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ISOLATION AND IDENTIFICATION OF DOMINATING VIABLE BACTERIAL SPECIES IN POTABLE WATER

PAVAN KUMAR PINDI *, B. RAVALI, S. PRASAD, K. ARCHANA, D. RAJENDAR, AND N.RANGAIAH

Department of Microbiology, Palamuru University, Mahabubnagar – 509001, A.P.

ABSTRACT

This paper aims at the analysis of viable bacteria in potable water**.** Bacteria are the most diverse living beings on earth and only a fraction of them have been identified. The study aims to analyze bacterial abundance in potable water. Sample collection was carried out in 13 different places of Mahabubnagar district of Andhra Pradesh, India. The species were identified based on morphology, biochemical, antibiotic sensitivity tests and 16S rRNA gene approach. 33 different morphotypes were found in the samples. Among them 4 dominating strains were considered for further studies. The 16S rRNA gene sequences revealed that Bacillus, Enterobacter, Exiguobacterium and Staphylococcus were dominating in these samples. Overall, 16S rRNA gene sequencing and biochemical tests revealed that these bacterial groups are potentially active in potable water.

KEY WORDS: Bacterial diversity, Drinking water, Identification methods, 16S rRNA gene.

PAVAN KUMAR PINDI
Department of Microbiology, Palamuru University, Mahabubnagar, A.P

***Corresponding author**

INTRODUCTION

Bacteria occur nearly everywhere in nature and occupy an important place in human view of life. Drinking water is an important resource all around the world. A very little attempt was made on identifying of viable bacteria in potable water. Potable water can be obtained from various sources, like surface water or ground water. The monitoring & identification of bacteria present in drinking water have primarily been achieved using a plating isolation strategy. The majority of these viable bacteria described in drinking water networks are included in the phylum Proteobacteria and a less extent in the phyla Actinobacteria, Firmicutes and Bacteriodates [1]. Some of the commonly detected genera include Enterobacter, Bacillus, Exiguobacterium and Staphylococcus. Overall, a common perception is that Enterobacter is the most abundant bacterial organism in water source.

Most studies concerning the quality of drinking water deal with the analysis of the DOC [2] or the determination of the viable count of different bacteria in the drinking water, mainly focusing on the viable and pathogenic bacteria [3] The present study describes the identification of the viable bacterial population in potable water of 13 villages of Mahabubnagar district, A.P, India. The bacterial diversity was determined based on their morphological, biochemical characteristics and 16S rRNA gene sequence**.**

MATERIALS AND METHODS

Source of samples

Water samples were collected from several sites from different villages of Mahabubnagar district, A.P, India during November, 2010. Water samples were collected in UV sterilized 1 litre water bottles from drinking water supplying tanks. The pH of the samples was measured immediately after sampling.

Physico-chemical tests

These tests have been done by using multi parameter water testing kit (WT-023 Himedia) for analysis of pH, turbidity, chloride content, fluoride content, total hardness, nitrate, iron and residual (free) chloride test.

Isolation method

The samples were collected from various places were brought to the lab and each 100ul of the water sample was placed on nutrient agar medium and incubated at 37 ˚C for overnight.

Identification Methods

A series of Morphological, Biochemical and Genetical methods were employed to identify these isolates at strain level.

Gram Staining

The gram staining, probably the most widely used technique in bacteriology is valuable because it enables to differentiate between two bacterial cultures which are morphologically indistinguishable yet of different species. The stain divides bacteria in to two large groups. Those that retain the primary stain crystal violet iodine complex (CVI) throughout the staining procedure, are termed as gram positive & those that loses the CVI complexes upon washing with alcohol but are stained with the color of counter stain (saffranin) are termed gram negative. The staining procedure can be performed on a bacterial smear prepared on glass slide. Smear is gently warmed using a spirit lamp and microscopically observed.

Biochemical Tests

Methyl Red Test

A pure culture is inoculated into 10ml buffered glucose broth incubate for 24 hrs at 37 ˚C. To 5ml incubated culture, 5 drops of methyl red indicator is added. A distinct red colour is positive & yellow is negative

Voges-Proskeur Test

A pure culture was inoculated into 10ml buffered glucose broth & incubated for 24hrs at 37 ˚C. After incubation period 0.6ml of alpha-naphthol solution & 0.2ml of KOH solution was added to 1ml of incubated broth, shaken vigorously for 10 seconds. Development of pink to crimson colour is for positive test and a copper or faint brown colour for negative test.

Citrate Test

A pure culture was inoculated into a tube of Simmons citrate agar, then streak the medium & incubated at 37 ˚C for 48 hrs. Examine agar tube for growth & colour change. A distinct blue colour in the presence of growth indicated a positive test; no colour change negative test.

H2s Production Test

A pure culture was inoculated by streaking the triple sugar iron (TSI) slant and it is incubated at 37 ˚C for 24 hrs. Black colour develop in the medium is positive for H2S Production.

Oxidase Test

Remove part of the culture from 18-24 hrs old nutrient agar slant & rub on the surface of filter paper, impregnated with 1 % aqueous solution of NNN 'N' tetra methyl –Para Phenylenediamine, a positive Dihydrochloride. A purple colour within 15-30 seconds constitutes a positive oxidase test & colourless constituted a negative oxidase test.

Fermentation of carbohydrates: (acid & Gas production)

It is not uncommon for two different bacterial cultures to be very similar in their morphological & cultural characteristics, yet show streaking difference in their ability to utilize various carbohydrates and in the end product produced from carbohydrate metabolism (fermentation). For example one culture may produce only a single acid or alcohols with or without production of gases such as CO_2, CH_4, and H_2S & H_2. This fermentation technique serves as one of the methods for identification of bacteria.

The fermentation procedure can be performed using oxidation & fermentation. The basal medium can be prepared by using substrates (Glucose, lactose, mannitol, fructose, sucrose) Beef extract 1gm, peptone 10gm, NaCl0.5 gm, phenol red 0.018g, dis. H_2O 1000ml, pH-7.

Then the test can be performed, the medium prepared in a test tube & containing small vial (Durhams tube) in an inverted position & stabilized. To this tube, 2 or 3 loopful of 18-24 hrs old broth culture was added & incubated for 24-48hrs at 37 ˚C & observed for the colour change in the medium (acid production) & gas collected in the inverted Durhams tubes (gas production) & the results can be confirmed.

Antibiotic Assay

This can be used to identify strains within a given species which is especially important in epidemiological studies. Among the variety of tests that are available, the disc method is probably simple to perform & interpret. Discs impregnated with antibiotic solution are placed on an agar plate uniformly and inoculated with an actively growing culture of the organism. The test organism grown in a lawn of confluent growth on the plate except for a clear & one around the antibiotic to which the antibiotic show no such inhibition they grow upto the very edge of the disc.

Genomic DNA Extraction

The genomic DNA from the bacterial cells was obtained by using modified method which described by sambrock *et al.,* (1989). The bacterial cells from pure culture were harvested by centrifugation (12,000rpm) for 2min, and the cell pellets mixed with 600µl of lysis buffer (10mM tris –HCl, 1mM EDTA [pH 7.5], 0.5% SDS, and 100/g/ml proteinase c) and incubated at 37 ˚C for 1h. After the addition of 100 µl 5 M NaCl, and 80µl CTAB NaCl, the samples were incubated at 65 ˚C for 10min. The samples were incubated at 65 ˚C for 10 min. the sample were cooled to room temperature, followed by extraction of the aqueous phase with an equal volume of

chloroform : isoamyl alcohol [24:11, v/v] and then with an equal volume of phenol: : chloroform : isoamylalcohol (25:24:1, v/v) which was centrifuged at 12,000rpm & 4 ˚C for 10 min. Isopropanol (0.6x) was mixed with the aqueous phase, and centrifuged at 12,000rpm and 4 ˚C for 10 min. The DNA pellets were dried under vacuum, and then dissolved in TE Buffer (10mM Tris-HCl, and 1mM EDTA [pH.7.5].

16S rRNA gene Sequencing

16S rRNA gene was amplified by using the universal primers and resulting amplicons were purified by using the DNA purification kit (Quiagen DNA purification kit) and the purified product was used for sequencing according to Lane DJ.1991. The obtained 16S rRNA gene sequence of the isolate was used for blast analysis to identify the nearest taxa [4].

RESULTS AND DISCUSSION

Four water samples were analyzed for different Physico-chemical parameter. i.e., turbidity, pH, hardness and chloride, fluoride, Nitrate and Iron concentrations. Among these Lingala water sample has shown high ion concentrations and it is rich in hardness. (Refer table: 1)

Table1
Multiparameter water test

S.No.	Water sample collected from	pH	Turbidity	Chloride test	Total hardness	Fluoride test	Nitrate test	Iron test	Residual free chloride
1	Lingala	8.0	10NTU	25PPM/2ml	685PPM	1PPM	10PPM	0.3PPM	Absent
2	Amangal	8.2	10NTU	50PPM/10ml	200PPM	1PPM	0PPM	0.3PPM	Absent
3	Ganapur	8.0	5NTU	50PPM/10ml	350PPM	1PPM	0PPM	0PPM	Absent
4	Papireddyguda	7.6	5NTU	40PPM/10ml	350PPM	0PPM	0PPM	0PPM	Absent
5	Jadcherla	7.3	5 NTU	20 PPM/10ml	225 PPM	0 PPM	0 PPM	0.2 PPM	Absent
6	Thimmapur	7.2	10 NTU	10 PPM/10ml	350 PPM	1 PPM	1 PPM	0.1 PPM	Absent
7	Kothur	7.4	5 NTU	20 PPM/10ml	225 PPM	0 PPM	0 PPM	0.2 PPM	Absent
8	Keshampet	7.6	5 NTU	20 PPM/10ml	250 PPM	1 PPM	0 PPM	0.3 PPM	Absent
9	Makthal	7.8	10 NTU	10 PPM/10ml	350 PPM	0 PPM	0 PPM	0.1 PPM	Absent
10	Balanagar	7.2	5 NTU	30 PPM/10ml	425 PPM	0 PPM	0 PPM	0.2 PPM	Absent
11	Gadwal	8.0	5 NTU	20 PPM/10ml	450 PPM	0 PPM	0 PPM	0.3 PPM	Absent
12	Nagarkurnool	7.8	10 NTU	30 PPM/10ml	350 PPM	0 PPM	0 PPM	0.1 PPM	Absent
13	Beejinapally	7.4	5 NTU	40 PPM/10ml	500 PPM	1 PPM	0 PPM	0.3 PPM	Absent

The potable water samples were collected from 13 different places of Mahabubnagar district, A.P, India. A total number of 33 bacterial pure cultures were isolated. Among these only 4 strains were showing high dominance in these sample, which were considered for further studies. Based on 16S rRNA gene sequencing results clearly indicate that these 4 strains belong to the genus of Bacillus, Enterobacter, Exiguobacterium, and Staphylococcus. (Refer table: 2 and 3)

Table: 2

Place Of Sample Collection	Strain Number	Type Of Water	Name Of Isolate	Homology	Gram's Nature
Lingala	L-I	TAP WATER	*Bacillus flexus*	99%	Gram positive
Amangal	A-II	TAP WATER	*Enterobacter cancerogenus*	98%	Gram negative
Ganapur	G-III	TAP WATER	*Staphylococcus arlettae*	99%	Gram positive
Papireddy Guda	P-IV	TAP WATER	*Exiguobacterium aestuarii*	98%	Gram positive

Table: 3

Isolate C3 Size 1506 bp NPA No AB021185.1| 99 *Bacillus flexus* strain IFO15715

CGGCTACCTTTGTTACGACTTCACCCCAATCATCTGTCCCACTTTAGGCGGCTGGCTCCATAAAGGTTACCCCACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGA
CGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCAGCCTACAATCCGAAC
TGAGAATGGTTTTATGGGATTGGCTTGACCTCGCGGTCTTGCAGCCCTTTGTACCATCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACG
TCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACA
TCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACTCTGTCCCCCGAAGGGGAACGCTCTATCTCTAGAGTTGTCAGAGGATGTCAAGACCTGGTAAGGT
TCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAA
TGCGTTAGCTGCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGC
GCCTCAGCGTCAGTTACAGACCAAAAAGCCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCGCTTTTCTCTTCTGCACT
CAAGTTCCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCT
TGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACAAGCAGTTACTCTTGTACTTGTTCTTCCCTAACAA
CAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGC
CGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTATGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGCCCATCTG
TAAGTGATAGCCGAAACCATCTTTCAATTTTCTCTTATGCAAGAAAAAATGTTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTGCCC
ACGTGTTACTCACCCGTCCGCCGCTAACGTCATAGAAGCAAGCTTCTAATCAGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCCTGAGCCAGG

Isolate G7 Size 1536 bp AB009933.1| 99 *Staphylococcus arlettae* strain ATCC 43957

TCCAGCCGCACCTTCCCGATACGGCTACCTTGTTACGACTTCACCCCAATCATTTGTCCCACCTTCGACGGCTAGCTCCATAAATGGTTACTCCA
CCGGCTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGACCCGGGAACGTATTCACCGTAGCATGCTGATCTACGATTAC
TAGCGATTCCAGCTTCATGTAGTCGAGTTGCAGACTACAATCCGAACTGAGAACAACTTTATGGGATTTGCATGACCTCGCGGTTTAGCTGCCCT
TTGTATTGTCCATTGTAGCACGTGTGTAGCCCAAATCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGT
CAACCTAGAGTGCCCAACTAAATGCTGGCAACTAAGTTTAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGA
CAACCATGCACCACCTGTCACTTTGTCCCCCGAAGGGGAAAGCTCTATCTCTAGAGTGGTCAAAGGATGTCAAGATTTGGTAAGGTTCTTCGCG
TTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGTCCCCGTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGT
GCTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTG
TTTGATCCCCACGCTTTCGCACATCAGCGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTGCGCATTTCACCGC
TACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAA
GAAACCGCCTACGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGC
TTTCTGATTATGTACCGTCAAGACGTGCACAGTTACTTACACGTTTGTTCTTCCCTAATAACAGAGTTTTACGAGCCGAAACCCTTCATCACTCAC
GCGGCGTTGCTCCGTCAGGCTTTCGCCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGG
CCGATCACCCTCTCAGGTCGGCTACGTATCGTTGCCTTGGTAAGCCATTACCTTACCAACTAGCTAATACGGCGCGGGTCCATCTATAAGTGAT
AGCAAAACCATCTTTCACTTTAGAACCATGCGGTTCTAAATGTTATCCGGCATTAGCCCCGGTTTCCCGGAGTTATTCCAGTCTTATAGGTAGGTT
ACCCACGTGTTACTCACCCGTCCGCCGCTAACGTCAAAGGAGCAAGCTCCTTATCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCG
TTCATCCTGAGCCAGGATCAAACTCT

Isolate H8 size 1505 bp AY594264.1| 98 *Exiguobacterium aestuarii* strain TF-16

GGCGGCGTGCCTAATACATGCAAGTCGAGCGCAGGAACCCGTCTGAACCCTTCGGGGGGACGACGGCGGAATGAGCGGCGGACGGGTGAGT
AACACGTAAAGAACCTGCCCATAGGTCTGGGATAACCACGAGAAATCGGGGCTAATACCGGATGTGTCATCGGACCGCATGGTCCGCTGATGA
AAGGCGCTTCGGCGTCGCCCATGGATGGCTTTGCGGTGCATTAGCTAGTTGGTGGGGTAATGGCCCACCAAGGCGACGATGCATAGCCGACC
TGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCT
GATGGAGCAACGCCGCGTGAACGATGAAGGCTTTCGGGTCGTAAAGTTCTGTTGTAAGGGAAGAACAAGTGCCGCAGGCAATGGCGGCACCTT
GACGGTACCTTGCGAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAA
AGCGCGCGCAGGCGGCCTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGCCATTGGAAACTGGGAGGCTTGAGTATAGGAGA
GAAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTTTGGCCTATAACTGACGCTG
AGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTGGAGGGTTTCCGCCCTT
CAGTGCTGAAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGGCTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGG
TGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAACTCTTGACATCCCCCTGACCGGTACAGAGATGTACCTTCCCCTTCGGGG
GCAGGGGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTG
CCAGCATTAAGTTGGGCACTCTAGGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGAGTTGG
GCTACACACGTGCTACAATGGACGGTACAAAGGGCAGCGAAGCCGCGAGGTGGAGCCAATCCCAGAAAGCCGTTCTCAGTTCGGATTGCAGG
CTGCAACTCGCCTGCATGAAGTCGGAATCGCTAGTAATCGCAGGTCAGCATACTGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGT
CACACCACGAGAGTTTGCAACACCCGAAGTCGGTGAGGTAACCGTAAGGAGCCAGCCGCCGAAGGTGGGGCAGATGATTGGGGTGAAGTCGT
AACAAGGTAGCCGTATCGGAAGGT

Isolate J10 Size 1537 bp JN644583.1 99 *Enterobacter cancerogenus* strain NB14_1A

CATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGTAACAGGAAGCAGCTTGCTGCTTCGCTGACGAGTGGCGGA
CGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGG
GGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTC
TGAGAGGATGACCAGCCACACTGAAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCT
GATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGTTGAGGTTAATAACCTCGGCGATT
GACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTA
AAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGA
GGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCT
CAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGT
GGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGG
TGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAA
CTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCA
GCGGTTAGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGG
CTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCT
GCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA
CACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAGTCGTAACA
AGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTTCTAGACCTGCAGA

There are many saprophytic species included in the Bacillus group. These bacteria are able to remain latent, which provides them adaptive characteristics to many environments[5] with mesophilic and thermophilic environments showing the greatest probability to the occurrence of the Bacillus genus [5, 6]. The genus Exiguobacterium was first proposed by Collins *et.al,* it has low G+C content and it is a gram positive, facultative anaerobe [7]. Staphylococcus is a gram positive organism, which is able to produce the enzyme coagulase (an enzyme causes blood to clot). Most species are harmless and reside normally on the skin and mucous membranes of humans. Some of them cause wound infections. Especially the species *Staphylococcus arlettae* was found to be able to degrade azodyes [8].

The genus Enterobacter is gram negative, facultative anaerobic bacteria. Several strains of these bacteria are pathogenic and cause opportunistic infections in immunocompromised host. Urinary and respiratory tract are the most common sites of infection. These bacterial species are susceptible to cephalosporin and Cefepime antibiotics.

Overall results suggest that the *Bacillus flexus* able to grow at the temperature of 27-48°C and the optimum temperature is 37 °C, *Enterobacter cancerogenus* able to grow at 27-42°C and the optimum temperature is 37 °C. *Staphylococcus arlettae* can grow best at 27-48 °C and the optimum temperature is 37 °C , *Exiguobacterium aestuarii* can grow at the temperature of 28 to 48 °C and the optimum temperature is 37 °C(refer table :4).

Table: 4
Effect of temperature

Isolate	Strain Number	4 °C	27 °C	37 °C	42 °C	48 °C	50 °C	55 °C
Bacillus flexus	L-I	-	+	+	+	+	-	-
Enterobacter cancerogenus	A-II	-	+	+	+	+	-	-
Staphylococcus arlettae	G-III	-	+	+	+	+	-	-
Exiguobacterium aestuarii	P-IV	-	+	+	+	+	-	-

All isolates were grown in the range of pH from 6-10**.** (Refer table: 5).

Table: 5
Effect of PH

Name of the isolate	Strain Number	PH-6	PH-8	PH-10	PH-11
Bacillus flexus	L-I	+	+	+	-
Enterobacter cancerogenus	A-II	+	+	+	-
Staphylococcus arlettae	G-III	+	+	+	-
Exiguobacterium aestuarii	P-IV	+	+	+	-

All strains showed tolerance to NaCl with range of 0.5% to 2.0 %. (Refer table: 6)

Table: 6
Effect of salt concentration

Name of the isolate	Strain Number	0.5%	1%	1.5%	2%
Bacillus flexus	L-I	+	+	-	-
Enterobacter cancerogenus	A-II	+	+	-	-
Staphylococcus arlettae	G-III	+	+	+	+
Exiguobacterium aestuarii	P-IV	+	+	+	+

Antibiotic assay revealed that three strains (*Bacillus flexus, Staphylococcus arlettae, Exiguobacterium aestuarii*) were shown sensitivity to most of the antibiotics and the strain *Enterobacter cancerogenus* has shown resistance to most of the antibiotics(Refer table:7).

Table: 7
Antibiotic sensitivity test

Name of the isolate	Strain number	K 10mg	S 25mg	AK 10mg	AMX 10mg	AX 10mg	FC 10mg	EX 10mg	CC 10mg	P 10mg	G 10mg	T 10mg	S 10mg	CHP 10mg
Bacillus flexus	L-I	S	S	S	S	S	S	S	S	S	S	S	S	S
Enterobacter cancerogenus	A-II	S	S	S	R	R	R	S	R	R	S	S	S	S
Staphylococcus arlettae	G-III	S	S	S	S	S	S	S	R	S	S	S	S	S
Exiguobacterium aestuarii	P-IV	S	S	S	S	S	S	S	S	S	S	S	S	S

Whereas S- Sensitive, R- Resistance.
K- kenamycin, S- streptomycin, AK- amikacin, AMX- amoxicillin, AX- ampicillin, FC- fusidic acid, EX- enroflaxacin, CC- clotrimezole, P- penicillin-g, G- gentamycin, T- tetracycline, CHP- chloramphenicol

In biochemical analysis, all these strains showed positive activity for Oxidase, Methyl red and negative for Gelatinase, Protease and Vogues Proskeur. **(Refer table: 8)**

Table 8
Bio-chemical tests

Name of the isolate	Strain Number	Gelatinase	Urease	Oxidase	Catalase	Lipase	protease	T.S.I.A	citrate	M.R	V.P	N.R. Test
Bacillus flexus	L-I	-	+	+	-	-	-	-	+	+	-	-
Enterobacter cancerogenus	A-II	-	-	+	+	+	-	-	+	+	-	-
Staphylococcus arlettae	G-III	-	-	+	+	-	-	+	+	+	-	+
Exiguobacterium aestuarii	P-IV	-	-	+	+	-	-	-	-	+	-	-

T.S.I.A → Triple Sugar Iron Agar; M.R → Methyl Red; V.P → Vogues Proskeur; N.R → Nitrate Reduction Test

CONCLUSION

Information about the bacterial contaminants and their viability in potable water is an important to take precautions to avoid water borne diseases. Study of bacterial diversity in potable water plays an important role to understand the quality of water and to identify viable pathogenic organisms. The results from this study further improve our understanding of the molecular diversity and bacterial population dynamics of potable water bacterial communities. Moreover, these results provide the sequence foundation for the development of molecular assay that target potential bacteria in potable water. The public must recognize that drinking water is not frequently monitored by health laboratories for acceptable quality.

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Competing Interests
Authors have declared that no competing interests exist.

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