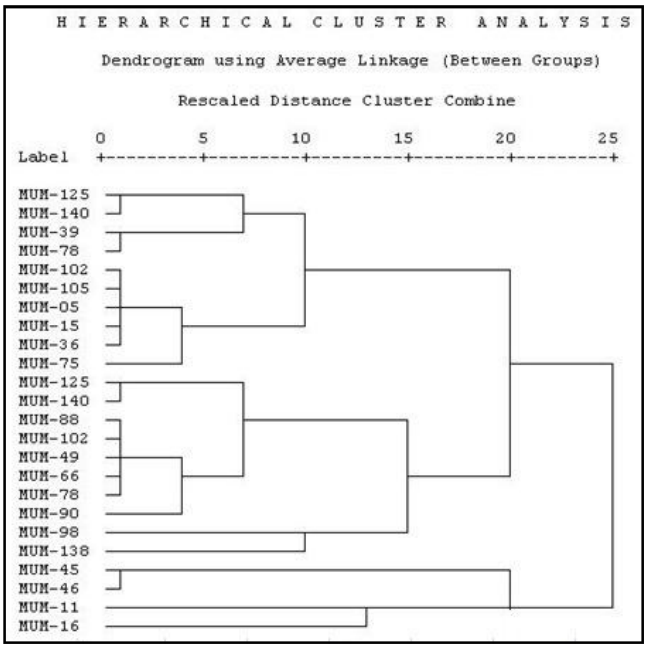

Fig. 6: Dendrogram of *Klebsiella species* on the basis of RAPD similarity matrix data by Hierarchical cluster analysis.

(73)and *P. fluorescens* (61), *E.coli* (97) and *Klebsiella* genera (83) including *K. pneumonia* (54) and *K. oxytoca* (29). Out of 18 oligonucleotide primer screening 07 primers showed 100% polymorphic band while 11 primers showed both single and polymorphic band patterns; therefore, 258 (90%) bands were polymorphic. This indicates that there is a significant genetic diversity among the isolates. DNA fingerprinting was resulted in multiple DNA products with 02 to 09 bands ranging from 130 to 940 bp. The high number of bands may be attributed to the presence of high number of primer annealing sites on the template DNA of the tested isolates. Polymorphisms at DNA level may occur as a result of several types of mutations, such as single base change in the primer-annealing site in the genome that prevents amplification by introducing a mismatch at 3' end of a DNA segment (Weeden *et al.,* 1992). The molecular markers including OPB-04 for *S. anginosus*, *S. pneumonia* and *S. bovis*, OPC-04, OPA-09, OPC-07 for *S. aureus*, *S. epidermidis,* and *S. saprophyticus*, OPA-06, OPC-03, OPC-04 for *P. aeruginosa*, OPC-03, OPC-04, OPC-05 for *P. oryzihabitans* and OPA-06, OPC-03, OPC-04, OPC-05, OPC-07 for *P. fluorescens*, OPC-01 for *E. coli*, OPA-03 and OPA-05 for *K. pneumonia* and *K. oxytoca* may be used in further study of genetic assortment of bacterial isolates from different sites, respectively. The unique bands may indicate as a fingerprinting or marker for these isolates and lead to the suggestion of using these primers to define these isolates in future studies.

Conclusion

The present research emphasizes the impact of good personal hygiene; and get off from the water bodies which are not fit for drinking, swimming and other domestic purpose use and adequate cleansing procedures applied to protect surfaces of bus handles and shop counters. It could be a step forward action to reduce hand contamination. Such kind of actions should be commenced in parallel with the public education on hygienic standards and hand washing procedures. Therefore, appropriate hygiene measures are required to suppress any potential microbial contamination. Our study focused on the prevalence of 05 common genera including *Streptococcus, Staphylococcus, Pseudomonas, Escherichia coli* and *Klebsiella.* Further study was focused on the bacterial species *Streptococcus anginosus*, *Streptococcus pneumonia*, *Streptococcus bovis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa*, *Pseudomonas oryzihabitans*, *Pseudomonas fluorescens*, *Escherichia coli*, *Klebsiella pneumonia* and *Klebsiella oxytoca*. These findings from our study are also distinguishing the need to take urgent measures to improve the sanitary conditions of public transport buses and shop counters. Therefore, removing soil, dust and dirt from them are necessary steps on the reduction of surface contamination by microbes.

Acknowledgement

The authors acknowledge their profound gratitude to Department of Science and Technology, New Delhi (Govt. of India) for financial support.

References

Surface Hygiene Guideline, BC Centre for Disease Control, Vancouver, British Columbia (2010); http://www.bccdc.ca /resource-gallery/Documents/Guidelines % 20 and % 20 Forms/Guidelines% 20 and % 20 Manuals/EH /FPS/Food/EnvMonitoringHygieneGuideforEHOs.pdf

Weeden, N.F, G.M. Timmerman and B.E. Kneecn (1992). Inheritance and reliability of RAPD markers, *In: Application of RAPD technologies plant breeding*, Crop. Sci. Society of American Madison, Press. U.S.A., 12-17.

WHO (2011); Guidelines for drinking-water quality, 4th Ed. Switzerland: WHO Library Cataloguing-in-Publication Data, *World Health Organization,* Geneva.