STUDENT STUDY PROJECT

ON

"STUDY AND IDENTIFICATION OF PATHOGENIC BACTERIA IN BORE WATER "

Department of Microbiology

Dr. BRR Government Collage, Jadcherla

Mahabubnagar– 509001

Accredited by NAAC with B^{++} Grade// An ISO 9001-2015 Institution

Mahabubnagar (Dist), Telangana State, India – 509301

Affiliated to Palamuru University

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CERTIFICATE

This is to certify that the project work entitled **"Study and Identification of Pathogenic Bacteria in Bore Water" Jadcherla, Mahabubnagar District, and Telangana.** "is a bonafide work done by the students of III MZC (EM) **Mr. A. Vinay, Mr. K. Rahul, Miss. P. Anitha, Miss. Safoora Tabasuum, Mr. Syed Sohail** under my supervision for the award of Project Work in Microbiology, Department of Microbiology, Dr. BRR Government College, Jadcherla and the work hasn't been submitted to any other College/University either in part nor in full, for the award of any degree.

Sumera Tabassum

Lecture of Microbiology

Signature Of HOD

26/8/23

Signature of external examiner

Signature of Internal Examiner

Signature of Principal

DECLARATION

We hereby declare that the project work entitled with **"Study and Identification of Pathogenic Bacteria in Bore Water" Jadcherla, Mahabubnagar District and Telangana.**"Is a genuine work done by us under the supervision of **K. NEERAJA,** for the Department of Microbiology, Dr.BRR. Government College and it has not been under the submission to any other Institute/University either in part or in full, for the award of any degree.

S.NO	NAME	CLASS	HALL TICKET NO	SIGNATURE
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ACKNOWLEDGMENTS

We express my heartfelt gratitude, respect indebtedness to **K. NEERAJA,** Incharge of Microbiology, Dr. BRR Government College, and Jadcherla for the valuable guidance, encouragement and timely suggestions and immense patience throughout the period of work, without which it would not have been possible to complete the work.

We express deep sense of gratitude to Dr. CH. APPIYA CHINNAMMA Principal Dr. BRR. Government Jadcherla, Mahabubnagar for her moral and technical support for the project work.

And also thanks to our research team for making this project successful and we continue the same for future Endeavour.

CONTENT

ABSTRACT

Water is essential to life, but many people do not have access to clean and safe drinking water and many die of waterborne bacterial infections. In this review a general characterization of the most important bacterial diseases transmitted through water—cholera, typhoid fever and bacillary dysentery—is presented, focusing on the biology and ecology of the causal agents and on the diseases' characteristics and their life cycles in the environment. The importance of pathogenic Escherichia coli strains and emerging pathogens in drinking water-transmitted diseases is also briefly discussed. Microbiological water analysis is mainly based on the concept of fecal indicator bacteria. The main bacteria present in human and animal feces (focusing on their behavior in their hosts and in the environment) and the most important fecal indicator bacteria are presented and discussed (focusing on the advantages and limitations of their use as markers). Important sources of bacterial fecal pollution of environmental waters are also briefly indicated. In the last topic it is discussed which indicators of fecal pollution should be used in current drinking water microbiological analysis.

INTRODUCTION

Water is essential for sustaining all life forms and access to clean and safe drinking water is a basic human need (United Nation Millennium Declaration. 2000). Safe drinking water for all is one of the major consumption. Groundwater constitutes 85% of the source of drinking water in India and none of the major Indian cities have a continuous water supply (National Institute of Urban Affairs, 2005). Ground water is considered much cleaner than surface water. Contamination of water resources is occurred due to poor water resources sanitation, animal manure and improper disposal of solid waste and domestic sewage. In many areas groundwater is polluted by human activities. In areas where material above the aquifer is permeable, pollutants can readily sink into groundwater supplies. If groundwater becomes polluted, it will no longer be safe to drink. The microbiological quality of groundwater is likely to arise from a variety of sources like leakage, infiltration and seepage of domestic sewage lines, household septic tanks, and infiltration from sewage treatment plants, earthen sewer lines, septic tanks, pits, lagoons, ponds, sanitary land filled areas and soak pits into the shallow aquifers. It is evidently important to control ground and surface water from the contamination. It is necessary to have a continuous monitoring on the water quality through microbial and chemical examinations. In general, safe drinking water should not have any infectious agents that dangerous to human health and should be aesthetically acceptable to the consumer. Infectious agents that find in drinking water in the first place are those caused by fecal contaminatio. Even after enactment of water (prevention and control of pollution) Act as early as in 1974, water quality continues to deteriorate in India. Therefore understanding the factors that can affect quality of ground water is of vital importance in managing this significant resource.

1) Escherichia Coli

Escherichia Coli (also known as E. Coli) can cause nausea, vomiting, abdominal pain and diarrhea if consumed in contaminated water. Symptoms usually appear within one to eight days.

2) Campylobacter Jejuni

Drinking water contaminated with Campylobacter jejuni can cause infections with symptoms of cramping, diarrhea, fever, and pain. Symptoms of infection appear between two and ten days after exposure.

3) Hepatitis A

Hepatitis A is a serious infection and can be present in your drinking water. Symptoms include dark urine, jaundice, stomach pain, fever, and fatigue. Hepatitis A has a lengthy incubation period and symptoms might not appear until 28 days after exposure.

4) Giardia Lamblia

Giardia Lamblia is actually a parasite which causes the infection, giardiasis. Symptoms include nausea, cramps, gas, and diarrhea. The incubation period for giardiasis is two weeks.

5) Salmonella

Salmonella is a common pathogen that causes chills, fever, headache, diarrhea, and pain. Salmonella contaminates water and food and symptoms occur in one to three days after consuming.

6) Legionella Pneumophila

Legionella pneumophila can cause serious bacterial infections known as Legionnaires disease. Some symptoms of legionnaires infection are fever, shortness of breath, cough, and muscle aches. Legionnaires is very serious and usually involves hospitalization or can even result in death.

7) Cryptosporidium

Cryptosporidium is actually a protozoan which works similar to a parasite. It causes severe and painful diarrhea and spreads through contaminated drinking water. Cryptosporidium can occur even in a city with clean water, and testing services are required to determine water quality and if these protozoa are thriving in your drinking water.

- To identify the Pathogenic bacteria in bore water.
- To analyze the microbial quality and quantity of bore water..

To propose key strategies to municipal authorities to maintain safe bore water. Supplies of Jadcherla

REVIEW OF LITERATURE

VessoniThereza Christina Penna'

A typical purification system that provides purified water which meets ionic and organic chemical standards must be protected from microbial proliferation to minimize cross-contamination for use in cleaning and preparations in pharmaceutical industries and in health environments.

Joao P.S.Cabral

It was concluded that safe drinking water for all is one of the major challenges of the 21st century and that microbiological control of drinking water should be the norm everywhere. Routine basic microbiological analysis of drinking water should be carried out by assaying the presence of *Escherichia coli* by culture methods.

CarrolSujakhu

The hygiene of environmental surfaces from college premises like laboratory, classrooms, staffroom, canteen, washroom, corridor, railings and miscellaneous sites play role in spreading different pathogenic bacteria.

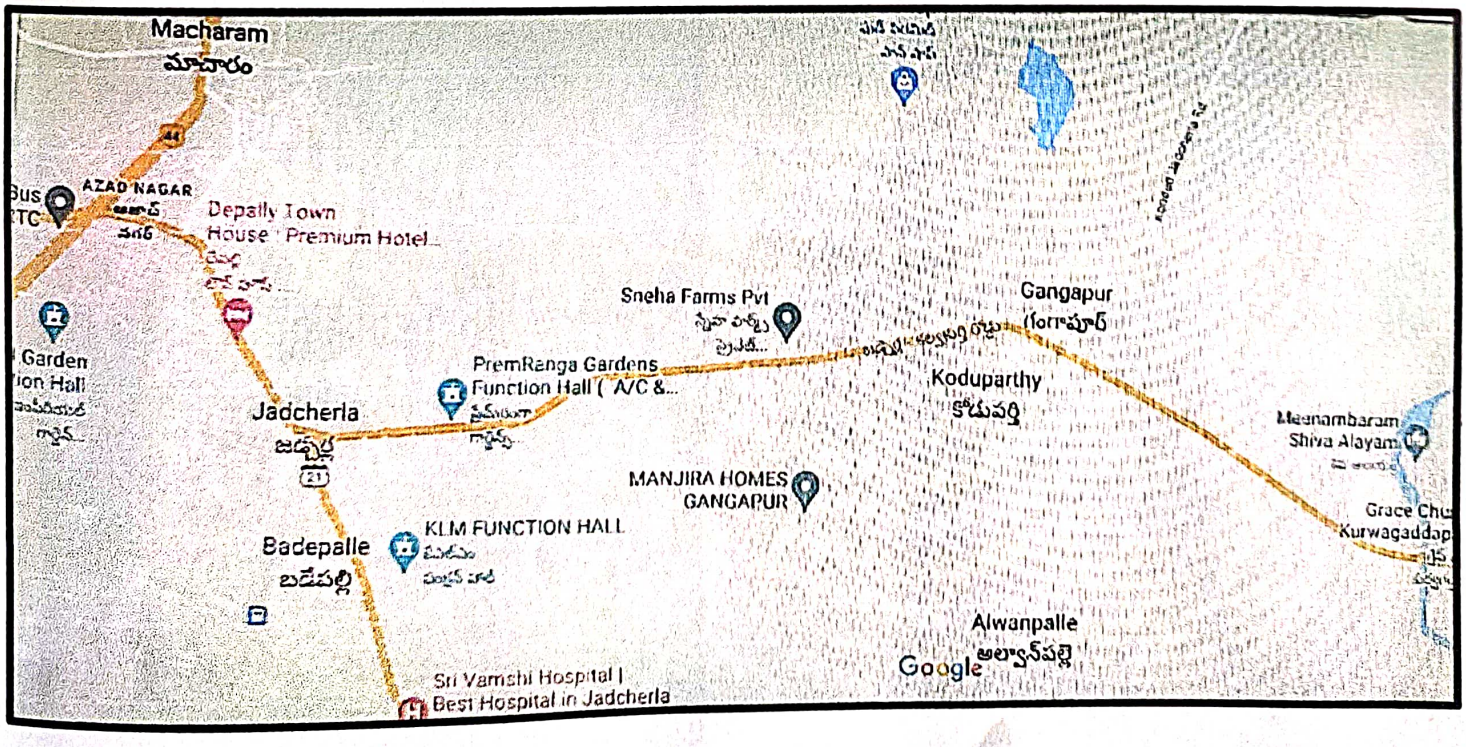
Linda varadi

This review highlights the challenges associated with the detection and identification of pathogenic bacteria, by providing an introduction to the techniques currently used, as well as newer techniques that are in development. Focusing on the chemical basis for these techniques, the review also provides a comparison of their advantages and disadvantages.

VessoniThereza Christina Penna,SilvaAlzira Maria Martins&PriscilaGavaMazzola vess found that research was required for the identification of gram-negative non-fermenting bacteria, which were isolated from drinking water and water purification systems, since *Pseudomonas* genera represents opportunistic pathogens which disperse and adhere easily to surfaces, forming a biofilm which interferes with the cleaning and disinfection procedures in hospital and industrial environments.

STUDY AREA

Jadcherla is **a census town in Mahbubnagar district of the Indian state of Telangana**. It is located in Jadcherla mandal in Mahbubnagar revenue division. In 2011, it was upgraded from village to a census town, along with 11 other villages. It is a historical town and is known for its cultural heritage. Bore water comes from groundwater which in turn comes from rain that has naturally seeped into the ground and is stored in spaces between soil and rocks. The layers and bodies of water in these underground spaces are known as aquifers. Groundwater is the water present beneath Earth's surface in rock and soil pore spaces and in the fractures of rock formations. About 30 percent of all readily available freshwater in the world is groundwater.[1] A unit of rock or an unconsolidated deposit is called an aquifer when it can yield a usable quantity of water. The depth at which soil pore spaces or fractures and voids in rock become completely saturated with water is called the water table. Groundwater is recharged from the surface; it may discharge from the surface naturally at springs and seeps, and can form oases or wetlands. Groundwater is also often withdrawn for agricultural, municipal, and industrial use by constructing and operating extraction wells. The study of the distribution and movement of groundwater is hydrogeology, also called groundwater hydrology.

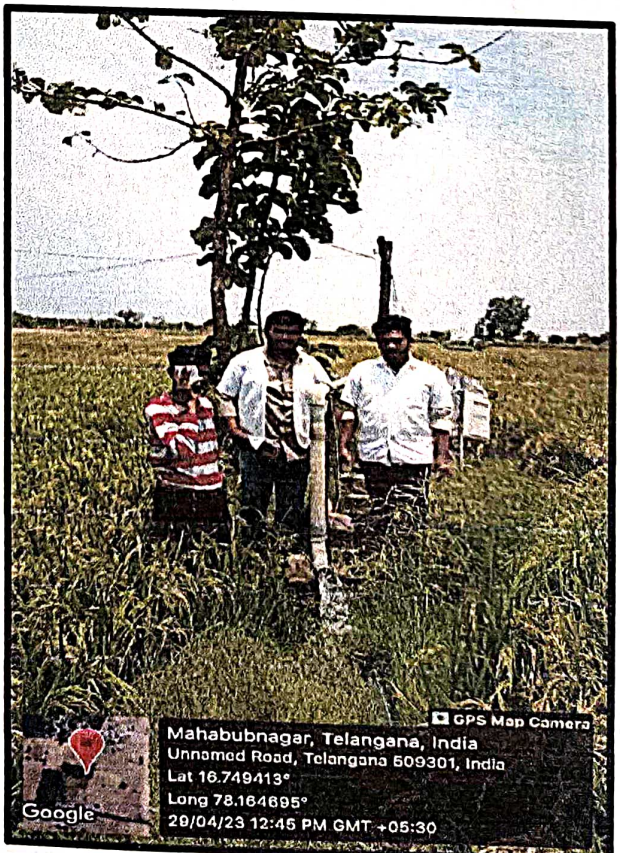


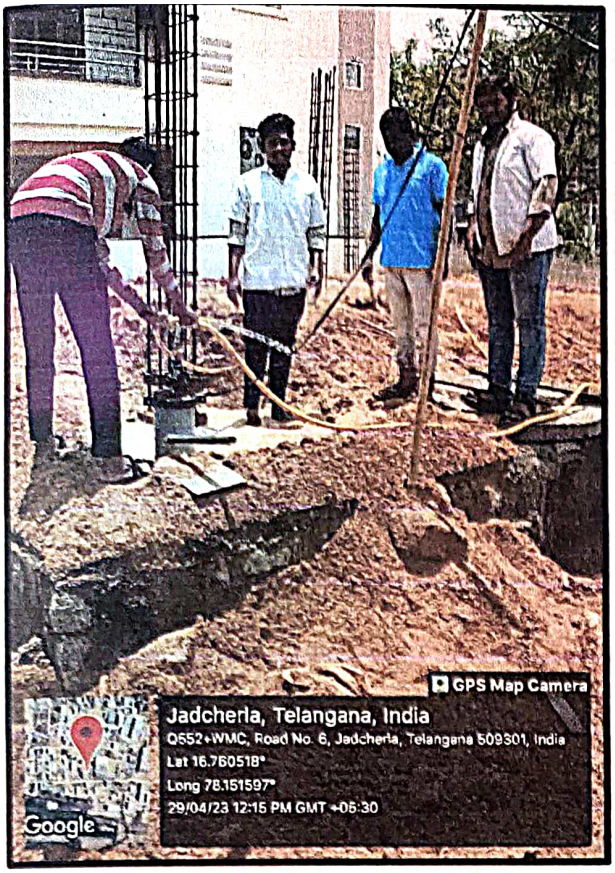
METHODOLOGY

SAMPLE COLLECTION

- we are collected 5 water samples from bore water.
- Identification of pathogenic bacteria.
- Collect water samples for microbiological testing only in sterile containers. Laboratory Services will supply sterilized sample containers on request.
- Do not rinse out the bottle.
- Never touch the neck of the sample container, or the inside of the cap or stopper.
- Never put the cap down on any surface and always replace the cap immediately the container has been filled with sample.
- Keep the sample container capped until immediately before filling. Avoid breathing, sneezing or coughing over the open sample container, or near the sampling point.

- SAMPLE-1 → BOTANICAL GARDEN OF BRR DEGREE COLLAGE
- SAMPLE-2→ GOURI SHENKAR COLONY
- SAMPLE -3→ BALAJI NAGAR COLONY
- SAMPLE -4→KIRSHNA REDDY COLONY
- SAMPLE- 5 → NEARBY CHAI CLUD

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Physical examination of water sample

- Colour- BLUE
- Temperature- 77° Fahrenheit (25° Celsius).
- PH - 5 to 7
- Odor -Naturally occurring hydrogen sulfide in your water supply may also cause this odor.

Chemical examination of water sample

- **PH**

Material required:

- PH paper.
- PH meter.
- Tissue paper.

Procedure:

The Procedure of Determination of pH Using pH paper:

- On a white tile place a clean pH paper strip.
- Drop of the sample on the pH paper using a clean dropper.
- Observe the change in the color of the pH paper.
- Now compare the color obtained on the pH paper with the color shades on the standard pH chart.

BIOLOGICAL EXAMINATION OF WATER SAMPLE

MPN METHOD (Multiple tube fermentation method)

AIM: To examine the microbiological quality of a bore water sample by multiple tube fermentation test for the presence of coliform bacteria.

PRINCIPLE:

Apparently clear looking water without any particulate matter may or may not be pure microbiologically. Some microorganisms naturally occur in water and some are acquired from the soil. Usually the natural micro flora of water in which pathogenic to humans and animals. The presence of coli form in water samples is mainly test two methods.

REQUIREMENTS:

- Test tubes
- Durham's tubes
- Pipettes
- Conical flasks
- Measuring cylinders
- Lactose broth media
- Water sample
- Other microbiological lab equipments

PROCEDURE:

Prepare 100ml of single strength lactose broth media by following the medium composition.

PREPAEATION OF TUBES:

- Disperse 10ml of single strength lactose broth into each of the 10 test tubes.
- In a similar way, disperse 10ml of double strength lactose broth into each of the 5 test tubes.
- Introduce 1 durham's tube in an inverted position into each of the 15 test tubes with broth medium without any air bubbles in it.

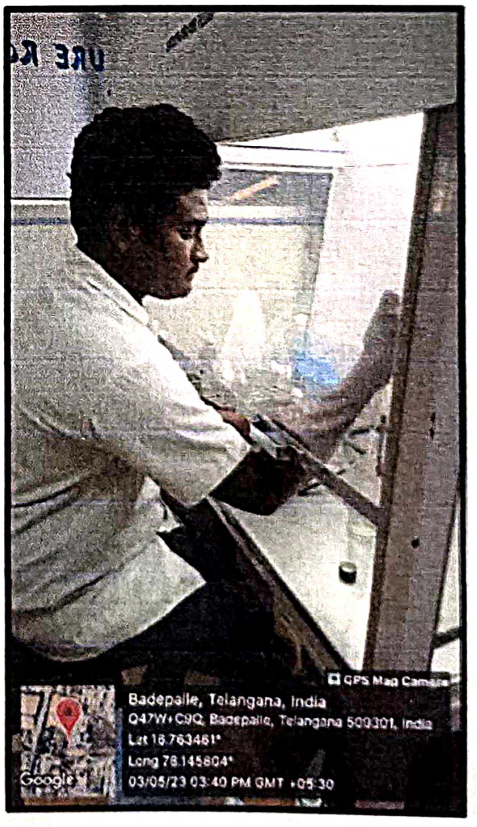
- Close the mouths of all the tubes with medium cotton plugs and sterilize in an autoclave.
- After sterilization, let the tubes cool in room temperature.
- Lable the 5 test tubes with DSLB as 10ml, 5 test tubes with SSLB as 1ml and the remaining 5 SSLB tubes as 0.1ml.

INOCULATION AND INCUBATION OF THE TUBES

- Inoculate all the tubes with water sample as per labelling, inoculate 5 test tubes of DSLB medium each 10 ml of water sample, and remaining 5 tubes each with 0.1 ml of water sample.
- Incubate all the inoculated tubes for 24 hrs at 37C in an electronically controlled water bath.

NOTE:

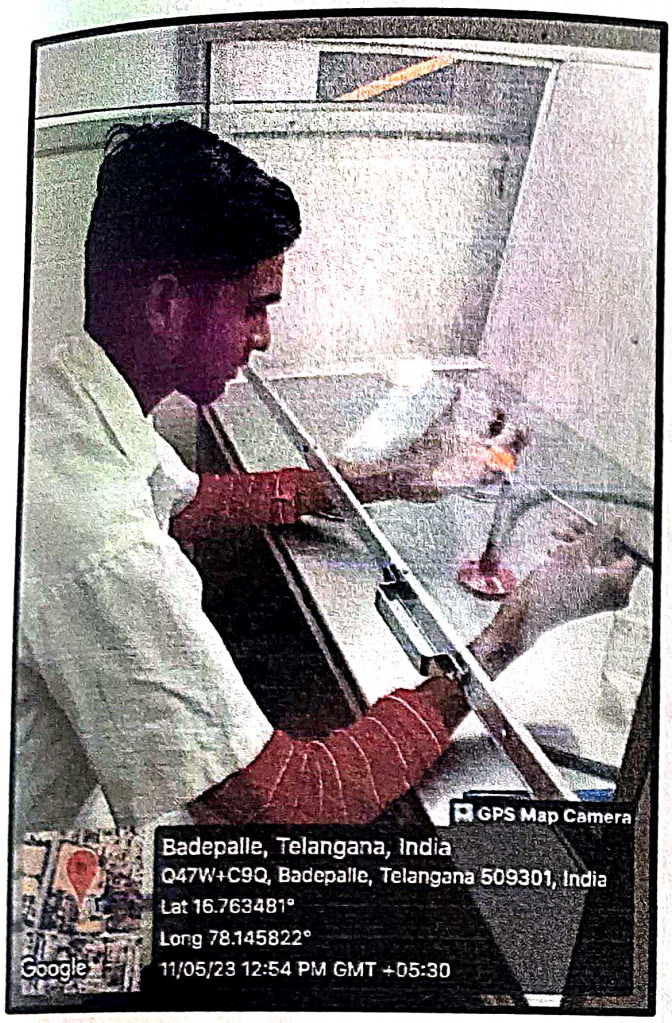
- All 5 samples are collected and done for MPN method and they are tested positive to gas and acid formation.



CONFIRMATIVE TEST

- Confirmative test is carried out for the sample produced gas and acid for further Media -Mac-conkey agar, EMB agar and XLD
- Plates-inoculated with samples tested positive
- Incubation – 24 to 48 hours

Observed colony formation and done Gram Staining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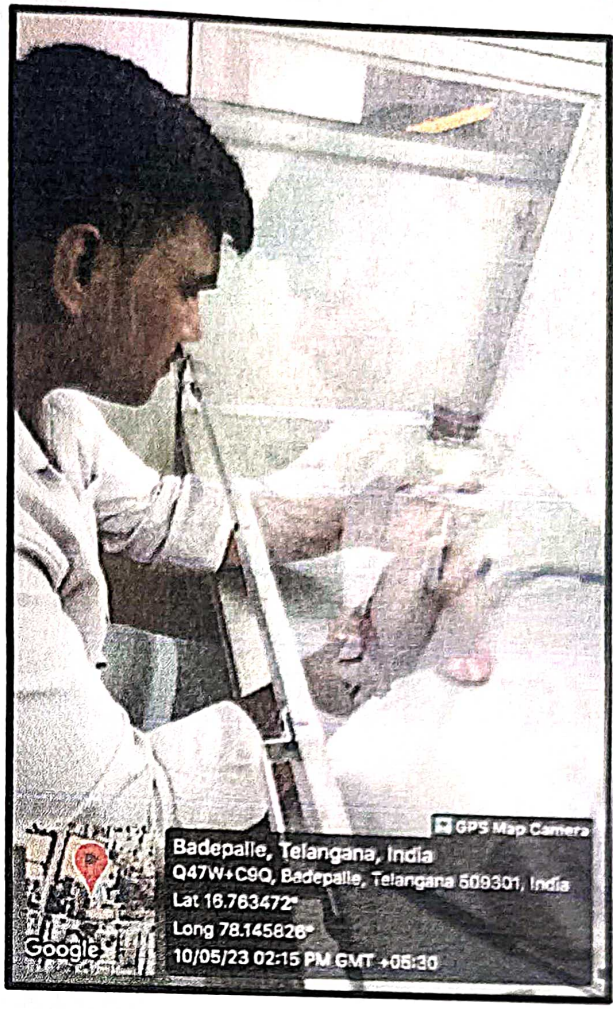
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GRAM'S IODINE

Biochemical tests

The culture are further tested for proper identification by doing following biochemical tests

1. Indole test
2. Methyl red test
3. Catalase test
4. Urease test

1. Indole Test

Principle:

Tryptophan is hydrolyzed by tryptophanase to produce three possible end products; indole, pyruvate, and ammonia. Indole production is detected by Kovac's or Ehrlich's reagent. Indole, if present, combines with the aldehyde in the reagent to produce a pink to red-violet quinoidal compound (if benzaldehyde reagent is used) or a blue to green color (if cinnamaldehyde reagent is used). The absence of enzyme results in no color production (i.e. indole negative).

Reagents:

Indole spot reagent :

- P –Dimethylaminocinamaldehyde (DMACA) 10.0 gm
- Hydrochloric Acid, 37% 100.0 ml
- Deionized Water 900.0 ml

Indole Kovacs Reagent :

- P –Dimethylaminocinamaldehyde 50.0 gm
- Hydrochloric Acid, 37% 250.0 ml
- Amyl alcohol 750.0 ml

Procedure:

Inoculate the tube of tryptone broth with a small amount of a pure culture. Incubate at 37°C for 24 to 48 hours. To test for indole production, add 5 drops of Kovác's reagent directly to the tube. A positive indole test is indicated by the formation of a pink to red color ("cherryred ring") in the reagent layer on top of the medium within seconds of adding the reagent. If a culture is indole negative, the reagent layer will remain yellow or be slightly cloudy.

Indole positive bacteria : E. coli, Vibrio cholera Indole negative bacteria : Klebsiella, Salmonella, Shigella sp.

2. Methyl red test [MR]

Principle:

Some bacteria have the ability to utilize glucose and convert it to a stable acid like lactic acid, acetic acid or formic acid as the end product. These bacteria initially metabolize glucose to pyruvic acid, which is further metabolized through the 'mixed acid pathway to produce the stable acid. The type of acid produced differs from species to species and depends on the specific enzymatic pathways present in the bacteria. The acid so produced decreases the pH to 4.5 or below, which is indicated by a change in the color of methyl red from yellow to red.

In the methyl red test (MR test), the test bacteria is grown in a broth medium containing glucose. If the bacteria has the ability to utilize glucose with production of a stable acid, the colour of the methyl red changes from yellow to red, when added into the broth culture.

The mixed acid pathway gives 4 mol of acidic products (mainly lactic and acetic acid), 1 mol of neutral fermentation product (ethanol), 1 mol of CO_2, and 1 mol of H_2 per mol of glucose fermented. The large quantity of acids produced causes a significant decrease in the pH of the culture medium.

Reagents

MRVP broth (pH 6.9)

Ingredients per liter of deionizer water:

Buffered peptone= 7.0 gm

Glucose= 5.0 gm

Dipotassium phosphate= 5.0 gm

Methyl red solution, 0.02%

Dissolve 0.1 g of methyl red in 300 ml of ethyl alcohol, 95%.

Add sufficient distilled water to make 500 ml.

Store at 4 to 8 degree C in a brown bottle. Solution is stable for 1 year.

Procedure

Prior to inoculation, allow medium to equilibrate to room temperature.

Using organisms taken from an 18-24 hour pure culture, lightly inoculate the medium.

Incubate aerobically at 37 degrees C. for 24 hours.

Following 24 hours of incubation, aliquot 1ml of the broth to a clean test tube.

Reincubate the remaining broth for an additional 24 hours.

Add 2 to 3 drops of methyl red indicator to aliquot.

Observe for red color immediately.

3. Catalase Test

Principle:

The enzyme Catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide, and the rapid elaboration of oxygen bubbles occurs. The lack of Catalase is evident by a lack of or weak bubble production. The culture should not be more than 24 hours old.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2 \text{ (gas bubbles)}$$

Bacteria thereby protect themselves from the lethal effect of Hydrogen peroxide which is accumulated as an end product of aerobic carbohydrate metabolism.

Reagents:

- 3% hydrogen peroxide

Procedure:

Place a microscope slide inside a Petri dish. Keep the Petri dish cover available. Using a sterile inoculating loop or wooden applicator stick, collect a small amount of organism from a well-isolated 18- to 24-hour colony and place it onto the microscope slide. Be careful not to pick up any agar. This is particularly important if the colony isolate was grown on agar containing red blood cells. Carryover of red blood cells into the test may result in a false-positive reaction. Using a dropper or Pasteur pipette, place 1 drop of 3% H2O2 onto the organism on the microscope slide. Do not mix. Immediately cover the petri dish with a 125 Bacterial Identification Tests MICROBIOLOGY MODULE Microbiology Notes lid to limit aerosols and observe for immediate bubble formation (O2 + water =

bubbles). Observing for the formation of bubbles against a dark background enhances readability.

4. Urease test

Principle:

Urea is the product of decarboxylation of **amino acids**. Hydrolysis of **urea** produces **ammonia** and **CO2**. The formation of **ammonia** alkalinizes the medium, and the pH shift is detected by the color change of **phenol red** from **light orange** at pH 6.8 to **magenta (pink)** at pH 8.1. Rapid urease-positive organisms turn the entire medium **pink** within 24 hours. *Weakly positive organisms may take several days, and negative organisms produce* **no color change** *or* **yellow** *as a result of* **acid production**.

$$(NH_2)_2CO + 2\,H_2O \xrightarrow{\text{Urease}} CO_2 + H_2O + 2\,NH_3$$

Urea — Carbon dioxide — Water — Ammonia

Procedure:

Christensen's Urea Agar (4, 5) Use a heavy inoculums from an 18- to 24-hour pure culture to streak the entire slant surface. Do not stab the butt as it will serve as a color control. Incubate tubes with loosened caps at 35oC. Observe the slant for a color change at 6 hours, 24 hours, and every day for up to 6 days. Urease production is indicated by a bright pink (fuchsia) color on the slant that may extend into the butt. Note that any degree of pink is considered a positive reaction. Prolonged incubation may result in a false-positive test due to hydrolysis of proteins in the medium. To eliminate protein hydrolysis as the cause of a positive test, a control medium lacking urea should be used. Rapidly urease-positive Protease (Proteus spp., Morganella morganii, and some Providencia stuartii strains) will produce a strong positive reaction within 1 to 6 hours of incubation. Delayed-positive organisms (e.g., Klebsiella or Enterobacter) will typically produce a weak positive reaction on the slant after 6 hours, but the reaction will intensify and spread to the butt on prolonged incubation (up to 6 days). The culture medium will remain a yellowish color if the organism is urease negative.

Physical examination results:

COLOUR: Colour less.

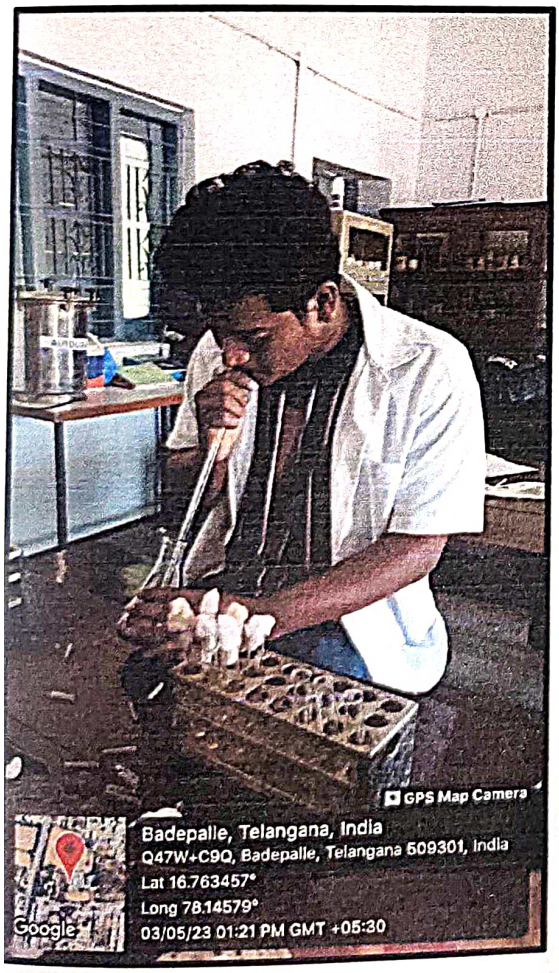
PH: 7

Odour: - No Smell

BOD RESULTS :

Sample: 1

S.No	DO of first day (D_1)	DO of After s5days (D_2)
1.	5.4	6.5
BOD ($D_2 - D_1$)	**6.5-5.4= 1.1 mg/L**	

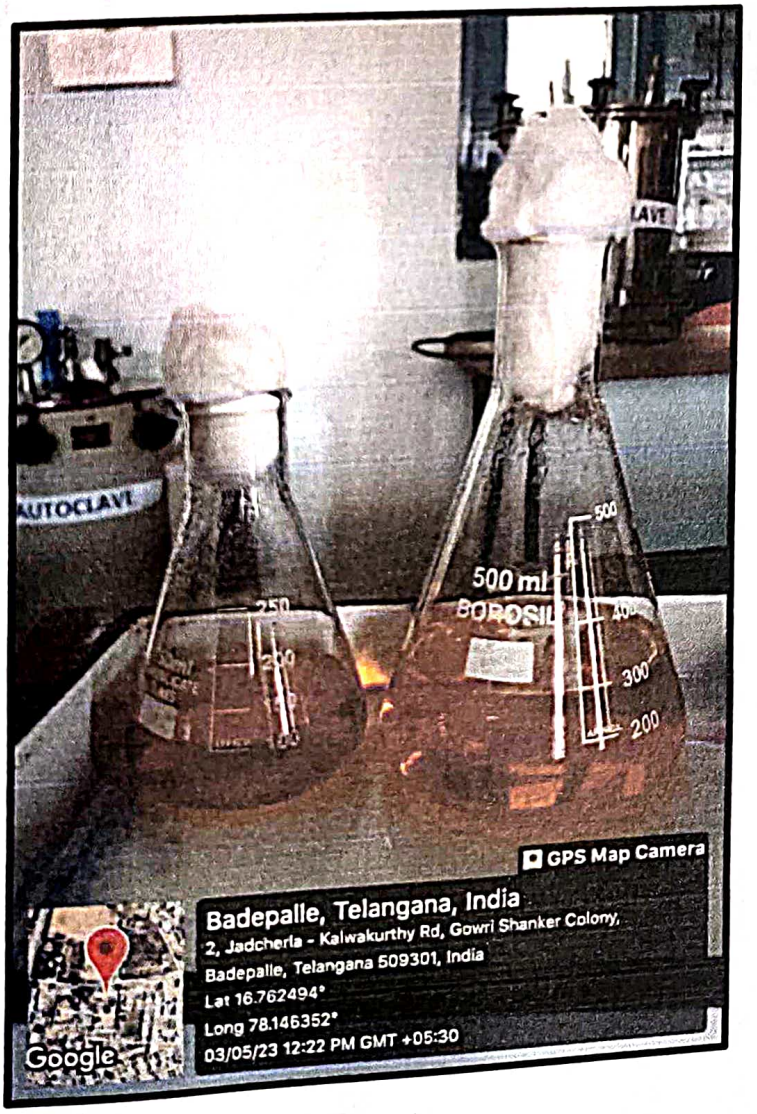


ample - 2

S.NO	DO of first day (D_1)	DO of After 5 days (D_2)
1.	6.1	9.4
BOD ($D_2 - D_1$)	**9.4-6.1=3.3 mg/L**	

Sample - 3

S.NO	DO of first day (D_1)	DO of After 5 days (D_2)
1.	7.3	9.6
BOD ($D_2 - D_1$)	**9.6-7.3=2.3mg/L**	



Sample - 4

S.NO	DO of first day (D_1)	DO of After 5 days (D_2)
1.	6.2	10.1
BOD ($D_2 - D_1$)	**10.1-6.2= 3.9mg/L**	

MPN Results:

Sample:1

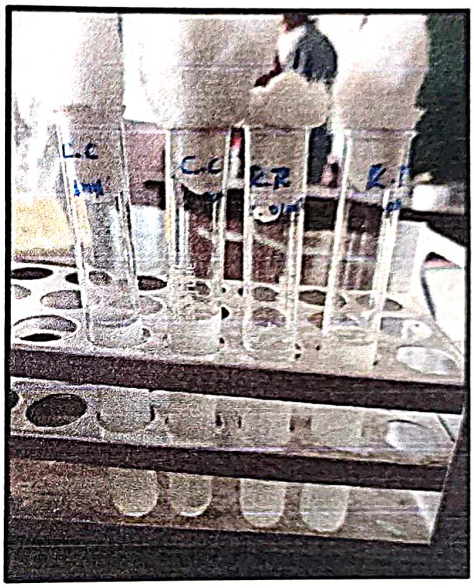
S.S (0.1)ml	S.S (1)ml	D.S (10) ml	MPN Method index (Upper value)
0	0	1	11

Sample: 2

S.S (0.1)ml	S.S (1)ml	D.S (10)ml	MPN Method index (Upper value)
0	1	2	18

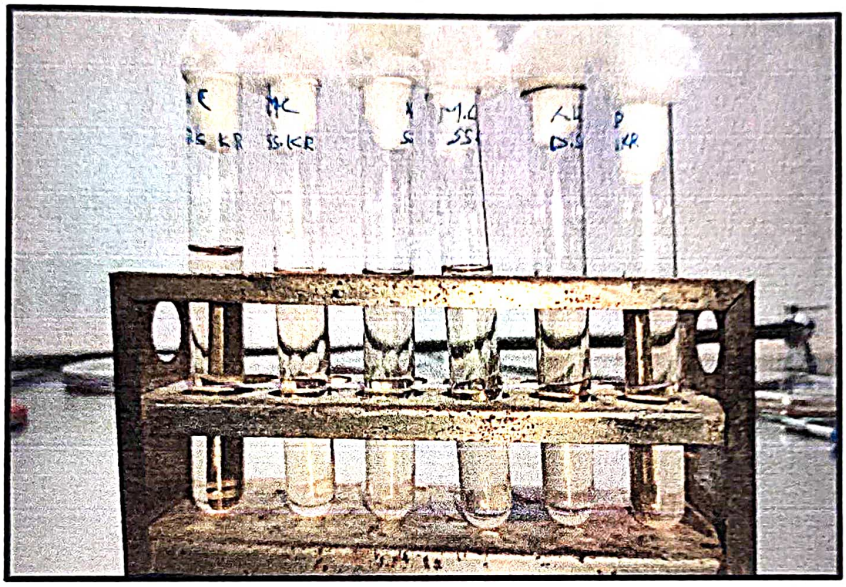
Sample: 3

S.S(0.1)ml	S.S (1)ml	D.S (10)ml	MPN Method index (Upper value)
0	1	2	18



Sample: 4

S.S(0.1)ml	S.S(1)ml	D.S(10)ml	MPN Method index (Upper value)
0	0	2	17



MEDIA AND CULTURES

SAMPLE 1 : [DR.BRR GOVT DEGREE COLLEGE]

MEDIA ;XLD,DS,CC

SAMPLE 2 ; [BALAJI NAGAR COLONY]

MEDIA; XLD,SS,KR

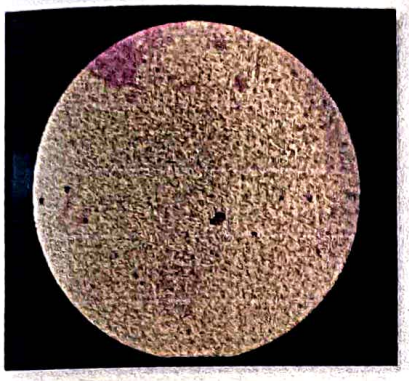
SAMPLE 3 ;[KRISHNA REDDY COLONY]

MEDIA;XLD,DS,KR

SAMPLE 4 ; [GOURI SHANKAR COLONY]

MEDIA; MC,DS,KR

GRAM STAINING

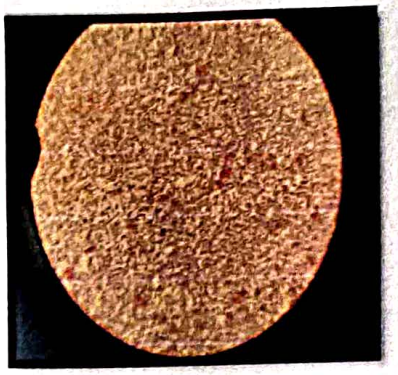


Sample :1 (Dr BRR Govt Dregree college) colony)

Name:Gram positive Salmonella

Shape: Round

Colour : Pink



Sample :2 (Gouri Shankar

Name ;Gram positive Shigilla

Shape :Rod

Colour :Red

Sample :3 (Sai nagar colony)

Name : Gram negative Steptococcous

Shape :Rod

Colour : Violet

Sample :4 (Gangapur Road)

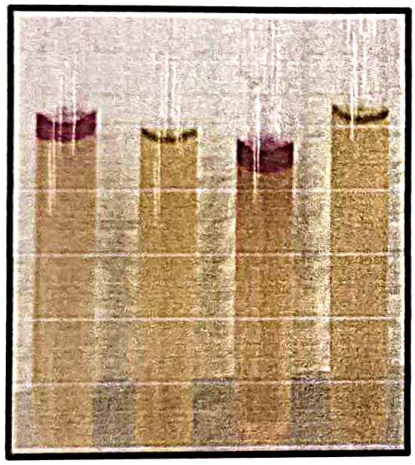
Name :Gram positive E -coli

Shape :Round

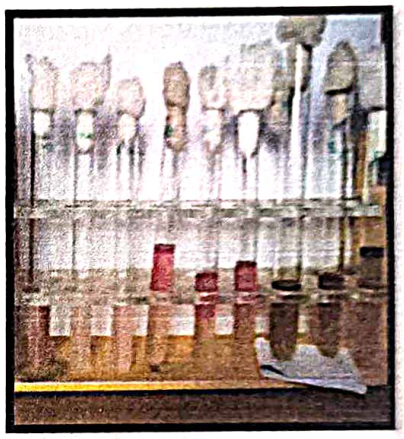
Colour :Pink

BIO CHEMICAL RESULTS:

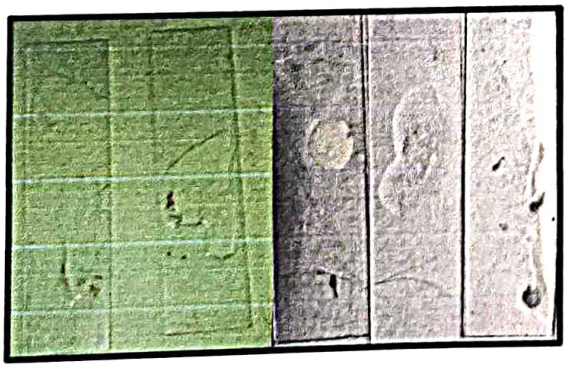
S.No	Name of the bacteria	Indole test	Methyl red test [MR	Catalase test	Urease test
1	Escheriachia coli	+	+	-	-
2	Salmonella	+	+	+	-
3	Shigella	+	-	+	-



Indole test



Methyl red test



Catalase test

Urease test

OBSERVATIONS & RESULTS

- All five bore water samples are tested and observed and are tested with presence of pathogenic bacteria
- Among five, potential pathogens are Identified are ***E. coli, Salmonella, shigella***

CONCLUSION

- Hence Bore water supplies is mild unsafe
- May cause Pathogenic bacteria infections and waterborne diseases.
- With this we have come to conclusion that bore water for all is one of the major challenges of the 21st century and that microbiological control of bore water should be the norm everywhere.
- Routine basic microbiological analysis of bore water should be carried out by assaying the presence of pathogenic bacteria.
- Submit the same report to municipal authorities for further extension of measures.
- We will extend our testing to other institutes and aware on safe consumption of BORE WATER

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