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Isolation and Biochemical Characterization of Bacteria in Surface Water of Ramsagar Pond Gaya

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Abstract: ***The major purpose of the current investigation is to identify the bacterial strains present in Ramsagar Pond water. Based on the morphological and biochemical investigation, it was discovered that the Ramsagar pond water contains pathogenic bacteria. The isolated bacteria were identified as E. Coli, K. pneumoniae, P. aeruginosa, and S. aureus. This contaminated water may harm the local population if used for residential purposes without sufficient treatment. The current study can help raise public awareness about the safety precautions and procedures that should be implemented to prevent contamination of water sources.***

Keywords: coliform, IMViC test, pathogenic bacteria, Gram's staining

1. Introduction

According to the World Health Organisation, disease-related water contamination kills over 3.4 million people each year [1]. According to UNICEF, over 4000 children die every day due to water poisoning. Water contamination affects both poor and developed countries. Thus, contamination of water resources with harmful germs that cause illness is a big worry around the world. Improving water quality is an important factor in reducing water-related disorders [2]. Water quality is typically defined using physical, chemical, and biological factors. Increasing population expansion, urbanisation, indiscriminate chemical use, and poor sewage discharge have all contributed to a decline in water quality [3]. Surface water is susceptible to bacterial contamination because it collects trash and pollution from humans and animals.

This contaminated water may put the local population's health at danger. Surface water is continually altered by human activity since it is utilised for swimming, tubing, surfing, and fishing, as well as being a repository for waste and waste water from the surrounding area [4].

Waterborne infections include typhoid fever, cholera, poliomyelitis, and viral hepatitis A and E. These pathogens enter water sources via faeces and sewage discharges. Bacteria and fungus dominate most aquatic environments. *Salmonella, Staphylococcus aureus, Pseudomonas aeruginosa, Clostridium botulinum, Vibrio cholerae,* and *Escherichia coli* are some of the most often detected bacteria. They serve a crucial role in trash degradation and recycling, but they are also harmful to humans.

The presence of coliform bacteria, specifically *E. coli*, in water bodies implies faecal pollution [5]. The current study has been done with all of the aforementioned factors in mind.

2. Materials and Methods

Study Area
Ramsagar Pond (24. 7735ºN and 85.0320° E) is a permanent manmade water body measuring 821.5 m2 with a mean depth of 2m. This pond had both floating and submerged aquatic weeds.

This pond was near densely populated areas.

Collection of Sample
The water sample was taken aseptically in a sterile bottle with a top. The bottle was branded with full information and stored in a hygienic environment.

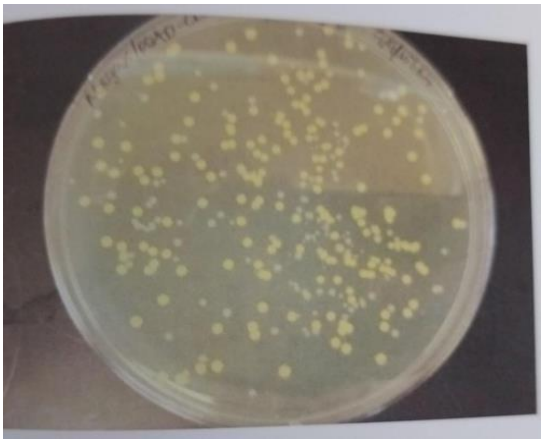
Isolation of bacteria from water sample
1mL of the collected water sample was taken and serially diluted with distilled water. The sample was serially diluted up to 10-5, and 100 uL of the diluted material was put on a nutrient agar plate after each dilatation.

Cultural and Mosphalagical Studies
The incubated plates reveal the colour and shape of the colonies. Observing the colony's perimeter and height revealed information about its shape. Gram's staining techniques were used to conduct morphological analyses.

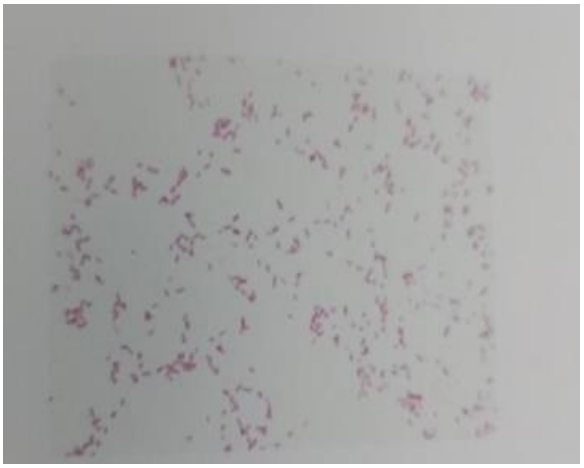
Biochemical Examination
Biochemical analysis was performed to determine the sorts of bacteria found in the study's water samples. Each isolate underwent six biochemical tests, including lactose fermentation, indole, methyl red, voges-proskauer, citrate utilisation, and catalase activity assays. These tests were performed using established procedures.

3. Result

In the current investigation, four (4) bacterial isolates were isolated by growing them on nutrient agar plates. The four isolates were identified as RSPW1, RSPW2, RSPW3, and RSPW4. The RSPW is an abbreviation for Ramsagar Pond Water.

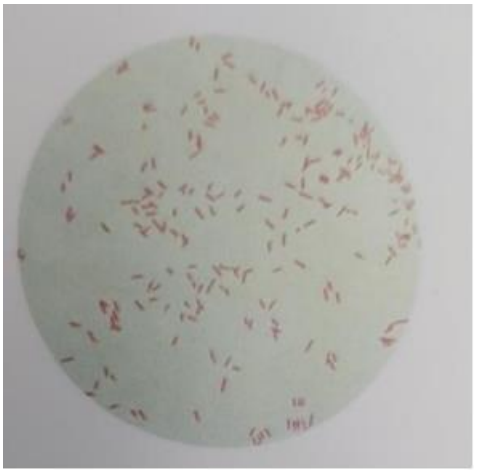


Culture of nutrient agar medium



Microscopic view of *K.pneumoniae*

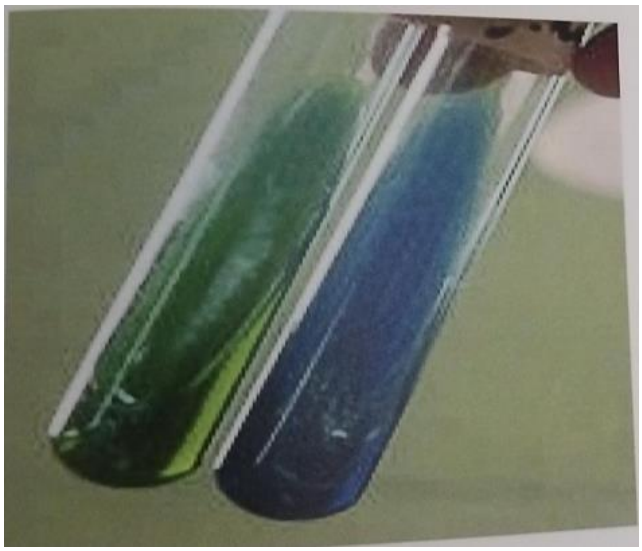
Microscopic view of *E.coli.*



Microscopic view of *P.aeruginosa*

IMViC test of *E.coli*

IMViC test of *K. pneumoniae*



Citrateutilisation test of *P. aeruginosa*

Morphological characterization of Bacteria:

The morphological properties of all four isolates' colonies, such as colour and nature, were determined in this study. Additionally, Gram's staining was used to evaluate all of the isolates. Table 1 shows the morphological characteristics of all four isolates.

Table 1: Morphological Characteristics of the isolates

Isolate No	color	nature of colony	Gram's staning	Test organism identified
RSPW1	Cream	Shiny,Mucoid,Entire	-ve,rod	E.Coli
RSPW2	White	Transparentmucoid,Flat	-ve,rod	K.pneumoniae
RSPW3	Light blue	Smooth,Flatmucoid	-ve,rod	P.aeruginosa
RSPW4	Light brown	Smooth,Flat,convex, circular	+ve,cocei	s. aureus

Out of the four isolates isolated from Ramsagar pond water three strains were Gram -negative rods and another one is Gram-positive cocci.

Biochemical characterization of bacteria isolated from Ramsagar pond water sample

In this investigation, four colonies were isolated from Ramsagar pond water samples. All the isolates were biochemically characterised (Table -2). RSPW1 isolates gave negative results to citrate and Voges-Proskauer tests. RSPW2 isolates showed negative results to indole and methyl red tests. Similarly, RSPW 3 isolates showed negative results to the indole, methyl red, VP, and lactose fermentation tests.

Table 2: Biochemical characteristics of the bacterial isolates

Name of the test	RSPW1	RSPW2	RSPW3	RSPW4
Catalase test	+	+	+	+
Indole test	+	-	-	-
Methylred test	+	-	-	+
Voges-Proskauer test	-	+	-	-
Citrate test	-	+	+	+
Lactose fermentation test	+	+	-	+

(+):Positive,(-): Negative.

The isolates RSPW4 Showed negative result for Indole test and VP test.

4. Discussion

In the current study, it was discovered that the Ramsager pond water is contaminated with various bacteria. Using Bergey's manual of determinative bacteriology, the bacteria isolated from Ramsager pond water, RSPW-4, were provisionally identified as *E. coli, K. pneumoniae, P. aeruginosa*, and *S. aureus* based on their morphological and biochemical characteristics. All bacteria found in the current

study are pathogenic. *E. Coli* and *K. Pneumoniae* were isolated in the current study, which was consistent with the findings of Panneerselvam A [6]. Gram negative bacilli were the most prevalent bacteria identified in the current investigation, which was consistent with Sengupta's findings [7].

E. Coli is a commensal bacterium that lives in the gastrointestinal tracts of warm-blooded animals, including humans, without producing disease except in immunocompromised hosts [8]. However, certain *E. Coli* gained virulence factors encoded on mobile genetic elements such as bacteriophages, plasmids, and transposons via horizontal gene transfer and became pathogenic. These pathogenic *E. coli* bacteria cause diarrhoea, urinary tract infections, sepsis, and newborn meningitis [9]. *K. pneumoniae*, the primary cause of hospital-acquired infections in the United States, has traditionally been regarded as an opportunistic pathogen since it often infects immunocompromised persons. *K. pneumoniae* causes severe infections such as pneumonia, UTIs, and bloodstream infections (10].

P. aeruginosa plays an important role in burn wound infections, accounting for roughly one-third of all burn-related infections. In animals, *P. aeruginosa* can cause a variety of dermatological illnesses, including hot spots and otitis infections [1]. *P. aeruginosa* ear infections can cause a painful illness known as otitis externa. This infection frequently occurs after being exposed to polluted water, such as in swimming pools or hot tubs [12]. *P. aeruginosa* catheter-related UTIs have significant clinical consequences since they are associated with Pyelonephritis. *P. aeruginosa* causes approximately 10% catheter-associated UTIS (CAUTI) and up to 16% UTIs in ICU patients [13].

S. aureus is a global concern in clinical care. Despite extensive research and development, no vaccine against *S. aureus* has been authorised. *S. aureus* can cause a variety of ailments, including miner's skin infections like pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, and abscesses, as well as potentially fatal diseases including pneumonia, meningitis, bacteremia, and sepsis. It is still one of the five most prevalent causes of hospital-acquired infections, and it contributes to wound infections after surgery [14].

5. Conclusion

The study concluded that Ramsagar pond water is contaminated by pathogenic microorganisms, particularly Gram-negative rods. If this water is used for residential purposes without sufficient treatment, it might cause serious sickness. The current inquiry also raises awareness in society about the extent of contamination and how damaging it is to humans living near Ramsagar Pond.

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