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Isolation and Characterization of Bacteria from Polluted Water of Kamrup Metropolitan District, Assam

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ABSTRACT
Water contains a various number of microorganisms in it .In this study, we have isolated and characterized a few dominant bacteria from some polluted sites. Water samples were collected from four polluted sites namely Deepor Beel, Bharalu River, Digholi Pukhuri, Jorpukhuri. Samples were isolated in nutrient Agar medium. Six Dominant bacterial isolates were evaluated by morpholpgical and biochemical tests. Gram staining of the bacterial isolates was also performed on selected four isolates. According to Biochemical test (Urease test, Starch Hydrolysis test, Catalase test) Isolate 1,2 and 4 showed positive against starch hydrolysis. Isolate 2 showed negative against urease and catalase test. Antibiotic sensitivity test were also done on the bacterial strains. Most of the bacterial strains isolated are resistant to Streptomycin and Gentamicn and found susceptible against Polymixin B, Tetracycline and Amikacin. This preliminary work aims to identify the pathogenic bacteria which are responsible for water contamination and to suggest preventive measures for control and management of this water bodies for sustainable development of aquatic climate of this regions.
Keywords: Water pollution, pathogenic bacteria, gram staining, Antibiotic sensitivity test.

INTRODUCTION

The water availability has always been of great importance for life and for every living organism. It has a life-sustaining role in welfare and growth of mankind ((Yassi et al., 2001). Sewage contains human faces and therefore contains human enteric pathogens i.e *Salmonella enteric, Salmonella enteritidis,Salmonella typhimurimum,Shigella dysentriae, Enterobactor*

spp.Vibrio spp. These pathogens are responsible for serious gastrointestinal illness which is a significant cause of waterborne health epidemics (Sharpe et al.,2003; Keinanen et al., 2004, Lynch et al. 2007).The infants are the most vulnerable targets of these diseases as Enterobacteriaceae and non-fastidious, Gram-negative rods constitute one of the major contaminants of water (Sigee, 2005;Suthar *et al.,* 2009, Berg et al. 2014). Enterobacteriaceae and non-fermentative Gram-negative rods, Gram-positive Staphylococci, Enterococci and Micrococci, and some genera of the Actinobacteria group (Benson,2001, Cabrera *et al*., 2016). Bacteria are considered to be the best bioremediator in boremediation ,indigenous microorganism of contaminated sites or new microorganisms can be used From various studies ,it showed that microorganisms such as *Pseudomonas* spp., *Modococci* spp., *Bacillus* spp., *Micrococcus* spp., *Rhodococcus* spp ., *Entrobacter* spp., *Mycobactena* spp.,are capable of degrading petroleum compound There are many studies that has been done on bacteria found in pollutes sites (May,2006,Szewzyk *et al*.2000, Martinez,2008). Bacteria found in polluted sites are used in bioremediation of heavy metals , petroleum ,petroleum derivatives ,etc.*Deinococcus radiodurans* is a radiation extremophile bacterium that is genetically engineered for the bioremediation of solvents and heavy metals (Touron,2007,Rudrangshu et al., 2021,Ogutcu et al., 2022). Recent studies emphasized the presence of antibiotic resistance bacteria and antibiotic resistance genes in drinking water (Lee *et al*., 2011, Vaz-Moreira *et al*.,2011, Panel, *et al*.,2013, Retnam *et al*.,2013, Deen, *et al*., 2014)

Microbial aquatic ecosystems, mainly those exposed to anthropogenic activities, represent important vehicles for the dissemination of human-associated microorganisms and may constitute a reservoir for the spread of resistance genes among bacterial communities (Amos,*et al*., 2015, Chen, *et al*.,2015)..For further study they selected pure culture of bacterial isolates were carried out a test for H_2S production ,starch hydrolysis ,catalase test,urease test,carbohydrate ferementation test and Antibiotic resistance. And concluded that the polluted water sample have *Bacillus* sp.by bacteriological analysis. They recommended for proper sanitation,regular treatment ,and supervision of water sources and regular bacteriological assessment of water sources for consumption (Chauhan *et al*.,2015, Cabrera, *et al*.,2016, Sirisha, *et al*.,2017, Chanda and Dey,2018, Musa and Si 2019, Aguilar, *et al*.,2020, Bhumbla, *et al*.,2020, Aqeel, *et al*.2021, Ogutcu, *et al*.2022).

MATERIALS AND METHODS

A. Description of study area:

The study area is located in Guwahati city, Kamrup Metropolitan District,Assam. Kamrup Metroplitan district occupies an area of 1528 sq kilometers. Guwahati city is located at Latitude 26.1158^0 N and Longitude 91.7086^0 E. The minimum temperature are normally around 11.1^0 C, while the maximum temperature is around 38.4^0 C. Water samples were collected from Guwahati city area such as Bharalu River(Sample A), Digholi pukhuri(Sample B), Deepor Beel(Sample C), Jorpukhuri (Sample D).

B. Cultural and morphological features of the bacteria isolates:

The serial dilution technique is followed for the isolation and enumeration of bacteria. The cultural and morphological features falls under the phenotypic characterization, which were studied by adopting standard methods. Morphology of colonies on plates is a characteristic feature of bacterium, which is quite useful in preliminary identification procedure. Different colony features such as configuration, elevation, margin, texture, consistency etc. were noted down by using a hand lens.Standard plate count method was used to enumerate the bacterial cultures. The colonies formed after the incubation were counted. The number of bacterial colonies in each was reffered to as a colony forming units (CFU). Colonies exhibition good variable growth was selected for further streaking on fresh plates. Further purification and multiplications of isolates were done by streaking on fresh plates. The CFU were determined by the following formula:

CFU/g= Average no. of colonies /Inoculation volume plated (ml) × Dilution factor

C. Gram Staining:

Gram staining is a differential staining technique that separates bacteria into two groups. Gram positive and Gram negative. A thin smear of the bacterial cultures was made on a glass slide . After rinsing with water, Grams iodine solution was added and kept for one minute. It then was washed with water and decolorized with 95% ethyl alcohol for 20 seconds. Rinsed with water, and then counter stain with safranin for one minute. Rinsed with water, blotted dried, and then observed under microscope. The nature of Gram's reaction was observed and morphological details were noted down. Cells were identified by the color observed purple or blue for Gram positive and pink or red Gram negative.

D. Biochemical characterization of bacterial isolates:

(i) Urease Test:

The urease test was performed by inoculating the bacterial isolates into Urease broath. Urea was sterilized by ultraviolet irradiation before adding sterilized medium containing in test tubes and observed after incubation. The indicator's color changes from clear to pink indicate a positive result due to the accumulation of ammonia which raises the p^H of the medium.

(ii) Starch Hydrolysis:

This test is used to differentiate based on their ability to hydrolyze starch with the enzyme alpha-amylase or oligo -1, 6-glucosidase by breaking the glycosidic linkages between the sugar subunits. The bacterial cultures were streaked on the starch agar plates and incubated at 30^0 C for 24-48 hours .After incubation, iodine solution was flooded on the petri plates. Iodine reacts with starch and produces a blue or dark brown color, therefore any microbial growth hydrolysis will be revealed as a clear zone surrounding the growth .The formation of the clear zone around the colony was taken as positive test.

(iii) Catalase Test: The enzyme catalase present in some microorganisms breaks down hydrogen peroxide to water and oxygen which helps them in their survival. The bacterial isolates were inoculated into Nutrient Agar (NA)and then incubated for 48 hours at 30+-2.0^0C.And after proper growth, catalase production was determined by introducing 5-6of H_20_2(20%)into each slides .Release of free oxygen gas ($0_{2)}$ bubbles indicated a positive catalase test.

(iv) Determination of Antibiotic Resistance:

To determine the antibiotic sensitivity of the bacterial isolates, antibiotic discs (Hi-media) were placed on freshly prepared lawns of each isolates on Mueller Hilton Agar plates. The isolates were tested for antibiotic sensitivity, according to Kirby-Bauer disc diffusion method (Bauer *et al*.,1966) to 4 antibiotics .The selected antibiotic were placed on the plates and incubated at 37^0C for 24 hours .The diameter of the inhibition zones were measured to the nearest mm and the isolates were classified as resistant (R),intermediate (I),and susceptible (S) following the standard antibiotic disc sensitivity testing method. A total of seven antibiotic discs were taken Gentamicin, Penicillin, Streptomycin, Vancomycin, Polymyxin B, Tetracycline and Amikacin.

RESULTS & DISCUSSION

Isolation and Maintenance of the Bacterial Isolates:

After isolation of bacteria from the collected samples, 8 bacterial isolates were selected from, 4 from each samples. Selected isolates were pure cultured on medium by streak plate method and these were taken for further study to study the morphology and colony characteristics It was observed that total number of bacterial C.F.U was very high in Sample A,B than C and D .The colonies from the isolated samples wee of various colours, various forms and margins. From both samples,4 bacterial isolates were selected and pure cultured for cultured for future studies. Out of all selected contaminate sites, Bharalu river showed highest cfu followed by Digholi pukuri, deepor beel. The least no of cfu was estimated in Jorphukuri (**Figure. 1)**.

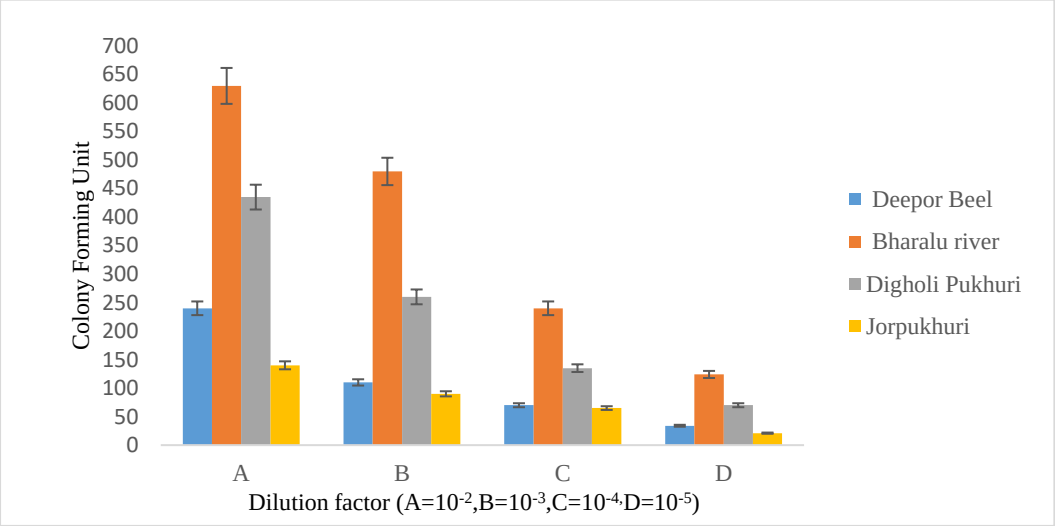


Figure 1 : C.F.U of isolated bacteria from the four selected study sites

Cultural characteristics of isolated bacteria:

Colony Morphology:

The bacterial colonies color, form, margin , elevation were observed in the culture plates with nutrient agar and recorded.(**Table 1)**

Table 1 : Colony morphology of isolated bacterial strains.

	Isolate No.	**Colony color**	**Form**	**Margin**	**Elevation**
Sample A	Isolate 1	White	Irregular	Lobate	Raised
	Isolate 2	White	Circular	Undulate	Flat
	Isolate 3	Yellow	Irregular	Entire	Convex
	Isolate 4	White	Punctiform	Erose	Raised
Sample B	Isolate 5	White	Irregular	Entire	Flat
	Isolate 6	Pink	Irregular	Undulate	Raised
	Isolate 7	Opaque	Rhizoid	Undulate	Pulvinate
	Isolate 8	Yellow	Circular	Entire	Convex
Sample C	Isolate 9	Pink	Irregular	Undulate	Convex
	Isolate 10	White	Irregular	Erose	Raised
	Isolate 11	Yellow	Rhizoid	Erose	Flat
	Isolate 12	White	Circular	Entire	Flat
Sample D	Isolate 13	Yellow	Circular	Entire	Raised
	Isolate 14	Yellow	Circular	Entire	Raised
	Isolate 15	White	Rhizoid	Undulate	Flat
	Isolate 16	White	Irregular	Undulate	Convex

Gram staining of the bacterial isolates:

Out of all 16 isolates, four isolates were taken as pure culture and Gram staining were done for the morphological identification. And it was observed that out of four isolates, isolate1, 2 and 3 were gram positive and Isolate 4 was gram negative (Table 3).

Biochemical Characterization:

Based on Gram Staining Test, the bacterial isolates were identified as Gram Positive and Gram Negative. For further characterization, different biochemical test were performed to identify the bacterial isolates. The results of the bacterial isolates are given in the table below. Bacteral Strain isolate1, isolate 3, Isolate 4 showed positive result against urease test and Catalase test while the isolate 2 showed negative result against urease and catalase test . Isolate 3 showed negative against starch hydrolysis. Isolates 1,2 and 4 showed positive test for starch hydrolysis (**Table 2;Photoplate 1**).

Table 2: Biochemical characteristics of isolated bacterial strains

Isolates	Gram staining	Urease Test	Starch Hydrolysis	Catalase Test
Isolate 1	+	+	+	+
Isolate 2	+	-	+	-
Isolate 3	+	+	-	+
Isolate 4	-	-	+	+

Antibiotic Sensitivity Test:

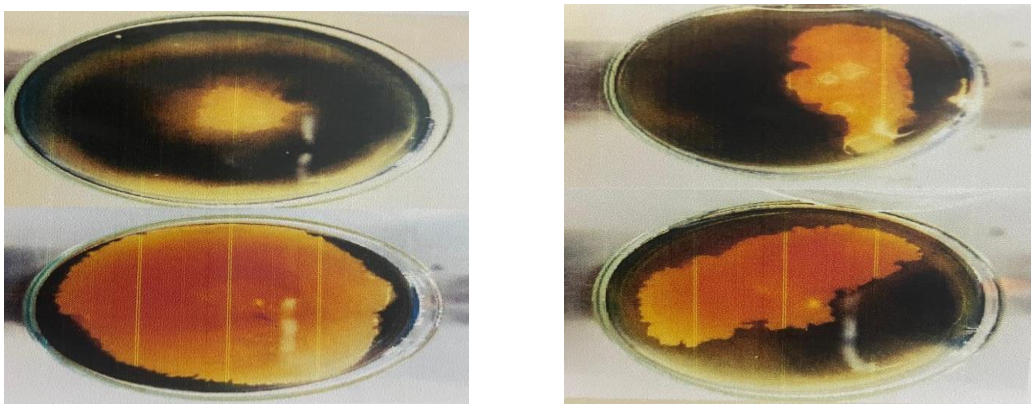
The bacterial isolates are tested for Antibiotc sensitivity. Most of the bacterial strains isolated are resistant to Streptomycin and Gentamicn and found susceptible against Polymixin B, Tetracycline and Amikacin. On the other hand, almost all the isolate showed no inhibition to Penicilin. The antibiotic zones were evaluated by Antibiotic zones scale and recorded. The recorded inhibition zones were measured to identify the susceptible, intermediate and resistance nature of all bacterial isolates (**Table 3, Photoplate 2**).

Table 3: Antibiotic sensitivity test of isolated bacterial strains

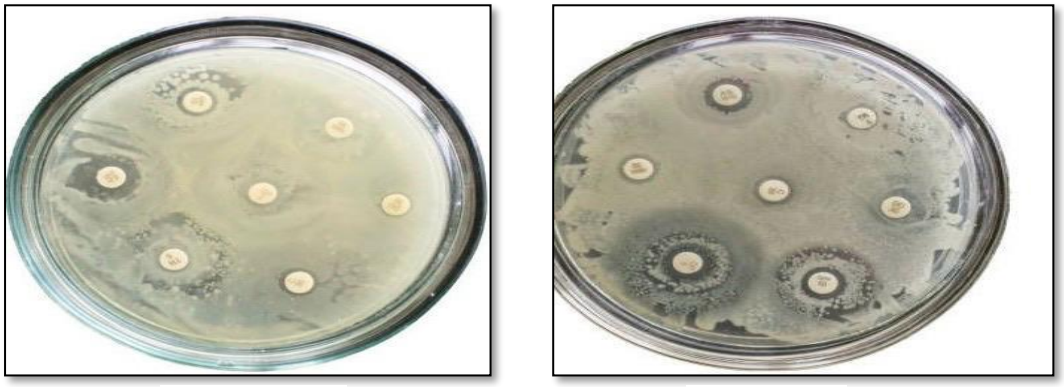
Isolates	Diameter of Inhibition Zone (mm)						
	Gentamicin	Penicillin	Streptomycin	Vancomycin	Polymyxin B	Tetracycline	Amikacin
Isolate 1	22 mm (I)	11 (S)	20 mm (R)	18 mm (I)	14(S)	34(S)	33(S)
Isolate 2	20 mm (I)	NI	18 mm (R)	17 mm (I)	20(S)	35(S)	34(S)
Isolate 3	24 mm (R)	NI	23 mm (R)	21 mm (R)	10(I)	24(S)	26(S)
Isolate 4	24 mm (R)	NI	24 mm (R)	20 mm (R)	12(S)	26(S)	29(S)

Diameter of Disc=6mm,

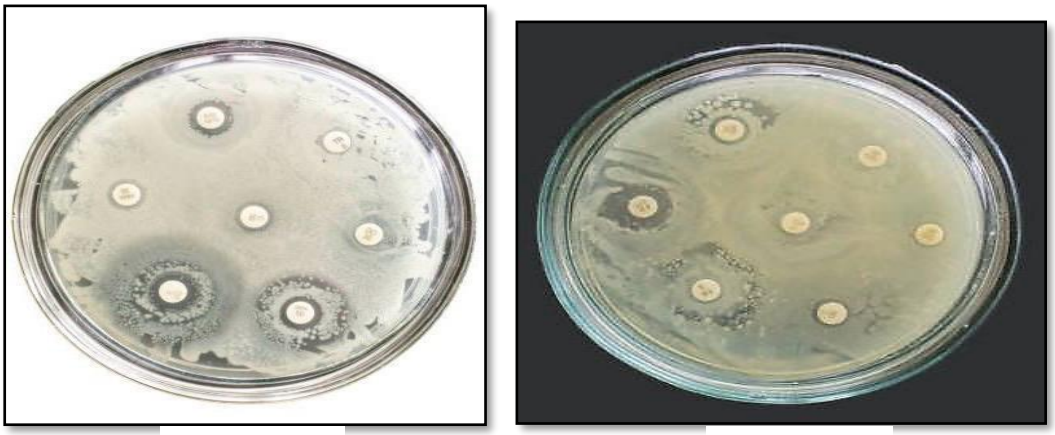
NI=No Inhibition; S= Susceptible; I= Intermediate; R=Resistant



PHOTOPLATE 1: Starch Hydrolysis test



Isolate 1 **Isolate 2**



Isolate 3 **Isolate 4**

PHOTOPLATE 2: Antibiotic sensitivity test

Water pollution is inevitable in these modern days. Various sites that we know of are polluted .Water are polluted by numerous pollutant, which can be organic or inorganic .There are vast numbers of soil pollutants including food wastage ,industrial waste, oil spillage ,household wastes, etc. .Even in these polluted sites microorganisms are present (Aqeel *et al*,2021).Numerous studies are being done on bacteria from polluted sites. Morphological ,Biochemical test are being done to characterize the bacterial strains .Bacteria found in contaminated water are studied upon for various purposes including biodegradation of kerosene, derivatives of kerosene , their tolerance against heavy metals, bioremediation ,etc (Tafesse and Assefa 2022; Ogutcu *et al*., 2022) Wang et al.(2020) isolated and studied bacterial strains from oil polluted sites and suggested few bacterial strains with potential of TPHs biodegradation .Ghoreishi et al .(2017) isolated bacteria from petroleum contaminated water with an aim to isolate bacteria for bioremediation of petroleum polluted water.

CONCLUSION

Water bacteria found in contaminated water are mostly pathogenic in nature. There are some bacteria which are useful to us. Some bacteria found in contaminated site can be altered and used in bioremediation, while some bacteria help in biodegradation toxic metals, chemicals etc. The isolated bacterial strains will be helpful for possible application of bioremediation of contaminated sites.

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