Godavari Puskaralu Dip is a Mass Immunization in Disguise

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***Abstract*— The present study was conducted to find the scientific microbial reason behind the Godavari Maha puskaralu dip as a mass immunization in disguise. The study was concentrated on the biological examination of water from three main ghats constructed on the eve of Mahapuskaralu on the bank of Rajamundry, Andhra Pradesh, India. There are broad variety of bacteria isolated from the water samples collected form three Ghats namely the entry Ghat-1(Koti lingalu Ghat), the Middle Ghat-7 (Puskara Ghat) and the Exist Ghat-15 (Ramalayam Ghat). Different spieces were identified in each Ghat.**

***Index Terms*— Godavari maha puskaralu, mass immunization, Ghats and bacteria**

I. INTRODUCTION

Godavari originates at Triumbakam, Nasik district of Maharastra State and flows through southern state of Andhra Pradesh and reaches the Bay of Bengal. Andhra pradesh state has many temple towns on the banks of river Godavari. Godavari has other name called Gowthami.

Figure 1. Course of the Godavari River through the South Indian Peninsula

Pushkarudu is the name after the name of pushkar god, who makes the river holy. The initial twelve days of Godavari pushkaram is called **Aadhi pushkaram** and the final twelve days of Godavari pushkaram is called **Anthya pushkaram**. These twenty four days are holy to devotees, as god travels during these days. "Maha" means "great" and Pushkaram refers to the worship of the river in which the god Pushkar appears every 12 years. The 2015 Godavari Pushkaram is believed to be a *Maha Pushkaram*, which takes place once in 144 years. During the year 2015, Jupiter enters Leo on **14 July 2015 at 6.26 AM**, when the Maha Pushkaram begins, and ends after 12 days on **25 July 2015**. The Governments of Andhra Pradesh have made all arrangements for this event by constructing Ghats for bathing.

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Many people all over India take a holy dip in Gangetic river. The piligrims becames a source of contamination (Semwal and Akolkar, 2006). Kishore and Hanumantrao, 2010 explored the physic chemical characteristics of drinking water sources of Tipparthy revenue sub-division, Nalgonda District, Andhra Pradesh, India and observed that chemical constituent was beyond the permissible limits. Mass Bathing, an old age ritual in India is one of the main causes for increasing pollution of the river. Mass bath deteriorates water quality. This may lead to several communicable diseases (Shivi Bhasin *et al.,* 2015).

Mass Bathing, an old age ritual in India is one of the main cause for increasing organic pollution of the river. High amount of organic matter is flooded in the river in form of soaps, detergents and washing of cloths. Out of the total domestic tourist, religious and pilgrimage tourist contribute 13.8% (MOI/NCAER, 2002). Several studies have been conducted on the impact of mass bathing on different water bodies in India (Sinha *et al.* 1991; Lal 1996; Dhote *et.al.* 2001; Chandra and Prasad 2005 and Kulshreshtra & Sharma, 2006).

The Achencovil River is in Kerala, India, formed towards the southern tip of the peninsula from the streams of the Rishimala River, Pasukidamettu River, and the Ramakkalteri river of Western Ghats. This river enriches the Pathanamthitta district of Kerala state. It joins with the Pamba River at Veeyapuram, in the Alappuzha district of Kerala in South India. This is a small river not more than 130 kilometers. Sabarimala is the largest annual pilgrimage in India with an estimated 45–50 million devotees visiting every year. The present investigation involves the analysis of water quality in relation to microbiological parameters of mid-stream of Achencovil river during Sabarimala Pilgrimage season and off seasons. During Sabarimala pilgrimage season, devotees come to Pandalam in large numbers to worship the deity of Valiyakoikkal Temple near the Pandalam Palace. Pilgrims use the water of Achencovil River for various sanitary purposes. The water quality is also disturbed by various other anthropogenic activities by the population living near the river. (Prakasan & Joseph, 2000).

River is being polluted by indiscriminate disposal of sewage, industrial waste and plethora of human activities, which affects its and microbiological quality (Koshy & Nayar, 1999). The River Godavari is the main source of water supply for Nashik city. Besides this it is used for industrial and domestic waste disposal. Beyond urban area, agricultural activities are carried out at a very large scale on both the banks of river Godavari. The growing problem of human activities on river bank has made it important to monitor water quality of rivers to evaluate their state of pollution. Being a holy river most of the religious activities are performed on the bank of river Godavari and that too at Ramkund. People from all the parts of country come to Ramkund for various

religious purposes and most of them take a holy dip in Ramkund. Since it is a part of river Godavari and most of the people take bath in Ramkund the water quality at this location is analyzed.

Cientific Reasons

1) The enhanced Electro magnetic field of earth due to sun spot cycle or solar magnetic activity cycle at the time pushkar magnetizes water in the river and enhances healing properties in water. It is observed that entire body system raised to condition where it is equal to the effect of meditation.

2) Water will acquire magnetic power from earth and stored until early hours of sun rise. When ever a person perform bathing ritual at that time will be relieved from several health disorders. (Roshen Dalal, 2014 and Shrikala Warrier, 2014). The aim of the present study is not to draw a picture of horror and discourage religious activities but the study will provide a base line data on microbiological aspects to maintain integrity and secrecy of the river in order to control water-born diseases. The present study is to find the scientific microbial reason behind the Godavari puskaralu dip as a mass immunization in disguise.

II. MATERIALS AND METHODS

STUDY AREA

The present study area was done along river Godavari from Ghat-1 to Ghat-15 covering 5 km stream in Rajamundry, Andhra Pradesh, India during Maha pushkar event

Figure 2: a. The Ghats of Rajahmundry,Andhra Pradesh on the eve of Maha pushkaram; b. Entry Ghat-1(Koti lingalu Ghat); c. Middle Ghat-7 (TTD or VIP Ghat) and d. The exist Ghat-15 (Ramalayam Ghat).

There are fifteen Ghats constructed on the bank of Godavari are shown in figure 2a. The Ghats include: 1.**Koti lingalu Ghat;** 2.Chithalamma Ghat; 3.Lakshmi Gana pathi; 4.Kanaka Durga Ghat; 5.Krishna Ghat ;6.Puskara Ghat;7.**TTD or VIP Ghat** ;8.shradha nandh Ghat; 9.Padmavathi Ghat;10.Vigram Ghat;11.Sarswathi Ghat;12.Viswaswara Ghat;13. Gauthami; 14.Sidhaswara Ghat and **15.Ramalayam Ghat**. The samples were collected from three ghats. Three ghats include the entry Ghat-1(Koti lingalu Ghat) shown in figure 2b, middle Ghat-7 (TTD or VIP Ghat) shown in figure 2c and the exist Ghat-15 (Ramalayam Ghat) shown in figure 2d.

WATER SAMPLE COLLECTION

Five liters of three water samples were collected in sterile bottles at the entry, middle and exit Ghats on 22nd of July, 2015. The samples collected from each Ghat in sampling bottles as per (standard method, 1998). The collected water samples were brought to the laboratory within 5hours to the laboratory and stored in the refrigerator at 4°C for Biological Examination of Water. All the collected water samples were immediately examined for Microbiological properties. All water quality parameters estimated by the standard methods given by APHA (1998).

Identification of isolates

The bacterial isolates were characterized by their cultural, morphological and biochemical characters by adopting standard techniques.

Colony characterization of the isolates grown on Nutrient Agar.

The size, pigmentation, form, margin, elevation and optical characteristics were assessed.

Morphological characterization

The shape and arrangement nature of the bacteria was studied by Gram staining. Light microscope was used to study the morphology of the bacteria.

Biochemical characterization

The physiological and biochemical products produced by isolated bacteria were identified by culturing in differential and selective media – Blood Agar, Chocolate Agar, Starch Agar, Lipid Agar, Milk Agar, Eosin Methylene Blue Agar, Cetrimide Agar, Bile Esculin Agar, Simmons Citrate Agar, Mannitol Salt Agar, Tryptone Glucose Beef Extract Agar, Levin Eosin Methylene Blue Agar, Triple Sugar Iron Agar, CLED Agar, Saline Nutrient Agar, Bennet's Agar, Pikova's Medium, Hugh Leifson Medium, Soya bean Casein Digest Agar, Indole Nitrate Medium, Anaerobic Agar, Kenknight & Munaier's Medium, Garrod Actinomyces Medium, Yeast Mannitol Agar, Czapek Dox Agar and Sabourand Agar. (all media were procured from Himedia,India).The series of tests demonstrated include: Indole test, Methyl red test, Vogusproskeur, Citrate test, Nitrate reduction test, Hydrogen sulphide test, Urease test, Gelatin liquification test, TSI test and Catalase test. The most common and useful staining procedure is the gram stain which separates bacteria into 2groups according to the composition of their cell walls and was done as described by (William *et al*,2001). A film was made on a clean slide by emulsifying part of a colony in loopful of distilled water. The film was then air dried and fixed by slight.

III. RESULTS AND DISCUSSION

There are broad variety of bacteria isolated from the water samples collected form three Ghats namely the entry Ghat-1(Koti lingalu Ghat), the Middle Ghat-7 (Puskara Ghat) and the exist Ghat-15(Ramalayam Ghat). The isolated bacteria are coded with respective to the selected media used for their screening. Water quality assessment of Godavari river was studied by Chavan et al., (2009), who reported the bad and medium water quality in the studied stretch of the river. Water quality assessment of Ninglad stream using benthic micro-invertebrates was studied by Sharma et al., (2008). Water quality profile of Kosi river in Uttrakhand has

been studied by Sharma *et al.,* 2009. Impact on industrial activities of an industrial area of Bangalore city was studied by Charmaine and Anitha (2010), who found that most of the water quality parameters were beyond the desired limits prescribed by BIS, thereby declaring water unsafe for human consumption. Water quality assessment of Chenab river during low flow season was studied by Bhatti and Latif (2011).

THE ENTRY GHAT-1(KOTI LINGALU GHAT)

Table1. Ghat-1(Koti lingalu Ghat)

S.No.	Code	Extension of code
1	HLM-1	Hugh Leifson Medium-1
2	BB-1	Bennet's Broth-1
3	SFB-1	Spore Forming Bacteria-1
4	SNA-1	Saline Nutrient Agar-1
5	SCDA-1	Soyabean Casein Digest Agar-1
7	INM-1	Indole Nitrate Medium-1
8	TSIA-1	Triple Sugar Iron Agar-1
9	TGB-1	Tryptophan Glucose Beef Extract Agar-1
10	Cho-A-1	Chocolate Agar-1
11	BA-Alfa-1	Blood Agar- Alfa-1

The bacteria isolated from the entry ghat -1 were coded with respective their selective media used for isolation. The coding of each isolate was shown in table1. Eleven isolated isolates include Hugh Leifson Medium-1, Bennet's Broth-1, Spore Forming Bacteria-1, Saline Nutrient Agar-1, Soyabean Casein Digest Agar-1, Indole Nitrate Medium-1, Triple Sugar Iron Agar-1, Tryptophan Glucose Beef Extract Agar-1, Chocolate Agar-1 and Blood Agar- Alfa-1.

Table 2. Colony Characterization of the isolates grown on Nutrient Agar Ghat-1

S.No.	Isolate code	Size	Pigmentation	form	Margin	Elevation	Consistency	Optical Characterizations	Nutrient Broth	Form on Slant
1	HLM-1	Small	Milky white	Circular	Entire	flat	Mucoid	Opaque	Uniform fine turbidity and sediment	Echinulate
2	BB-1	Small	Milky white	Irregular	Undualate	Flat	Mucoid	Translucent	Pellicle and Sediment	Effuse
3	SFB-1	Small	Milky white	Irregular	Lobate	Flat	Mucoid	Opaque	Uniform, Fine Turbid and Sediment	Echinulate
4	SNA-1	Small	Gray	Circular	Entire	Flat	Mucoid	Opaque	Pellicle and sediment	Echinulate
5	SCDA-1	Small	Cream	Circular	Entire	Flat	Butyrous	Opaque	Pellicle and sediment	Effuse
6	INM-1	Small	Milky white	Circular	Entire	Flat	Stringy	Translucent	Pellicle and sediment	Beaded
7	TSIA-1	Small	Milky white	Irregular	Lobate	Flat	Mucoid	Opaque	Pellicle, sediment and Uniform fine turbidity	Effuse
8	TGB-1	Pin point	Light Yellow	Irregular	Lobate, Undulate	Raised	Mucoid	Translucent	Uniform, Turbid and Sediment	Filiform
9	Cho-A-1	Small	Light Yellow	circular	Entire	Raised	Mucoid	Opaque	Uniform, fine turbidity and sediment	Echinulate
10	BA-Alfa-1	Pin point	Cream	Irregular	Lobate	Convex	Butyrous	Translus cent	Uniform fine turbidity and sediment	Echinulate
11	EMB-1	Small	cream	circular	Entire	Convex	Mucoid	Opaque	Uniform fine turbidity and small sediment	Echinulate

Colony characterization of the isolates grown on Nutrient Agar

Size, pigmentation, form, margin, elevation, consistency, optical characterization, nutrient broth and form on slant are the colony characteristics studied for the isolates grown on nutrient agar was shown in table 2. The size ranges from pin point to small. The pigmentation was light yellow for isolated TGB-1 and Cho-A-1rest of them were milky to cream in color. The form was circular to irregular. The Margin was entire, lobate and undulate. The elevation observed as flat, raised and convex. The consistency was mucoid for all but INM-1 and BA-Alfa-1 showed stringy and butyrous respectively. The optical characterization was opaque, translucent and transparent. The nutrient broth showed uniform, fine turbidity and sediment. The form on slant was shown as echinulate, effuse, beaded and filiform.

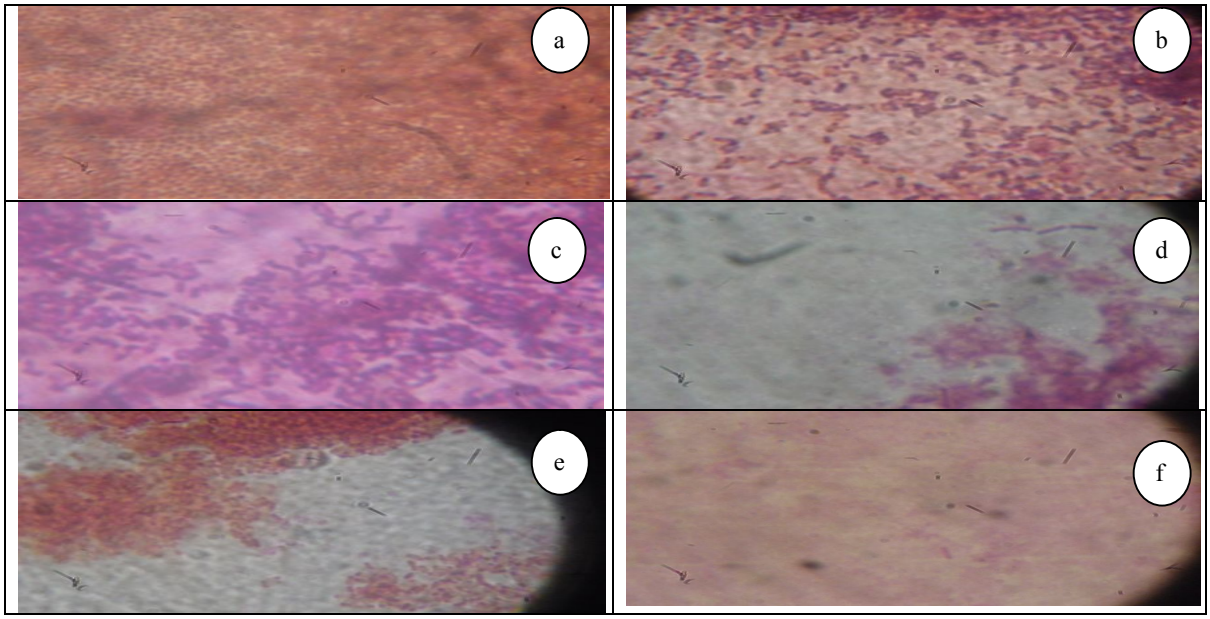

Figure 3: a. Hugh Leifson Medium-1 (HLM-1); b. Bennet's Broth-1 (BB-1); Spore Forming Bacteria-1 (SFB-1); d. Saline Nutrient Agar-1 (SNA-1); e. Soyabean Casein Digest Agar-1 (SCDA-1) and f. Indole Nitrate Medium-1 (INM-1).

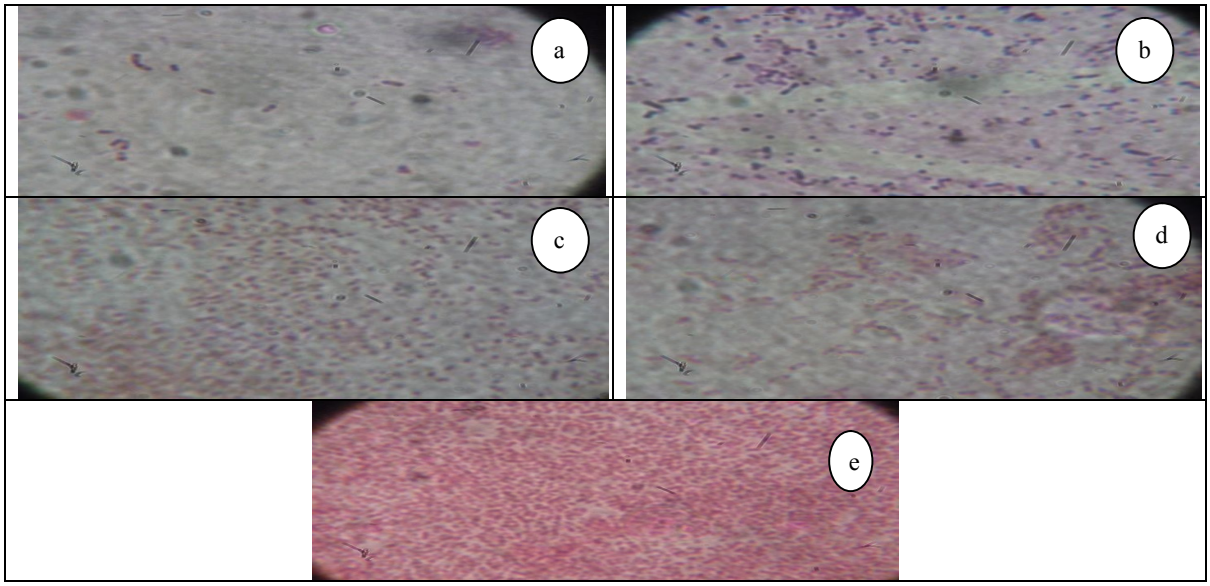

Figure 4: a. Triple Sugar Iron Agar-1 (TSIA-1); b Tryptophan Glucose Beef Extract Agar-1 (TGB-1); c. Chocolate Agar-1 (Cho-A-1); d. Blood Agar- Alfa-1 (BA-Alfa-1) and e. Eosin Methylene Blue Agar (EMB-1).

Table3. Morphological characterization of isolates isolated from Ghat-1

S.No.	Isolate code	Shape	Arrangement	Gram staining	Spore staining	Identification at spieces level
1	HLM-1	Cocci	cocci	Gram Positive	Non sporulating	Enterococcus spp.
2	BB-1	Bacillus	Bacillus	Gram Positive	Biterminal spores	Nocardia Spp.
3	SFB-1	Bacillus	Bacillus	Gram Positive	Non sporulating	Clostridium spp.
4	SNA-1	Bacillus	Bacillus	Gram Positive	Non sporulating	Actinomyces spp.
5	SCDA-1	Bacillus	Bacillus	Gram Positive	Bipolar spores	Bacillus spp.
6	INM-1	Bacillus	Bacillus	Gram Negative	Non sporulating	Enterobacteriacae spp.
7	TSIA-1	Bacillus	Bacillus	Gram Positive	Biterminal spores	Bacillus spp.
8	TGB-1	Cocci	Cocci	Gram Positive	Non sporulating	Streptococcus spp.
9	Cho-A-1	Bacillus	Bacillus	Gram Positive	Non sporulating	Eubacterium spp.
10	BA-Alfa-1	Bacillus	Bacillus	Gram Negative	Terminal and biterminal spores	Bacillus spp.
11	EMB-1	Bacillus	Bacillus	Gram Negative	Non sporulating	Enterobacteriacae

Morphological characterization of isolates

The shape and the arrangement of all the isolates were bacilli except for HLM-1 and TGB-1. All isolates were Gram positive except for INM-1, BA-Alfa-1and EMB-1. BB-1, SCDA-1, TSIA-1 and BA-Alfa-1 were sporulating isolates. The isolates were coded as HLM-1, BB-1, SFB-1, SNA-1, SCDA-1, INM-1, TSIA-1, TGB-1, Cho-A-1, BA-Alfa-1, EMB-1were identification at spieces level Enterococcus spp., Nocardia Spp., Clostridium spp., Actinomyces spp., Bacillus spp., Enterobacteriacae spp.,

Bacillus spp., Streptococcus spp., Eubacterium spp., Bacillus spp. and Enterobacteriacae spp., respectively basing on the selective media used for isolation. The morphological characterization of the isolates were shown in Figure 3[a. Hugh Leifson Medium-1 (HLM-1); b. Bennet's Broth-1 (BB-1); Spore Forming Bacteria-1 (SFB-1); d. Saline Nutrient Agar-1 (SNA-1); e. Soyabean Casein Digest Agar-1 (SCDA-1) and f. Indole Nitrate Medium-1 (INM-1)] and in figure 4 [a. Triple Sugar Iron Agar-1 (TSIA-1); b Tryptophan Glucose Beef Extract Agar-1 (TGB-1); c. Chocolate Agar-1 (Cho-A-1); d. Blood Agar- Alfa-1 (BA-Alfa-1) and e. Eosin Methylene Blue Agar (EMB-1)].

Table 4. Biochemical characterization of Isolates isolated from Ghat-1

S.No.	Isolate code	Indole Test	Methyl red Test	Vogusproskeur	Citrate Test	Nitrate Reduction Test	Hydrogen sulphide Test	Urease Test	Gelatin Liquification Test	TSI Test	Catalase Test
1	HLM-1	-ve	Positive	-ve	Positive	-ve	-ve	-ve	-ve	Red/Yellow	-ve
2	BB-1	-ve	-ve	Positive	Positive	-ve	-ve	-ve	-ve	Red/Yellow	-ve
3	SFB-1	-ve	-ve	Positive	Positive	-ve	-ve	-ve	-ve	Red/Yellow	-ve
4	SNA-1	-ve	-ve	Positive	Positive	-ve	-ve	-ve	-ve	Red	-ve
5	SCDA-1	-ve	-ve	Positive	Positive	-ve	-ve	Positive	-ve	Red/Yellow	-ve
6	INM-1	-ve	-ve	Positive	-ve	-ve	-ve	-ve	-ve	Red/Yellow	-ve
7	TSIA-1	-ve	-ve	Positive	-ve	-ve	-ve	-ve	-ve	Yellow	-ve
8	TGB-1	-ve	Positive	-ve	Positive	-ve	-ve	-ve	-ve	Yellow	-ve
9	Cho-A-1	-ve	Positive	-ve	Positive	-ve	-ve	-ve	-ve	Red/Yellow	-ve
10	BA-Alfa-1	Positive	Positive	-ve	Positive	-ve	Positive	-ve	-ve	Red/Yellow	Positive
11	EMB-1	-ve	Positive	-ve	Positive	-ve	-ve	-ve	-ve	Red/Yellow	-ve

Biochemical characterization of Isolates

Indole test was negative for all isolates except for BA-Alfa-1. Methyl red test was positive for HLM-1, Cho-A-1, BA-Alfa-1 and EMB-1. Vogusproskeur was positive for BB-1, SFB-1, SNA-1, SCDA-1, INM-1 and TSIA-1. Citrate test was negative for INM-1 and TSIA-1 but rest of the isolates were positive. Nitrate Reduction test was negative for all isolates. BA-Alfa-1isolate was the only positive for hydrogen sulphide test. SCDA-1was the only positive for urease test. All the isolates isolated from Ghat-1 showed negative for gelatin liquefaction test. All isolates produced acid in triple sugar iron test but SNA-1produced alkali. BA-Alfa-1 was the only positive for catalase test. The detail of the biochemical characterization of isolates is depicted in table 4.

THE Middle Ghat-7 (TTD/VIP GHAT)

Table 5. Middle Ghat-7(TTD/VIP Ghat)

S.No.	Code	Extension of the code
1	LFA-2	Lipid Agar
2	SFB-2	Spore Forming Bacteria-2
3	SNA-2	Saline Nutrient Agar-2
4	SCDA-2	Soyabean Casein Digest Agar-2
5	TSIA-2	Triple Sugar Iron Agar-2
6	Cho-A-2	Chocolate Agar-2
7	KMM-2	Kenknight & Munaier's Medium-2
8	BA-Beta-2	Blood Agar- Beta-2
9	SCA-2	Simmons Citrate Agar
10	Pk-2	Pikova's Medium
11	KMM-1/2	Kenknight & Munaier's Medium-1/2
12	GAM-1/2	Garrod Actinomyces Medium-1/2

The bacteria isolated from the middle ghat-2 were coded with respective their selective media used for isolation. The coding of each isolate was shown in table 5. Twelve isolated isolates includes Lipid Agar, Spore Forming Bacteria-2, Saline Nutrient Agar-2, Soyabean Casein Digest Agar-2, Triple Sugar Iron Agar-2, Chocolate Agar-2, Kenknight & Munaier's Medium-2, Blood Agar- Beta-2, Simmons Citrate Agar, Pikova's Medium, Kenknight & Munaier's Medium-1/2 and Garrod Actinomyces Medium-1/2.

Table 6. Colony Characterization of isolates grown on Nutrient Agar Ghat-7

S. No.	Isolate code	Size	Pigmentation	form	Margin	Elevation	Consistency	Optical Characterizations	Nutrient Broth	Form on Slant
1	LFA-2	Pinpoint	Gray	Circular	Entire	Raised	Mucoid	Translucent	Pellicle and uniform turbidity	Effuse
2	SFB-2	Small	Milky white	Irregular	Undulate	Flat	Mucoid	Translucent	Uniform, Fine Turbid and Sediment	Effuse
3	SNA-2	Pinpoint	Light yellow	Irregular	Lobate	Raised	Mucoid	Opaque	Sediment	Filiform
4	SCDA-2	Small	Cream	Irregular	Lobate	Flat	Mucoid	Opaque	Pellicle and Sediment	Effuse
5	TSIA-2	Small	Light Yellow	Irregular	Serrate	Raised	Butyrous	Translucent	Uniform fine turbidity	Filiform
6	Cho-A-2	Pinpoint	cream	circular	Entire	Convex	Mucoid	Translucent	Pellicle, Uniform turbidity and small sediment	Echinulate
7	KMM-2	Pin point	Light Yellow	Irregular	Lobate	Flat	Mucoid	Opaque	Flocculent sediment	Effuse
8	BA-Beta-2	Moderate	Light yellow	Irregular	Lobate	Flat	Mucoid	Opaque	Uniform fine turbidity	Effuse
9	SCA-2	Small	cream	Irregular	Lobate	Convex	Butyrous	Translucent	Uniform fine turbidity	Beaded
10	PK-2	Moderate	Cream	Irregular	Undulate	Flat	Butyrous	Translucent	Pellicle and small sediment	Effuse
11	KMM-1/2	Small	Cream	Irregular	Lobate	flat	Butyrous	Opaque	Pellicle, small sediment and uniform fine turbidity	Echinulate
12	GAM-1/2	Small	Light Yellow	Irregular	Lobate	Flat	Mucoid	Opaque	Flocculent, uniform fine turbidity and small sediment	Effuse

Colony characterization of the isolates grown on Nutrient Agar

Size, pigmentation, form, margin, elevation, consistency, optical characterization, nutrient broth and form on slant are the colony characteristics studied for the isolates grown on nutrient agar was shown in table 6. The size ranges from pin point to moderate. The pigmentation was light yellow for isolated SNA-2, TSIA-2, KMM-2, BA-Beta-2 and GAM-1/2 rest of them were milky to cream in color. The form was circular to irregular. The Margin was entire, lobate, undulate and serrate. The elevation observed as flat, raised and convex. The consistency was mucoid and butyrous. The optical characterization was opaque and translucent. The nutrient broth showed uniform, fine turbidity and sediment. The form on slant was shown as echinulate, effuse, beaded and filiform.

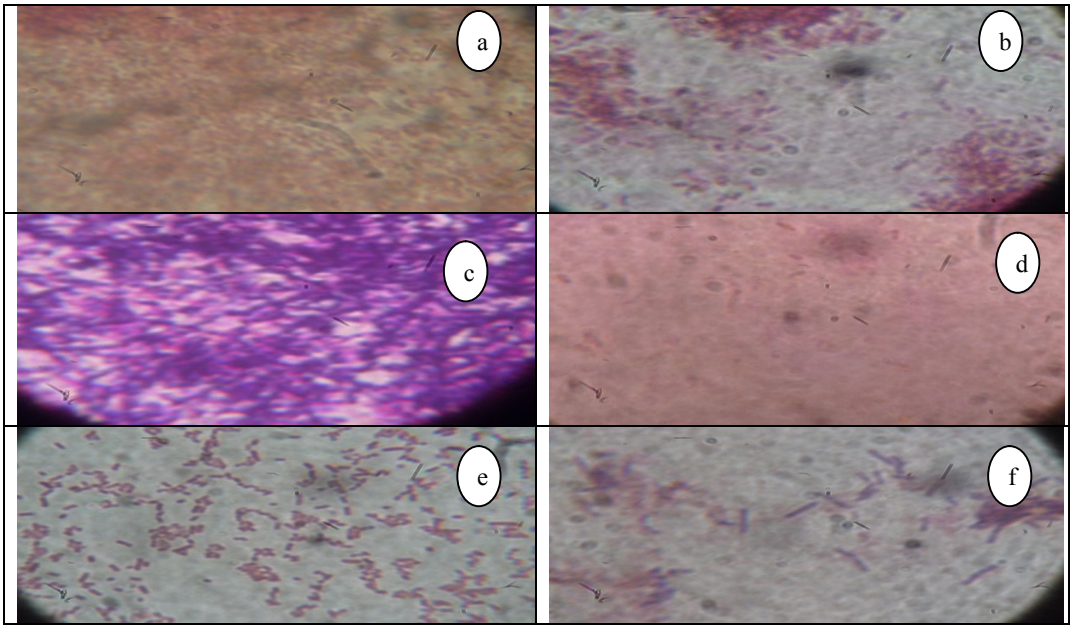

Figure 5: a. Lipid Agar (LA-2); b. Spore Forming Bacteria-2 (SFB-2); c. Saline Nutrient Agar-2 (SNA-2); d. Soyabean Casein Digest Agar-2(SCDA-2); e. Blood Agar- Beta-2 (BA-Beta-2) and f. Triple Sugar Iron Agar-2 (TSIA-2).

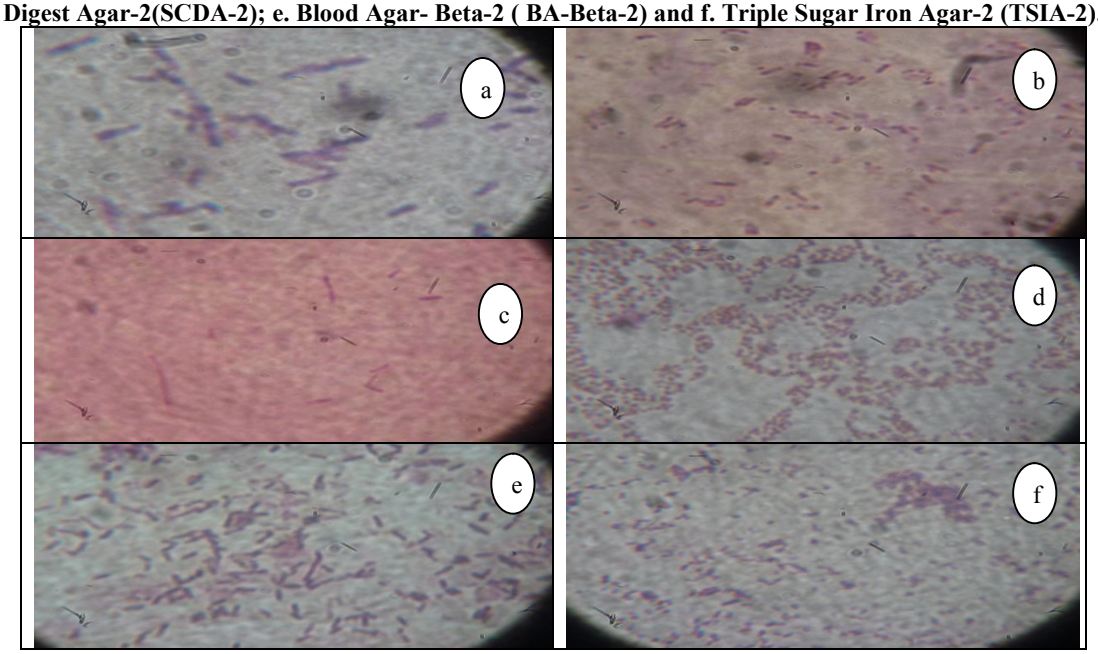

Figure 6: a. Chocolate Agar-2 (Cho-A-2); b. Pikova's Medium (PK-2); c. Simmons Citrate Agar (SCA-2); d. Garrod Actinomyces Medium-1/2 (GAM-1/2); e. Kenknight & Munaier's Medium-1/2 (KMM-1/2) and f. Kenknight & Munaier's Medium-2 (KMM-2).

Table 7. Morphological characterization of isolates isolated from Ghat-7

S.No.	Isolate code	Shape	Arrangement	Gram staining	Spore staining	Identification at spieces level
1	LFA-2	Cocci	cocci	Gram Positive	Non sporulating	Enterobacteraceae spp.
2	SFB-2	Bacillus	Bacillus	Gram Negative	Terminal and Biterminal spores	Clostridium spp.
3	SNA-2	Bacillus	Strepto bacilli	Gram Positive	Non sporulating	Eubacterium spp.
4	SCDA-2	Bacillus	Bacillus	Gram Positive	Bipolar spores	Bacillus spp.
5	TSIA-2	Bacillus	Streptobacilli	Gram Positive	Non sporulating	Eubacterium spp.
6	Cho-A-2	Bacillus	Streptobacillus	Gram Positive	Non sporulating	Streptomyces spp.
7	KMM-2	Bacillus	Dumble shape Bacilli	Gram Positive	Non sporulating	Actinomyces spp.
8	BA-Beta-2	Bacillus	Bacillus	Gram Positive	Non sporulating	Bacillus spp.
9	SCA-2	Bacillus	Streptobacillus	Gram Positive	Non sporulating	Streptomyces spp.
10	PK-2	Bacillus	Bacillus	Gram Positive	Terminal and biterminal spores	Bacillus spp.
11	KMM-1/2	Bacillus	Bacillus	Gram Positive	Non sporulating	Actinomyces spp.
12	GAM-1/2	Bacillus	Long chains of Bacillus	Gram Negative	Non sporulating	Actinomyces spp.

Morphological characterization of isolates

The shape and the arrangement of all the isolates were bacilli except for LFA-2. All isolates were Gram positive except for SFB-2 and GAM-1/2. SFB-2 and PK-2 were sporulating with Terminal and bi terminal spores is shown in table 7. The isolate codes include LFA-2, SFB-2, SNA-2, SCDA-2, TSIA-2, Cho-A-2, KMM-2, BA-Beta-2, SCA-2, PK-2, KMM-1/2and GAM-1/2 were identification at spieces level as Enterobacteraceae spp., Clostridium spp., Eubacterium spp., Bacillus spp., Eubacterium spp., Streptomyces spp., Actinomyces spp., Bacillus spp., Streptomyces spp., Bacillus spp., Actinomyces spp.,and Actinomyces spp. respectively basing on their growth on selective media is shown in table 7. The morphological characterization of the isolates were shown in Figure 5 [a. Lipid Agar (LA-2); b. Spore Forming Bacteria-2 (SFB-2); c. Saline Nutrient Agar-2 (SNA-2); d. Soyabean Casein Digest Agar-2(SCDA-2); e. Blood Agar- Beta-2 (BA-Beta-2) and f. Triple Sugar Iron Agar-2 (TSIA-2) and Figure 6 [a. Chocolate Agar-2 (Cho-A-2); b. Pikova's Medium (PK-2); c. Simmons Citrate Agar (SCA-2); d. Garrod Actinomyces Medium-1/2 (GAM-1/2); e. Kenknight & Munaier's Medium-1/2 (KMM-1/2) and f. Kenknight & Munaier's Medium-2 (KMM-2)].

Table 8. Biochemical characterization of Isolates isolated from Ghat-7

S.No.	Isolate code	Indole Test	Methyl red Test	Vogusproskeur	Citrate Test	Nitrate Reduction Test	Hydrogen sulphide Test	Urease Test	Gelatin Liquification Test	TSI Test	Catalase Test
1	LFA-2	-ve	-ve	Positive	Positive	-ve	-ve	-ve	-ve	Red/Yellow	-ve
2	SFB-2	-ve	-ve	Positive	Positive	-ve	-ve	-ve	-ve	Red/Yellow	-ve
3	SNA-2	-ve	Positive	-ve	-ve	-ve	-ve	-ve	-ve	Red/Yellow	-ve
4	SCDA-2	-ve	-ve	Positive	Positive	-ve	-ve	-ve	-ve	Red/Yellow	-ve
5	TSIA-2	-ve	-ve	Positive	-ve	-ve	-ve	-ve	-ve	Yellow	-ve
6	Cho-A-2	Positive	Positive	-ve	Positive	-ve	Positive	Positive	-ve	Red	-ve
7	KMM-2	-ve	-ve	Positive	Positive	-ve	-ve	Positive	-ve	Red/Yellow	-ve
8	BA-Beta-2	Positive	Positive	-ve	Positive	-ve	Positive	-ve	-ve	Red/Yellow	Positive
8	SCA-2	-ve	Positive	-ve	Positive	-ve	-ve	-ve	-ve	Red/Yellow	-ve
9	PK-2	-ve	-ve	Positive	Positive	-ve	-ve	-ve	-ve	Red/Yellow	-ve
11	KMM-1/2	-ve	-ve	Positive	Positive	-ve	-ve	Positive	-ve	Red/Yellow	-ve
12	GAM-1/2	-ve	-ve	Positive	Positive	-ve	-ve	Positive	-ve	Red/Yellow	-ve

Biochemical characterization of Isolates

Indole test was negative for all isolates except for Cho-A-2 and BA-Beta-2. Methyl red test was positive for SNA-2, Cho-A-2, BA-Beta-2 and SCA-2. Vogusproskeur was negative for SNA-2, Cho-A-2, BA-Beta-2 and SCA-2. Citrate test was negative for SNA-2 and TSIA-2 but rest of the isolates were positive. Nitrate Reduction test was negative for all isolates. Cho-A-2 and BA-Beta-2 isolates were positive for hydrogen sulphide test. Cho-A-2, KMM-2, KMM-1/2 and GAM-1/2 were positive for urease test. All the isolates isolated from Ghat-7 showed negative for gelatin liquefaction test. All isolates produced acid in triple sugar iron test but Cho-A-2 produced alkali. BA-Beta-2 was the only positive for catalase test. The detail of the biochemical characterization of isolates is depicted in table 8.

THE EXIST GHAT-15 (RAMALAYAM GHAT)

Table 9. Ghat-15 the exist Ghat-15 (Ramalayam Ghat).

S.No.	Code	Extension of the code
1	HLM-3	Hugh Leifson Medium-2
2	SIM-3	Simmons Citrate Agar
3	BB-3	Bennet's Broth-2
4	MSA-3	Mannitol Salt Agar
5	GAM-3	Garrod Actinomyces Medium-3
6	BA-Gamma-3	Blood Agar- Gamma-3
7	GAM-2/3	Garrod Actinomyces Medium-2/3
8	INM-3	Indole Nitrate Medium-2
9	TGB-3	Tryptophan Glucose Beef Extract Agar-2

The bacteria isolated from the middle ghat-15 were coded with respective their selective media used for isolation. The coding of each isolate was shown in table 9. Nine isolated isolates includes Hugh Leifson Medium-2, Simmons Citrate Agar, Bennet's Broth-2, Mannitol Salt Agar, Garrod Actinomyces Medium-3, Blood Agar- Gamma-3, Garrod Actinomyces Medium-2/3, Indole Nitrate Medium-2 and Tryptophan Glucose Beef Extract Agar-2.

Table 10. Colony Characterization isolates grown on Nutrient Agar Ghat-15

S. No.	Isolate code	Size	Pigmentation	Form	Margin	Elevation	Consistency	Optical Characterizations	Nutrient Broth	Form on Slant
1	HLM-3	Small	Milky white	Circular	Entire	flat	Butyrous	Opaque	Uniform fine turbidity , Pellicle and sediment	Effuse
2	SIM-3	Small	Milky white	Circular	Entire	flat	Mucoid	Opaque	Uniform fine turbidity and sediment	Arborescent
3	BB-3	Moderate	Milky white	irregular	Lobate	Flat	Mucoid	Translucent	Sediment	Effuse
4	MSA-3	Pin point	Cream	Curricular	Entire	Flat	Mucoid	Opaque	Pellicle and sediment	Echinulate
5	GAM-3	Pin point	Light yellow	Irregular	Lobate	Raised	Mucoid	Opaque	Pellicle, Uniform fine turbidity and sediment	Effuse
6	BA-Gamma-3	Moderate	Gray	Irregular	Lobate	Convex	Mucoid	Translucent	Uniform fine turbidity and small sediment	Effuse
7	GAM-2/3	Small	Light Yellow	Irregular	Entire	Flat	Mucoid	Opaque	Flocculent, uniform fine turbidity and small sediment	Echinulate
8	INM-3	Pinpoint	cream	irregular	Entire	convex	Butyrous	Opaque	Uniform, Fine Turbid and Sediment	Echinulate
9	TGB-3	Pin point	Light Yellow	Ciricular	Entire	Convex	Mucoid	Translucent	Fine turbidity and sediment	Echinulate

Colony characterization of the isolates grown on Nutrient Agar

Size, pigmentation, form, margin, elevation, consistency, optical characterization, nutrient broth and form on slant are the colony characteristics studied for the isolates grown on nutrient agar was shown in table 10. The size ranges from pin point to moderate. The pigmentation was light yellow for isolated GAM-3, GAM-2/3 and TGB-3 rest of them were milky to cream in color. The form was circular to irregular. The Margin was entire and lobate. The elevation observed as flat, raised and convex. The consistency was mucoid for all but HLM-3 and INM-3 showed butyrous. The optical characterization was opaque and translucent. The nutrient broth showed pellicle, uniform, fine turbidity and sediment. The form on slant was shown as echinulate, effuse and arborescent.

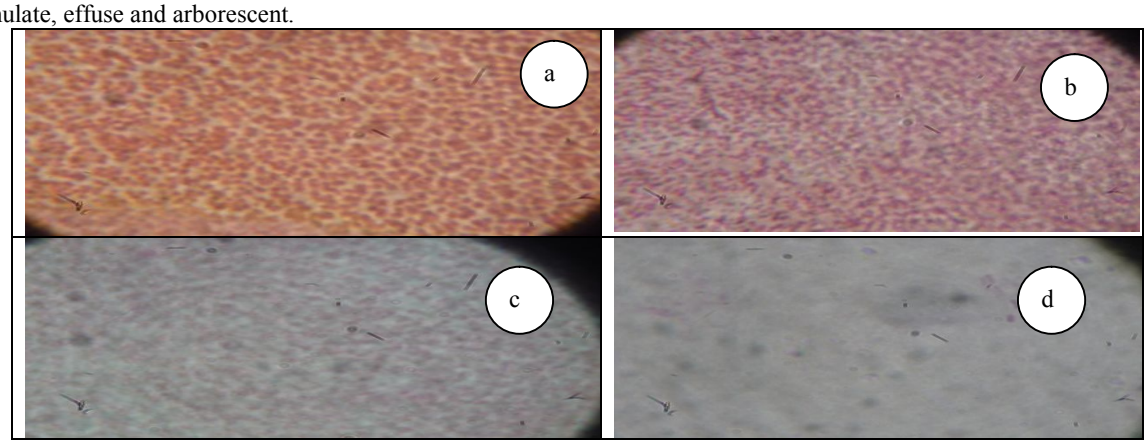

Figure 7: a. Hugh Leifson Medium-3 (HLM-3); b. Blood Agar- Gamma-3 (BA-Gamma-3); c. Simmons Citrate Agar (SIM-3) and Garrod Actinomyces Medium-2/3 (GAM-2/3).

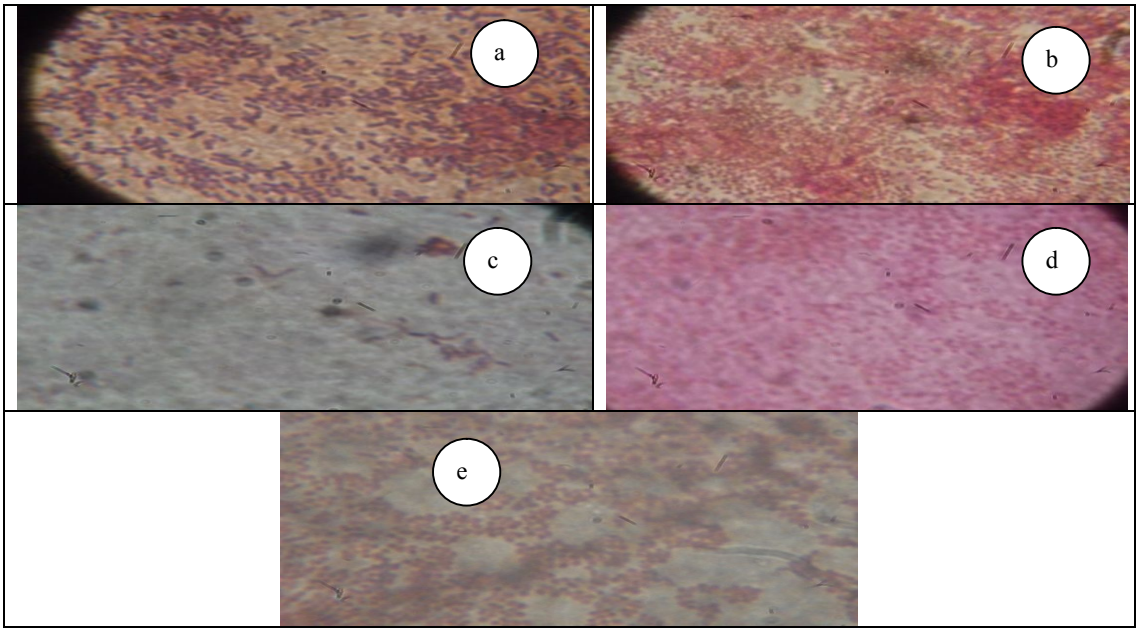

Figure 8: a. Bennet's Broth-3(BB-3); b. Indole Nitrate Medium-3 (INM-3); c. Mannitol Salt Agar (MSA); d. Tryptophan Glucose Beef Extract Agar-3 (TGB-3); and e. Garrod Actinomyces Medium-3 (GAM-3).

Table 11. Morphological characterization isolates isolated from Ghat-15

S.No.	Isolate code	Shape	Arrangement	Gram staining	Spore staining	Identification at spieces level
1	HLM-3	Cocci	Cocci	Gram Negative	Non sporulating	Neisseria spp.
2	SIM-3	Bacillus	Bacillus	Gram Negative	Non sporulating	Citrobacter spp.
3	BB-3	Bacillus	Diplo Bacillus	Gram Positive	Non sporulating	Micromonospora spp.
4	MSA	Bacillus	Bacillus-Rod shaped	Gram Positive	Non sporulating	Staphylococcus spp.
5	GAM-3	Bacillus	Long chains of Bacillus	Gram Negative	Non sporulating	Actinomyces spp.
6	BA-Gamma-3	Bacillus	Bacillus	Gram Positive	Non sporulating	Bacillus spp.
7	GAM-2/3	Bacillus	Bacillus	Gram Positive	Non sporulating	Actinomyces spp.
8	INM-3	Cocci	Cocci	Gram Negative	Non sporulating	Neisseria spp.
9	TGB-3	Cocci	Cocci	Gram Negative	Non sporulating	Neisseria spp.

Morphological characterization of isolates

The shape and the arrangement of all the isolates were bacilli except HLM-3, INM-3and TGB-3. All isolates were Gram negative except BB-3, MSA, BA-Gamma-3 and GAM-2/3. All the isolates were non sporulating. The isolate codes include HLM-3, SIM-3, BB-3, MSA, GAM-3, BA-Gamma-3, GAM-2/3, INM-3 and TGB-3 were identified at spieces level as Neisseria spp., Citrobacter spp., Micromonospora spp., Staphylococcus spp., Actinomyces spp., Bacillus spp., Actinomyces spp., Neisseria spp. and Neisseria spp. respectively basing on their growth on selective media is shown in table 11. The morphological characterization of the isolates were shown in figure 7 [a. Hugh Leifson Medium-3 (HLM-3); b. Blood Agar-Gamma-3 (BA-Gamma-3); c. Simmons Citrate Agar (SIM-3) and Garrod Actinomyces Medium-2/3 (GAM-2/3)] and figure 8 [a. Bennet's Broth-3(BB-3); b. Indole Nitrate Medium-3 (INM-3); c. Mannitol Salt Agar (MSA); d. Tryptophan Glucose Beef Extract Agar-3 (TGB-3); and e. Garrod Actinomyces Medium-3 (GAM-3)].

Table 12. Biochemical characterization of Isolates isolated from Ghat-15

S.No.	Isolate code	Indole Test	Methyl red Test	Vogusproskeur	Citrate Test	Nitrate Reduction Test	Hydrogen sulphide Test	Urease Test	Gelatin Liquification Test	TSI Test	Catalase Test
1	HLM-3	-ve	Positive	-ve	Positive	-ve	-ve	-ve	-ve	Red/Yellow	Positive
2	SIM-3	-ve	Positive	-ve	Positive	-ve	-ve	-ve	-ve	Red/Yellow	-ve
3	BB-3	-ve	-ve	Positive	Positive	-ve	-ve	-ve	-ve	Red/Yellow	-ve
4	MSA	-ve	Positive	-ve	Positive	-ve	-ve	-ve	-ve	Red/Yellow	-ve
5	GAM-3	-ve	Positive	-ve	Positive	-ve	-ve	-ve	-ve	Yellow	-ve
6	BA-Gamma-3	Positive	Positive	-ve	Positive	-ve	Positive	Positive	-ve	Yellow	-ve
7	GAM-2/3	-ve	Positive	-ve	Positive	-ve	-ve	-ve	-ve	Yellow	-ve
8	INM-3	-ve	Positive	-ve	Positive	-ve	-ve	-ve	-ve	Yellow	-ve
9	TGB-3	-ve	Positive	-ve	Positive	-ve	-ve	-ve	-ve	Red/Yellow	-ve

Biochemical characterization of Isolates

Indole test was negative for all isolates except for BA-Gamma-3. Methyl red test was positive for BB-3. Vogusproskeur was negative for all but positive for BB-3. Citrate test was positive for all isolates. Nitrate Reduction test and gelatin liquefaction test were negative for all isolates. BA-Gamma-3 was the only positive for hydrogen sulphide test and urease test. GAM-3, BA-Gamma-3, GAM-2/3and INM-3 were only acid producing but rest of them produced alkali and acid in triple sugar iron test. HLM-3 was the only positive for catalase test. The detail of the biochemical characterization of isolates is depicted in table 12.

CONCLUSION

The study was concentrated on the biological examination of water from three main ghats constructed on the eve of mahapuskaralu on the bank of Rajamundry, Andhra Pradesh, India.There are broad variety of bacteria isolated from the water samples collected form three Ghats namely the entry Ghat-1(Koti lingalu Ghat), the Middle Ghat-2 (Puskara Ghat) and the exist Ghat-3(Ramalayam Ghat). The isolated bacteria ware coded with respective to the selected media used for their screening. It was found that eleven,tweleve and nine were isolated from Ghat-1, Ghat-7 and Ghat-15 respectively. Different spieces were identified in each ghat. In Ghat-1 Actinomyces spp., Bacillus spp., Enterobacteriacae spp., Enterococcus spp., Nocardia Spp., Clostridium spp., Streptococcus spp., and Eubacterium spp. were identified. In Ghat-7 Actinomyces spp., Enterobacteraceae spp., Clostridium spp., Eubacterium spp., Bacillus spp. and Streptomyces spp. and in Ghat-15 Bacillus spp., Actinomyces spp.,Neisseria spp., Staphylococcus spp., Micromonospora spp., and Citrobacter spp..

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