Microbial investigation through Isolation and identification of water bodies from selected hotels of Indore city

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ABSTRACT

The goal of this study is to use the MPN and IMViC Test to identify any Microorganism that may be present in water bodies. Water samples from the affected areas, where there is a high likelihood that raw or waste water will mix with water bodies, were taken for this study. Coliforms, which may be harmful in nature and are the cause of illnesses including cholera, dysentery, TB, and others in people and domestic animals, were checked for using MPN testing. In few hotels from Indore, microorganisms were isolated from the drinking and tap waters. The harmful bacteria can result in cholera, dysentery, typhoid fever, gastrointestinal infections, and other ailments like E. coli. The findings of a microbiological examination performed on several waters are discussed in the report. It was discovered that the water samples from the various hotels in Indore had large contaminants, severe degradation, and noteworthy diversity. In the Microbiological, analysis, coliform group of bacteria are differentiated by the Most Probable Number (MPN) test. After performing these tests Gram negative bacteria, were rod shaped coliform bacterium and non-spore forming found in water sample, having pink colonies on EMB agar indicated negative result. The isolates was identified as E.Coli, Enterobacteria, klebsiella, Salmonella are Gram negative bacteria etc. Therefore this work is recommended on the need to control the microorganisms' contamination of water bodies.

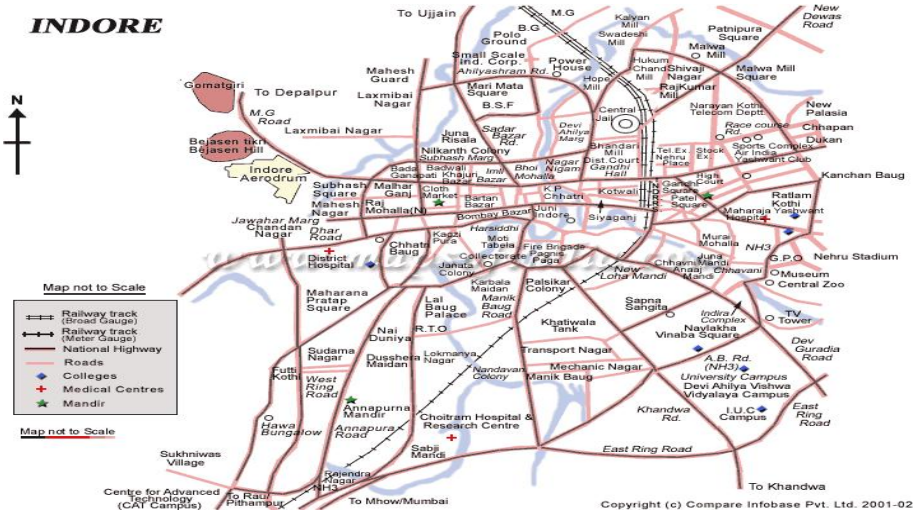
Keywords: Microorganisms, Contamination, Gram negative Bacteria, Most Probable Number, IMViC

1. INTRODUCTION

One of the most fundamental needs of every living thing on earth is water. Water was needed for human everyday activities. The usage of it by plants, animals, microbes, and people is exceedingly effective. For the specific reason that man cannot survive without water, no one bacterium has been found in the microbial world to be active at the severe scarcity of water; it is crucial to check tap water supply. [1].Water too plays an important role in human life. All living organisms required organic and inorganic compounds for their growth, repair, maintenance and reproduction[2]. Water is the molecule rich various inorganic compounds such as water (H_2O) and the hydrochloric acid (HCL), vital to living organisms [3]. Although statistics vary, as the World Health Organization (WHO) [4] reports that approximately 36% of urban and 65% of rural Indian's were without access to safe drinking water WHO Press,[4].Tap water can be recycled and used for domestics and for drinking also. One technique for determining if water is safe to drink is to look for signs of sickness. Bacteria are dispersed in water borne illnesses by sewage that has not been properly or adequately cleaned [5]. Typhoid fever, cholera, and bacillary dysentery are only a few of the many water-borne illnesses. Water availability has become a serious and urgent issue in many developing nations, which is of significant worry to families and communities who rely on non-public water delivery systems [6].Increase in human population exerts an enormous pressure on the provision of safe drinking water especially in developing countries [7].

Water in nature is sometimes totally pure. Water is contaminated as it drops to earth, the combustion of fossil fuel put sulphur compound responsible for acid rainfall in the air. Water that moves below the ground's surface undergoes filtration that removes most organisms 8]. For this logic, water from springs is generally of good quality. The most dangerous organisms found in water like Escherichia coli enter the water supply. When contamination drinking water *E.coli* can cause many diseases [9]. Pathogens example are *Salmonella spp, Vibrio cholera* and *E. coli* found in water samples. Indicator organisms are commonly used to check the microbiological quality of surface waters. faecal coliforms are the most commonly used bacterial indicator of faecal impurity (South Africa, 1998).The microbiological quality of drinking water is of monitoring must be given highest lead.[10] Extent investigations have find Microbiological analysis of domestic water of selected hotels of Indore city. As several disease outbreaks to low treated water containing bacterial pathogens that have been isolated from surface water in various sources as related.

2. STUDY AREA



3. MATERIAL AND METHODS

Collection of samples

Sampling followed the guidelines set forth by the American Public Health Association. 02 Water samples were gathered for microbial examination. Water samples were taken in sterile borosilicate glass containers. Tap water and drinking water samples were collected from a few hotels in Indore, Madhya Pradesh.

Most probable number (MPN) Test

Use the Most Probable Number (MPN) method to determine how many faecal coliforms are present in a water sample. If present, the microorganism fermented the lactose in the water sample to create acid, CO2, and gas. Three tests—the presumptive test, the confirmatory test, and the finished test—were conducted for the MPN. MacConkey broth was used as a presumptive test, BGLB (bright green lactose broth) was used as a confirming test, and EMB (eosin methylene blue) agar was used as the final test. The motility-indole-urease test, the methyl red-voges-proskauer (MRVP) test, and the citrate utilisation agar tests were performed on pure colonies of the isolates. The biochemical testing provided confirmation of the microorganism isolates (IMViC test).

i). Presumptive test: The first test, also known as the presumptive test, is a screening test that determines if coliform bacteria are present in a water sample. The water sample is inoculated into a number of lactose fermentation tubes. If the presumptive test returns a negative result, no additional testing is carried out since the water supply is considered microbiologically safe. However, if any tube in the sequence displays gas and acid, the water is considered dangerous, and the confirmatory test is carried out on the tube showing a positive result. The most likely number (MPN), or concentration of coliform organisms in the water sample, is another purpose of the presumptive test

Procedure: The presumptive test is identification coliform in a water sample
a) Make the mocConkey broth and divide it among three bottles.
b) Prepare three sets of test tubes, each containing five tubes. One set should be filled with double strength (DS), while the other two should be filled with single strength (SS).
c) Dhurum' s tube are added in bottle at Autoclave at 121°C for 15min.
d) Using sterile pipettes, take 15 tubes of mocConkey broth and label in 5 tubes of single strand inoculate 0.1ml sample of water, in 5 tubes of double strand inoculate 1ml sample of water, and in 1 tube of double strand inoculate 10ml water sample.
e) All contaminated tubes should be incubated at 37 degrees Celsius for 24-48 hours.
f) After incubation, observe the Durham's tube's gas production and the tubes' altered colour.
g) Presumptive coliform count per 100 ml of water sample may be determined by counting the positive findings from each set and compared them to the standard chart.

ii) **Confirmed test:**A second screening technique that uses a gram-negative selective medium is called the verified test (like EMB). This makes it possible to distinguish between colonies that don't include coliform, which gives off a green metallic shine.

Procedure:
a) Formulate BGLB (Brilliant green lactose broth).
b) Sterilized in autoclave at 121°C for 15mins
c) Inoculate the BGLB tubes with the inoculums from mocConkey broth positive presumptives test
d) Incubate at 37°c for 24 to 48 hrs

iii) Completed test: The test is completed on a typical, well-isolated colony to confirm lactose gas production and to identify the morphology of the isolate from a nutrient agar slant metabolic diversity

To perform IMVIC tests
The physiological features of bacteria from the Family Enterobacteriaceae, particularly *Escherichia* and *Enterobacter* produced from EMB colonies, are studied using biochemical IMViC tests. For the Indole test, Methyl Red and Voges Proskauer tests, and the Citrate test, the green sheen colony is inoculated into tryptone broth, MR VP broth, and a Simmons Citrate slant, respectively.

i) Indole test
The indole test detects between specific strains of bacteria that metabolize the amino acid tryptophan into indole, which increases in the medium and changes its pH. Tryptophanase is the name of the enzyme that is used in the procedure. This indole changes the broth's colour from yellow to cherry red when it interacts with Kovac's reagent. Due to the insolubility of the amyl alcohol included in the red colouring, an oily layer may be seen on the broth's top surface.

Procedure:

a) Fill a sterile test tube with 4 mL of tryptophan broth.
b) Aseptic conditions inoculate the tube with the growth from 18 to 24 hours culture.
c) Incubate the tube for 24-28 hours at 37°C.
d) To the broth culture, add 0.5 mL of Kovac's reagent.
e) Look for the presence or absence of a ring.

Methyl Red:

MRVP, a glucose-peptone medium including phosphate buffer, is used for both procedures. Each test is carried out independently once the bacteria have grown in this medium. Half of the culture is then placed into a different tube. A few drops of the dye methyl red are added to one portion of the culture to perform the Methyl Red test. Methyl red is yellow in neutral temperatures and red in acidic conditions (this is the reverse of the previously Phenol Red). A red colour indicates a positive test when there is enough acid production to exceed the phosphate buffer.

Procedure:

a) Allow the medium to equilibrate to room temperature before inoculating.
b) Carefully inoculate the medium with organisms from an 18-24 hour pure culture.
c) Incubate aerobically at 37° C for 24 hours.
d) After 24 hours, transfer 1ml of the broth to a clean test tube.
e) Re-incubate the remaining broth for another 24 hours.
f) To the sample, add 2 to 3 drops of methyl red indicator.
g) Immediately look for red.

Voges Proskauer reaction tests:

For the presence of acetoin, an intermediate in the fermentation of butane diol (acetyl methyl carbinol). This test must be performed within the first 24 hours of development; otherwise, butane diol will be produced, for which there is no simple colour test. After continuous shaking, the two Voges Proskauer reagents are introduced, and a successful test is the appearance of a wine-red colour. For a period of 10 to 20 minutes, the tube should regularly be stirred (to provide oxygen into the fluid) in place to enable the reaction

Procedure:

a) Bacterium is inoculated into glucose phosphate broth and cultured for at least 48 hours for testing.
b) The test broth is shaken with 0.6 ml of alpha-naphthol.
c) The broth is shaken with 0.2 ml of 40% KOH. Allow the tube to stand for 15 minutes.
d) The presence of red colour is considered a positive test.
e) The negative tubes must be retained for one hour since optimal colour development occurs within one hour of reagent addition.

Citrate Test:

The capacity to grow using citrate as the only carbon source. Brom-thymol blue, a dye that becomes yellow in acid and blue in alkaline solutions, is present in the medium. Half yellow and half blue make up the dye's green colour when the pH is neutral. There is no green; the green colour or unvaccinated medium is an optical illusion. As the organism utilises the citrate, sodium ions stay in the medium, making it basic. As a result, a blue colour appears. Citrate is introduced to the medium as sodium citrate.

Procedure:

a) Bacterial colonies are picked up with a straight wire, inoculated into Simmon's citrate agar slope, and incubated overnight at 37º C.
b) If the organism can use citrate, the medium changes colour from green to blue.

4. RESULTS AND DISCUSSION

Microorganisms were isolated from domestic water sample of selected hotels of Indore. The temperatures were taken for studies as 30, 40, 55 and 65°C. Growth rate decreased as the temperature was increase. The temperatures were taken for studies as 30, 40, 55 and 65°C The tube and plates were incubated at 37°C for 24 to 48 hrs. Lactose fermentation in the tubes was shown the pink colonies on EMB Plate present negative. Microorganism isolates that produced gas at 37°C, were found Gram negative, non-spore forming and rod-shaped bacteria. Different water samples using the MacConkey broth and high temperature 37°C incubation period, followed by plating for isolation on EMB and different agar plate. It shows combination of domestic water into drinking water and concludes that the selected hotel water samples in Indore is not suitable for kind of purpose and that should be monitored by Indore Municipal corporation day to day for reducing impurities, disease and hazards.

Presumptive test displayed (Table-1 & figure-1) Colour change in broth from purple to yellow *i,e* bacteria fermented lactose and produce acid and co_2. Production of acid and co_2 Show positive test and colour change in tubes from purple to yellow MPN of sample is (92/100ml).

Table 1: Presumptive test Colour Changes in selected water sample

Water Sample	Type of water	Media	Temperature Incubation time & temperature	observations			positive control	Negative control
				10ml	1ml	0.1ml		
AT 1	Tap Water	MocConkey Broth	37°C for 24-48 hours	4	3	4	Positive	Negative
	Drinking Water			-	-	1		
LD 2	Tap Water			-	-	-		
	Drinking Water			5	3	4		

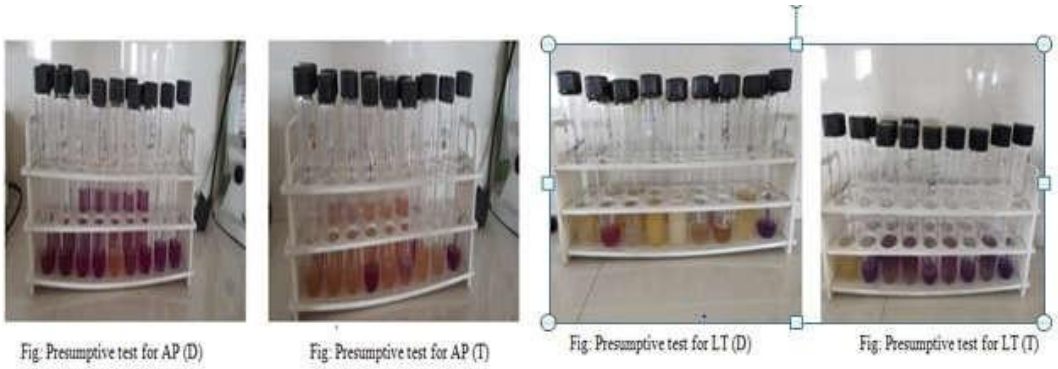


Figure 1: Presumptive test Colour Changes in selected water sample

Confirmation test-(Table 2 Figure 2 Gram staining reveals gram negative short rods after colour change in BGLB and development on NAM, XLDA, MSA, Cet. Mac, SCA, and EMB plates. The test is verified when the colour changes and microscopic observations reveal gram-negative short rods.

Table 2: Confirmation test showed growth in selected water sample

Water samples	Type of Water samples	Microbial Analysis					
		MLT (Microbial growth)					
		NAM	SCA	Cet.	Mac.	XLDA	EMB
AP 1	Drinking Water	+	+	+	+	+	+
	Tap Water	+	+	+	+	+	+
LT 2	Drinking Water	+	+	+	+	+	+
	Tap Water	+	+	+	+	+	+

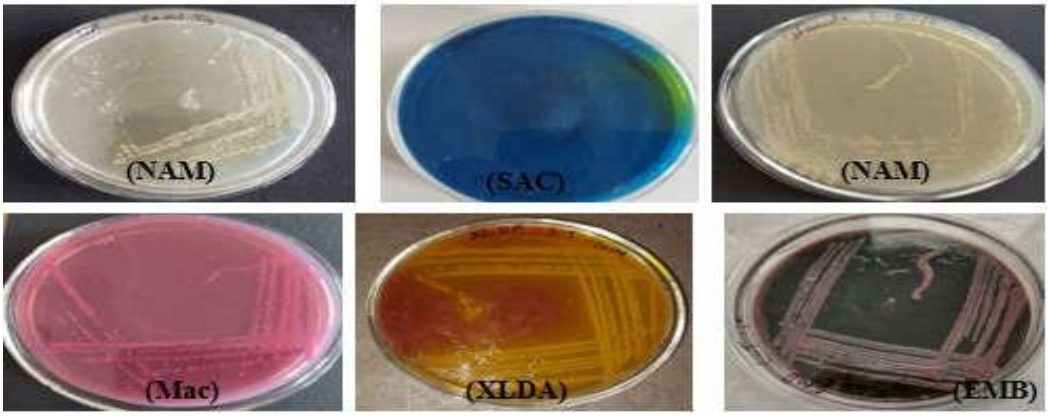


Figure 2: Confirmation test showed growth in selected water sample

Complete test: After gram-negative rods were stained, a pink colony was seen on the EMB plate. This indicates that the test was successful because there was no green metallic colony seen on the plate. Fig in Table No. 2 Media nutrient agar, Simmons citrate media, Cetrimide media, MacConkey agar media, XLDA agar and Eosin methylene blue

***IMViC* Results:** To get the results of these four tests—tryptone broth (indole test), methyl red - Voges Proskauer broth (MR-VP broth), and citrate—four test tubes were infected. The Enterobacteriaceae family members were identified and distinguished using IMViC testing. Also looked at the absence of E. coli and the medium Gram Negative Klebsiella pneumonia's appearance of green to blue colour in the Citrate Utilization Test.

Table 3: *IMViC* Result

S.No	Biochemical Testing	Results		
1	Indole Test	Appearance of yellow coloured ring	Absence of E.coli	(-)
2	Methly Red Test	Appearance of Yellow Coloured in methyl red.	Absence of E.coli	(-)
3	Voges Proskaur Test	Appearance of brownish coloures in Voges Proskaur.	Absence of E.coli	(-)
4	Citrate Utilization Test	Appearance of green colour or blue colour in the medium GreenNegative. Blue- Positive	Klebsiella pneumonia	(+)

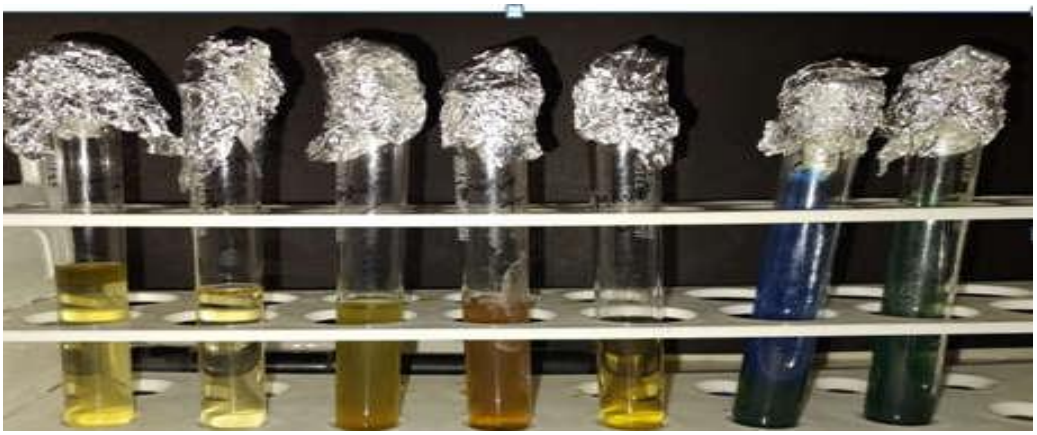


***Figure 3: IMVIC* Results**

5. CONCLUSION

This investigation came to the conclusion that additional manufacturing is required to improve the water quality distributed to a few Indore hotels and to reduce the quantity of microorganisms discharged into spread systems. Drinking contaminated water exposes people to many waterborne illnesses. Thus, water treatment and quality improvement are required before water may be used for drinking. Effective management and upkeep are advised in order to reduce the acute problem of water-related diseases that are local to human health for faecal contamination. Drinking water indicator organisms offer a highly sensitive means of estimating quality. Much work has to be done to raise awareness of the dangers of drinking polluted water and the best ways to do so. This examination also establishes the value of the study for the consumer or consumers who obtain their drinking water from separate sources. The study advised hotel management to utilize municipal corporation-supplied clean water for drinking and tab purposes. This would assist to prevent customer illnesses connected to contaminated water as well as harmful organisms in the water.

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