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Microbiological Quality of Available Water Sources in and around Tribal Areas of Araku Valley Mandal, Visakhapatnam District, AP, India

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Abstract

In the study microbiological analysis was performed in the water sources like spring, tank well and bore water of Tribal Villages of Araku valley Mandal, Visakhapatnam, (District) Andhra Pradesh, India. Eighteen samples had been collected during the period 2014-2015 in pre and post monsoon and analysed microbiologically by MPN/100ml, Faecal coliform, HPC and faecal streptococci it was observed that 85% of water sources were not up to the mark of Bureau of Indian Standards BIS & World Health Organization (WHO) limit. The water that was collected from spring and well was mostly contaminated, Most Probable Number (MPN) count range between 39-1100MPN/100ml. The mean counts of Faecal coliform in spring, tank and well are found to be high than bore and the HPC range from 2.22×10^4 CFU to 6.02×10^4 CFU and faecal streptococci range from 0.037×10^4 CFU/100ml to 1.32×10^4 CFU /100 ml, in spring, tank, well and bore samples. Hence it is resulted, the samples of well, spring and tank were above the prescribed limit of BIS, 2006. Isolated and identified organisms were Escherichia, staphylococci, salmonella, shegeila species, vibrio species, pseudomonas species, aeromonas etc. Thus the results revealed that the frequency of water borne diseases in the area is high in post monsoon season this may be due to the water consumed. Thus the findings of the microbiological water quality suggest that the water sources have direct effect on the health conditions of the tribes. Hence the water from this area is mostly contaminated and suggested to treat properly before it consumed or to look for alternative sources for drinking.

Keywords: Water quality, Spring, Samples, Water born diseases, Source and tribes.

Introduction

Water is a crucial unique solvent prerequisite for existence, to the extent that extra-terrestrial life is sought by identifying whether a planet or heavenly body has water. Water becomes essential for human survival in various forms: direct consumption, pre-digestion (cooking) of food, agriculture, livestock farming, manufacturing and industrial processes. The National Water Policy (2002) (of India) states that water is a prime natural resource, basic human need, and a precious national asset. The consequence of urbanisation and industrializations leads to disturb water quality, for various purposes the ground and surface water is explores in rural areas especially where other sources like dam and rivers are not available[1]. Over one billion people worldwide have no access to safe drinking water[2].

The quality of water is essential to identify the major impurities and the extent of pollution in such water, so that remedial measures can be taken towards better human health and efficient industrial processes. Taking into account the limited band of pollution acceptable for human consumption, surface water and groundwater are the primary sources for human consumption, farming and certain industries, while sea water is restricted to certain industries, that too as a cooling medium and for certain types of aqua culture. Water uses with the highest demands for quantity often have the lowest demands for quality, whereas drinking water needs to be of the highest quality, but in relatively small quantities[3]. Even these small quantities aggregate to very high volumes, when factored with our burgeoning population. The spread of diarrhoeal diseases especially in infants is because of contamination of drinking water with organisms of faecal origin[4].

Unsafe water is responsible for major types of water borne diseases. It has been estimated that 30% of mortality and 50% of morbidity to infectious disease in Indians are responsible for major types of water borne diseases[5]. Water born diseases spread due to the microbiologically contaminated source is the major challenged being faced by tribal areas of Araku Valley Mandal, Diarrhoea, dysentery, worms, typhoid, jaundice are some of the transmitted diseases, the deaths due to these diseases are considerable. Excess concentration of various toxic metal ions and nutrients in drinking water many often causes serious health hazards.

In India, about 80% of the diseases are believed to be water related and the World Health Organization has reported that nearly five million human deaths occur every year through polluted drinking water[6]. There are many areas in India where more than 90% of the population depends on groundwater for drinking and other purposes[7], while the subsurface has come to

serve as the receptor for much rural, urban and industrial wastewater and for solid waste disposal. There are increasingly widespread indications of degradation in the quality and quantity of groundwater, serious or incipient, caused by excessive exploitation and / or inadequate pollution control. The scale and degree of degradation varies significantly with the susceptibility of local aquifers to exploitation-related deterioration and their vulnerability to pollution.

In an UN survey, scoring-1.31 water quality indicator values, India occupied 120th rank out of total 180 countries which was surveyed for water quality. According to official records of ministry from health and family welfare, Government of India in 11th five year plan, the state of Andhra Pradesh prevailed in the first place with 17846 Hepatitis cases, 1, 35, 550 Typhoid cases and prevailed in second place with 12,15,659 cases among the states of the nation regarding water sanitation.

Some studies have shown massive and widespread faecal contamination and the simultaneous presence of a multitude of pathogens, including coliform, and Camplylobacteria in groundwater's, suggesting that human wastes are the source of the contamination originating from wastewater facilities and septic tank effluent discharges[8]. Faecal coliform are thus a very convenient indicator of the presence of other pathogens).

In Araku valley Mandal, the tribal people face many problems for scarcity and supply of good quality water and therefore mostly the tribal population consume water from spring, open well and bore water. The microbiological quality of water from the sources have been observed to be high with coliform count which crossing the limit prescribed by WHO and BIS. According to the survey in the remote villages many people suffer with water born diseases include viral fever, typhoid, malaria, gastroenteritis and cholera.

Purpose of the study: The Araku Valley Mandal is 112 km away from Visakhapatnam District, Andhra Pradesh. The main source of drinking water in Araku valley region is open wells and Kundi's (spring water storage device). Natural springs (Oota) are the only source available for drinking water as well as utility purpose in remote villages. The remote villages have only spring water as a source for drinking and utility. The tribal population mostly drinks water without treatment under unhygienic conditions, the impact of the developmental activities on drinking water sources has not been explored, and hence there is a need to estimate the degree of microbial contamination in the water.

Aim and objectives: i. To examine the most probable number (MPN) of *coliform and E. coli* present in different drinking water sources. ii. To confirm characteristics of bacterial species by using standard microbiological methods.

Methodology

Study Area: The study area is scattered in Araku valley Mandal which is on the north-eastern part of Visakhapatnam district, Andhra Pradesh India. The Araku division consist of the hills and valleys covered by Eastern Ghats with an altitude of about 900 meters above by several peaks exceeding 1200 meters above the sea level. The Climatic condition is cool, due to green vegetation, elevation and thick forest. The temperature of this area goes down on the period of south west monsoons and the temperature reaches to a mean minimum of 4ºC by January of every year, after which there is a increase of temperature to mean maximum of 34ºC till April and May. During the month of June the monsoons begins with average rainfall of 178.1cm on every year.

Sample Collection: Spring, well, bore and tank water were collected by simple Random sampling Method from various villages of remote habitation in Araku Valley Mandal of Visakhapatnam District, Andhra Pradesh. India. The samples were collected in clean sterile plastic cans of 5 litres and stored in a ice box of below 4^{o}C shifting to the laboratory of Department of Environmental Sciences Andhra University, by following the precautions laid by standard methods of APHA[9]. Microbial examinations were performed as soon the sample carried to the laboratory.

Microbiological Analysis: The Bacteriological quality of the water samples were analysed by standard most probable method (MPN) method. The total coliform count was determined by taking necessary dilution in the individual samples. The sample was taken 10 ml in three test tubes which contain double strength lactose broth 1ml of lactose broth was taken in three test tubes and incubated at 37^{0}C for 24-48 hrs. When the gas formation was observed in the Durham tubes after incubation the most probable number (MPN) was determined for coliform number using the MPN index table (APHA, 2005). Specific media was used for the bacteriological analyses, which are Plate count agar (PCA), nutrient agar (NA), and Lactose broth (LB), Eosin Blue Agar (EMB) .For presumptive tests for coliform and total viable count, serial dilution method was followed. To determine *heterotrophic bacteria, salmonella and shigella, vibrio cholera* salmonella shigella agar, thiosulphate citrate bile sugar agar were used respectively.

Results and Discussion

In this study 18 drinking water samples collected from Araku valley Mandal of Visakhapatnam were analysed for the bacteriological quality. It was found that all microbiological quality was above the prescribed limit of BIS[10] and WHO[11-12].

Microbial groups namely i. *Heterotrophic bacteria* ii. *Total coli form* iii. *Faecal coliform* iv. *Faecal streptococci* were analysed to identify the present state of quality with environmental significance. The microbiological examination of water is a direct indicator of faecal contamination and its extent of risk to human health. Selected indicator organisms are routinely monitored to indicate the probability of pathogenic population in water.

The Most Probable Number (MPN): The Microbiological water quality was done by monitoring the presence of microbiological population mostly *faecal coliform bacteria* (FC). The results are summarized in Table-1, 2, and 3. *Coliform* are also routinely found diversified natural environments, as some of them are of telluric origin, but drinking water is not a natural environment for them, as a result their presence in drinking water must be considered as harm to human health[13]. The observed values were then compared with the standard methods for the examination of water, (APHA, 2005)[14], Bureau of Indian Standards (BIS), World Health Organization (WHO). Presence of *coliform* group of microbes as a whole is recognized as a suitable indicator for drinking water. *Total Coliform* counts in Bore waters were found maximum in the range of 15 to 75 MPN/100ml with mean of 34.87 MPN/100ml, in spring and tank water range from 93 to 1100 MPN/100ml ,with mean of 393.22 and in well water MPN ranged from 93 to 1100 MPN/100ml,with mean of 457.66 MPN/100ml.

Table-1
MPN/100ml in spring and tap water, *faecal coliform count*, HPC and *faecal Streptococci*

S.No	Name of the village	Source	MPN/100ml	*Faecal coliform count* CFU/100ml	HPC/ CFU/100ml.	*Faecal Streptococci* CFU/100
1	Karsaliguda	Spring	460	3.2×10^4	6.36×10^4	1.20×10^4
2	Old post office	Spring	240	3.56×10^4	7.82×10^4	1.23×10^4
3	Madagada	Spring	1100	3.20×10^4	7.76×10^4	1.10×10^4
4	Kinang guda	Spring	1100	3.21×10^4	7.68×10^4	1.32×10^4
5	Janamguda	Spring	93	2.68×10^4	5.68×10^4	0.46×10^4
6	Kumbaraveedi	Spring	210	2.96×10^4	5.41×10^4	0.22×10^4
7	Ravalaguda	Tank/tap	93	0.56×10^4	6.36×10^4	0.056×10^4
8	Panirangini	Tank/tap	150	1.23×10^4	4.36×10^4	0.86×10^4
	min		93	0.56×10^4	4.36×10^4	0.056×10^4
	max		1100	3.56×10^4	7.82×10^4	1.32×10^4
	mean		393.22	2.47×10^4	6.36×10^4	0.782×10^4

Table-2
MPN/100ml in bore water, *faecal coliform count*, HPC and *faecal Streptococci*

S.No	Name of the village	Source	MPN/ 100ml	*Fecal coliform count* CFU/100ml	HPC/ CFU/100ml	*Fecal Streptococci* CFU/100ml
1.	Sarbaguda	Bore	21	0.42×10^4	3.32×10^4	0.045×10^4
2.	b-coloney	Bore	15	0.21×10^4	1.52×10^4	0.021×10^4
3.	Bosubeda	Bore	20	0.54×10^4	1.42×10^4	0.06×10^4
4.	Balluguda	Bore	43	0.86×10^4	1.52×10^4	0.032×10^4
5.	Yendapallivalasa	Bore	15	0.16×10^4	1.28×10^4	0.030×10^4
6.	Padmapuram	Bore	75	0.72×10^4	3.72×10^4	0.031×10^4
	min		15	0.16×10^4	1.28×10^4	0.021×10^4
	max		75	0.86×10^4	3.72×10^4	0.06×10^4
	mean		34.87	0.491×10^4	2.22×10^4	0.037×10^4

Table-3
MPN /100ml in well water, *faecal coliform count*, HPC and *faecal Streptococci*

S.No.	Name of the village	Source	MPN/100ml	*Fecal coliform count* CFU/100ml	HPC/ CFU/100ml	*Fecal Streptococci* CFU/100ml
1.	Tangulaguda	Well	240	2.56×10^4	5.68×10^4	0.32×10^4
2.	M hattaguda	Well	1100	3.42×10^4	7.82×10^4	1.22×10^4
3.	Sunkarametta	Well	120	2.42×10^4	5.58×10^4	0.65×10^4
4	Sukurguda	Well	93	1.52×10^4	4.63×10^4	0.05×10^4
	minimim		93	1.52×10^4	4.63×10^4	0.05×10^4
	max		1100	3.42×10^4	7.82×10^4	1.22×10^4
	mean		457.66	2.47×10^4	6.02×10^4	0.585×10^4

On comparing the, bore, spring, tank and bore samples minimum of 34.87 MPN/100ml was observed in bore water sample and maximum 457.66 MPN/100ml in well water. The spring, tank and Well water are found to be more contaminated in terms of MPN index. Hence it is apparent from the results obtained that the bore water is faintly safer than the spring, tank and well water sources in the absences of alternative sources. *Coliform* in ground water might be originated from sanitation facilities located too close to the wells[15].

Heterotrophic Plate Count: This HPC will give the total bacterial count present in 100 ml of water. They form the colony forming units by counting this CFU/100ml. In the study the minimum HPC count was 1.28×10^4 CFU/100ml in Yendapallivalasa Bore water and maximum count was observed at Padmapuram bore 3.72×10^4 CFU/100ml water sample. HPC mean value in bore water was 2.22×10^4 CFU/100ml. In well water minimum was recorded at Sukuruguda well i.e. 4.63×10^4 CFU/100ml and maximum was at M.Hattaguda well, 7.82×10^4 CFU/100ml with mean of6.02×10^4 CFU/100ml. In spring and tank sample minimum of 4.36×10^4 CFU/100ml and maximum was observed at Madagada spring sample i.e.7.82×10^4 CFU/100ml.

The heterotrophic plate count of the samples in well, and spring were found to be more than the standard limit of 1.0×10^4 CFU/100 ml (EPA, 2002)**.** On comparing the results of HPC of all the sampling sources, minimum count was observed in bore sample i.e. 2.22×10^4 CFU/100ml and maximum count was observed in spring and well water. The presence of heterotrophic bacteria in drinking water is not an indication that the water presents the health risk but poses significant health risk in immune compromised individuals[16]. The total plate count (TPC) indicated that none of the samples were found in drinking water according to the WHO standards (100 CFU/ml).

Faecal Coliform: In the spring and tank water samples on EMB agar plate the *Faecal Coliform* counts ranged between l0.56×10^4 to 3.56×10^4 CFU/100ml with means of 0.491×10^4 CFU/100ml. In bore water samples *faecal coliform* ranged from 0.16×10^4 CFU/100ml to 0.86×10^4 CFU/100ml with mean of 0.491×10^4CFU/100ml and in well water it ranged from 1.52×10^4 CFU/100ml to 3.42×10^4 CFU/100ml with mean of2.47×10^4 CFU/100ml. Hence it is concluded that the load of *Faecal coliform* count in bore water is comparatively lesser than the well, tank and spring water, and found to be little safer than the other sources in (Table-1) (spring and well).

Similar studies dealing with the assessment of ground water[17-19], indicate that *faecal coliforms* are one the three parameters that can effect, the others being the nitrates and chlorides. Certain analytical procedures, such as the Colilert method, are popular to verify the count of even chlorine-injured bacteria[20], even after a lapse of four weeks. Poor sanitation, low level of hygiene, uncontrolled treatment parameters are the causes for contamination.

Faecal streptococci: *Streptococcus* is one of the indicators for faecal contamination in drinking water. In the present study the count of *Faecal Streptococci* ranged from of 0.021×10^4 to 0.06×10^4 CFU/100ml bore water with mean of 0.037×10^4 CFU/100ml and in well water count ranged from 0.05×10^4 CFU/100ml to1.22×10^4CFU/100ml with mean 0.585×10^4 CFU/100ml. In spring and tank water count ranged from 0.056×10^4 CFU/100ml to1.32×10^4 CFU/100ml with mean of 0.782×10^4 CFU/100ml. The bore water sources are found to somewhat safer, during rainy season than the well and spring sources. The presence of bushes and shrubs around water bodies makes it likely and possible that some individuals may have been coming around to drink water thereby passing out faeces into the stream water[21]. Hence the load of *faecal Streptococci* count observed to be higher in count in spring and well water

samples than the other sources may be due to the mixing of surface runoffs during rainy seasons in to the open well and springs.

Characterization and Identification of Bacterial Species: In the study area different bacterial species were identified based upon the morphological characteristics of isolates obtained from the water samples on nutrient agar(NA) and different selective medias as shown in table 05 The identified isolates include *Escherichia, staphylococci, salmonella, shigella species, Salmonella sp, vibrio species, pseudomonas species, Enterobacter aerogenes* and *Aeromonas sp.,* (Table-4 and 5). The isolated bacteria species were identified to be same with those commonly encountered in water of Logos State, Nigeria[22].

The isolated enteric bacterial species were identified to be with same with those commonly encountered in water reported in study on river sources of rural Venda South Africa[22], similar findings revealed that pathogens such as *Salmonella spp., Vibro sp*., *Gardia lamblia* and *Crytosporidium parvum* appeared occasionally in water samples[23].

Pseudomonas does not harm a healthy individual but cause problem in individual with weak immune system[24]. However, it is more reliable and safe if the drinking water does not show the presence of *Pseudomonas* spp, suggested that the source of the presence of *Pseudomonas* spp in the water is due to contamination by human themselves[25].

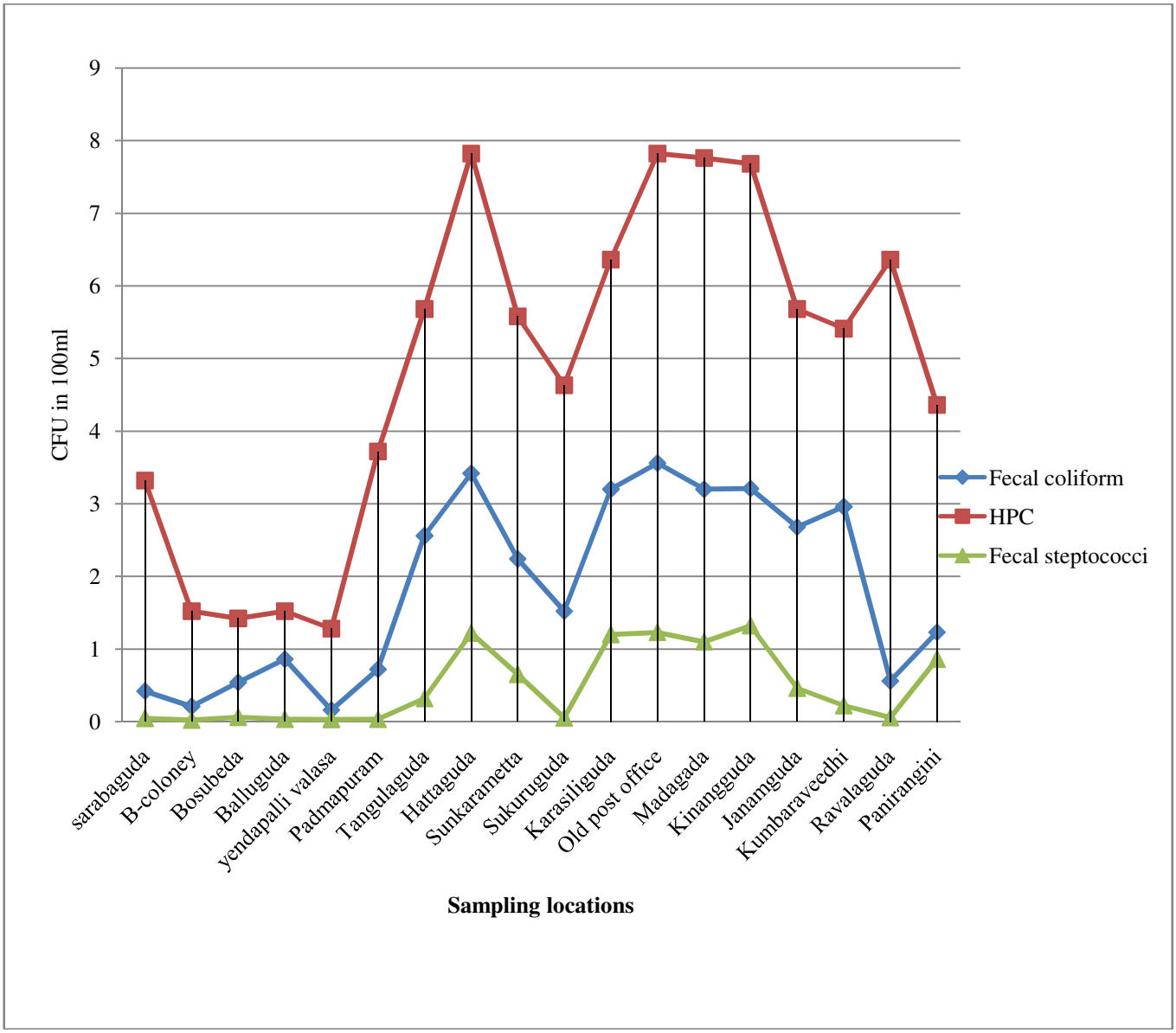


Figure-1
***HPC, Faecal streptococci and Faecal Coliform* count in sampling location**

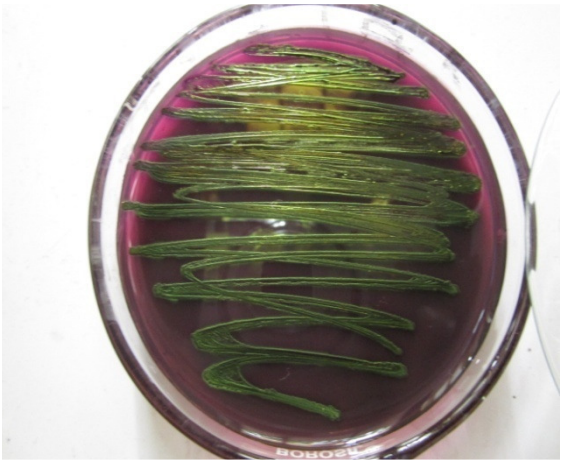
Table-4
Morphological characteristics of micro-organisms

Isolate	Morphological Characteristics	Organism
C1	Non- spore forming and non- motile, gram positive cocci, circular, low convex with entire margin, smooth, medium, opaque colony on Nutrient Agar, Yellow colure colonies on Mannitol Salt Agra Media grown at pH 7 and 37^0 C	*Staphylococcus sp*.
C2	Gram positive cocci, thin, even, growth on Nutrient Agar, black or brown colure colonies on Bileesilin Agar.	*Group D Streptococcus,*
C3	Gram positive rod, spore forming, abundant, opaque, white waxy growth on Nutrient Agar.	*Bacillus Sp.*
C4	Gram negative rod, circular, low convex, with entire margin, mucoid, opaque, growth on Nutrient Agar, green metallic sheen colony on Eosin Methylene Blue (EMB) Agar.	*E. coil*
C5	Gram negative rod, thin, blue gray, spreading growth on Nutrient Agar.	*Proteus Sp.,*
C6	Gram negative curved rod, abundant, thick, mucous white colure colonies on Nutrient Agar. Yellow colure colonies on TCBS agar	*Vibrio cholera*
C7	Gram negative curved rod abundant, thick, mucous white colure colonies on Nutrient Agar. Green colure colonies on TCBS agar	*Vibrio .parahaemolytics*
C8	Gram negative rod, thin even greyish growth on Nutrient Agar	*Salmonella sp*
C9	Gram negative rod, thin even greyish growth on Nutrient Agar	*Shigella*
C10	Gram negative rod, abundant thick, white glistening growth on Nutrient Agar	*Enterobacter aerogenes*
C11	Gram negative, non spore forming rod shaped, facultative anaerobic bacteria. Thick, mucous white colure colonies on Nutrient Agar. Light yellow to light to tan homogenous free flowing powder on Starch Ampicillin Agar	*Aeromas sp.,*

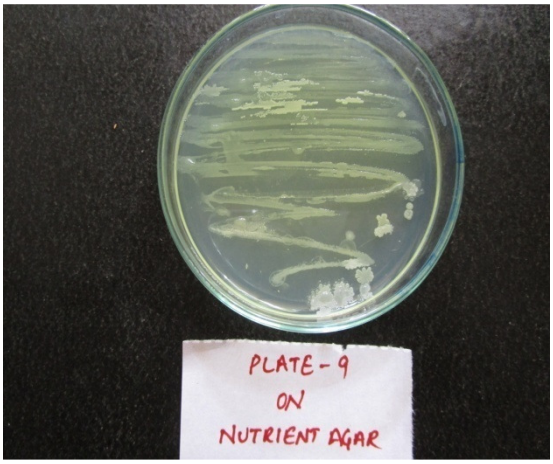
Table-5
Biochemical Characteristics of isolates
C1-Staphylococcus, C2-Streptococcus, C3- Bacillus Sp., C4- E. Coil, C5-, Proteus Sp.,C6- Vibrio cholera sp., C7- Vibrio parahemolytic., C8- Salmonella sp., C9- Shigella, C10- Enterobacter aerogenes, C11- Aeromas sp.,

Test	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
Catalase	P	n	P	P	P	P	P	P	N	n	P
Oxidase	N	N	N	n	N	P	N	N	N	N	P
Motility	N	N	P	P	N	P	N	N	P	N	P
Indole	N	N	N	P	P	P	N	P	N	N	P
MethylNred	N	P	N	P	P	N	P	P	(+)	N	-/+
VogeNProskauer	P	N	P	N	N	P	N	N	P	N	P
Citrate UtilizatioN	N	N	N	N	N	P	P	N	P	P	P
Urease	P	N	N	N	P	N	N	N	P	P	P
HydrogeN sulphide	N	N	P	N	P	A	P	N	N	N	N
Starch hydrolysis	N	N	P	N	N	N	N	N	N	N	P
Nitrate UtilizatioN	N	N	P	P	P	P	P	P	P	+-	N
GelatiN liquificatioN	N	N	P	N	P	P	N	N	(+)	P	N
Lactose fermeNtatioN	N	A	N	AG	N	AG	N	N	AG	N	AG
Glucose fermeNtatioN	A	A	A	AG	AG	AG	AG	A	AG	N	AG
Sucrose fermeNtatioN	A	A	A	A(+)	AG+-	AG	AG	A+-	N	N	AG

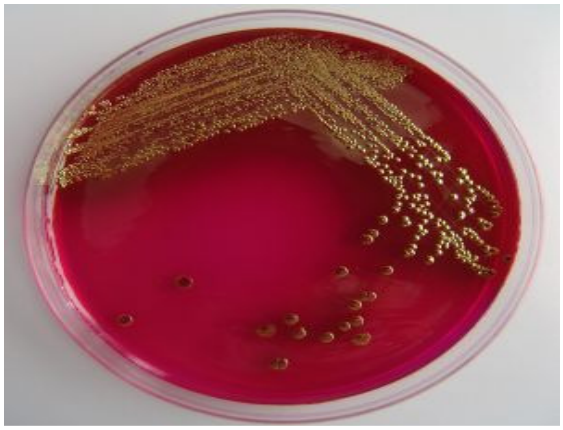
A- Acid production only; AG - Acid and gas production; ± =Variable reaction; P – Positive ; N = Negative ; (+) – Late Positive



Green Metallic Sheen on EMB Agar (*E.coli)*



Heterotrophic Plate Count on Nutrient Agar



***E.coli* (Dark Pink) on Endo agar plate**

Brown colonies on Bileesulin Agar *(Fecal Streptococci,)*

Positive Tube (Yellow in colure Acid/gas),

Negative (red in colure No gas/acid)

Figure-2
Images of microbiological results: PLATE-1

Conclusion

Microbiological analysis through MPN index count in 100 ml of sample resulted that the 85% of the water samples from various sources, exceed the standards laid by Bureau of Indian Standards (BIS) and world Health Organisation (WHO)[11-26]. The spring and the well samples were highly tainted, and the Most Probable Number (MPN) index range between 393.22-1100MPN/100ml. The mean counts of *Faecal coliform* in spring, tank and well are found to be higher than bore and the HPC range from 2.22×10^4 CFU to 6.02×10^4 CFU/100ml and *faecal streptococci* range from 0.037×10^4 CFU/100ml to 1.32×10^4 CFU /100 ml, in spring, tank, and well and bore samples. Hence it is concluded, that the samples of well, spring and tank were exceeding the standard limit (BIS, 2010)[10]. From this study it is evident that, the concentrations of faecal coliform, HPC(heterotrophic plate count) in spring and well water found higher and observed to be unfit for human consumption unless and until it is treated. Hence the bore water

is preferred for drinking for the local tribal community than the spring and well water, in the absence of other alternative sources. Water protection and good hygiene practices can improve the quality of household drinking water where disinfection is not available. Awareness programmes must be conducted by the government to educate the tribes on hygienic and sanitation of water resources.

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