DETECTION OF MULTIDRUG-RESISTANT ORGANISMS FROM WASHROOM ENVIRONMENTS.

A DISSERTATION SUBMITTED FOR PARTIAL FULFILLMENT OF THE DEGREE OF BACHELORS OF SCIENCE IN VOCATIONAL BIOTECHNOLOGY

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CERTIFICATE

This is to certify that the undersigned have assessed and evaluation to the research project entitled **"Detection of Multidrug- Resistant Organisms from Washroom Environments".** Submitted by **Miss. Urmila Chelaram Choudhary**. The project has been accepted for the partial fulfillment of the degree of Bachelor of Science in Vocational Biotechnology.

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Candidate's Declaration

I hereby declare, the project titled "Detection of multidrug-resistant organisms from washroom environment" was conducted under the guidance of Mr. Pranav Tambe at Abeda Inamdar Senior College.

I confirm that all sources of information used in this project have been acknowledged. The results presented are accurate, and ethical guidelines were followed throughout the study. I affirm that this project has not been submitted elsewhere.

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DEDICATION

This work is dedicated with all appreciation to my family and friends. Your unwavering support has been my back throughout this challenging journey of my project. Thank you for standing by me and believing in me every step of the way.

1. ABBREVIATIONS

- **MDR – Multidrug- resistant**
- **NA-Nutrient agar**
- **NB- Nutrient broth**
- **DW- Distilled water**
- **MH- Mueller Hinton**
- **MAC- MacConkey agar**
- **MR- Methyl red test**
- **VP- Vogus Prosker test**
- **TSI- Triple sugar iron test**
- **OD – Optical Density**
- **PBS – Phosphate- Buffered Saline**
- **LF - Lactose fermenting**
- **NLF - Non lactose fermenting**

ABSTRACT

1. ABSTRACT

Background :

Multidrug resistant organisms (MDROs) are microorganisms that have developed resistance to multiple antibiotics, making them difficult to treat with conventional antimicrobial therapies. The washroom environment is a common place where people come into contact with various microorganisms, including MDROs. In recent years, there has been a growing concern about the presence of MDROs in washroom environments, including toilets, sinks, and floors.

Objective:

The objective of studying multi-drug resistance organisms (MDROs) from washroom environments is to understand the presence, distribution, and transmission of MDROs in these environments, and to develop effective strategies to prevent and control the spread of these organisms.

Materials & Methods:

Outlines the experimental approach, which involves sample collection from washroom environment, processing and testing of the sample. In which Gram staining of isolated colonies was done to distinguish between the Gram positive and Gram negative bacteria. Also motility test was performed. After the basic procedures the biochemical characterization was done to identify and characterize the isolated organisms. After identification the Antibiotic susceptibility testing was done to determine the resistance and sensitivity of the identified organisms. The biofilm formation of the identified organisms was done. Based on results obtained from the above procedures the organism found to be MDR were further proceed for RT- PCR for genetic sequencing of the organisms.

Results:

The study successfully identified multidrug-resistant organisms (MDROs) in washroom environments, particularly from toilets, sinks, and floors. After isolating and characterizing the organisms using Gram staining, motility tests, and biochemical analysis, the identified organisms included *Pseudomonas spp.*, *Staphylococcus spp.*, and *Serratia spp*. Antibiotic susceptibility testing revealed varying resistance patterns, with some organisms showing significant resistance to common antibiotics. Biofilm formation was observed, suggesting the potential for increased persistence and transmission of these MDROs. Genetic analysis via RT-PCR is currently in process to further confirm the multidrug resistance traits of these organisms. Preliminary results indicate that *Pseudomonas spp.* and *Serratia spp.* are the primary MDROs present in the washroom environment, highlighting the need for improved hygiene and antimicrobial control measures in these high-risk areas.

Conclusion:

This study underscores the significant presence of multidrug-resistant organisms (MDROs) in washroom environments, highlighting a potential public health risk due to frequent human contact with contaminated surfaces. The isolation of *Pseudomonas spp.*, *Staphylococcus spp.*, and *Serratia spp.*, alongside their varying resistance profiles, suggests that common washroom areas may serve as reservoirs for these resistant pathogens. Biofilm formation further complicates the issue, as it enhances the persistence and spread of MDROs. While antibiotic susceptibility testing provides insight into the resistance patterns, the ongoing RT-PCR analysis will offer a deeper understanding of the genetic mechanisms behind this resistance. This research emphasizes the need for improved sanitation protocols and more effective antimicrobial strategies to limit the spread of these organisms in high-risk environments, such as public washrooms. Furthermore, regular monitoring and further studies are essential to track the dynamics of MDROs in such settings and to inform better hygiene practices.

Future research should focus on developing novel disinfectants or antimicrobial coatings specifically designed to target biofilm-forming MDROs in washroom environments. Additionally, exploring the genetic mechanisms of resistance through further RT-PCR studies could provide insights for developing targeted interventions to combat these pathogens effectively.

KeyWords:

Multidrug-resistant organisms (MDROs), washroom environment, antibiotic resistance, biofilm formation, Gram staining, motility test, biochemical characterization, RT-PCR, genetic sequencing, infection control.

INTRODUCTION

2. INTRODUCTION

2. INTRODUCTION

The washroom environment is a common place where people come into contact with various microorganisms, including bacteria, viruses, and fungi. Unfortunately, some of these microorganisms can be multi-drug resistant, posing a significant threat to public health. In this introduction, we will explore the concept of multi-drug resistance organisms (MDROs) and their presence in the washroom environment. [3]

What are Multi-Drug Resistance Organisms (MDROs)?

MDROs are microorganisms that have developed resistance to multiple antibiotics, making them difficult to treat with conventional antimicrobial therapies. These organisms can be bacteria, viruses, or fungi, and they can be found in various environments, including hospitals, communities, and even in the washroom environment.[1]

Why Should We Be Concerned?

Although we typically think of washrooms as places where we "freshen up" and take care of personal hygiene, they can also act as breeding grounds for harmful pathogens, including methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE), which are known for their resistance to antibiotics. [4]

These MDROs can spread through direct contact, like touching a contaminated surface, or indirect contact, such as when bacteria transfer from one surface to another. What's more, certain bacteria can even become airborne under the right conditions, which means they can spread to other areas within the washroom or beyond.[7]

Types of MDROs Found in Washroom Environment [5]

Several types of MDROs have been identified in the washroom environment, including:[9]

1. **Methicillin** - Resistant Staphylococcus aureus (MRSA): A type of bacteria that is resistant to methicillin and other antibiotics.

2. **Vancomycin**- Resistant Enterococci (VRE): A type of bacteria that is resistant to vancomycin and other antibiotics.

3. **Extended- Spectrum Beta-Lactamase (ESBL)-Producing Bacteria**: A type of bacteria that is resistant to multiple antibiotics, including penicillins, cephalosporins, and carbapenems.

4. **Carbapenem -Resistant Enterobacteriaceae (CRE):** A type of bacteria that is resistant to carbapenems and other antibiotics.

Sources of MDROs in Washroom Environment [11]

MDROs can be found in various sources in the washroom environment, including: [22]

1. Toilet Handles: Toilet handles can harbor MDROs, which can be transferred to hands and other surfaces.

2. Sinks: Sinks and faucets can be contaminated with MDROs, which can be spread through water and hands.

3. Floors: Floors can be contaminated with MDROs, which can be spread through foot traffic and cleaning activities.

4. Air: The air in washrooms can also be contaminated with MDROs, which can be spread through aerosolization and inhalation.

Transmission of MDROs in Washroom Environment[15]

MDROs can be transmitted in the washroom environment through various routes, including:[10]

1. **Direct Contact**: Direct contact with contaminated surfaces, hands, or other objects can transmit MDROs.

2. **Indirect Contact**: Indirect contact with contaminated surfaces, hands, or other objects can also transmit MDROs.

3. **Airborne Transmission**: MDROs can be transmitted through the air, particularly in areas with poor ventilation.

Prevention and Control of MDROs in Washroom Environment [17]

Preventing and controlling the spread of MDROs in the washroom environment requires a multi-faceted approach, including: [21]

1. Proper Hand Hygiene: Frequent hand washing and use of hand sanitizers can help prevent the spread of MDROs.

2. Cleaning and Disinfection: Regular cleaning and disinfection of surfaces, particularly high-touch areas, can help reduce the transmission of MDROs.

3. Proper Ventilation: Improving ventilation in washrooms can help reduce the transmission of MDROs through the air.

4. Education and Awareness: Educating users about the risks of MDROs and the importance of proper hygiene practices can help prevent the spread of these organisms.

Application of Multi-drug Resistant (MDR). [18]

The application of Multi-Drug Resistance (MDR) is a complex and multifaceted field that has significant implications for various aspects of healthcare, research, and public health. Here are some of the key applications of MDR: [23]

1. **Antibiotic Development**: Understanding the mechanisms of MDR is crucial for the development of new antibiotics that can effectively target and kill MDR bacteria. [8]

2. **Infection Control**: MDR has significant implications for infection control practices in healthcare settings, including the use of personal protective equipment, isolation protocols, and environmental cleaning and disinfection. [27]

3. **Public Health:** MDR has significant public health implications, including the potential for outbreaks and epidemics of MDR infections, and the need for surveillance and monitoring of MDR bacteria. [19]

4. **Clinical Practice**: MDR has significant implications for clinical practice, including the need for clinicians to be aware of the potential for MDR infections and to use appropriate diagnostic and treatment strategies. [13]

5. **Research**: MDR is an active area of research, with scientists working to understand the mechanisms of MDR, develop new diagnostic and treatment strategies, and identify new targets for antibiotic development. [11]

6. **Pharmaceutical Industry**: The pharmaceutical industry is actively involved in the development of new antibiotics and other therapies to combat MDR infections. [18]

7. **Government Agencies**: Government agencies, such as the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO), play a critical role in monitoring and responding to MDR infections, and in developing and implementing policies and guidelines to prevent and control the spread of MDR bacteria. [6]

8. **Hospital and Healthcare Settings**: MDR has significant implications for hospital and healthcare settings, including the need for infection control practices, antibiotic stewardship programs, and education and training for healthcare workers. [25]

Overall, the application of MDR has significant implications for various aspects of healthcare, research, and public health, and is an active area of research and development. [5]

Public washrooms are often a hub of microbial contamination and the examination of bacterial contamination in these facilities can serve as an important indicator of the transmission of infectious diseases. [24] This study was conducted to determine the prevalence of bacterial contamination in public washrooms based on the economic class of the building. Samples were collected from various spots from public washroom . The findings showed that the level of contamination was seen in the washroom. The most dominant Gram-positive bacteria were of the staphylococci species to identified Gram-positive isolates. The most dominant Gram-negative bacteria identified *Serratia marcescens* (S. marcescens) and *Pseudomonas aeruginosa* (P. aeruginosa). The antibiotic sensitivity test results revealed the presence of multidrug-resistant bacteria among the Gram-positive and negative isolates [1]

HYPOTHESES

Hypotheses

1. **Washrooms help spread strong germs:** We think that washrooms, especially busy ones, help strong germs grow and spread.

2. **Touching washroom surfaces can make you sick:** We believe that touching things like toilet handles, sinks, and faucets in washrooms can give you strong germs and make you sick.

3. **Bad air in washrooms helps germs spread:** We think that if the air in washrooms is not fresh, it helps strong germs spread through the air.

4. **Good hygiene keeps germs away:** We believe that washing your hands often and cleaning surfaces properly can stop strong germs from spreading in washrooms.

5. **New cleaning tools can fight strong germs**: We think that creating and using new cleaning tools and special coatings can help stop strong germs from growing and spreading in washrooms.

Literature Review

Introduction

When we think of washrooms, we usually associate them with hygiene and cleanliness. However, these spaces can be breeding grounds for harmful microorganisms, including multidrug-resistant organisms (MDROs). These bacteria, fungi, and viruses have become resistant to multiple antibiotics, making them difficult to treat and posing a growing public health threat. Public washrooms, with their high traffic and frequent contact with surfaces like sinks, faucets, and toilets, can be hotspots for these resistant microbes. This literature review aims to shed light on the presence of MDROs in washroom environments, their impact on human health, and strategies to control their spread.

- **Prevalence of MDROs in Washroom Environments**

  Numerous studies have highlighted the concerning presence of MDROs in washrooms. A study by **Zhao et al. (2019)** found that washrooms in public places, especially in high-traffic areas, have a variety of harmful organisms like *Methicillin-resistant Staphylococcus aureus* (MRSA), *Vancomycin-resistant Enterococci* (VRE), and **Extended-Spectrum Beta-Lactamase** (ESBL)-producing bacteria. These pathogens are often found on commonly touched surfaces such as door handles, faucets, and toilet seats, which increase the risk of transmission to individuals who come into contact with them.

  Kramer et al. (2006) also identified that both **Gram-positive** and **Gram-negative bacteria** are prevalent in washrooms. These include *Staphylococcus aureus* and *Pseudomonas aeruginosa*, two bacteria known for their antibiotic resistance. The study emphasized that washrooms with heavy foot traffic were more likely to be contaminated with a higher load of these pathogens, demonstrating the link between human activity and microbial contamination.

- **Sources and Routes of Transmission**

  MDROs in washrooms are transmitted mainly through direct and indirect contact. Surfaces like **toilet handles**, **sinks**, and **faucets** can harbor these resistant organisms, which are then transferred to hands. People can unknowingly spread these microorganisms when they touch other surfaces or parts of their body, such as the mouth or eyes, which are common entry points for infections.

A study by **Wong et al. (2016)** found that touching contaminated surfaces, particularly **toilet flush handles** and **door handles**, increased the chances of MDRO transmission. Moreover, in some situations, when toilets are flushed, water droplets can become aerosolized, potentially spreading MDROs through the air. This makes poor ventilation in washrooms a significant risk factor for airborne transmission.

- **Biofilm Formation and Persistence of MDROs**

  One of the most worrying features of MDROs in washrooms is their ability to form **biofilms**. Biofilms are clusters of bacteria that stick to surfaces and form a protective barrier, making them resistant to antibiotics and cleaning efforts. **Pseudomonas aeruginosa** and *Staphylococcus aureus* are known for their biofilm-forming abilities, allowing them to survive on washroom surfaces for extended periods.

  According to **Nielsen et al. (2015)** and **Ghosh et al. (2017)**, these biofilms can make it much harder to remove these harmful organisms from surfaces. Even thorough cleaning might not eliminate biofilm-associated bacteria, which means they can continue to spread and cause infections. This persistent presence of bacteria in washrooms, even after cleaning, complicates infection control efforts.

- **Antibiotic Resistance Patterns**

  MDROs are concerning because they are resistant to multiple antibiotics, which limits treatment options. For example, **Staphylococcus aureus** and **Enterococcus faecalis** found in washrooms are resistant to methicillin, vancomycin, and penicillin. Similarly, **Pseudomonas aeruginosa**, **Serratia marcescens**, and other **Gram-negative bacteria** show resistance to broad-spectrum antibiotics, such as carbapenems.

  Research by **Bautista et al. (2018)** demonstrated that MDROs isolated from washroom surfaces exhibited high resistance to the commonly used antibiotics, making these infections more challenging to treat. Furthermore, **Gonçalves et al. (2020)** reported that some bacteria, like *Serratia marcescens*, not only displayed resistance to antibiotics but also to common disinfectants used in washrooms. This highlights the urgent need for new methods of disinfecting these spaces and treating infections caused by these organisms.

- **Prevention and Control Strategies**

  Given the significant presence of MDROs in washrooms, preventing their spread is crucial. The primary methods for controlling the transmission of these pathogens include **proper hand hygiene**, **regular surface cleaning**, and **improved ventilation**.

  Hand hygiene is essential in reducing the spread of MDROs, and frequent hand washing with soap or the use of hand sanitizers can help prevent contamination. Regular cleaning and disinfecting of high-touch areas like faucets, toilet handles, and floors are also critical. Studies by **Prussin et al. (2017)** suggest that enhancing **ventilation** in washrooms can reduce the airborne transmission of these pathogens, particularly in poorly ventilated spaces.

  Public health campaigns that educate the public on the importance of good hygiene practices are also vital. **Antimicrobial coatings** on frequently touched surfaces and **biofilm-targeting disinfectants** may help mitigate the spread of MDROs by targeting these resilient bacteria.

- **Conclusion**

  The presence of MDROs in washroom environments is a growing public health concern. These organisms, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, and others, exhibit resistance to commonly used antibiotics, making them difficult to treat. Biofilm formation further complicates efforts to eliminate these organisms from surfaces. To reduce the transmission of MDROs in washrooms, comprehensive infection control measures such as improved cleaning practices, enhanced ventilation, and better public education on hand hygiene are needed.

  Further research is necessary to develop more effective disinfectants that target biofilm-forming MDROs and to explore new ways to combat antibiotic resistance in these environments. Continued monitoring and innovative strategies will help curb the spread of these resistant organisms, ultimately safeguarding public health.

MATERIALS AND METHODS

Material & Methods

1. Sample – A sample was collected from a washroom environment.

2. **Processing and testing of the sample-**

-The swabs were transported to the lab in saline and were spread on Nutrient agar plate. A loopful of sample suspended in saline was spread on NA plate. The plates were incubated at 37°C for 24 hours. Isolated colonies were observed on the plates after incubation at 37 °C for 24h, selected colony was subcultured on the NA plate for further process.

The organism were also visualized and detected on the Mac medium to identify LF (Lactose fermenting) and NLF (Non lactose fermenting)

3. Gram Staining-

Principle-

Gram staining is a differential staining technique used in microbiology to classify bacteria into two groups: **Gram-positive** and **Gram-negative**, based on their cell wall composition. This method involves the application of a series of stains and reagents that interact differently with bacterial cell walls, allowing for their identification under a microscope.

1. **Primary Staining with Crystal Violet**

   Initially, all bacterial cells are stained with a **primary stain, crystal violet**, a basic dye that penetrates the cell wall and cytoplasm, giving all bacteria a deep purple color.

2. **Fixation with Mordant (Iodine Solution)**

   The application of **iodine** (mordant) follows, which forms an insoluble **crystal violet-iodine (CV-I) complex** inside the bacterial cells. This complex enhances the retention of the crystal violet stain within the bacterial cell wall, making it less soluble.

3. **Decolorization with Alcohol (Ethanol or Acetone-Alcohol Solution)**

   This is the most critical step that differentiates bacteria into Gram-positive and Gram-negative groups. The effect of the alcohol-based decolorizer depends on the bacterial cell wall structure:

4. **Counterstaining with Safranin**

   To visualize the now colorless Gram-negative bacteria, a **secondary stain, safranin**, is applied. This basic dye penetrates both Gram-positive and Gram-negative cells:

- **Gram-positive bacteria** still appear **purple or blue** because the crystal violet stain remains dominant.
- **Gram-negative bacteria** take up the **saffranin stain** and appear **red or pink** under a microscope.

- **Requirements-**
    1. Primary stain- Crystal violet
    2. Secondary stain- Saffranine
    3. Decolorizer- Acetone alcohol
    4. Mordant- Gram's iodine
    5. Given bacterial suspension.
    6. Clean and grease free slide and Nichrome wire loop

Protocol-

Prepare a thin uniform smear on a grease free slide, air dry, heat fix

↓

Stain with crystal violet for 1 minute and remove excess stain under running tap water

↓

Flood the slide with Gram's iodine solution and allow it to react for 1 minute. Drain the excess iodine solution.

↓

Decolorize with decolorize till violet color stops coming out.

↓

Immediately rinse with tap water

↓

Counter stain with saffranine for 2 minutes, rinse, air dry, heat fix and observe under oil-immersion lens (100X).

4. Motility –

Principle:-

The motility test is based on the principle that motile microorganisms can move using their flagella or other motility structures. The test medium is designed to provide a suitable environment for the microorganisms to move, and the movement is observed and recorded.

Requirements –

1. Clean grease free cavity slide.
2. Clean grease free cover slip.
3. A loopful of suspension
4. Microscope

<u>Protocol</u> –

In aseptic condition take a loopful of suspension on a clean grease free cavity slide

↓

With the help of lubricant place a clean grease free cover slip on cavity

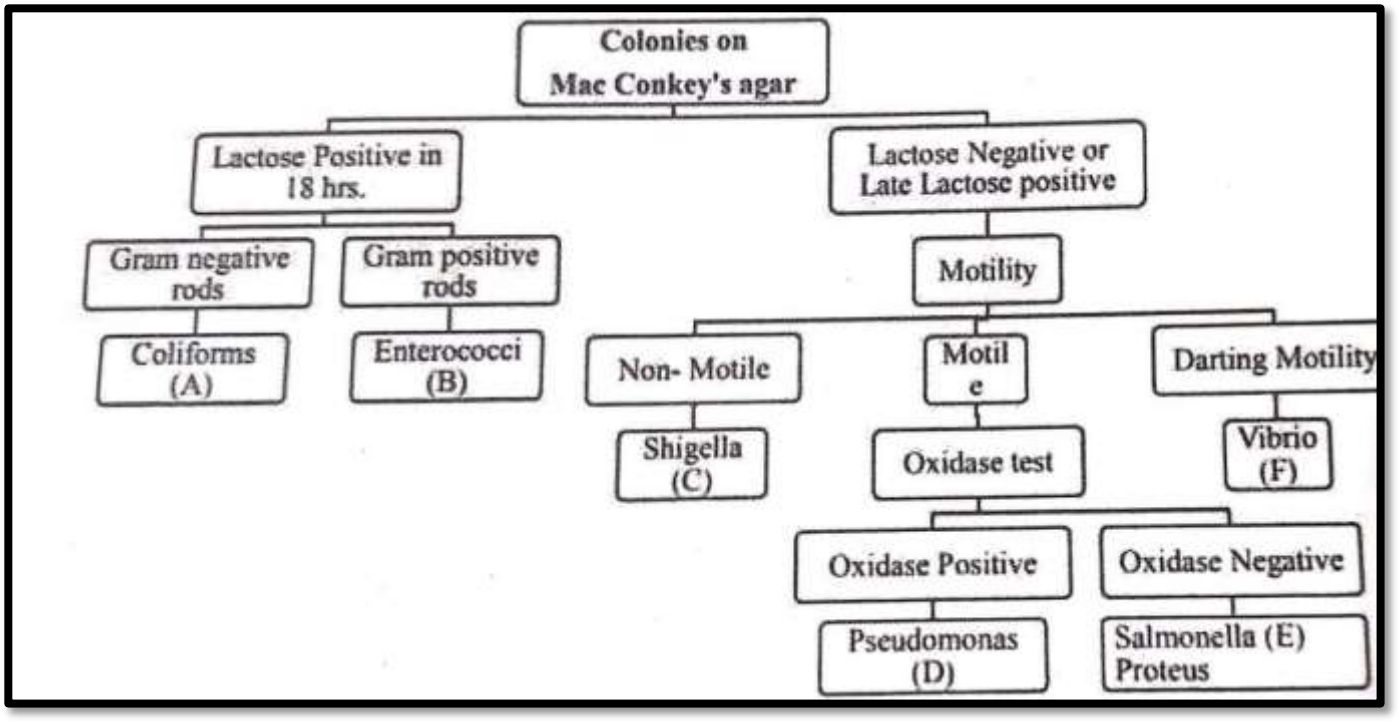
↓ Slide.

Observe under microscope at objective lens of 10 X , 45 X

BIOCHEMICAL CHARACTERIZATION

BIOCHEMICAL CHARACTERIZATION

- Key used to perform further biochemical test of isolated organisms.



KEY FOR INOCULATING BIOCHEMICALS

Sr.NO	Name of Organism	Code	Biochemical Tests
1	*E. coli/ Klebsiella*	A	Indole, MR, VP, Citrate, TSI
2	*Enterococci: Streptococcus faecalis*	B	Glucose, 40% Bile
3	*Shigella*	C	Glucose, Indole, Mannitol, TSI
4	*Pseudomonas*	D	Oxidase, Catalase, Gelatinase, Nitrate reduction, Glucose, Dettol Agar, decarboxylase (lysine, ornithine, arginine)
5	*Salmonella /Proteus*	E	Glucose, xylose, Mannitol, citrate, TSI, H_2S, Urease, gelatinase
6	*Vibrio*	F	Glucose, Mannitol, oxidase, gelatinase, Indole, Choline reduction test
7	*Staphylococcus*	G	Glucose (aerobic, anaerobic), DNAse, Catalase, coagulase, Mannitol, VP
8	*Streptococcus*	H	Glucose, Mannitol, VP, catalase, hemolysis, Glucose azide broth

4. Biochemical Characterization-

Certain biochemical tests were performed crucial for the identification of pathogen which helped to distinguish between different species and strains of microorganisms based on their metabolic capacities. By checking how microorganisms utilize specific substrates or produce particular byproducts, these tests provide valuable clues for identifying unknown isolates. Referring to Ananthanarayan and Picnicker's Textbook of Microbiology, the following biochemical tests were performed for the identification.

IMViC Tests

1. **Indole Test:** Determines if bacteria can break down tryptophan into indole. A positive result appears red after adding Kovac's reagent

Principle- Tryptophan is an amino acid that can undergo deamination and hydrolysis by bacteria that express tryptophanase enzyme. Indole is generated by reductive deamination from tryptophan via the intermediate molecule indolepyruvic acid. Tryptophanase catalyzes the deamination reaction, during which the amine (-NH2) group of the tryptophan

molecule is removed. Final products of the reaction are indole, pyruvic acid, ammonium (NH_4^+) and energy. Pyridoxal phosphate is required as a coenzyme.

When indole is combined with Kovac's reagent (which contains hydrochloric acid and p-dimethylaminobenzaldehyde in amyl alcohol) the solution turns from yellow to cherry red. Because amyl alcohol is not water soluble, the red coloration will form in an oily layer at the top of the broth.

Requirements-

1. Medium- sterile 1% tryptone water or 2% peptone.
2. Reagents- Kovac's reagent and xylene.
3. Test culture.

Protocol-

Inoculate loopful of given culture in 1% tryptone or 2% peptone water.

↓

Incubate the tube at 37°C for 24 hours.

↓

Add xylene reagent to the tube to extract indole.

↓

Add a few drops of Kovac's reagent. Observe for a cherry red ring.

2. **Methyl Red-** Detects strong acid production from glucose fermentation. A red color indicates a positive test.

Principle-

Methyl red test commonly known as MR test is used to determine the ability of an organism to produce and maintain stable acid end products from glucose fermentation. Organism metabolizing pyruvic acid by the mixed acid pathway will produce more acid end product such as lactic acid and acidic acid and maintain an acidic environment. The methyl red detects the mixed acid fermentation that lowers the pH of the broth. The MR indicator is added after incubation which is red at ph 4.4and yellow at ph6.2. if the organism produces a large number of organic acids from glucose fermentation, the broth medium will remain red after the addition of methyl red, a ph indicator. MR-negative organisms further metabolize the initial fermentation product by decarboxylation to produce neutral acetyl methylcarbinol(acetoin), which result in decreased acidity in the medium and raises the ph towards neutrality (ph6.0 or above). For those organisms which do not produce the acid end products, the broth medium will change to yellow coloration indicating a negative test.

Requirements-

1. Medium- sterile glucose phosphate broth
2. Reagent- methyl red(pH indicator)
3. Test culture.

Protocol-

Inoculate a loopful of the test culture in the sterile medium.

Incubate at 37°C for 24 hours.

Add few drops of methyl red to the tube.

Observe the change in color.

3. **Voges-Proskauer Test**- Identifies the production of acetoin, a fermentation byproduct. A red color after Barritt's reagent indicates a positive result.

Principle-

The Voges-Proskauer (VP) test is used to determine if an organism produces acetyl methyl carbinol from glucose fermentation. If present acetyl methyl carbinol is converted to diacetyl in the presence of alpha naphthol, strong alkali (40%KOH), and atmospheric oxygen. The alpha naphthol was not part of the original procedure but was found to act as a color intensifier by Barrits and must be added first. The diacetyl and quanidine containing compounds found in the peptones of the broth then condense to form a pinkish red polymer.

Requirements-

1. Medium- Sterile glucose phosphate broth

2. Reagent- Barrits reagent, alpha napthol and 1% KOH.

3. Test culture

Protocol-

Inoculate a loopful of the test culture in the sterile medium.

↓

Incubate at 37°C for 24 hours.

↓

Add few drops of Barrits reagent to the tube.

↓

Observe the change in color.

4. **Citrate Utilization Test-** Determines if bacteria can use citrate as their sole carbon source. A positive test changes the medium from green to blue.

Principle- Citrate agar is used to test an organism's ability to utilize citrate as a source of energy. The medium contains citrate as the sole source of carbon and inorganic ammonium salts($NH_4H_2PO_4$) as the sole source of nitrogen. Bacteria that can grow on this medium produce an enzyme, citrate-permease, capable of converting citrate to pyruvate. Pyruvate then can enter the organism's metabolic cycle for the production of energy. Growth is indicative of utilization of citrate, an intermediate metabolite in the Krebs's cycle. When the bacteria metabolize citrate, the ammonium salts are broken down to ammonia, which increases the alkalinity. The shift in ph turns the bromothymol blue indicator in the medium from green to blue above ph 7.6.

Requirements-

1. Medium- sterile Kosers citrate broth or sterile Simmons citrate agar slant
2. Reagent- bromothymol blue (pH indicator)
3. Test culture.

Protocol-

Inoculate a loopful of given culture in sterile peptone water, sterile GPB and citrate medium

↓

Incubate at 37°C for 24 hours

↓

Add reagent (Bromothymol blue)

↓

Record the result

5. **Triple Sugar Iron Test**

Principle-

The TSI test is based on the principle that enteric bacteria can ferment sugars, such as glucose, lactose, and sucrose, and produce acid, which turns the medium yellow or red. The test also detects the production of hydrogen sulfide, which is indicated by the formation of a black precipitate.

Requirements-

1. TSI agar slant
2. Sample
3. Incubator

Protocol-

Inoculate TSI agar slant with bacterial suspension.

↓

Incubate at 37°C for 18-24 hours.

↓

Observe slant and butt for color change Yellow/Red / Acid production.

↓

Observe for black precipitate Black / H2S production.

↓

Record the result

5. Biofilm:

Biofilm formation is a process where microorganisms, such as bacteria, adhere to surfaces and produce a protective and adhesive matrix made of polysaccharides, proteins, and nucleic acids. This matrix allows the microorganisms to stick together and to the surface, creating a structured community**.**

Principle:

Biofilm formation occurs in several stages, including:

1. **Initial Attachment**: Bacteria attach to a surface, often through weak electrostatic or hydrophobic interactions.
2. **Adhesion**: Bacteria produce adhesins, such as proteins or polysaccharides, that help them attach to the surface more securely.
3. **Colonization:** Bacteria multiply and form a microcolony, which can lead to the formation of a biofilm.
4. **Maturation:** The biofilm matures and becomes more complex, with the production of EPS and the formation of a three-dimensional structure.
5. **Dispersion**: Bacteria can disperse from the biofilm, either through active processes, such as swarming or swimming, or through passive processes, such as erosion or sloughing.

The principle of biofilm formation is crucial for the development of effective strategies to prevent and control biofilm-related problems in various fields, including medicine, industry, and environmental science.

Requirement:

- Nutrient broth (NB)
- Sterile tubes or 96-well plates
- Bacterial colony (freshly cultured)
- Crystal violet solution (0.1%)
- Phosphate-buffered saline (PBS), sterile
- Acetic acid (33%)
- Sterile pipette tips and micropipette
- Incubator (37°C)
- Microplate reader (for OD measurement)

Protocol:

Standard Biofilm Formation Protocol

Objective:

This protocol is designed to assess biofilm formation by bacterial colonies on a sterile surface. It includes the inoculation, staining, and optical density (OD) measurement to quantify biofilm development.

Procedure:

1. **Preparation of the Inoculum:**

- Take 2 to 5 mL of sterile Nutrient Broth (NB) in a sterile surface such as tubes or a 96-well plate, ensuring sterility is maintained throughout the process.
- Inoculate a single bacterial colony (from a fresh agar plate) into the NB. Use a sterile inoculating loop or pipette tip to transfer the colony into the broth.

2. **Incubation:**

- Place the inoculated tubes or plates in an incubator at 37°C for 48 hours to allow the bacteria to form biofilms on the surface.

3. **Crystal Violet Staining:**

- After incubation, carefully remove the excess NB from the tube or plate to avoid disturbing the biofilm formed.
- Flood the surface with 0.1% crystal violet solution, ensuring the surface is completely covered with the stain.
- Allow the crystal violet to bind to the biofilm for approximately 10 minutes. During this period, the crystal violet will stain the biofilm, making it visible for analysis.

4. **Washing:**

- After 10 minutes, gently remove the excess crystal violet by aspirating or decanting the stain.
- Wash the surface thoroughly with sterile PBS to remove any unbound crystal violet. Take care to avoid disrupting the biofilm during the washing process.

5. **Biofilm Quantification:**

- To quantify the biofilm, add 33% acetic acid to dissolve the crystal violet that is bound to the biofilm.
- Gently agitate the solution for a few minutes to ensure the complete release of the dye from the biofilm.

6. **Optical Density (OD) Measurement:**

- Using a microplate reader or spectrophotometer, measure the optical density (OD) of the acetic acid-diluted biofilm at a wavelength of 590 nm.
- Record the OD values and use the blank (acetic acid alone) as a reference to subtract background absorbance.

6. Enzyme detection test-

All biochemical reactions that occur both outside and inside the cell are precisely controlled by some governing factors, the enzymes. An enzyme is biochemical catalyst, a substance that accelerates the rate of specific chemical reaction. Enzymes are either exoenzymes (Extracellular) or endoenzymes (Intracellular). Exoenzymes which are few in number are released from the cell and act on the substrate. These are mainly hydrolytic enzymes. Endoenzymes are utilized by the cell for further metabolic degradation of carbohydrates. These enzymes are either constitutive or induced in nature.

- Constitutive enzymes: Continuous synthesis of enzymes irrespective of requirement. For e.g. Glucokinase.

- Induced enzymes: These enzymes are synthesized only when there is some induction impulse (like specific substrate). For e.g. β-galactosidase. This enzyme is induced when lactose is present in the medium.

1. **Catalase detection**

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 formation. The culture should not be more than 24 hrs. old. Bacteria there by protect themselves from the lethal effect of Hydrogen Peroxide which is accumulated as an end product of aerobic carbohydrate metabolism.

Requirements-

1. Culture of test organism.

2. Sterile Nutrient Agar slant.

3. 3%H_2O_2

Protocol:

Take a loopful of suspension and streak it on a sterile NA slant.

Incubate at 37 0 C for 24 hrs.

Add 3% H_2O_2 to the slants.

Check for production of gas bubbles**.**

2. Oxidase detection-

Principle-

Cytochrome containing organisms produce an intracellular Oxidase enzyme. This Oxidase enzyme catalyzes the oxidation of Cytochrome c. Organism which contain cytochrome c as part of their respiratory chain do not oxidize the reagent, leaving it colorless within the limits of the test and are Oxidase negative. Oxidase positive bacteria possesses Cytochrome Oxidase or indophenols Oxidase (an iron containing haemoprotein). Both of these Catalase the transport of electrons from donor compounds (NADH) to electron acceptor (usually oxygen). The test reagent NNN'N tetramethyl-paraphenylenediamine dihydrochloride act as an artificial electron acceptor for the enzyme Oxidase. The oxidized reagent forms the colored compound indophenol blue.

Requirements-

1. Culture of test organism.
2. Sterile Nutrient Agar slant.
3. 1% NNN'N tetra methyl para-phenylenediaminedihydrochloride

Protocol-

1. Take a loop full of suspension and streak it on a sterile NA slant.

2. Incubate at 37 0 C for 24 hrs.

3. Take a filter paper strip and slightly moisten it.

4. Take growth from the slant on the filter paper.

5. Add few drops of reagents and observe.

3. Coagulase Test:

Principle:

Coagulase is an enzyme produced by certain bacteria that catalyzes the conversion of fibrinogen to fibrin, causing the blood plasma to clot. The coagulase test helps identify *Staphylococcus aureus*, which produces coagulase, as opposed to other *Staphylococcus* species, which do not. Coagulase-positive bacteria form a clot in the plasma, whereas coagulase-negative bacteria do not.

Requirements:

1. Culture of test organism.
2. Sterile Rabbit or Human plasma.
3. Sterile test tubes.
4. Incubator set at 37°C.
5. Sterile loop or pipette.

Protocol:

1. Take a loop full of the test organism and inoculate it into a sterile test tube containing a small amount of rabbit or human plasma.

2. Gently mix the plasma and the inoculum to ensure proper distribution.

3. Incubate the test tube at 37°C for 4 hours to allow the reaction to occur.

4. After incubation, observe for the presence of clot formation in the plasma.

7. Antibiotic sensitivity-

Antibiotic sensitivity testing for multi-drug resistance organisms (MDROs) is a laboratory test used to determine the effectiveness of various antibiotics against a specific MDRO isolate. The test helps to identify which antibiotics the MDRO is sensitive to, and which ones it is resistant to.

- **Requirements-**

i. Mueller-Hinton agar plates for antibiotic sensitivity testing
ii. Antibiotic discs
iii. Bacterial suspension

- **Protocol-**

Take a loop full of suspension and spread it on a sterile MH plate.

↓

Antibiotic discs of 12 antibiotics were kept in the culture plates aseptically and incubated at 37°C for 24 hours.

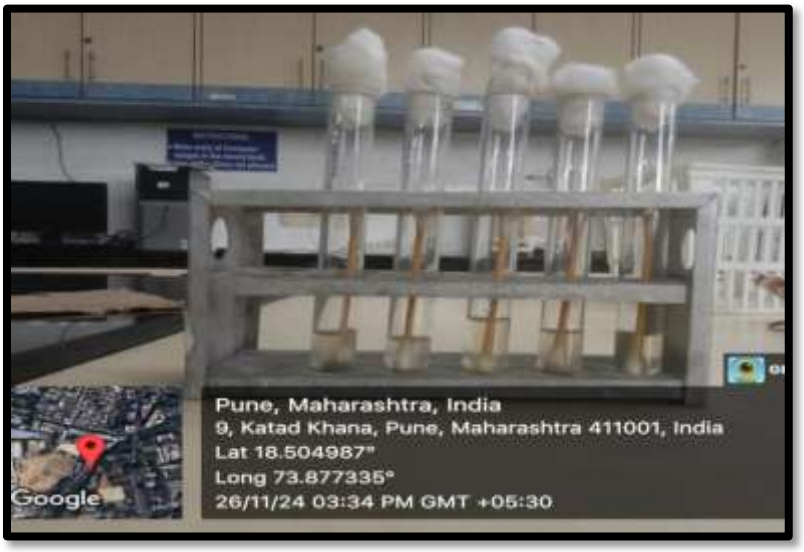
↓

The zone of inhibition was measured and the results were interpreted.

RESULTS

a. Results-

- **Sample collection:**



- **Growth of samples collected:**

- **Master plate of isolated colony:**

  - **Isolate 1**

  - **Isolate 2**

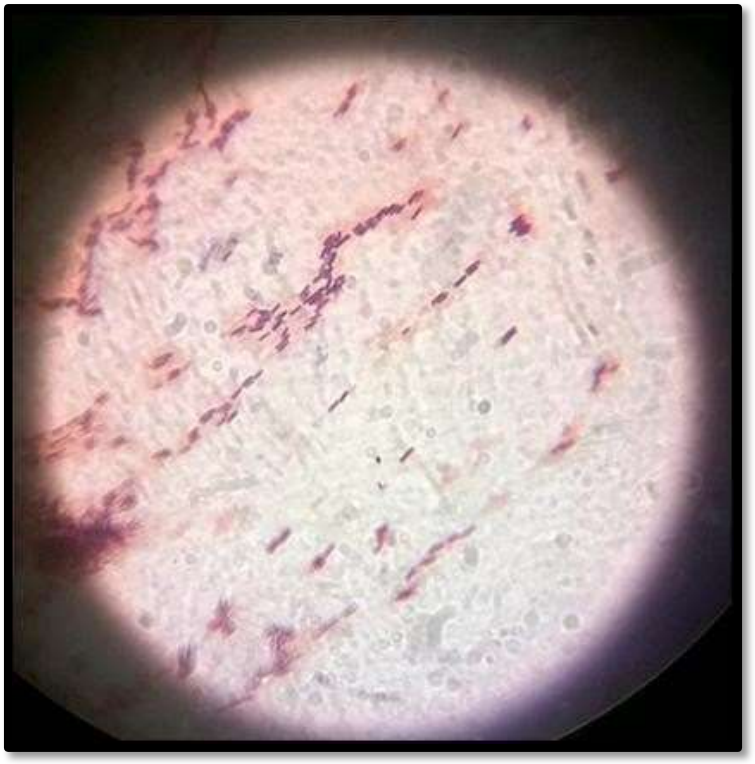
- **Isolate 3**

➢ **Gram Staining :**

- **Isolate 1**

- **Isolate 2**

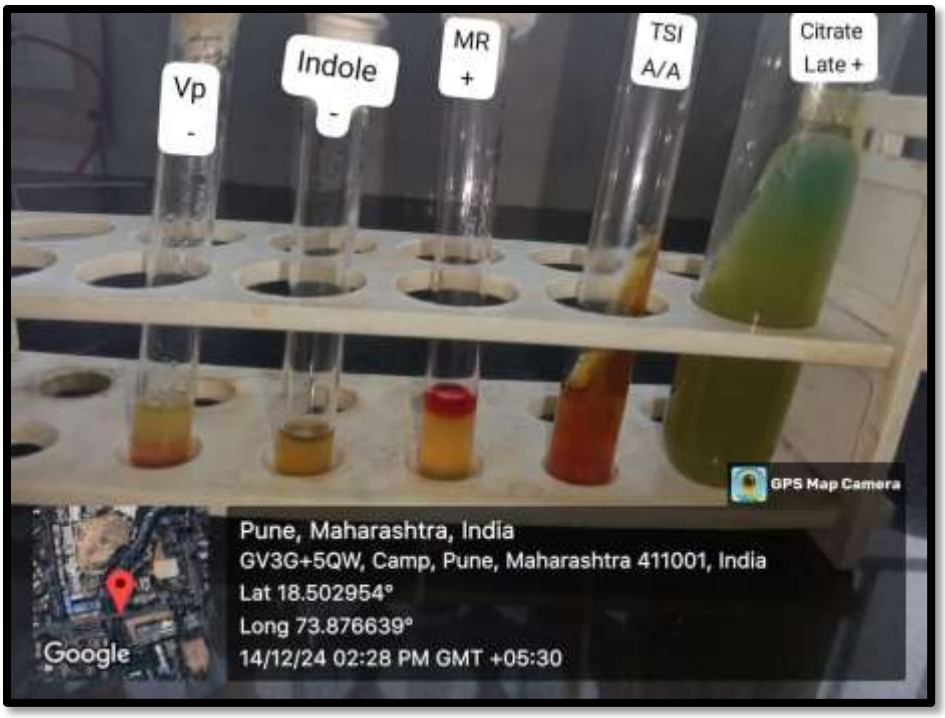
- **Isolate 3**



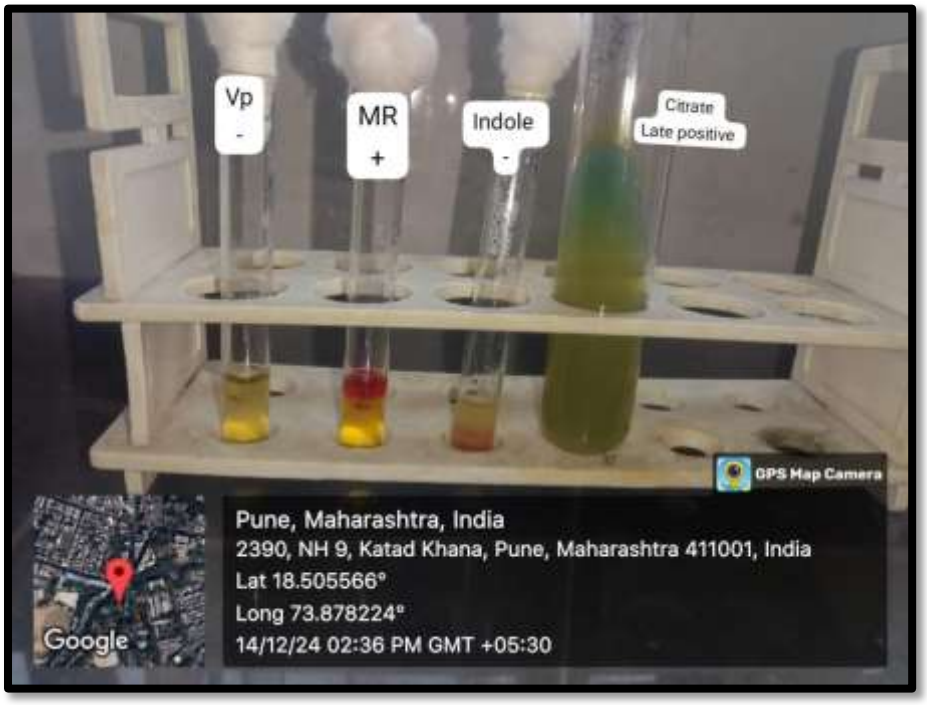
Column1	Isolate 1	Isolate 2	Isolate 3
Size	1-2 mm	1-3 mm	1-3 mm
Shape	Round	Circular	Circular
Margin	Entire	Entire	Entire
Opacity	Opaque	Opaque	Opaque
Colour	Yellow	Pink	Greenish-blue
Elevation	Convex	Convex	Convex
Consistency	Creamy	Mucoid	Mucoid
Gram Staining	Gram +ve	Gram –ve	Gram –ve
Motility	Non motile	Motile	Motile

➢ Biochemical characterization:

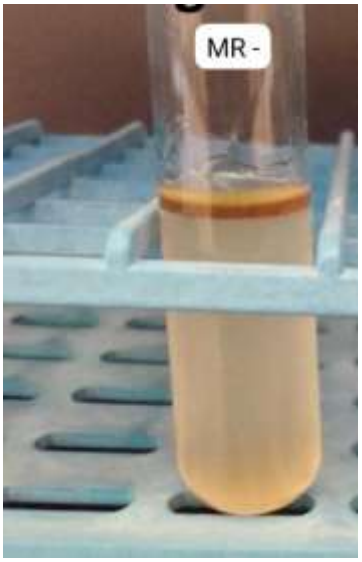
- **Isolate 1**

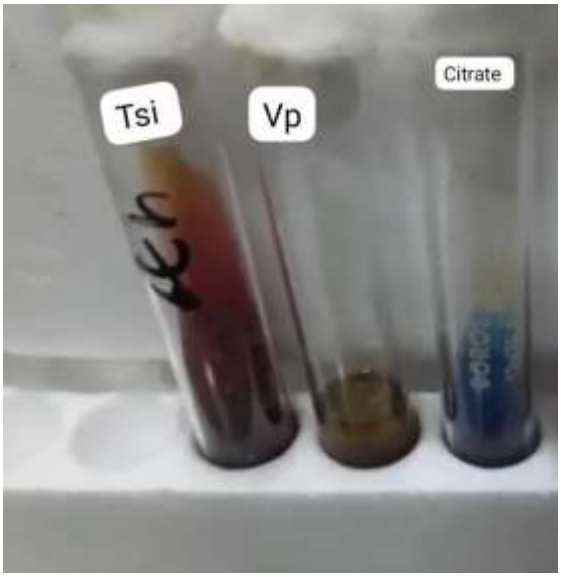


- **Isolate 2**



- **Isolate 3**





Isolate No.	IMViC				Oxidase	Catalase	Gram character	Motility
	In	MR	VP	C				
1	-	+	-	+	-	+	Gram +ve	Non-motile
2	-	+	-	+	-	+	Gram -ve	Motile
3	-	-	-	+	+	+	Gram -ve	Motile

Coagulase test:

Coagulase test was negative for *Staphylococcus.*

- **Biofilm:**

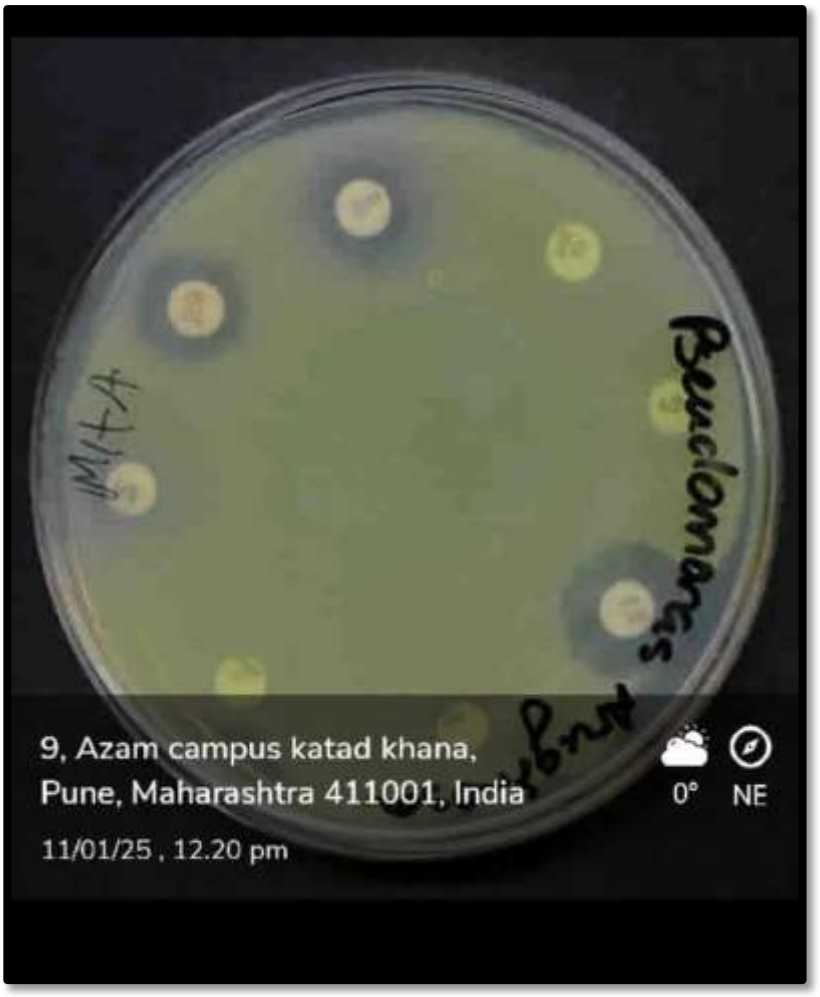
Biofilm formation was observed for *Seeratia* and *Pseudomonas* . In which biofilm formation of *Pseudomonas* was much more compared to *Seeratia.*

- **<u>Antibiotic sensitivity test results-</u>**

1. *<u>Staphylococcus</u>*

2. *<u>Serratia</u>*

3. <u>Pseudomonas</u>



- These are the antibiotics used for antibiotic sensitivity test.

Sr. No.	Name of antibiotic
1	Ampicillin
2	Neomycin
3	Kanamycin
4	Colis
5	Nalidixic acid
6	Penicillin G
7	Erythromycin
8	Novobiocin
9	Tetracyclin
10	Norfloxacin
11	Trimethoprim
12	Vancomycin

DISCUSSION

Discussion-

The findings from this study shed light on an important yet often overlooked issue—multidrug-resistant organisms (MDROs) present in public washrooms. While washrooms are places we frequently visit and interact with, they are not always seen as hotspots for bacterial contamination. However, the reality is that these environments harbor various microorganisms, some of which pose serious health risks due to their resistance to multiple antibiotics.

MDROs, as we discovered, are particularly concerning because of their ability to evade treatment. With organisms like *Pseudomonas spp.*, *Staphylococcus spp.*, and *Serratia spp.* being isolated from various washroom surfaces, it becomes clear that our daily habits—using public toilets, washing our hands, or even just walking on washroom floors—can expose us to these pathogens. What's troubling is the fact that these bacteria often form biofilms, which enhance their resistance and persistence on surfaces, making them harder to eliminate with regular cleaning.

The detection of *Staphylococcus aureus* (especially the methicillin-resistant variant, MRSA) and *Serratia marcescens* is significant because these organisms are notorious for causing hospital-associated infections, but their presence in public washrooms suggests a broader, community-level transmission. This is particularly worrying for individuals who have weakened immune systems, elderly people, or those who are regularly exposed to public facilities. Given that these organisms can spread from surfaces to hands and ultimately to individuals, the public health risks are substantial.

A major concern raised by this study is the phenomenon of antibiotic resistance. Many of the isolated organisms showed resistance to commonly used antibiotics, meaning that if someone were to become infected, the standard treatments may not be effective. The resistance profiles observed in our study, combined with the potential for biofilm formation, underscore the fact that simple hygiene practices may not always be enough to protect us from these resistant pathogens.

It is also crucial to note that the washroom environment, with its frequent human interaction and high-touch surfaces, plays a significant role in the transmission of these organisms. Areas like toilet handles, faucets, and floors act as reservoirs, allowing bacteria to transfer from one person to

another through direct contact or even indirectly when hands come into contact with contaminated surfaces. This highlights the importance of regular cleaning and disinfection, particularly in public washrooms, to reduce the spread of MDROs.

Moving forward, it’s clear that strategies to control these organisms in public washrooms need to evolve. Simple interventions like improving hand hygiene (through frequent washing and hand sanitizing) and ensuring adequate ventilation could help reduce airborne transmission. Moreover, increased public awareness about the potential dangers of these organisms is essential. Encouraging individuals to wash their hands properly, as well as promoting the use of disinfectants in high-touch areas, could make a significant impact.

The study also emphasizes the importance of research in understanding the mechanisms behind MDROs' resistance. RT-PCR genetic sequencing, currently underway in this study, promises to provide valuable insights into the genetic factors that allow these organisms to survive and resist common antibiotics. The more we understand about their genetic makeup, the more targeted we can be in developing new treatments or interventions.

In addition, there is an urgent need for the development of antimicrobial agents that are specifically designed to target biofilm-forming bacteria in public spaces, particularly in washrooms. Traditional disinfectants may not be enough to fully eliminate these organisms, and the application of antimicrobial coatings could help reduce their persistence on surfaces. This type of innovation could be a game-changer in terms of preventing the spread of MDROs.

Lastly, this study serves as a call to action for governments, public health organizations, and healthcare providers. Establishing stricter guidelines for cleaning and disinfection in public washrooms, coupled with stronger surveillance and monitoring systems, could significantly reduce the risk of MDRO transmission. Ultimately, it’s about ensuring that we protect vulnerable populations and prevent future outbreaks by addressing the root causes of resistance and transmission.

In conclusion,

MDROs in public washrooms are more than just an inconvenience—they are a public health issue that requires immediate attention. This study has made it clear that multidrug-resistant pathogens can thrive in washroom environments, making them potential reservoirs for resistant infections. The presence of these organisms and their ability to form biofilms complicate efforts to combat their spread. In light of these findings, it's imperative that we take stronger actions to ensure washrooms are properly maintained and that public hygiene practices are improved. As we continue to understand more about the resistance mechanisms of these pathogens, we can develop better interventions and ultimately reduce the threat of MDROs to public health.

FUTURE PROSPECTS

Future prospects-

The future of dealing with MDROs in washrooms is full of promise. With smarter cleaning products, faster ways to track bacteria, and a greater public understanding of hygiene, we can start reducing the spread of these dangerous organisms. The real key, though, is taking action—whether it's through better technology, stronger public awareness, or more coordinated efforts from researchers. Each step we take brings us closer to safer, cleaner environments for everyone. By tackling the problem together, we can make public spaces healthier and help prevent the spread of these "superbugs" that are a growing concern in our everyday lives.

1. Creating Better Cleaning Products

2. Smarter Ways to Track MDROs

3. Public Health Campaigns and Hygiene Education

4. Building Better Hygiene Practices in Public Spaces

5. Long-Term Studies on the Public Health Impact

6. Global Effort to Tackle the Problem

Summary

Public washrooms, while essential for personal hygiene, can also become place for harmful microorganisms, including multidrug-resistant organisms (MDROs). These organisms have developed resistance to multiple antibiotics, making them harder to treat and posing significant public health risks.

MDROs, such as Methicillin-resistant Staphylococcus aureus (MRSA), Vancomycin-resistant Enterococci (VRE), and Pseudomonas aeruginosa, can often be found in high-traffic washrooms, particularly on surfaces like toilet handles, sinks, and floors. These bacteria are resilient and can spread easily through direct or indirect contact with contaminated surfaces, or even through the air when flushed toilets aerosolize water droplets.

One concerning feature of these MDROs is their ability to form biofilms—clusters of bacteria that stick to surfaces, making them even harder to remove with regular cleaning or antibiotics. This means that even thorough sanitation may not fully eliminate the bacteria, allowing them to persist in washrooms for long periods.

Studies have shown that MDROs are not only resistant to antibiotics but may also resist disinfectants, which complicates infection control efforts. The research highlights the need for better hygiene practices in washrooms, such as frequent hand washing, more regular cleaning, and improved ventilation to reduce the spread of these resistant pathogens.

This study shines a light on an often overlooked issue: multidrug-resistant organisms (MDROs) in public washrooms. While we typically think of washrooms as places for hygiene, they can also harbor harmful bacteria, some of which are resistant to multiple antibiotics, making them hard to treat.

The bacteria discovered in this study—like *Pseudomonas spp*., *Staphylococcus spp*., and *Serratia spp*.—are particularly concerning because they can resist standard antibiotics. What's even more troubling is that these bacteria often form biofilms, which act like a shield, making them tougher to eliminate even with cleaning. The study also found well-known bacteria like Staphylococcus aureus (including the resistant MRSA strain) and *Serratia marcescens*, which are typically linked to hospital infections but are now showing up in public spaces. This raises

serious concerns about the spread of infections in the community, particularly for vulnerable groups like the elderly or people with weakened immune systems.

The resistance of these bacteria to common antibiotics, combined with their ability to stick to surfaces and survive on them for long periods, makes it clear that everyday hygiene practices may not always be enough to protect us. Areas like toilet handles, faucets, and floors in washrooms are major hotspots for bacteria transmission, either through direct contact or when hands touch contaminated surfaces. This highlights the need for regular cleaning and disinfection in public washrooms to limit the spread of these dangerous organisms.

The study also emphasizes the importance of public education on hand hygiene and the need for better cleaning protocols. Small steps, like frequent handwashing and using hand sanitizers, along with improved ventilation in washrooms, could help reduce the spread of these bacteria. The ongoing research, like the genetic sequencing being done in this study, promises to give us more insights into why these bacteria are so resistant and how we can develop better treatments.

Looking ahead, the future of tackling MDROs in public washrooms seems promising. There is potential for smarter cleaning products, more effective ways to track bacteria, and a better understanding of hygiene practices. With a coordinated global effort, we can create cleaner, safer public spaces and reduce the risks posed by these "superbugs" in our everyday lives.

In short, MDROs in public washrooms are not just an inconvenience—they’re a real public health risk. This study serves as a wake-up call for stronger cleaning measures, better public hygiene education, and ongoing research to fight these resistant organisms and protect public health.

APPENDIX

APPENDIX

- **Nutrient agar-**

Composition:

Peptone	0.15g
Yeast extract	0.6g
NaCl	0.15g
Agar	4.5g
DW	300ml
PH	7.0 – 7.2

- **Mueller- Hinton medium-**

Composition :

Beef extracts	2.0g/ml
Casein hydrosylate	17.5g/ml
Starch	1.5g/ml
Agar	1.7g/ml

- **Methyl Red Test**

MR-VP broth (Glucose phosphate broth)

Composition:

Peptone	5.0 g
K2HPO	4 5.0 g
Distilled water	1000 ml
Glucose 10% solution	50 ml
PH	7.6

Preparation: Dissolve the peptone and phosphate. Adjust the pH Filler. Distribute in 5

ml amounts and steam sterilize and at 121°C for 15 minutes. Sterilize the glucose

solution by filtration and add 0.25 ml to each tube. (final concentration 0.5%).

- **Citrate agar**

(Simmon's citrate medium)

Composition***:***

Koser's (modified) broth	1000 ml
Bromothymol blue	(0.2%) 40.0 ml
Agar	20.0 g

Preparation: Add the indicator in the broth. Dissolve agar. Distribute in the tubes, steam sterilize at 12 1°C for 15 min. and allow to set as slants.

- **Crystal Violet staining solution**:

Dissolve 2 g of Crystal Violet powder dye in 20 ml of 95% ethanol (Histanol 95) and mix with 80 ml of 1% aqueous solution of ammonium oxalate.

- **Gram's Staining**

Solutions required:

a. Crystal violet

Solution X

Crystal violet (90% dye content) chloride 2.0 g

Ethyl alcohol (95%) 20.0 ml

Solution Y

Ammonium oxalate 0.8 g

Distilled water 80.0 ml

Dissolve crystal violet in ethyl alcohol and the ammonium oxylate in distilled water. Mix solutions X and Y.

b. Gram's iodine (Lugol's iodine)

Iodine 1.0 g

Potassium iodine 2.0 g

Distilled water 300.0 ml

Dissolve potassium iodide in distilled water then add iodine crystals. Dissolve.

c. Ethyl alcohol (95%)

Ethyl alcohol (1 00%) 95.0 ml

Distilled water 5.0 ml

d. Safranin

Safranin (2.5% solution in 95% ethyl alcohol 10.0 ml

Distilled water 100.0 ml

Filter the solution before use.

e. Basic fuchsin stain

Basic fuchsin	0.5 g
Distilled water	1000.0 ml

Note: Never use dilute carbol fuchsin because it tends to stain gram negative bacteria so intensely that they may appear gram positive.

- **Decolorizing Agent**

Ethanol,	95% (vol/vol)

*Alternate Decolorizing Agent

Some professionals prefer an acetone decolorizer while others use a 1:1 acetone and ethanol mixture. Commercially, a variety of mixtures are available, most using 25 – 50% acetone with the ethanol. A few include a small quantity of isopropyl alcohol and/or methanol in the formulation.

Acetone,	50 ml
Ethanol	(95%), 50 ml

- **MacConkey Agar (MAC)**

  Composition:

Peptones (Protease Peptone)	17.0 g/L
Sodium chloride (NaCl)	5.0 g/L
Lactose	10.0 g/L
Bile salts (usually sodium deoxycholate)	1.5 g/L
Crystal Violet	0.001 g/L
Neutral Red (pH indicator)	0.03 g/L
Agar	13.5 g/L
Water	1 liter

- **Nutrient Broth**

  Composition:

Peptone	5.0 g/L
Beef extract	3.0 g/L
Sodium chloride (NaCl)	5.0 g/L
Agar (if preparing Nutrient Agar instead of Broth)	15.0 g/L
Water	1 liter

- **Triple Sugar Iron (TSI) Agar**

Composition:

Peptones	10.0 g/L
Sodium chloride (NaCl)	5.0 g/L
Sodium thiosulfate ($Na_2S_2O_3$)	0.3 g/L
Ferric ammonium citrate	0.1 g/L
Glucose (D-glucose)	1.0 g/L
Lactose	10.0 g/L
Sucrose	10.0 g/L
Agar	12.0 g/L
Distilled water	1 liter.

pH:

- The final pH of the medium before autoclaving is around **7.4**, which may change during fermentation, becoming more acidic (pH < 7) or more alkaline (pH > 7) based on bacterial activity.

- **Indole Test**
-

Composition:

Tryptone	10.0 g/L
Sodium chloride (NaCl)	5.0 g/L

Distilled water To make up the final volume of 1 liter.

pH:

- The pH of the medium is generally around **7.4** before autoclaving.

- **Saline Solution**

  Composition:

  NaCl

  Water

- **Phosphate Buffered Saline (PBS)**

  Composition:

Sodium chloride (NaCl)	8.0 grams
Potassium chloride (KCl)	0.2 grams
Disodium phosphate (Na_2HPO_4)	1.44 grams
Monopotassium phosphate (KH_2PO_4)	0.24 grams
Distilled water	1 liter

pH:

- The pH of PBS is typically **7.4**, which is close to the physiological pH of blood and cells.

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