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Isolation And Evaluation Of Antibiotics Susceptibility Of Environmental Bacterial Isolate

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ABSTRACT

Antibiotic resistance is a growing global issue because some bacteria have developed the ability to survive even when antibiotics are used. This makes infections more difficult to cure. Natural environments like soil and water can act as reservoirs where these resistant bacteria and their resistance traits exist and spread.

In this study, samples were taken from different environmental sources and bacteria were grown in the laboratory using basic microbiological methods. Techniques such as serial dilution were used to reduce the number of bacteria in a sample, making it easier to isolate individual types. The bacteria were then cultured on different growth media to obtain pure colonies. After isolation, the bacteria were identified by examining their shape, size, and appearance, along with performing biochemical tests to understand their characteristics..

To determine how the bacteria respond to antibiotics, the disk diffusion technique was performed. In this method, bacteria are spread evenly on a growth medium, and small discs containing different antibiotics are placed on the surface. During incubation, the antibiotics diffuse into the medium and interact with the bacteria.

After some time, the areas around the discs are examined. If a clear region appears around a disc, it means the bacteria are sensitive to that antibiotic. If there is little or no clear area, the bacteria are considered resistant. The results showed a mix of responses: some bacteria were affected by the antibiotics, while others were not. Notably, certain bacteria were resistant to several antibiotics at once, indicating multidrug resistance, which can make infections more difficult to treat..

The findings of this study indicate that bacteria present in the environment can play a major role in the spread of antibiotic resistance. This shows that resistance is not only a problem in hospitals but can also develop in natural surroundings such as soil and water. For this reason, it is important to study environmental bacteria to better understand how resistance begins and spreads.

To examine how these bacteria react to antibiotics, the disk diffusion method was used. In this process, small discs containing antibiotics are placed on a surface where bacteria are growing. The effectiveness of each antibiotic is judged by whether it can stop bacterial growth around the disc. The results showed that different bacteria responded in different ways. Some were easily controlled by antibiotics, while others were less affected or not affected at all. A few bacteria were resistant to several antibiotics at the same time, making them more difficult to treat.

These results suggest that environmental bacteria are not just passive organisms but actively contribute to the development and spread of antibiotic resistance. Therefore, regular study and monitoring of these bacteria are necessary. It also highlights the importance of using antibiotics carefully, managing waste properly, and maintaining good environmental practices to reduce the spread of resistance and protect human health..

Key Words: Bacteria, Antibiotics, environment, Environment bacteria, Resistant bacteria, Public health

INTRODUCTION

Microorganisms exist in almost all natural habitats, especially in soil and water. They are essential for maintaining ecological balance by breaking down waste materials, returning nutrients to the environment, and supporting various life processes. Among them, bacteria are very diverse and can be both helpful and harmful, which makes them important in nature as well as in human health.

In recent years, the increase in bacteria that are no longer affected by antibiotics has become a serious global issue. This makes infections harder to treat and control. Earlier, this problem was mainly linked to hospitals, but it is now known that natural environments also play a key role. Soil, water, and other surroundings can act as storage sites where resistant bacteria and their resistance traits are maintained and spread over time. Bacteria found in the environment can develop resistance either naturally over time or through exposure to antibiotics and pollutants released from human activities, such as farming, industrial discharge, and improper disposal of medicines. These resistant bacteria can pass their resistance traits to other bacteria, including those that cause diseases, which increase the spread of antibiotic resistance. To study this issue, bacteria are collected from environmental samples and isolated using laboratory methods like dilution and culturing to obtain pure colonies. These isolates are then identified through their physical appearance and biochemical behaviour. Their response to antibiotics is tested using standard techniques to determine whether they are sensitive or resistant. Such studies are important for understanding how antibiotic resistance develops and spreads outside clinical settings, and they help in creating better strategies for controlling resistance, promoting responsible antibiotic use, and protecting both public health and the environment.

Bacteria in the environment can develop resistance naturally over time through genetic changes, or they can acquire it when exposed to antibiotics and other harmful chemicals present in their surroundings. Human actions play a major role in this process. For instance, antibiotics used in farming, release of untreated industrial waste, and improper disposal of medicines all add antibiotic residues to soil and water. These residues create conditions that promote the survival and spread of bacteria that are resistant, allowing them to persist in the environment and potentially reach humans and animals..

Once bacteria become resistant, they can pass these resistance traits to other bacteria using different genetic methods. This means that even bacteria that are normally harmless in the environment can give resistance genes to harmful bacteria that cause disease. This gene transfer accelerates the spread of resistance, making it a serious public health concern. Because of this, it is important to study bacteria in natural environments to understand how antibiotic resistance starts and spreads outside of hospitals and clinics.

To study environmental bacteria, scientists first collect samples from sources like soil and water. These samples are then handled using standard laboratory techniques. One common method, serial dilution, reduces the concentration of microorganisms so that individual bacterial cells can grow into separate colonies. These colonies are subsequently grown on appropriate growth media to obtain pure bacterial isolates, which are essential for studying the characteristics of each type accurately.

Once the bacteria have been isolated, they are identified by examining both their physical traits and their biochemical behaviour. Features such as the shape, size, colour, and texture of the colonies give initial clues about the type of bacteria. Biochemical tests are then used to study their metabolic activities, which helps confirm their exact identity. This process is important because it allows scientists to know which bacterial species are present in the environment and understand their potential roles.

The next stage is to examine how the isolated bacteria react to different antibiotics. A common method for this is the disk diffusion test. In this technique, small discs containing specific antibiotics are placed on a surface where bacteria are growing. As the antibiotics spread into the surrounding area, their ability to stop bacterial growth can be observed. Based on the results, bacteria are categorized as sensitive (easily affected), moderately resistant (partially affected), or resistant (not affected) to the antibiotics..

Studies like this are important for monitoring antibiotic resistance in natural environments. They allow scientists to understand how resistance emerges and moves beyond clinical settings, such as hospitals. The results emphasize the importance of using antibiotics responsibly, managing waste properly, and taking steps to protect the environment. These measures help limit the spread of resistant bacteria and safeguard public health.

COLLECTING OF WATER SAMPLE PHOTOS

Fig 1: Foul Water

Foul water is wastewater produced from household activities, including toilets, sinks, baths, and laundry. It contains substances such as human waste, food scraps, grease, soaps, and detergents, which make it heavily contaminated. Due to these pollutants, foul water can be harmful to both human health and the environment if it is not treated or disposed of properly. Unlike surface water, such as rainwater or river water, foul water is much more polluted and requires careful management..

Managing foul water correctly is crucial for preventing diseases and protecting water sources. Foul water is usually transported through a network of sewers to wastewater treatment plants. At these plants, it goes through a combination of physical, chemical, and biological treatments that remove contaminants, reduce harmful substances, and eliminate disease-causing microorganisms. This treatment ensures the water is safer before it is released back into rivers, lakes, or groundwater. If foul water is not treated, it can pollute natural water bodies and pose serious health and environmental risks.

In most cities, foul water is carried through sewer systems that are separate from storm water drains, though older systems sometimes combine the two. Keeping these sewer systems in good condition through regular maintenance is important to prevent blockages, leaks, and overflows. People can also contribute by not throwing unsuitable materials, like plastics, oils, or other waste, into drains. Proper management of foul water is essential for protecting public health, preserving the environment, and ensuring access to clean water.

Fig 2: Impure Water

Impure water is water that contains contaminants such as soil particles, chemicals, microorganisms, or dissolved minerals, which make it unsafe for drinking or other purposes. These impurities can come from natural sources, like soil and rocks, or from human activities, including industrial discharge, sewage, and runoff from farms. Contaminated water may look, smell, or taste unpleasant and can be harmful to human health if consumed. Here's a fully original explanation:

Impurities in water can cause a range of health issues. Harmful microorganisms, such as bacteria, viruses, and parasites, can lead to illnesses like diarrhea, cholera, and typhoid. Chemical contaminants, including pesticides or heavy metals, may have serious long-term effects on health. For these reasons, it is essential to make sure that water is clean and properly treated before it is used for drinking or other purposes.

To make contaminated water safe for use, it needs to be treated through purification processes. Common ways to clean water include filtering out particles, boiling to kill microorganisms, adding

disinfectants like chlorine, or using advanced methods such as reverse osmosis. These treatments remove harmful substances and improve the quality of the water. Providing access to safe, clean water is crucial for health, daily activities, and preventing the spread of waterborne illnesses in communities.

LITERATURE REVIEW

1. Introduction

Soil and water contain many types of microorganism, especially bacteria. These bacteria can be helpful, harmless, or harmful. Scientist are now paying attention to them because some bacteria are becoming resistant to antibiotics. This means the medicines used to treat infections may stop working.

Antibiotics used in hospitals, farming, and animal production often end up in the environment through waste and runoff. When this happens, it creates conditions that allow resistant bacteria to grow and spread. That is why studying a bacterium in the environment is very important.

2. Distribution of Bacteria in soil and water

Bacteria are found everywhere in soil and water. Soil usually contains a large number of bacteria because it has nutrients and organic matter that support their growth. Water sources like rivers, lakes, and groundwater also contain many bacteria. Some of these bacteria occur naturally, while others come from human activities. These bacteria can survive for a long time and adjust to difficult conditions, including exposure to antibiotics.

3. Sources of Bacterial Contamination

3.1 Human-Related Sources

Many bacteria enter soil and water because of human activities. These include:

- Sewage disposal
- Wastewater from hospitals and industries
- Agricultural activities like the use of manure and fertilizers.
- Urban and industrial runoff

These sources not only introduce bacteria but also bring antibiotics into the environment, which encourages resistance.

3.2 Natural Sources

Some bacteria naturally live in soil and water. They help in important processes like breaking down organic matter and recycling nutrients. However, even these natural bacteria can develop resistance over time.

4. Isolation and Identification of Bacteria

- Dilute the samples
- Grow bacteria on special media
- Allow them to grow under controlled conditions

After growth, the bacteria are identified by observing their appearance and performing laboratory tests. Advanced methods like genetic analysis are also used for more accurate identification.

5. Antibiotics Susceptibility Testing

After isolation bacteria, scientists test how they react to antibiotics.

Disk Diffusion Method

Antibiotic discs are placed on bacteria, and the area where bacteria cannot grow (clear zone) shows how effective the antibiotic is.

Minimum Inhibitory Concentration (MIC)

This method finds the smallest amount of antibiotic needed to stop bacterial growth.

Molecular Methods

These methods detect specific genes that make bacteria resistant to antibiotics.

6. Patterns of Antibiotic Resistance

Many environmental bacteria are resistant to more than one antibiotic(multidrug resistance). This happens because:

- They are constantly exposed to small amounts of antibiotics.
- They can share resistance genes with other bacteria
- They adapt to harsh environmental conditions.

7. Public and Environmental Health

Antibiotics-resistant bacteria in soil and water can affect human health. They can spread through:

- Drinking contaminated water
- Eating contaminated food
- Direct contact with the environment

This can lead to infections that are difficult to treat. It can also harm ecosystems and agriculture. That is why this issue is considered important for human, animal, and environmental health together.

8. Research Gaps (Explanation)

There are still some areas that need more sturdy:

- Not enough data from developing countries
- Lack of long term monitoring
- No standard methods for testing environmental bacteria
- Limited understanding of how resistance spreads in nature.

9. Conclusion

Studying bacteria from soil and water and testing their response to antibiotics is very important. These environments act as storage places for resistant bacteria, which can spread to humans and animals. To control this problem, better waste management, regular monitoring, and careful use of antibiotics are needed.

METHODS AND MATERIAL

Studying how environmental bacteria respond to antibiotics involves two main steps.

1. Isolation of bacteria from environmental samples – This step involves collecting samples from places like soil, water, or other natural environments and separating individual bacterial species. Techniques such as dilution and culturing on specific growth media are used to obtain pure bacterial colonies for study.

2. Assessment of antibiotic susceptibility or resistance – Once the bacteria are isolated, their response to different antibiotics is tested. Standard methods, like the disk diffusion test, help determine whether bacteria are sensitive, partially resistant, or fully resistant. This step provides important information about how resistance develops and spreads in environmental bacteria.

Below is a simple, step-by-step overview of the methods most often used:

This introduces a structured explanation of the standard procedures or techniques, showing each stage in order to make the process easy to understand. It helps readers follow how experiments or analyses are carried out in a logical sequence.

1. Isolation of Environmental Bacterial Isolate.

A. Sample Collection

* Sources: Environmental water sources such as rivers, lakes, ponds, wells, or wastewater.

* Sterile Handling: Collect water using clean, sterile containers and equipment to prevent contamination.

* Storage: If the water cannot be processed immediately, keep the samples refrigerated at around 4°C to preserve the bacteria until analysis.

This ensures that the collected water accurately represents the bacterial community present in the environment.

B. Serial Dilution and Plating

* Serial Dilution: Gradually dilute the water sample in steps (for example, from 10^{-1} to 10^{-6}) to reduce the number of bacteria. This makes it easier to isolate individual colonies.

* Plating: Spread the diluted samples onto different types of growth media:

* **NUTRIENT AGAR for general bacterial growth.**

* SELECTIVE MEDIA like MacConkey agar to specifically grow Gram-negative bacteria.

* Incubation: Keep the plates at an appropriate temperature (usually between 28°C and 37°C depending on the sample source) to allow bacteria to grow into visible colonies.

This process helps obtain well-isolated colonies that can be studied further for identification and antibiotic susceptibility.

C. Pure Culture Isolation

* Selecting Colonies: Choose individual, well-separated colonies from the growth plates.

* Streaking: Transfer these colonies onto fresh agar plates using techniques like quadrant streaking to separate individual bacterial cells and allow them to form new colonies.

* Confirming Purity: Check the resulting colonies under a microscope and observe their physical characteristics, such as shape, size, and colour, to ensure that only a single type of bacterium is present.

This step is essential to obtain pure bacterial cultures for accurate identification and further testing.

2. Identification of Isolates

Physical (morphological) features: Bacteria are first identified by looking at their visible characteristics, such as the shape and colour of the colonies. Gram staining is also performed to group them into Gram-positive or Gram-negative types based on their cell wall structure.

Biochemical characteristics: Further identification is done by testing how the bacteria behave chemically. Tests like catalase and oxidase show whether certain enzymes are present, while IMViC tests help distinguish between different types of bacteria based on their metabolic reactions.

- **Together, these observations help determine the identity of the bacteria.**

*** Molecular methods:** These techniques identify bacteria by studying their genetic material. One of the most reliable approaches is 16S rRNA gene sequencing, which analyses a specific gene present in all bacteria. Because this gene has both common and unique regions, it helps accurately determine the type of bacteria and is considered a standard method for identification.

*** PCR-based identification:** Polymerase Chain Reaction (PCR) is used to amplify specific DNA sequences of bacteria. By targeting particular genes, PCR helps detect and identify bacterial species quickly and accurately, even when only a small amount of DNA is present.

These molecular techniques provide precise results and are often used to confirm findings from other identification methods.

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3. Antibiotic Susceptibility Testing (AST)

A. Disk Diffusion Method (Kirby–Bauer Test)

This is one of the most commonly used techniques to check how bacteria respond to antibiotics.

Procedure:

A uniform layer of bacteria is first spread over the surface of Mueller-Hinton agar.

Small paper discs containing different antibiotics are then placed on the agar.

- The plate is incubated for about 16–24 hours to allow bacterial growth and antibiotic action.

- **Observation:**

After incubation, clear areas may appear around some discs where bacterial growth has been stopped. These are called zones of inhibition.

Interpretation:

- The size of these clear zones is measured and compared with standard guidelines (such as CLSI or EUCAST) to determine whether the bacteria are sensitive, intermediate, or resistant to each antibiotic.
- This method helps in selecting effective antibiotics and understanding resistance patterns.

B. Broth Dilution Method

Used to determine **MIC (Minimum Inhibitory Concentration)**

- **Macro/microdilution**
- Serial antibiotic concentrations in broth
- Observe bacterial growth
- MIC = lowest concentration preventing visible growth

C. E-test (Gradient Method)

- Plastic strip with antibiotic gradient
- Gives precise MIC value
- Easy but more expensive

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4. Studying Evolution of Antibiotic Susceptibility (Resistance Development)

This is the key part for your topic.

A. Serial Passage Experiment

- Grow bacteria in sub-inhibitory antibiotic concentrations
- Gradually increase antibiotic levels over time
- Monitor changes in MIC

 Mimics natural selection and resistance evolution

B. Adaptive Laboratory Evolution (ALE)

- Long-term culturing under controlled antibiotic stress
- Periodic sampling
- Track phenotypic and genotypic changes

C. Mutation Frequency Assay

- Plate bacteria on high antibiotic concentrations
- Count resistant colonies
- Estimate mutation rates

D. Whole Genome Sequencing (WGS)

- Compare:
  - Initial strain vs evolved strain
- Identify:
  - Resistance genes
  - Mutations (e.g., efflux pumps, target modification)

E. Horizontal Gene Transfer Studies

- Detect resistance via:
  - **Plasmids**
  - **Transposons**
- Methods:
  - Conjugation assays
  - Plasmid isolation

5. Detection of Resistance Mechanisms

- **Enzyme production** (e.g., β-lactamases)
- **Efflux pump activity assays**
- **Target modification analysis**
- PCR for resistance genes (e.g., bla, tet, mecA)

6. Data Analysis

- MIC changes over time
- Growth curves under antibiotic stress
- Statistical comparison of susceptibility profiles

Key Considerations

- Use proper controls (sensitive reference strains)
- Maintain aseptic techniques
- Follow biosafety guidelines
- Replicate experiments for reliability

Summary Flow

1. Collect environmental sample
2. Isolate pure bacterial cultures
3. Identify isolates
4. Test baseline antibiotic susceptibility
5. Apply selective pressure (antibiotics)
6. Track evolution (MIC, genetics, phenotype)

MATERIALS REQUIRED:

Here's a **clear, structured list of materials required** specifically for **isolation and experimental evolution of antibiotic susceptibility in environmental bacterial isolates**. I've grouped them so you can easily use this in a lab record or report.

MATERIALS REQUIRED

1. Sample Collection Materials

- Sterile sampling containers (falcon tubes, bottles)
- Sterile spatula / scoop (for soil)
- Sterile swabs (for surfaces)
- Ice box or cooler (for transport, if needed)
- Labels and marker

2. Culture Media

General Growth Media

- Nutrient agar (NA)
- Nutrient broth (NB) or Luria–Bertani (LB) broth

Selective/Differential Media (optional)

- MacConkey agar
- Eosin Methylene Blue (EMB) agar

Susceptibility Testing Media

- Mueller–Hinton agar (MHA)
- Mueller–Hinton broth (for MIC testing)

3. Antibiotics

(Choose based on study design)

- Ampicillin
- Tetracycline
- Ciprofloxacin
- Chloramphenicol
- Kanamycin (optional)

Available as:

- Antibiotic discs (for disk diffusion)
- Powder/stock solutions (for MIC & evolution experiments)

4. Glassware & Plasticware

- Petri plates (sterile)
- Test tubes / culture tubes
- Conical flasks
- 96-well microtiter plates (for MIC)
- Micropipette tips (sterile)
- Spreaders (L-rod)
- Inoculating loops/needles

5. Equipment

- Incubator (30–37°C)
- Autoclave
- Laminar airflow / biosafety cabinet
- Micropipettes
- Vortex mixer
- Spectrophotometer (for OD600, optional but useful)
- Colony counter (optional)

6. Reagents & Solutions

- Sterile saline (0.85% NaCl)
- Distilled water
- Ethanol (70%) for sterilization
- Gram staining kit:
  - Crystal violet
  - Iodine
  - Safranin
  - Alcohol (decolorizer)

7. Storage Materials

- Glycerol (for preparing glycerol stocks, ~15–20%)
- Cryovials
- Freezer (-20°C or -80°C if available)

8. Antibiotic Susceptibility Testing Materials

For Disk Diffusion

- Antibiotic discs
- Forceps (sterile)
- McFarland turbidity standard (0.5)

For MIC Determination

- Antibiotic stock solutions
- Microdilution plates
- Plate reader (optional)

9. Materials for Evolution Experiment

- Broth media (large quantity)
- Antibiotic stock solutions (accurate concentrations)
- Sterile culture tubes/flasks for serial passaging
- Shaker/incubator (preferred for uniform growth)

10. Documentation & Analysis

- Lab notebook
- Marker pens
- Data sheets / computer with spreadsheet software

11. Safety Materials

- Gloves
- Lab coat
- Face mask
- Biohazard disposal bags
- Disinfectants (bleach, ethanol)

Below are some of the materials that were used for the experiment:

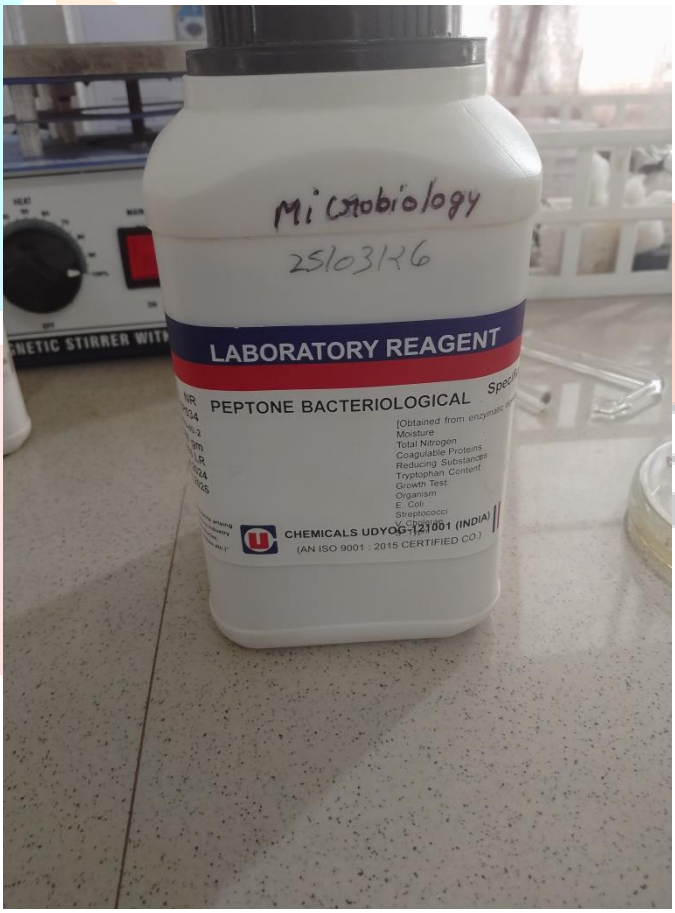


Fig 1: Peptone Bacteriological

Peptone (Bacteriological) is a nutrient-dense material derived from proteins that have been partially broken down through chemical or enzymatic methods. This process converts complex proteins into smaller, soluble molecules such as amino acids, short peptides, and other growth-promoting substances, which are easily absorbed and utilized by microorganisms. These components provide the essential building blocks required for the growth, metabolism, and reproduction of bacteria, fungi, and other microbial species.

Due to its high nutritional value, bacteriological peptone is widely employed in microbiology to prepare culture media, including both liquid broths and solid agars, creating an environment that consistently supports microbial growth under laboratory conditions. It serves as a reliable source of

nitrogen and other key nutrients, helping microorganisms thrive efficiently. Beyond laboratory use, peptone is also important in industrial microbiology, where it is utilized in fermentation processes to produce products such as enzymes, antibiotics, vitamins, and other microbial-derived compounds. Its combination of solubility, nutrient richness, and stability makes it a fundamental component in applications that require dependable microbial cultivation.

Fig 2: Agar Agar Powder

Agar agar powder is a plant-based substance extracted from certain types of red algae, making it completely suitable for vegetarian and vegan applications. It consists primarily of polysaccharides, a form of complex carbohydrate, which gives it the distinctive ability to form a firm gel when dissolved in hot water and cooled. This characteristic makes it highly versatile across various industries.

In the food sector, agar agar powder is widely used to thicken, stabilize, and set items such as jellies, puddings, custards, and other desserts. In laboratories, it acts as a dependable medium for cultivating bacteria, fungi, and other microorganisms because it creates a solid surface that most microbes cannot break down. Additionally, it finds use in pharmaceuticals, cosmetics, and other industrial processes that require a reliable, non-toxic gelling agent. Its natural origin, neutral taste and odor, and strong gelling properties contribute to its broad utility and make it a highly valued ingredient in both scientific and commercial contexts.

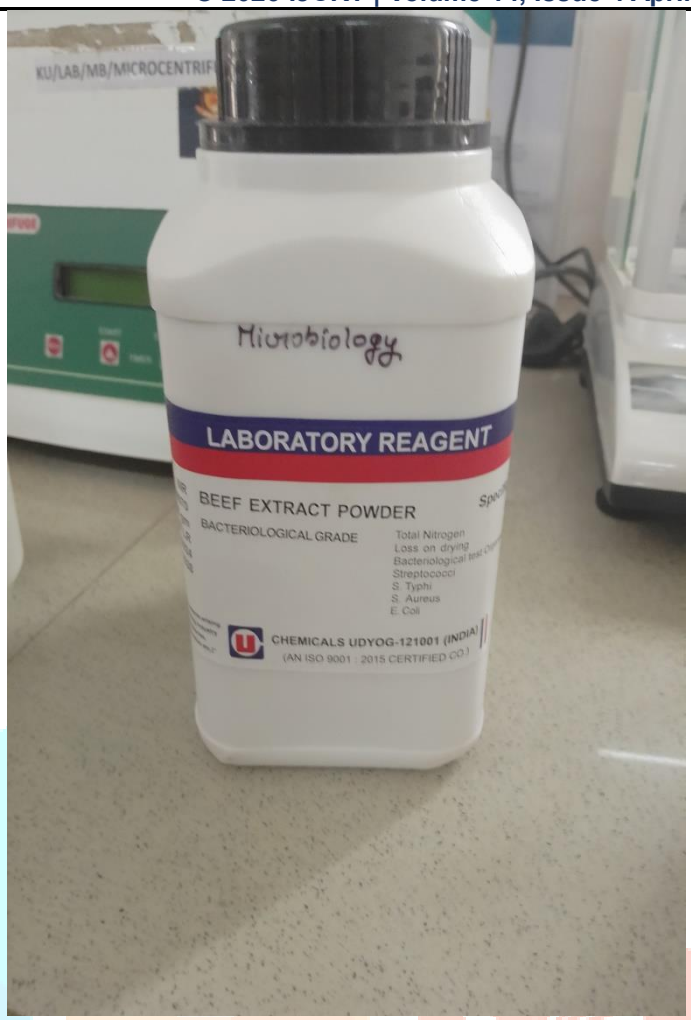


Fig 3: Beef Extract Powder

Beef extract powder is a processed form of meat in which the useful nutrients from beef are preserved in a concentrated and easy-to-use form. To make it, beef is first treated with water so that important soluble components—such as proteins, amino acids, vitamins, and minerals—are released into the liquid. The remaining solid parts are removed, leaving behind a nutrient-rich solution that is then thickened to increase its concentration.

This concentrated liquid is later dried using methods like evaporation or spray drying, which removes most of the water and turns it into a fine powder. Drying helps improve its shelf life and makes it more convenient to handle, store, and transport. Even after processing, the powder still contains much of the nutritional value of the original meat, which is why it is commonly used in laboratory culture media, food products for flavor enhancement, and other applications that require a dependable source of nutrients.

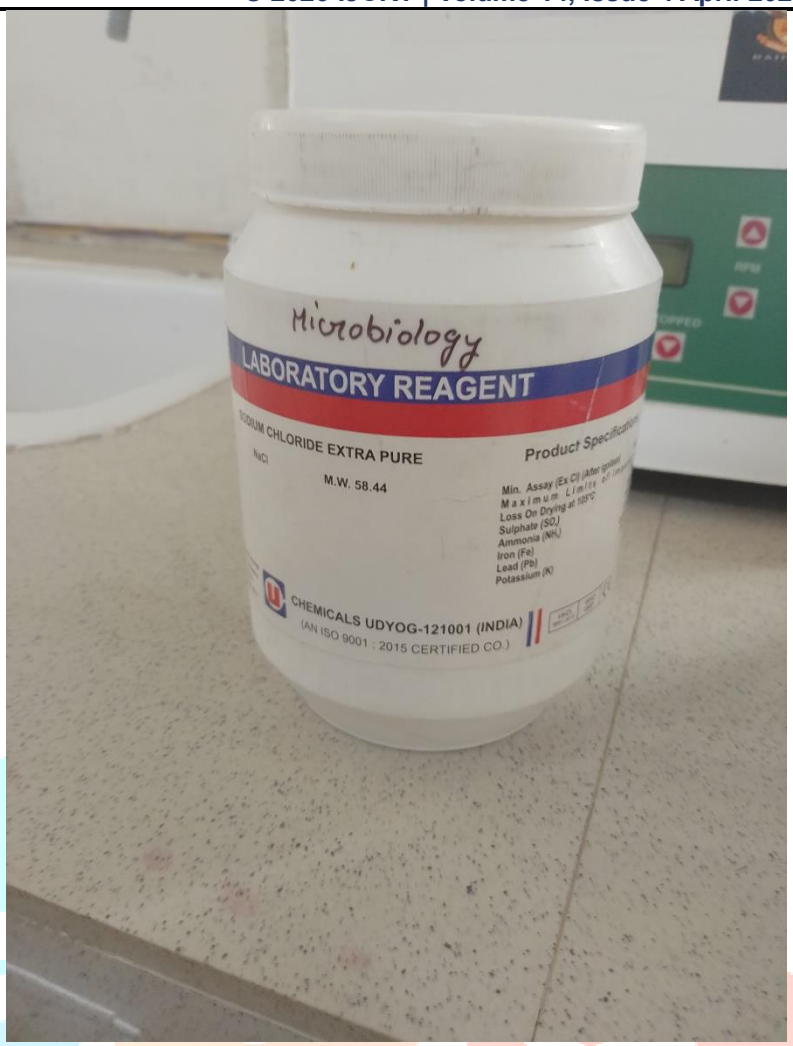


Fig 4: Sodium Chloride Extra Pure (Nacl)

Sodium Chloride Extra Pure (NaCl) is a highly purified version of ordinary salt that has been processed to remove nearly all unwanted substances. While everyday table salt may include added materials like iodine or agents that prevent clumping, this form is produced with great care so that it contains almost only sodium chloride, with very little contamination.

Due to this high level of purity and uniform composition, it is especially valuable in laboratory work. Scientists use it in experiments, chemical testing, and for preparing precise solutions, where even tiny impurities could interfere with accurate results. It is also important in certain industrial applications that demand consistent and dependable raw materials. The label “extra pure” indicates that the product meets strict quality standards, ensuring it can be used in situations where precision and minimal contaminations are critical.

Fig 5: Laminar Air Flow

Laminar airflow describes a situation in which a fluid moves in a very orderly and controlled way. The particles of the fluid do not move randomly or collide chaotically; instead, they travel in neat, well-defined paths. These paths form layers that stay separate from one another, with each layer sliding smoothly alongside the next without mixing.

In this type of flow, every layer maintains a steady speed and direction, which makes the movement easy to predict and stable over time. Because there is very little disturbance between the layers, the flow remains calm and consistent.

This is quite different from turbulent flow, where the motion is irregular and chaotic. In turbulence, the fluid forms swirls and eddies, causing different parts to mix together and creating constant changes in speed and direction.

RESULT AND DISCUSSION

Bacteria were successfully isolated from the water sample, as evidenced by visible growth on the agar plate. The isolate was subjected to the **Kirby–Bauer disk diffusion test** using four antibiotics: cefixime (CFM 5 µg), amoxicillin–clavulanate (AMC 30 µg), gentamicin (GEN 10 µg), and streptomycin (S 10 µg).

- A **distinct zone of inhibition** was observed around the **AMC (30 µg)** disc, indicating susceptibility.

- **Little to no zones of inhibition** were observed around **CFM (5 µg), GEN (10 µg), and S (10 µg)** discs, indicating resistance or low susceptibility.
- Bacterial growth was otherwise widespread across the plate, suggesting a viable and active isolate.

Summary of susceptibility:

- **Amoxicillin–clavulanate (AMC):** Sensitive
- **Cefixime (CFM):** Resistant
- **Gentamicin (GEN):** Resistant/Intermediate
- **Streptomycin (S):** Resistant

The findings show that the bacteria isolated from the water sample are **resistant to multiple antibiotics**, meaning they are not easily killed by several different drug types. This is known as multidrug resistance (MDR). Since the bacteria did not respond to at least three antibiotics tested, it suggests they have developed mechanisms to survive exposure to these drugs.

However, the bacteria were still affected by amoxicillin–clavulanate. This likely means they produce enzymes called **β-lactamases**, which normally break down certain antibiotics and make them ineffective. Clavulanic acid works by blocking these enzymes, allowing the antibiotic to function properly again.

The lack of response to cefixime, a cephalosporin antibiotic, may indicate more advanced resistance mechanisms. These could include enzymes similar to extended-spectrum β-lactamases (ESBLs), which can inactivate a wider range of antibiotics. Other possibilities include changes in the bacterial cell that prevent the drug from entering effectively, or systems that actively pump the antibiotic out of the cell.

Resistance to gentamicin and streptomycin suggests that the bacteria may also have ways to modify these drugs or alter their internal targets, such as ribosomes, so the antibiotics can no longer work.

Finding antibiotic-resistant bacteria in environmental water is a concern because it suggests contamination and the spread of resistance. This can happen due to:

- Waste from households or sewage entering water sources
- Agricultural runoff carrying antibiotics or resistant bacteria
- Transfer of resistance genes between different bacteria in the environment

Even though the exact type of bacteria was not identified, common water contaminants like those associated with Escherichia coli infection or other coliform bacteria often show similar resistance patterns. This is important because such bacteria can pose health risks if the water is used for drinking, cooking, or other domestic activities.

RESULE WITH TABLE FORMAT

Table 1: Visible colonies appeared on the **cultured agar plate**, showing that bacteria from the water sample were able to grow and had been successfully separated for study. This confirms that the isolation process worked. The isolated bacteria were then exposed to four different antibiotics using the **Kirby–Bauer disk diffusion method**, where antibiotic-impregnated discs are placed on the plate to observe how the bacteria respond to each drug.

Antibiotic	Disc Code	Zone of Inhibition (approx.)	Interpretation
Amoxicillin-clavulanate	AMC 30	Visible clear zone (~noticeable)	Sensitive
Cefixime	CMF 5	No/very small zone	Resistant
Gentamicin	GEN 10	Very small/unclear zone	Resistant/Intermediate
Streptomycin	S 10	No visible zone	Resistant

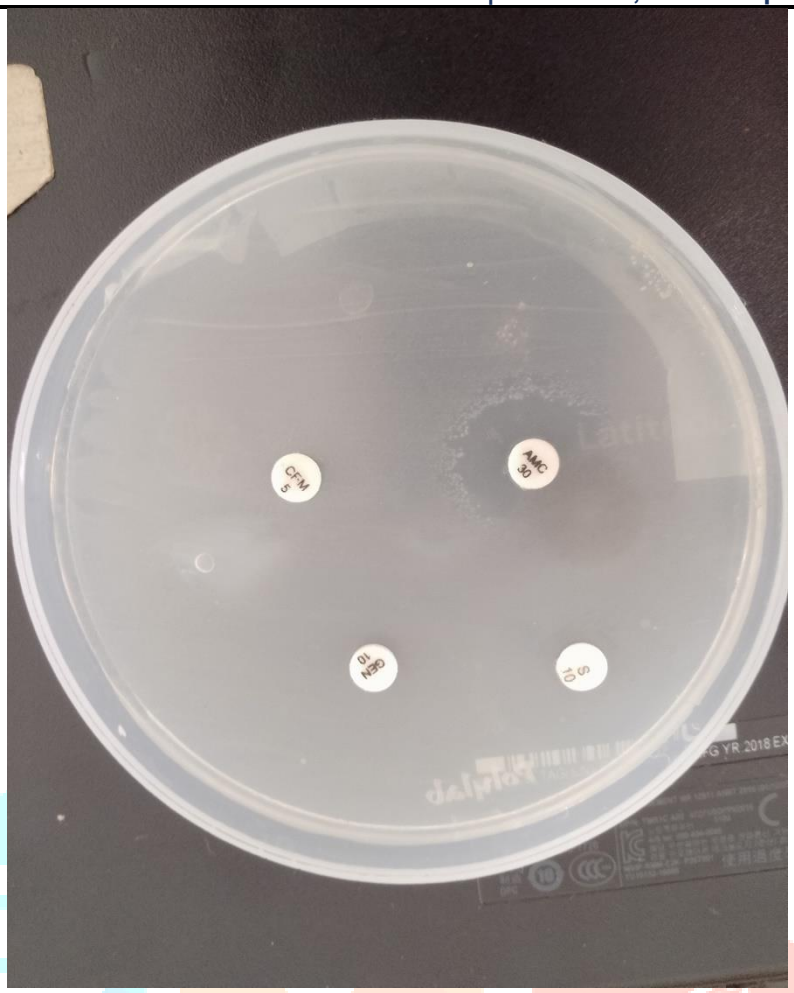
Note: Exact measurements in mm are recommended for standard interpretation.

The bacteria isolated from the water sample did not respond the same way to all the antibiotics tested, showing that their sensitivity varies depending on the drug. A noticeable clear area around the amoxicillin–clavulanate disc indicates that this antibiotic combination was effective in stopping bacterial growth. This may mean the bacteria produce β-lactamase enzymes that normally break down antibiotics, but in this case, clavulanic acid blocks those enzymes, allowing the drug to work.

On the other hand, the bacteria were not affected by cefixime, which suggests they have developed resistance to cephalosporin antibiotics. This resistance could be due to modified β-lactamase enzymes or structural changes in the bacterial cell that limit the entry of the drug. Similarly, the lack of effectiveness of gentamicin and streptomycin implies that the bacteria may inactivate these antibiotics or alter their internal targets, such as ribosomes, preventing the drugs from interfering with protein synthesis.

The detection of antibiotic-resistant bacteria in environmental water points to possible contamination from sources like sewage, farm runoff, or poor waste management. Such conditions can encourage the exchange of resistance genes between bacteria through processes like horizontal gene transfer, which can spread resistance traits widely.

Even though the exact bacterial species was not identified, microorganisms commonly found in polluted water—such as those associated with Escherichia coli infection—often display similar resistance patterns. This suggests that the bacteria may have adapted to environments where antibiotics or resistant strains are present.



- AMC **30 (amoxicillin-clavulanate)**: clear zone present → bacteria are **susceptible**
- CFM **5 (cefixime)**: little to no clear zone → likely **resistant**
- GEN **10 (gentamicin)**: minimal/unclear zone → possibly **resistant or intermediate**

- S **10 (streptomycin)**: little to no zone → **resistant**

CONCLUSION

The findings of this study demonstrate that the examined water sample harbors bacteria that are resistant to several commonly used antibiotics, while showing susceptibility only to amoxicillin–clavulanate. This pattern indicates the presence of **multidrug-resistant microorganisms**, which are capable of surviving exposure to multiple antimicrobial agents. Such resistance is particularly concerning because it limits treatment options if these bacteria are associated with infections. The detection of these organisms in water suggests that the source may be contaminated and not suitable for direct consumption without proper treatment.

The presence of antibiotic-resistant bacteria in environmental water poses a significant **public health risk**, as water is a major route through which humans can be exposed to harmful microorganisms. If consumed or used domestically, contaminated water may lead to infections that are more difficult to treat due to reduced antibiotic effectiveness. In some cases, these bacteria may also transfer their resistance traits to other microorganisms, including disease-causing species, further increasing the risk of widespread antimicrobial resistance.

This situation emphasizes the importance of implementing effective **water treatment methods**, such as filtration, chlorination, or boiling, to eliminate microbial contamination before use. Regular monitoring and microbiological testing of water sources are also necessary to detect contamination early and ensure safety standards are maintained. Additionally, the responsible and controlled use of antibiotics in healthcare, agriculture, and animal farming is crucial to reduce the selective pressure that drives the development and spread of resistant bacteria.

Overall, addressing antibiotic resistance in environmental water requires a combined effort involving proper sanitation, environmental management, and public awareness to safeguard both ecosystem health and human well-being.

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