ISOLATION OF BACTERIA FROM BIOMETRIC MACHINES AND THEIR CHARACTERIZATION

Project Report in partial fulfilment of the requirement for the degree of

Master of Science

In

Microbiology

By

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Under the supervision of

Dr. Rahul Shrivastava

to

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Solan – 173234, Himachal Pradesh

Candidate's Declaration

I hereby declare that the work presented in this report entitled "**ISOLATION OF BACTERIA FROM BIOMETRIC MACHINES AND THEIR CHARACTERIZATION**" in partial fulfilment of the requirements for the award of the degree of **Masters of Science** in **Microbiology** submitted to the Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology, Waknaghat is an authentic record of my own work carried out under the supervision of **Dr. Rahul Shrivastava** (Associate Professor, Department of Biotechnology and Bioinformatics).

The matter embodied in the report has not been submitted for the award of any other degree or diploma.

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Finally, I must acknowledge with due respect the constant support and patience of my parents.

Damini Singh (217855)

CERTIFICATE

This is to certify that the work reported in the M.Sc. thesis entitled "**ISOLATION OF BACTERIA FROM BIOMETRIC MACHINES AND THEIR CHARACTERIZATION**" submitted by **Damini Singh (217855) at Jaypee University of Information Technology, Waknaghat, India,** is a bonafide record of her original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ABBREVIATION	FULL FORM
%	Percent
°C	Degree Celsius
µg	Microgram
µl	Microliter
µM	Micromolar
CFU	Colony Forming Units
ANOVA	Analysis of variance
g	Grams
g/l	Grams per liter
KOH	Potassium Hydroxide
Hrs	Hours
BDs	Biometric machine
mL	Milliliter
DSW	Distilled water
Min.	Minutes
Sec.	Seconds
NA	Nutrient Agar
NB	Nutrient Broth
GPB	Glucose phosphate broth

LIST OF FIGURES

17	Brown colony (Main Gate from evening sample)
18	Pale yellow colony (Main gate from evening sample)
19	Orange colony (Main gate from morning sample)
20	White colony (Geeta bhawan from morning sample).
21	Simple streaking of each sample for further use (stored at 4°C).
22	Staining of White round colony
23	Staining of Pale-yellow colony
24	Staining Orange colony
25	Staining Brown colony
26	Different colonies shown on Blood agar.
27	yellow colony shows result after 7 days of incubation.
28	Different colonies shown on MacConkey agar
29	Result of Citrate utilization test
30	Methyl red test result of isolates
31	Indole test result of isolate

LIST OF TABLES

ABSTRACT

Countless microbes are everywhere, and these microbes represent an important part in various environmental, industrial, and medical phenomena. Potential infections that form biofilms and spread through biometric machines should not be ignored as microbial biofilm growth has been reported on practically all solid surfaces. Consequently, the present study leads to evaluate the bacterial flora was detected on fingerprint sensors of biometric attendance machines. The results of the current investigation demonstrated that numerous microorganisms, the majority of which related to the ecosystem of the body of an individual, were present on all cell phones, computers, and biometric devices used in the workplace and in schools. This indicates that it's imperative to After using a phone, laptop, or other essential biometric device, wash your hands thoroughly to prevent the spread of disease. In the study the results shows that all isolates are gram negative and biochemical tests are also done to see the biochemical activity of the isolates which we found in biometric machine.

CHAPTER-1

INTRODUCTION

1.1 INTRODUCTION

Microbes were widespread and have a role in a variety of atmospheric, economic, and medical occurrences. It is common to get contamination on objects & locations. Microbes are readily obtained by human hands from ambient things, and humans hands are the primary source of microbe spread [1]. The majority of studies demonstrate that microbes are present in the atmosphere or on objects. As a outcome of the accelerating human growth & business expansion. The widespread usage of electronics now is a significant source of microbial contamination. Microbes can thrive on a huge range of area, including those in clinical environments & cookhouse, eating place, also on common office supplies as laptops, biometrics and mobile phones around the globe [2].

Although advancements in modern theories and therapies, infections acquired in clinical facilities continue to be the central problem for global health systems. Usually individuals don't aware that germs can be discovered on a variety of everyday items in the environment, in workplaces, as well as in houses. ATM keyboards, computer keyboards, washbasins, work tables, stair railing, elevators, grocery store & trolley handle are a few examples of these kind of items. Individuals have a false sense of safety because individuals think that germs were exclusively found in laboratories, pharmacies, and health centers. Serious issues could result from unawareness of the underlying pathogens [3].

In clinics, health care-associated illnesses are a major reason for morbidity and mortality [4]. Harmful microbes constitute a severe health hazard since they are ubiquitous [5]. Several research discuss the possibility for computers to harbour possibly dangerous bacteria, and they detail the survivability of certain Gram negative & other Gram positive organisms in different ecological situations [6]. On surfaces like phone and table handsets, many germs may persist for week or even days and are contagious at quite less concentrations. The majority of microbes are found for such large quantities in natural, moist, surroundings, but few can also endure in arid situations. Despite the fact that dangerous microbes can be found in the Air, fluids, & then on objects, determining how they contribute to illness and sickness is difficult [7].

1.2 Microorganism Present on Cell Phone:

Utilization of smart phones is steadily rising throughout India. India is now the second-largest telecom worldwide. Persons can now communicate more easily by messaging, email and call-

ing, thanks to mobile phones. Nowadays mobile phones offer a variety of applications, including web surfing, games, email, social media accessibility, videos and audio apps, radios, E-books, dictionary, and more. Most users fall within the 15-25 age range. Today's telephones are widely used, inexpensive, or freely obtainable to everyone. Telephones are currently the most important hands - free device for efficiency.

It serves as a daily communication tool for families, workers, medical professionals, & others everywhere. There are currently No strict regulations prohibiting telephones in intensive care units (ICUs), in which the risk of contamination is greater. Reasonable pricing have enormously increased the public reliance on telephones. The interaction of telephones with human bodies and other objects is causing ongoing health issues. Even cleaning wifi might be a laborious task. 77.11% of the smartphones in the test showed microbes contamination [12]. With the mobile phone's achievements and benefits, its risk to health is not immediately apparent; this goes even against theory that many users may not observe personal hygiene & the no. of people who might use same cellphones.

The consistent handling of the cellphone through numerous users exposes it to a wide range of bacteria and provides it a good carrier for microbes, especially that connected to the epidermis, resulting in the spreading of diverse pathogens via customer to customer. Our research has demonstrated that a smartphone may pose a health concern due to the enormous variety of microbes who reside on every square inch of the device. According to Microbiologists, the consistent attention and heat from cellphones provide a perfect environment for some germs that are usually located on the epidermis to grow [13]. The most important preventive step is ensuring the sterility of palms to preserve a safe distance from contamination and cross-contamination.

Our hands frequently connect using our smartphones in this manner, acting like an attractive factor. 74percent of total of the smartphones in a clinical study found to be contaminated. Enhancing prevention strategies, including as routinely disinfecting telephones, can reduce that disease's impact. By using appropriate cleaning techniques for the cellphone, it is easy to use antibacterial treatments to prevent the growth of microbes. Worry may occur due to the utilization of guarded materials in the manufacture of smartphones that prevent growth of microbes. The warmth generated by smartphones, which are always in usage, creates an environment that is ideal for the growth of bacteria. Having smartphones being maintained within close proximity or in direct touch with our faces, palms, and mouths, there is a considerable

danger of infection [14]. People currently from a normal flora of body, the digestive and respiratory systems, and *Staphylococcus* epidermidis in particular, are *Staphylococci*. Twenty-five percent of people carry *Staphylococcus aureus* in their nasopharynx. *Staphylococci* are routinely discovered on bedding, clothes, as well as people surroundings.

Up to 25percent of healthy people and animals have the common microbe *Staphylococcus aureus* on their surface and in their nostrils. This close family member of methicillin-resistant *Staphylococcus aureus* also cause illnesses like pimples, pneumonia, boils(MRSA), and meningitis. This is carried in sustenance while cooking by hands, which is known as staphylococcus. The hands is a vital way of spreading several microbes, particularly gastrointestinal bacteria. The among the Gram negative microbes more frequently found in medical instances is *Proteus mirabilis*. It can result in a variety of germs that can be acquired from the population or an urgent care, such as those that affect the respiratory system, urinary tract, wounds and intake, neonatal meningoencephalitis, bacteremia, empyema, and osteomyelitis, *P. mirabilis*, which accounts for 3percent of hospital - acquired infections in the Combined Areas, is the second Enteric bacteria species routinely isolated in European clinical microbiology labs after *E. coli*. *Pseudomonas aeruginosa* is a metabolically flexible - Proteobacterium that inhabits settings with a connection to the earth, the ocean, living things, people, and plants. In our investigation, we looked at the microbial infection of cell phones in order to identify the dangerous microbes that are frequently associated with them. There are suggestions made regarding how to enhance the treatment of others. India's population of smartphone users has steadily increased to 5 million individuals a year [15].

1.3 Microorganism present on the surface of laptops and computers:

In the investigations, a lot of microbes were discovered the computer areas, including the private staff computers, public computer monitors, as well as other areas. The amount of microorganisms on keyboards has been discovered to be considerable, and public computers typically get much more microorganisms than the ones utilized just by single individual. Many individuals are probably to find themselves in places in which a large number of individuals pass out and in, such as colleges, hospitals or businesses, where they may introduce new microorganisms which will probably move mostly on keypad via atmosphere or via personal interaction. The laptop keyboard may be the cause of harmful bacteria due to improper body

washing and unclean surroundings, which may then lead in the unintentional spread of harmful infections. The majority of widely used devices are systems. The majority of systems contain more than one hundred one unique buttons, making it challenging yet time-consuming to cleaning. The majority of owners typically do not even clean and cleanse the system for this reason. Computer gaming systems have also been identified as a possible source of attractive controllers. Considering that computers aren't regularly maintained, the opportunity for the spread of filthy microbes is probably amazing. The keyboard and laptop terminal have a really special feature. So At end of the day, it's likely that the bacteria that reside on our nails, hands, skin and other body parts may transfer more tiny creatures towards machine. Especially in environments where lots of people go and arrive , like an urgent care, college, or workplace, there will undoubtedly be a certain amount of people who pass away, bringing with them fresh microorganisms that will unavoidably select device via air or by direct touch. The keypad of computer or laptop may be the source of microbe contamination, leading to the indirect spread of possible diseases, for 2 purposes: improperly conducted basic hygiene and unsterilized surroundings. Microbial contamination is also brought on by consuming close to gaming system. Spillages may land on as well as b/w keys, and the nutriment supplies encourage the growth of numerous tiny species. Remaining substances can retain moisture, allowing any existing microbes on the device to flourish. The ongoing investigation was conducted to evaluate the bacterial contamination of gaming system and their resistance to commonly used disinfecting swabs containing active ingredients chlorhexidine digluconate and Triclosan.

The illustrations used in this study weren't really chosen from urgent care equipment instead of basic technology used by teachers and employees involved in microbial laboratories. The objective of this study was to demonstrate that smartphones can potentially cause illness outside of urgent care. Microbes could harm individuals can be transmitted via smartphones and other digital equipment. Most people require that they be informed that, the transfer of these harmful microorganisms can take place in routine daily activities and minimize the cleaning of such devices. But several people think that microbe spread is only harmful in care environments. Using disinfecting cloths frequently on systems and cellular telephones can help prevent infectious diseases from becoming contaminated and transmitting via these devices.[16]

1.4 Biometric devices:

The utilization of Biometric machines (BDs) has increased above all comprehension and is an essential part of modern life. The achievement of a Indian Aadhar scheme provides proof that biometrics is fundamentally prepared for widespread implementation. The Unique Identification Authority of India (https://uidai.gov.in/) established a project in 2010 well with aim of giving each of India's 1.2 billion inhabitants a special twelve-digit count connected to biometric data that would offer as a recognition systems "devoid of any categorization of creed, caste, religion, and geographical." The use of biometric biometric verification is growing today across all industries. Systems for identity verification are becoming more prevalent. [6].

Although using biometric systems widely has the ability to have substantial bad impacts, it does provide major benefits to businesses, individuals, and government bodies. By physically touching the individual's epidermis to the smartphone's area during fingertip capture, the BDs can identify the user. Several people place their fingertips sequentially on the exact same specific hardware area. A variety of germs could be transmitted from these ecological gadgets to people as a result of this behaviour. Whenever these gadgets are utilized by medical professionals, experts, and pet managers whom bodies are much more likely to carry infections as well as other microbes, the danger of spreading pathogens might rise [9–10]. The epidermis serves as the home for a bacterial ecology that is primarily composed of gram-positive microbes, which is an essential point to remember. The body's microflora is maintained in part by heat, dampness, and epidermal biology [11]. The spreading of microorganisms via lifeless items is referred to as indirectly transferral from person to person in the medical setting of illness spreading, and the associated things are known as germs [10]. Numerous bacteria can be found on clear palms, and the majority are capable of live on lifeless items for a long time while still remaining alive in the palms for much more over twenty mins. Research have revealed that direct palm touch among non-living things can spread diseases in human [6]. We currently studied the presence of different microbial flora on the surface of BDs installed in Jaypee University of information and technology, wakhnaghat, solan and the risk of transfer of microbes through these fingerprinting devices.

1.5 Surface conditioning films:

When fresh surfaces are subjected to in-vitro and natural fluids comprising organic compounds, surface conditioning, films are quickly adsorbed. Fick's law of diffusion, which describes molecular diffusion in quiescent, is the main cause of the transport of organic products out from solid matrix to the surface. According to theoretical and practical research, molecular diffusion happens quickly and produces sizable organic layers in just 15 minutes. For instance, it has been demonstrated that molecular films produce between 0.8 and 15 mg of organic matter per square metre of uncovered material surface, or films between 30 and 80 nm thick**.** The formation of organic films may constitute polymers of proteins, glycoproteins, proteins, and possibly humic acids, which all may comprise as many as 100,000 small molecules units per chain, according to findings from experiments using Multiple Attenuated Internal Reflectance Infrared Spectroscopy (MAIR-IR), Fourier-Transformed Infrared Spectroscopy (FTIR), and Infrared Spectroscopy (IR). As a result, there would be a large number of reactive interaction sites available for additional reactions, whether with solute molecules with various sizes either with compounds produced on microbial surfaces. Surfaces exposed to organic chemicals have seen a variety of observable changes to their initial surface characteristics**.** For instance, both +positively as well as negatively charged regions gained net negative charge on the surface, and the zeta potentials, cohesion and friction angles, and free energy all change depending on the surface energy of the surface. With the exception of a few particular cases, the mechanism by which molecular films affect the binding of bacteria to surfaces is still unknown; however, the general consensus is that chemical groups on the organic coating interact with chemical structures on microbial appendages, such as flagella, pili, or bacterial exopolysaccharides (EPS). Such extensions penetrate the free energy hurdle to contact the extracellular matrix, where the structure might create reversible short-range electrostatic, covalent, or hydrogen connections. Another explanation for the chemotactic mobility and preferred location of several species of bacteria on solid surface is the occurrence of organic compounds on surfaces [8].

CHAPTER-2

REVIEW OF LITERATURE

2. Review of Literature

2.1 Possible Disease Spread through Pathogenic Deposits on the surface of Fingerprint Scanners.

Via disease-carrying stuff (fomites) or materials, microbes, including pathogens [19], may be transported from living sources to inanimate surroundings. Because of this, germs can move between people by coming into contact with shared surfaces in public spaces [20], such as fingerprint scanner surfaces, which may begin the spread of contagious diseases. The spread of infectious diseases through fingerprint scanners is categorised as an indirect method. Certain pathogens are capable of surviving for only a few sec. or min. until dying, dependent on the infectious microbe and given that the medium or droplets of aerosol stay living on the surface. Other diseases, on the other hand, depend on the frequency of use and the frequency of preventive surface disinfection to remain a continuous source of spread for days to days, weeks, or even months at a time [23] while waiting for a potential new host. For instance, studies [6,24] show that depending on the ambient temperature, humidity, and inoculum, the new coronavirus known as SARS-CoV-2, which produces COVID-19, can stay alive and pathogenic in aerosols for hrs and on the top layer for as long as days. These factors make long-lasting infections that are present in bodily fluids and spread by fingertips a subject of worry when using fingerprint scanners.

Table 1 provides a partial list of fluids from the body and fluids that could be applied to or collected through scanners that take fingerprints, along with some of the pathogenic isolates that are frequently linked with them.

Table 1. List of body secretions and fluids and other commonly associated microbial isolates capable of causing disease.

Body secretion/fluids	Implicated pathogenic isolates
Saliva	SARS-Co-2, *filoviruses, E. coli*.
Nasal mucus fluid	SARS-Co-2, *streptococcus, Actinomyces, filoviruses, Haemophilus influenzae*
Sweat	*Ebola virus, Staphylococcus species, Ebola virus*

Ear wax	mostly sterile, but yeast cells may be present.
Pus	*Klebsiella species, Pseudomonas species, Staphylococcus, E. coli*
Urine	*Klebsiella species, Candida albicans, Staphylococcus saprophyticus, Zika virus*, Yeast cells
Semen	*Ebola virus, Marburg virus, Lassa virus, E. coli, Hepatitis C*
Vaginal swab	*Trichomonas vaginalis, Candida albicans, E. coli, Neisseria gonorrhoeae*, yeast cells.
Eye secretion	*Pseudomonas aeruginosa, Staphylococcus aureus, Neisseria gonorrhoeae*.

Different pathogenic isolates from typical bodily secretions and fluids described in Table 1 may be kept on regularly touched surfaces, raising serious hygienic issues including the possibility for spreading of viruses that affect the respiratory system [106]. However, it is necessary to explicitly determine whether these infections can spread through compromised biometric fingerprint surfaces.

2.2 Sources of Fingerprint Scanner Surface Contamination

In essence, individuals who have Staphylococcus aureus contaminated hands, are nasal carriers of the bacteria, or exude the bacteria from skin's lesions like boils, infected cuts, pimple or wounds may contaminated the fingerprints biometric device surfaces by contact. Surfaces can be contaminated by contact with an uncovered wound that exudes pus-like or transparent fluids comprised of fibrin, the blood, and white cells. Additionally, it has been demonstrated that those with hepatitis A in the incubation stage, those with norovirus infections, those who have come into touch with the excreta of people who have cholera or salmonellosis, and those who are intestinal transmitters of Shigella species can all cause contamination [21].

2.4 STREAK PLATE METHOD

Streaking basically means “thin or long line”. It is a microbiological technique in which inoculum is taken and spread it on media plate in thin and line form on the surface f the media plate. This technique is used to isolate pure colonies of bacteria from a huge amount of population of the bacteria. You can also grow yeasts by this method. Basically, it is used for isolating pure colonies. In this method inoculum with lots of bacterial load spread or diluted on the agar plates and in the endless bacterial cells are isolated. This is method is used from old time. This method was identified by Loeffler and Gaffky in the laboratory of the Koch. This method has to be done in sterile conditions. For this method tools are used that is inoculating loop, cotton swab and metal stick are used for spreading on agar plate. Everything should be contamination free. Hence, isolated colonies are successfully seen. Principle of this method is by scatter it across the surface of agar plates, the inoculum is diluted. The inoculum gets diluted while streaking in consecutive parts of the plate until the point where just one bacterial cell appears on the top of the agar plate every less millimetres. When these single bacterial cells split and produce hundreds of additional microbial cells, a distinct colony is created. Selecting carefully separated colonies and re-streaking them on new and fresh plate of agar will give you Pure cultures. Materials that is required for this method is tissue paper or cotton, burner, loop, Agar plate, matchstick and ethanol. The agar surface can be used to separate each cell of bacteria using a variety of different streaking patterns. There are four fundamental kinds of streaking techniques- Continuous streak, Quadrant streak, T-streak and Radiant streak.

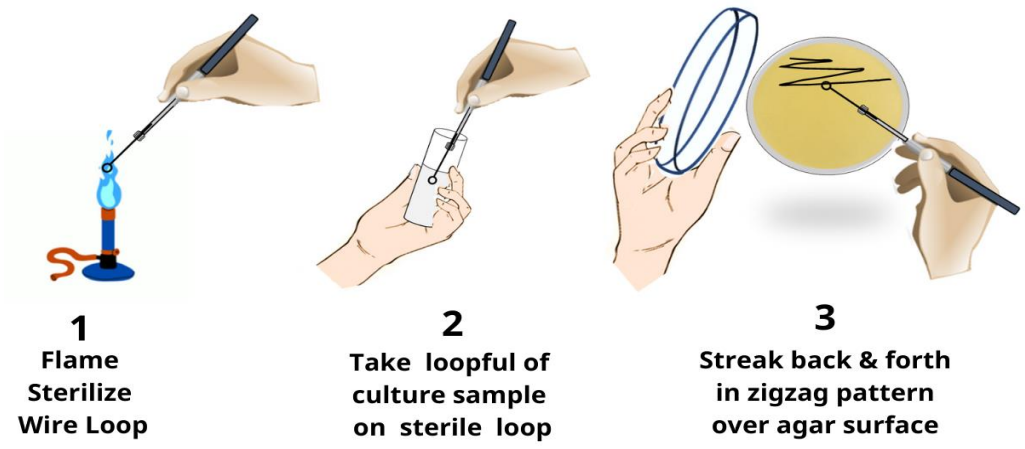

Fig 1 – Streak Plate Method Steps [18]

2.5 CONTINUOUS STREAK (ZIGZAG STREAK)

Zigzag streaks are used as an inoculation pattern on samples with few microorganisms. When isolation is not required and pure culture needs to be inoculated, a continuous streak is used. A sterilised cotton swab can also be used for this process.

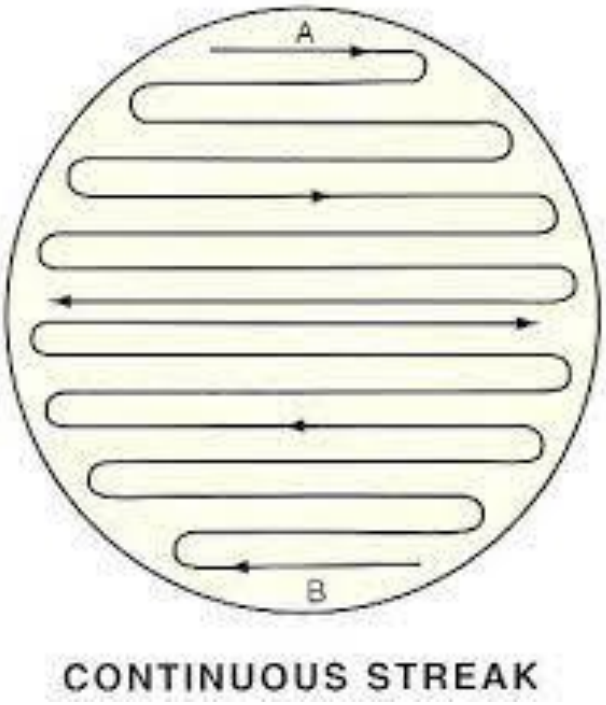

Fig 2 – Continuous Streaking Method [17]

2.6 QUADRANT STREAK

The most often used and preferred method for obtaining isolated bacterial colonies is this one. Due to the streaking of four identically sized regions on Agar plates, this streaking also referred to as the four sectors technique or four-quadrant streak. The four-quadrant approach is used to isolate bacterial colonies when a sample has a high concentration of bacterial cells. The sterile metal loop is used to streak a sample with a lot of bacterial cells. A single cell deposits most of its material onto the agar surface due to the way the loop of wire is streaked over it.

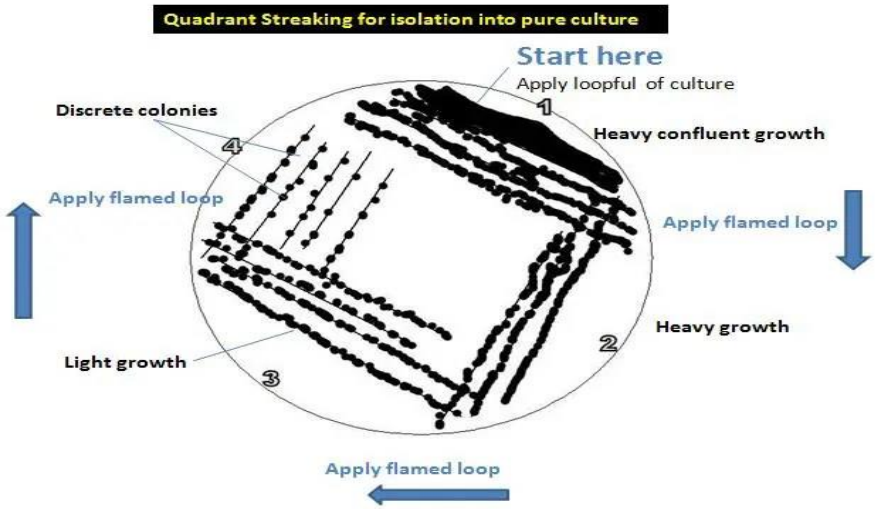

Fig 3 – Quadrant Streaking Method [18]

2.7 T- STREAK

This approach uses three sectors. Three portions of the agar plates have been streaked in this way. Three pieces make up the agar plate. A sterilised wire loop can be used for this procedure. Utilising a sterile wire loop, a full loop of a liquid sample holding a combination of bacteria is obtained, and three sections of the sample are sequentially dispersed onto the surface of the agar in Petri plates. Due of this, it is known as the "Three Sector Technique." In the initial portion, there is significant confluent growth. The second segment has fewer cells overall. Growth is relatively slow in the last section, and we see solitary colonies.

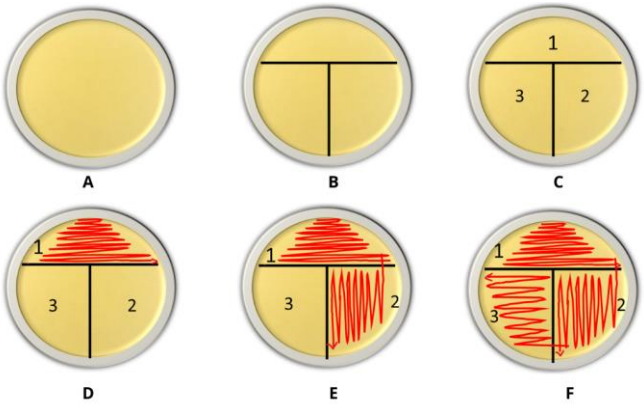

Fig 4 – T – Streaking Method [18]

2.8 RADIANT STREAK

The loopful of growth sample is spread out in area 1 of this streak pattern, close to the border of the agar plate. From this edge, the sample streaks in a radially manner, dragging straight lines in the direction of the area's opposing side1. In usually, 7 or 8 consecutive streaks are executed. The next step is to streak straight lines close to area 1 with a flame sterile loop.

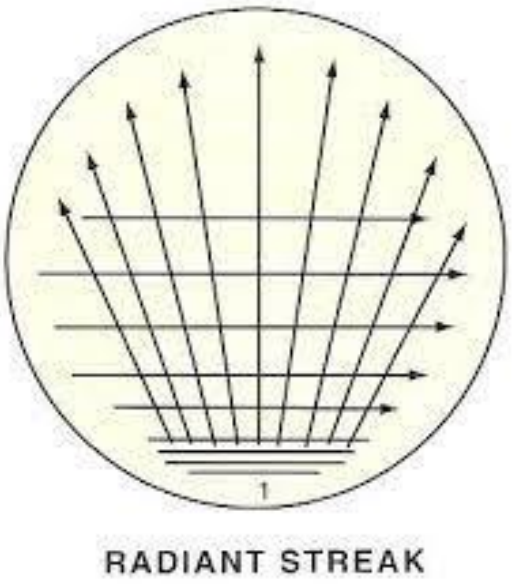

Fig 5 - Radiant Streaking Method [17]

2.9 GRAM STAINING

The method of gram staining proposed by Dr. Hans Christian Gram in 1884. Gram staining help us to determine a bacterium into gram negative and gram-positive classification according to their cell membrane composition. Gram stain was prepared by various dyes and reagents along with composition. Use of primary stain, Decolorising agent, counter stain and mordant. Gram positive bacteria have cell wall made up of peptidoglycan cell and Gram-negative bacteria is having lipid rich outer membrane. The formation of crystal violet- iodine complex because crystal violet complex are insoluble complex that is hard to remove especially after the use of mordant. Decolorising agent like alcohol dehydrates the cell wall and block the complex into the cell wall in Gram positive bacteria but in the Gram-negative bacteria the lipid rich wall will dissolve and remove the stain from the wall.

2.10 STRING TEST

It is also called Potassium Hydroxide test. The objective of string test is to differentiate b/w Gram negative and Gram positive bacteria. Like a gram stain this test is also based on chemistry of the cell wall of bacteria. As name suggest potassium hydroxide is a key chemical which is used for performing the string test. The function of potassium hydroxide is to dissolve or degrade the layer of peptidoglycan of the cell wall of Gram-ve bacteria. As a result, it makes bacterial smear thick and stringy. In Gram -ve bacteria, these bacteria will not be affected by potassium hydroxide, because gram negative bacteria have thicker peptidoglycan layer then gram +ve bacteria. Thus, cell will not lyse and no thick or stringy smear was observed.

2.11 MEDIA USED FOR BACTERIAL ISOLATION AND STUDY

2.11.1 NUTRIENT AGAR

Nutrient Agar is a basic culture media that are used for non-fastidious microorganisms. Nutrient Agar contains different nutrients like beef extract, agar and peptone. For more nutrients, some trace ingredients and vitamins are also added. Because of these nutrients a huge range of microorganisms can grow without a need of specific supplements and nutrients. Now let see about the benefits of the components which are add to the nutrient agar. Peptone is a good source of protein or nitrogen which leads to a source of amino acid for the microorganisms. Beef extract is also a good source of carbon which leading to the formation of carbohydrates in the microorganism. Beef extract contain other ingredients like minerals, vitamins, salts and organic compounds which help the growth of microorganisms. Sodium chloride is also a component in the beef extract which maintain osmotic equilibrium of the media and during growth phase it maintain the pH level. Distilled water is added so that nutrients will dissolve for the better absorption of the bacteria. Agar also holds main role in the nutrient agar as it provides solidifying agents to the media because of this we get to see colony morphology and numbering of the microorganism. Add all these ingredients with their correct quantities. Autoclave it and pour it onto plates.

2.11.2 BLOOD AGAR

Blood Agar comes under enriched medium that gives various nutrients with supplementation with blood. It comes under Basal media that can be used for growth media. This media is good for fastidious bacteria. Around 5% of defibrinated mammalian blood (Sheep, horse or human) is required. It gives medium to the fastidious bacteria but inhibits the growth of some microorganism like *Haemophilus* and *Neisseria*. When the blood agar heated up it will become Chocolate Agar and chocolate agar gives medium to the microorganism like Neisseria and Haemophilus. You can also check the Hemolysis activity of the bacteria by blood agar. There are 3 types of Hemolysis, Alpha Hemolysis, Beta hemolysis and Gamma hemolysis. When brownish or greenish-grey discoloration arund colony is found then it means it is a Alpha hemolysis like *Streptococcus pneumonia*. When there is clear zone around the colony it means it is a Beta hemolysis β-hemolytic bacteria include group B *streptococcus* like *S. agalactiae* and group A *streptococci* like *S. pyogenes*. When there is no change in color or no zone is found around the colony then it is called Gamma hemolysis like *Neisseria meningiditis*. It is made up of Blood, tryptose and peptone which gives nitrogen, vitamins, carbon and amino acids to the bacteria to grow. Sodium chloride is also a component in the beef extract which maintain osmotic equilibrium of the media and during growth phase it maintain the pH level.

2.11.3 MACCKONKEY AGAR

MacConkey agar was developed by Alfred Theodore MacConkey. It comes under both differential and selective media. It allows the growth of non- fastidious and gram-negative bacteria and also allow to grow *Pseudomonas* from the family of Enterobacteriaceae. By the help of MacConkey Agar medium, we can differentiate b/w lactose fermenting and lactose non-fermenting gram-negative bacteria. It contains Proteose peptone (meat and casein), peptone (Pancreatic digest of gelatin), bile salts, lactose monohydrate, crystal violet, neutral red, Distilled water and agar. Proteose peptone (meat and casein) and peptone (Pancreatic digest of gelatin) gives the required vitamins, minerals and nitrogen that are required for the growth of the organisms. Lactose monohydrate is the fermentable and good source of carbohydrates. Most of the gram positive bacteria cannot grow in bile salts and crystal violet. Sodium chloride is also a component in the beef extract which maintain osmotic equilibrium of the media and during growth phase it maintain the pH level. Neutral red gives pH indication by changing colour to the red (below 6.8) and goes colorless when pH above 6.8. As a result, if it gives

red or pink colour then it means it is a lactose fermenting bacteria and if it is colorless then it means it is lactose non-fermenting bacteria.

2.11.4 SIMMON'S CITRATE AGAR

Simmons Citrate Agar is that medium which can help in differentiation of Enterobacteriaceae based on the utilisation of citrate as a source of energy by bacteria. It is developed by Koser.

Sodium Citrate is a source of carbon in the medium. Dipotassium phosphate acts like a buffer. Magnesium sulfate provides various metabolic reaction. Brothymol blue use for an indicator which indicated colour from green to blue above pH 7.6. Sodium chloride is also a component in the beef extract which maintain osmotic equilibrium of the media and during growth phase it maintain the pH level. Agar also holds main role in the nutrient agar as it provide solidifying agents to the media because of this we get to see colony morphology and numbering of the microorganism.

2.11.5 PEPTONE WATER BROTH

Peptone water is a broth medium which is used for growth of the microorganism. This broth medium is also used in indole test. It contain sodium chloride and peptone. Peptone gives carbon, nitrogen, vitamins and carbon to the bacteria for the growth. Sodium chloride is also a component in the beef extract which maintain osmotic equilibrium of the media and during growth phase it maintain the pH level. It is best for indole production test. Peptone water is also used for carbohydrate fermentation. For the test put indicator like phenol red is added which shows the change in color in the medium. If growth is turbid then it is a positive result and if there is no growth means it is a negative result.

2.11.6 GLUCOSE PHOSPHATE BROTH

Methyl red is used to know about the ability of the microorganism to produce mixed acids by fermentation of glucose. It contains Glucose, proteose peptone, dipotassium phosphate and distilled water. It is used to perform methyl red test for microorganism.

2.11.7 NITRATE BROTH

Nitrate broth is a broth medium. It is used for nitrate reduction test and also tells about Enterobacteriaceae that reduce nitrate (gram negative bacteria) It recognise species like *Moraxella, Kingella* and *Neisseria.*

2.12 BIOCHEMICAL TEST USED FOR STUDY

2.12.1 Growth check on MacConkey Agar

MacConkey agar was developed by Alfred Theodore MacConkey. It comes under both selective and differential media. It allows the growth of non- fastidious and gram negative bacteria and also allow to grow Pseudomonas from the family of Enterobacteriaceae. By the help of MacConkey Agar medium we can differentiate b/w lactose non-fermenting gram negative bacteria and lactose fermenting bacteria.

Proteose peptone (meat and casein) and peptone(Pancreatic digest of gelatin) gives the required vitamins, minerals and nitrogen that are needs for the growth of the organisms. Lactose monohydrate is the fermentable and good source of carbohydrates. Most of the gram positive bacteria cannot grow in bile salts and crystal violet. Sodium chloride is also a component in the beef extract which maintain osmotic equilibrium of the media and during growth phase it maintain the pH level. Neutral red gives pH indication by changing colour to the red (below 6.8) and goes colorless when pH above 6.8. Distilled water is added so that nutrients will dissolve for the better absorption of the bacteria. Agar also holds main role in the nutrient agar as it provides solidifying agents to the media because of this we get to see colony morphology and numbering of the microorganism. Add all the components into distilled water. Then autoclaved it and pour into the sterile plates. As a result, if it gives red or pink colour then it means it is a lactose fermenting bacteria and if it is colorless then it means it is lactose non-fermenting bacteria.

2.12.2 Growth check on Blood Agar

Blood Agar comes under enriched medium that gives various nutrients with supplementation with blood. It comes under Basal media that can be used for growth media. This media is good for fastidious bacteria. Around 5% of defibrinated mammalian blood (Sheep, horse or

human) is required. It gives medium to the fastidious bacteria but inhibits the growth of some microorganism like *Haemophilus* and *Neisseria*. When the blood agar heated up it will become Chocolate Agar and chocolate agar gives medium to the microorganism like *Neisseria* and *Haemophilus*. You can also check the Hemolysis activity of the bacteria by blood agar. There are 3 types of Hemolysis, Alpha Hemolysis, Beta hemolysis and Gamma hemolysis. When brownish or greenish-grey discoloration arund colony is found then it means it is a Alpha hemolysis like *Streptococcus pneumonia*. When there is clear zone around the colony it means it is a Beta hemolysis β-hemolytic bacteria include group B *streptococcus like S. agalactiae* and group A *streptococci like S. pyogenes*. When there is no change in color or no zone is found around the colony then it is called Gamma hemolysis like *Neisseria meningiditis*. It is made up of Blood, tryptose and peptone which gives nitrogen, vitamins, carbon and amino acids to the bacteria to grow. Sodium chloride is also a component in the beef extract which maintain osmotic equilibrium of the media and during growth phase it maintain the pH level. Distilled water is added so that nutrients will dissolve for the better absorption of the bacteria. Agar also holds main role in the nutrient agar as it provide solidifying agents to the media because of this we get to see colony morphology and numbering of the microorganism. Add all these ingredients with there correct quantities and add 5% sterile mammalian blood. Autoclave it and pour it onto plates.

2.12.3 Indole Production Test

It is a biochemical test done on microbes. Indole test determines the potential of the organism to convert tryptophan into indole.

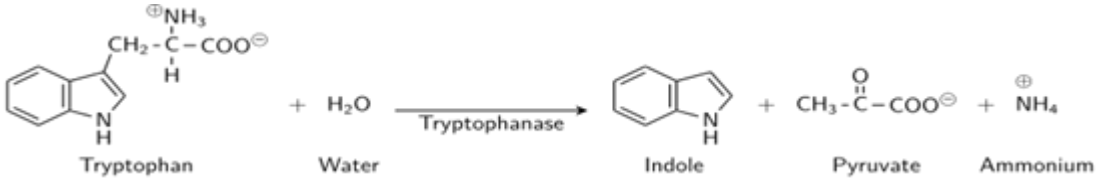

Fig 6 – Indole Reaction

It helps in identify Enterobacteria like E.coli P.rettgeri and P. vulgaris. Tryptophan is an amino acid that secreate by bacteria that contain tryptophanase enzyme. For performed this test it needs peptone water broth and kovac's reagent. For doing this test inoculate the bacteria into

Peptone water broth. Then place it for incubation at 37 C for 4-5 days. After growth are seen in the broth add 0.5ml (500 ul) and shake slowly. If red/pink colour is formed then it is a positive result and broth remains yellow then it is a negative result.

2.12.4 Methyl Red Test

Methyl red tells about the microorganism whether it is mixwd acid fermentation and produces stable acid end products. Methyl red is a dye which indicates color. pH of this is below 4.5. Methyl red test detects the acid production.

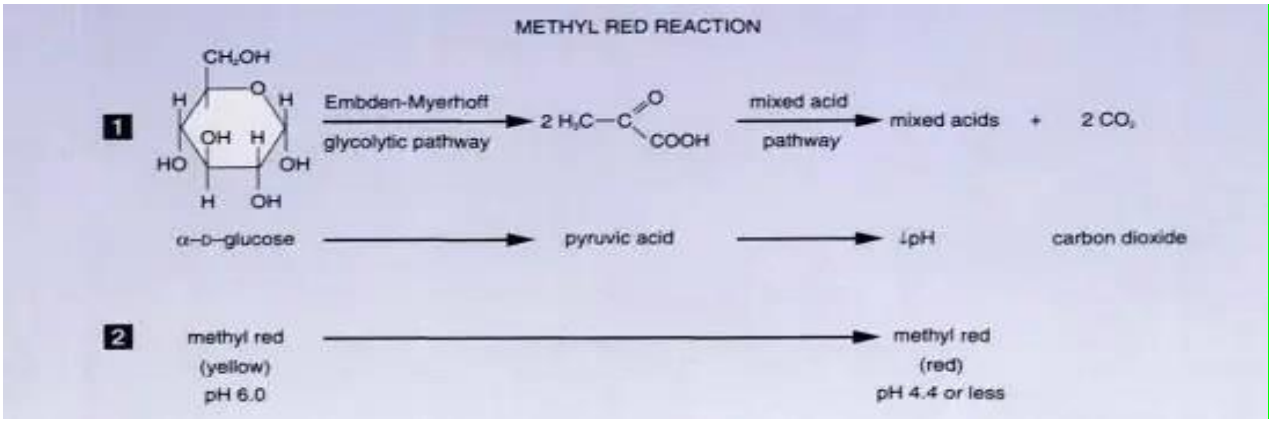

Fig 7 – Methyl red reaction.

For doing this test, firstly inoculate the microorganism in glucose phosphate broth. Then incubate it at 37 C for 3-5 days. Then add 4-5 drops of methyl red solution. Mix throughout and see the result. If it gives bright red colour then it means it isa positive result but if it remains yellow then it means it is a negative result.

2.12.5. Citrate utilisation Test

Citrate utilisation test is used to differentiate between Gram -ve bacilli in the family Enterobacteriaceae. Simmons Citrate Agar is that medium which can help in differentiation of Enterobacteriaceae based on the utilisation of citrate as a source of energy by bacteria. It is developed by Koser. It contains sodium citrate, sodium chloride, dipotassium phosphate, ammonium dihydrogen phosphate, bromothymol blue, magnesium sulfate and agar. Sodium Citrate is a source of carbon in the media. Ammonium dihydrogen phosphate provides nitrogen.

Dipotassium phosphate acts like a buffer. Magnesium sulfate provides various metabolic reaction. Brothymol blue use for an indicator which indicated colour from green to blue above pH 7.6. Sodium chloride is also a component in the beef extract which maintain osmotic equilibrium of the media and during growth phase it maintain the pH level. Agar also holds main role in the nutrient agar as it provide solidifying agents to the media because of this we get to see colony morphology and numbering of the microorganism.

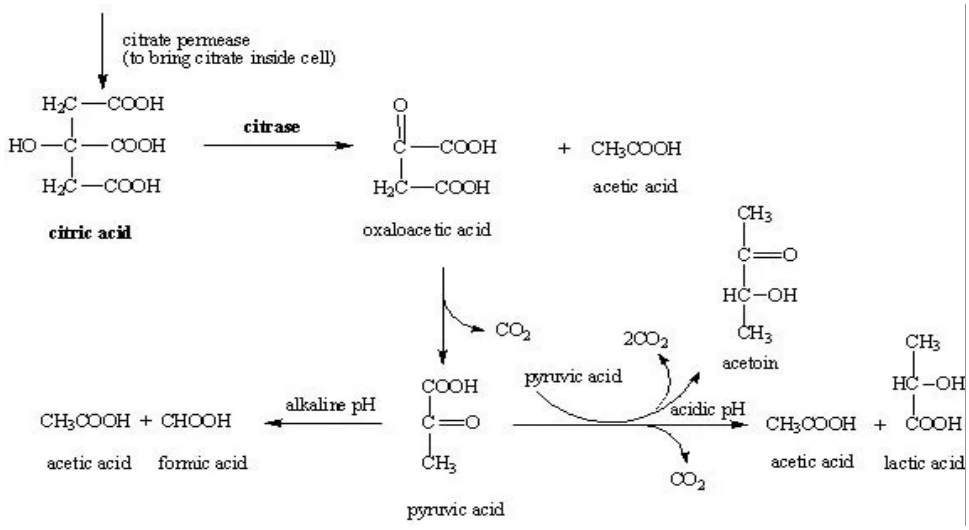

Fig 8 - Citrate reaction

For doing this test, firstly inoculate the microorganism directly on to the simmon's citrate agar plates. This media also contain indicator bromthymol blue. Place it on incubation at 37 C for 3-4 days. If growth give blue color then it is a positive result but if growth shows no change in colour and remains green in colour then it means it is negative result.

CHAPTER-3

Methodology

3. Methodology

3.1 Study Area

The study was carried out in Jaypee university of information & technology at microbiology department.

3.2 Study Period

The study was conducted out in the year 2023 between January to May.

3.3 Sample collection

- Sample collection from biometric machine of JUIT taken in morning 10a.m and in evening 5:30pm. Take sterile wet cotton swabs for collection and swab it on the scanner in all the direction and keep away from other parts of biometric devices.

Fig 9- Sterile Cotton swab.

- All swabs were collected on the same day & time.
- Here is the table which is showing time & location of sample collection.

TABLE 1. Showing time & location of sample collection.

S.no	Location	Sample collection timing
1.	MG (main gate)	M1(9:30PM) E2 (5:30PM)
2.	GB (geeta bhawan)	M1(9:30PM) E2 (5:30PM)
3.	AB (academic block)	M1(9:30PM) E2 (5:30PM)
4.	MD (mandir)	M1(9:30PM) E2 (5:30PM)

Process of swab samples-

- Take sterile cotton swab and dip it into saline water.

Fig 10 – Sterile cotton swab into saline water.

- Wet sterile cotton swabs were rubbed on the scanner in all the direction avoiding any touch of swabs with other parts of biometric devices.

Fig 11 – Cotton swab rubbed on the scanner for sample collection.

- Then directly spread it on nutrient agar plate.

Fig 12 – Spreading the inoculum from BDs on the plate.

3.4 ISOLATION OF BACTERIA

Hence, spreading is done. Now place the plates in incubator for 2-3 days at 37°C. When growth is fully visible, took the inoculum from these plates and further done quadrant streaking for pure colonies.

3.5 PURIFICATION OF ISOLATED COLONIES

Individual colonies was selected and purified using streak plate technique on nutrient agar plate and incubated at 37°C for 48 hrs for pure culture.

3.6 IDENTIFICATION OF BACTERIA

For identification of bacteria firstly selected isolates grown on the MacConkey Agar and Blood Agar and also observe phenotypic characterization like form, margin, color. Morphology can be studied under the observation of microscope after doing Staining and different Biochemical test like Indole test, Catalase test, citrate utilization test and Methyl red test.

3.7 GRAM STAINING

The process of Gram staining, firstly take a clean slide and place one drop of water on to the slide. Then take bacterial inoculum and make thin smear on the slide. Air dry the smear & heat fix the smear. Now, add four or five drops of crystal violet (primary stain) and kept it for one to two min followed by Wash it with tap water (it will remove extra stain on slide). Then add iodine solution (Mordant) and formation of Violet-iodine complex forms. Kept it for 1-2 min and wash with tap water. Now add Methanol (decolorizer) over the smear for 15-20 seconds then wash it with tap water. Now add safranin (counter stain) for 30 second to 1 minute and wash with tap water. Now leave the slide for air dry. After air dry the slide do microscopic examination. If bacteria is gram-negative then it will shows pink colour but if bacteria is gram-positive it will shows violet colour.

Table 2 – Composition of stains which are used in Gram Staining.

Gram Crystal Violet	0.5%
Gram Iodine	
Potassium Iodide	2%
Resublimed Iodine	1%
Gram Decolorizer	
Methanol	80%
Acetone	20%
Gram Safranine	1%

3.8 STRING TEST

For doing this string test, Firstly you need clean slide and 3% potassium hydroxide (3% means 3 grams in 100 ml of distilled water). Now place a drop of 3% potassium hydroxide on to the slide. Then take inoculum from plate and emulsify the inoculum on to the slide (having a drop of KOH). Emulsify it for a dense suspension. Stir it for 60 sec and gently pull the loop

away from the suspension which is on slide. If it become stringy and thick then it is Gram positive bacteria. But if it does not become stringy and thick then it is a Gram negative bacteria.

3.9 MEDIA WHICH ARE USED IN THIS EXPERIMENT

3.9.1 NUTRIENT AGAR

- Weigh and put 28g of nutrient agar powder into 1L of distilled water. Dissolve and mix thoroughly. Autoclaved it 121°C for 15 minutes. Let cool down it and then pour the media onto media agar plates (process done in LAF). Wait for the media to solidify. Place them into incubator at 37°C for 24 hrs.

Fig 13 – Nutrient Agar

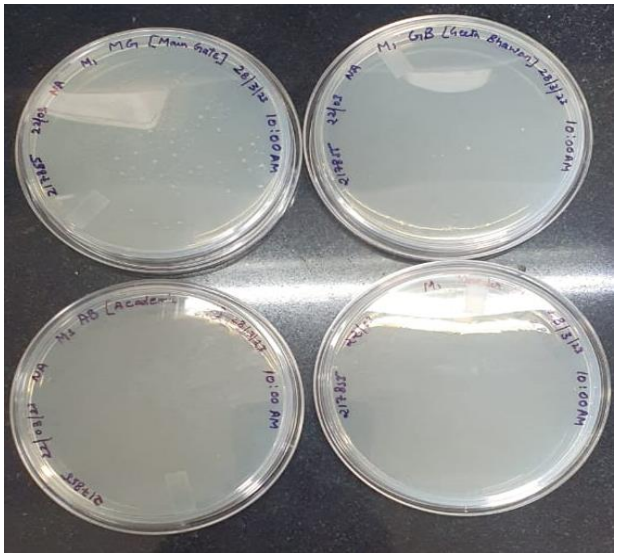

Fig 14 - Nutrient Agar Plates

3.9.2 BLOOD AGAR

Distilled water is added so that nutrients will dissolve for the better absorption of the bacteria. Agar also holds main role in the nutrient agar as it provide solidifying agents to the media because of this we get to see colony morphology and numbering of the microorganism. Add all these ingredients with there correct quantities and add 5% sterile mammalian blood. Autoclave it and pour it onto plates.

Table 3 – Composition of Blood Agar

Ingredients	**Gram/liter**
Peptone	10.0
Tryptose	10.0
Sodium chloride	5.0
Agar	15.0
Sterile mammalian blood	5%

3.9.3 MACCKONKEY AGAR

Distilled water is added so that nutrients will dissolve for the better absorption of the bacteria. Agar also holds main role in the nutrient agar as it provide solidifying agents to the media because of this we get to see colony morphology and numbering of the microorganism. Add all the components into distilled water. Then autoclaved it and pour into the sterile plates. As a result, if it gives red or pink colour then it means it is a lactose fermenting bacteria and if it is colorless then it means it is lactose non-fermenting bacteria.

Table 4 – Composition of MacConkey Agar

Ingredients	**Amount**
Peptone (Pancreatic digest of gelatin)	17 gm
Proteose peptone (meat and casein)	3 gm
Lactose monohydrate	10 gm
Bile salts	1.5 gm
Neutral red	0.03 gm
Crystal Violet	0.001 gm
Agar	13.5 gm
Distilled Water	Add to make 1 liter
Sodium chloride	5 gm

3.3.4 SIMMON'S CITRATE AGAR

It contains sodium citrate, sodium chloride, dipotassium phosphate, ammonium dihydrogen phosphate, bromothymol blue, magnesium sulfate and agar.

Table 5 – Composition of Simmon Citrate Agar

Ingredients	Amount
Sodium Chloride (NaCl)	5.0 gm
Ammonium Dihydrogen Phosphate	1.0 gm
Sodium Citrate (dehydrate)	2.0 gm
Dipotassium Phosphate	1.0 gm
Bromothymol Blue	0.08 gm
Magnesium Sulfate (heptahydrate)	0.2 gm
Agar	15.0 gm

- Dissolve all the salts which are mentioned in the table in distilled water. Adjust the pH (6.9). Add Bromothymol blue and agar. Autocalved it 121°C at 15psi. Let cool down it and then pour the media onto media agar plates (process done in LAF). Wait for the media to solidify. Place them into incubator at 37°C for 24 hrs.

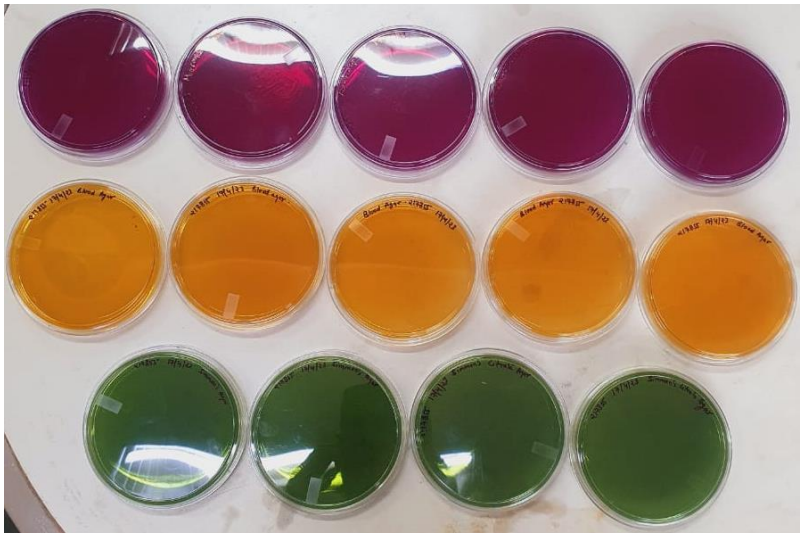

Fig 15 – a) MacConkey Agar b) Blood Agar c) Simmon's Citrate Agar

3.9.5 PEPTONE WATER BROTH

Add peptone and sodium chloride (in required quantities) into distilled water. Then auto-claved it.

Table 6 – Composition of Peptone Water Broth

Ingredients	**Amount**
Peptone	10.0 gm
Sodium Chloride	5.0 gm

- Dissolve 15.0 grams in 1000 ml distilled water.
- Dissolve and mix thoroughly.
- Autoclaved it 121°C for 15 minutes.

- Let cool down it and then pour the media onto media agar plates (process done in LAF).
- Wait for the media to solidify.
- Place them into incubator at 37°C for 24 hrs.

3.9.6 GLUCOSE PHOSPHATE BROTH

Methyl red is used to know about the ability of the microorganism to produce mixed acids by fermentation of glucose. It contains Glucose, proteose peptone, dipotassium phosphate and distilled water. It is used to perform methyl red test for microorganism.

Table 7 – Composition of Glucose Phosphate Broth

Ingredients	Amount
Proteose Peptone	5 gm
Glucose	5 gm
Dipotassium phosphate	5gm
Distilled water	1000 mL

- Dissolve all salts in 1000 ml distilled water.
- Dissolve and mix thoroughly.
- Autoclaved it 121°C for 15 minutes.

- Let cool down it and then pour the media onto media agar plates (process done in LAF).
- Wait for the media to solidify.
- Place them into incubator at 37°C for 24 hrs.

3.9.7 NITRATE BROTH

Nitrate broth is a broth medium. It is used for nitrate reduction test and also tells about Enterobacteriaceae that reduce nitrate (gram negative bacteria) It recognise species like *Moraxella*, *Kingella* and *Neisseria*.

Table 8 – Composition of Nitrate Broth

Ingredients	Amount
Potassium nitrate	1.000 gm
Sodium chloride	30.000
Meat exract	3.000
Peptic digest of animal tissue	5.000
Distilled water	1000 mL

- Dissolve all salts in 1000 ml distilled water.
- Dissolve and mix thoroughly.
- Autoclaved it 121°C for 15 minutes.

- Let cool down it and then pour the media onto media agar plates (process done in LAF).
- Wait for the media to solidify.
- Place them into incubator at 37°C for 24 hrs.

3.10 Biochemical Test performed in this experiment

3.10.1 Growth check on MacConkey Agar

MacConkey Agar contains Proteose peptone (meat and casein), peptone (Pancreatic digest of gelatin), bile salts, lactose monohydrate, crystal violet, neutral red, Distilled water and agar.

3.10.2 Growth check on Blood Agar

Blood Agar comes under enriched medium that gives various nutrients with supplementation with blood. It comes under Basal media that can be used for growth media. This media is good for fastidious bacteria. Around 5% of defibrinated mammalian blood (Sheep, horse or human) is required. It gives medium to the fastidious bacteria but inhibits the growth of some microorganism like *Haemophilus* and *Neisseria*.

3.10.3Indole Production Test

Indole test is a biochemical test done on bacterial species. Indole test determines the ability of the organism to convert tryptophan into indole. It helps in identify Enterobacteria like *E.coli P.rettgeri* and *P. vulgaris.* For performed this test it needs peptone water broth and kovac's reagent. For doing this test inoculate the bacteria into Peptone water broth. Then place it for incubation at 37°C for 4-5 days.

3.10.4 Methyl Red Test

Methyl red tells about the microorganism whether it is mixwd acid fermentation and produces stable acid end products. For doing this test, firstly inoculate the microorganism in glucose phosphate broth. Then incubate it at 37°C for 3-5 days. Then add 4-5 drops of methyl red solution. Mix throughout and see the result. If it gives bright red colour then it means it isa positive result but if it remains yellow then it means it is a negative results.

3.10.5 Citrate utilisation Test

Citrate utilisation test is used to differentiate between Gram-Negative bacilli in the family Enterobacteriaceae. It contains sodium citrate, sodium chloride, dipotassium phosphate, ammonium dihydrogen phosphate, bromothymol blue, magnesium sulfate and agar. For doing this test, firstly inoculate the microorganism directly on to the simmon;s citrate agar plates. This media also contain indicator bromthymol blue. Place it on incubation at 37°C for 3-4 days. If growth give blue color then it is a positive result but if growth shows no change in colour and remains green in colour then it means it is negative result.

CHAPTER-4

RESULT AND DISCUSSION

4. RESULT AND DISCUSSION

4.1 ISOLATED COLONIES-

For obtaining isolated colonies kept plate in incubator and after 2 days visible growth are seen and further done quadrant streaking to obtain pure colonies. This table tells about the colony type which is taken from different location.

Yellow colony- Y , Irregular white colony- R , Orange colony- O , White round colony- W , Brown colony- B.

Table 9 – Colonies obtained from BDs.

MAIN GATE	**COLONY TYPE**
10:00 A.M	144 yellow colony 132 Irregular white colony 41 Orange colony
5:30 P.M	148 White round colony 16 Irregular white colony 10 Yellow colony 1 Brown colony 1 Orange colony

ACADEMIC BLOCK	**COLONY TYPE**
10:00 A.M	12 yellow colony 8 white round colony
5:30 P.M	20 White round colony 22 yellow colony

GEETA BHAWAN	**COLONY TYPE**
10:00 A.M	17 yellow colony 2 Orange colony 6 White round colony
5:30 P.M	1 Big circular colony 1 Small circular colony

MANDIR	COLONY TYPE
10:00 A.M	15 yellow colony 7 White round colony 1 Big white colony
5:30 P.M	22 white round colony 2 Big round colony 1 Yellow colony 1 Orange colony

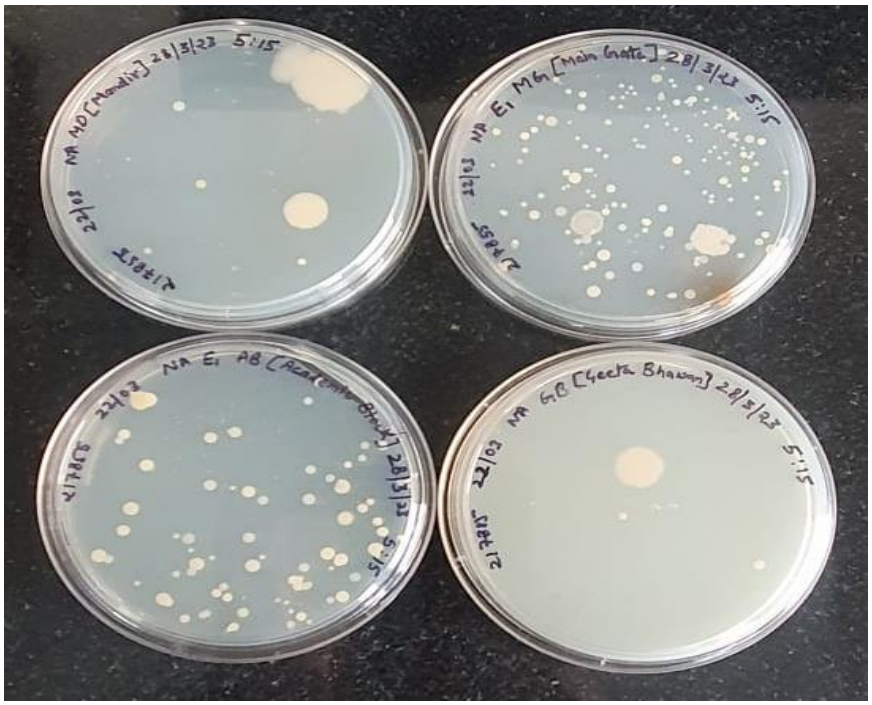

Fig 16 - Different colony growth took in morning and evening from BDs

4.3 PURIFICATION OF ISOLATED COLONIES

After doing quadrant streaking pure colonies are seen also there are having different shape and colour from each other.

Fig 17 – Brown colony (Main Gate from evening sample)

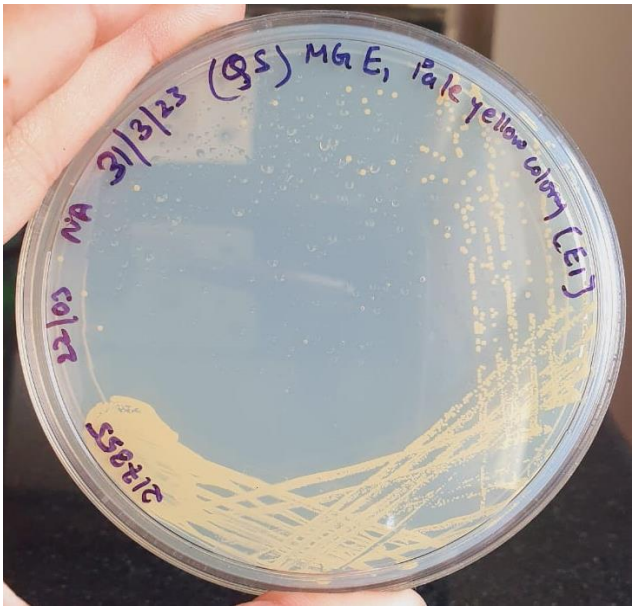

Fig 18 – Pale yellow colony (Main gate from evening sample)

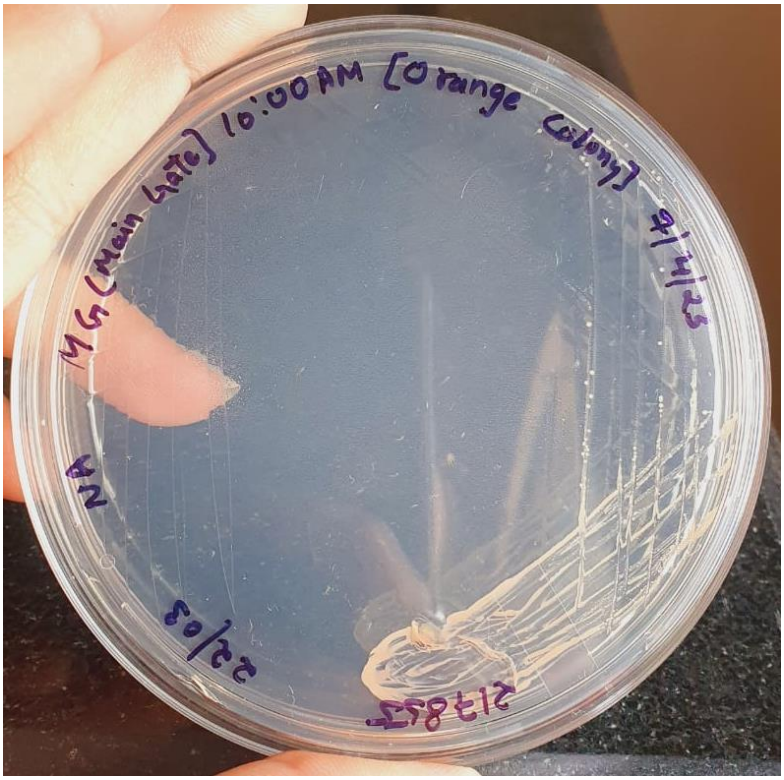

Fig 19 – Orange colony (Main gate from morning sample)

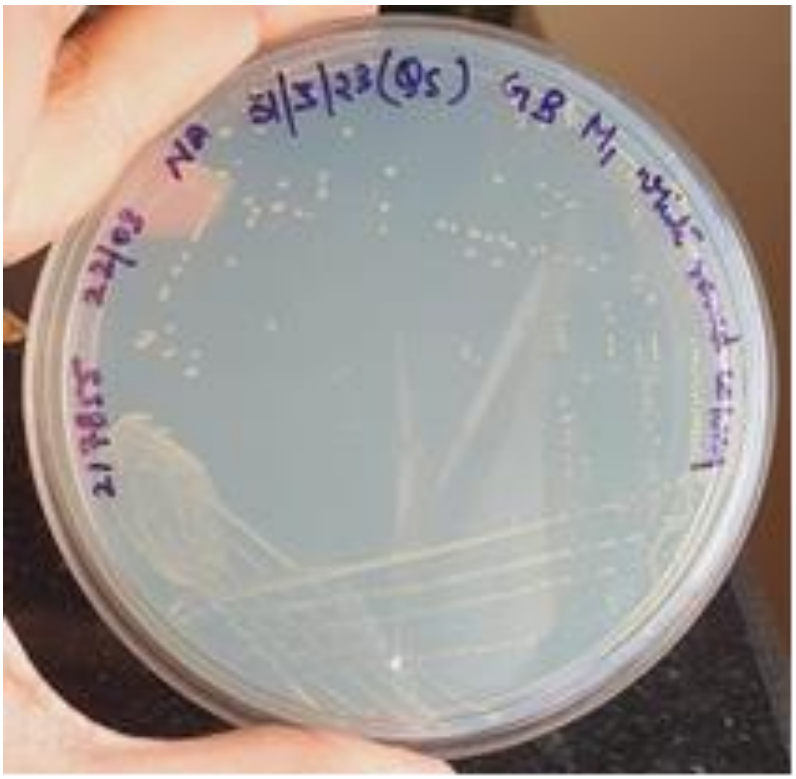

Fig 20 – White colony (Geeta bhawan from morning sample).

Table 10 – Colony characteristics on solid medium.

COLONY	SHAPE	EDGE	SURFACE	TEXTURE	CONSISTENCY
Yellow colony	Circular	Entire	Glistening	Smooth	Buttery
White colony	Irregular	Undulate	Rough	Dry	Dry
Orange colony	Circular	Entire	Glistening	Creamy	smooth
Brown colony	Circular	Entire	Rough	Smooth	Dry
White colony	Round	Entire	Glistening	smooth	Buttery

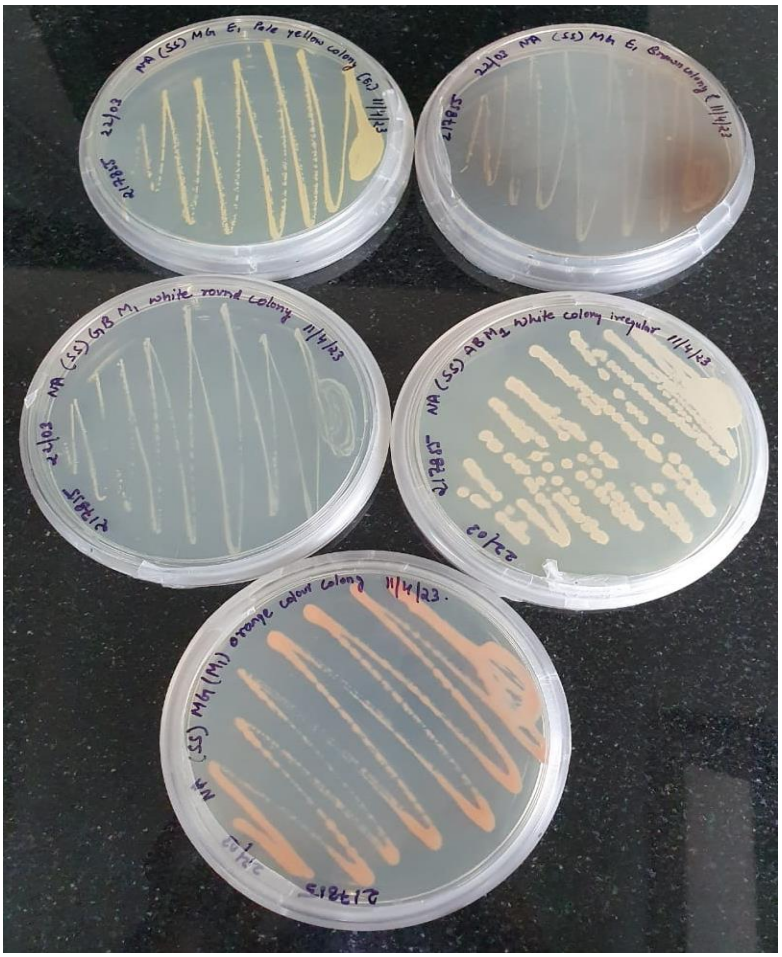

Fig 21 – Simple streaking of each sample for further use (stored at 4°C).

4.4 GRAM STAINING

All bacteria are showing pink colour which means these are gram negative bacteria.

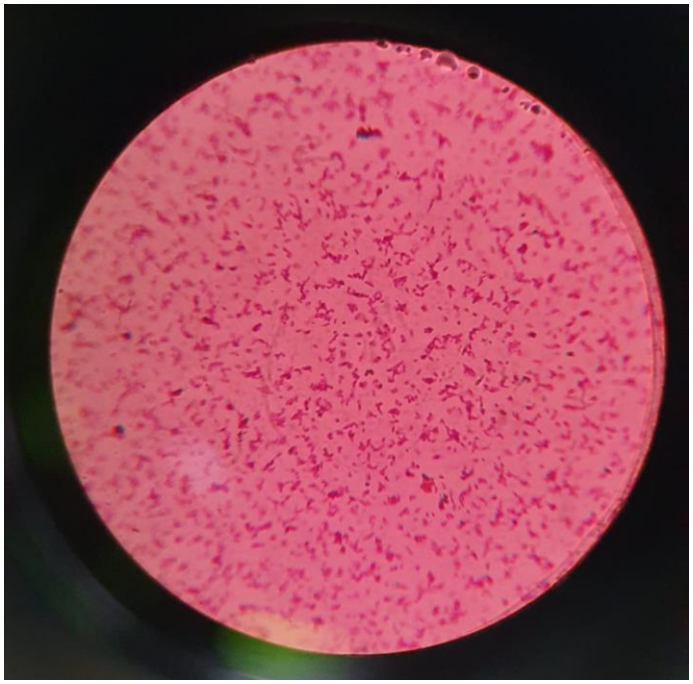

Fig 22 – Staining of White round colony

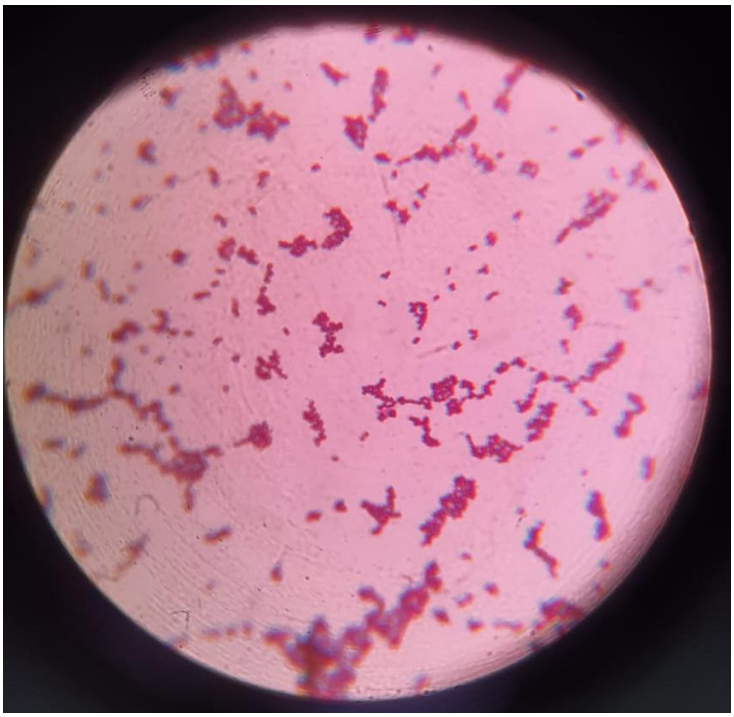

Fig 23- Staining of Pale-yellow colony

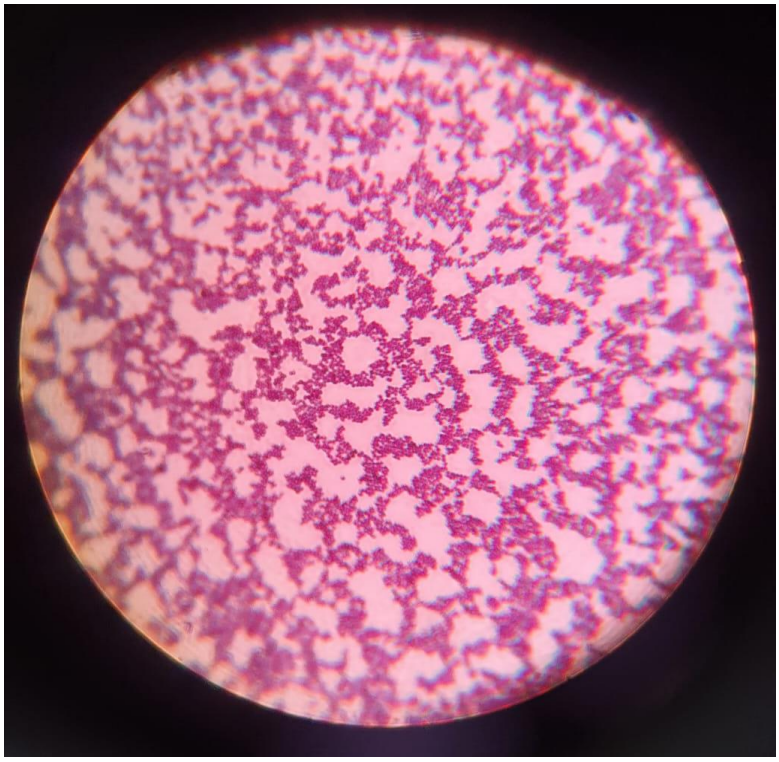

Fig 24 – Staining Orange colony

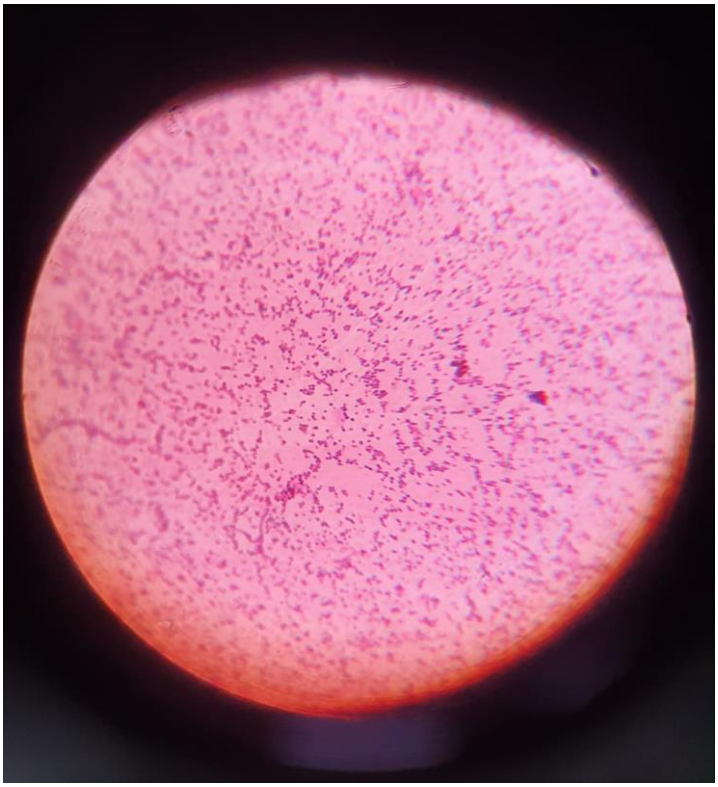

Fig 25 – Staining Brown colony

4.5 HEMOLYTIC ACTIVITY RESULT

Isolates are showing Different type of Haemolysis. All are showing Beta Hemolysis.

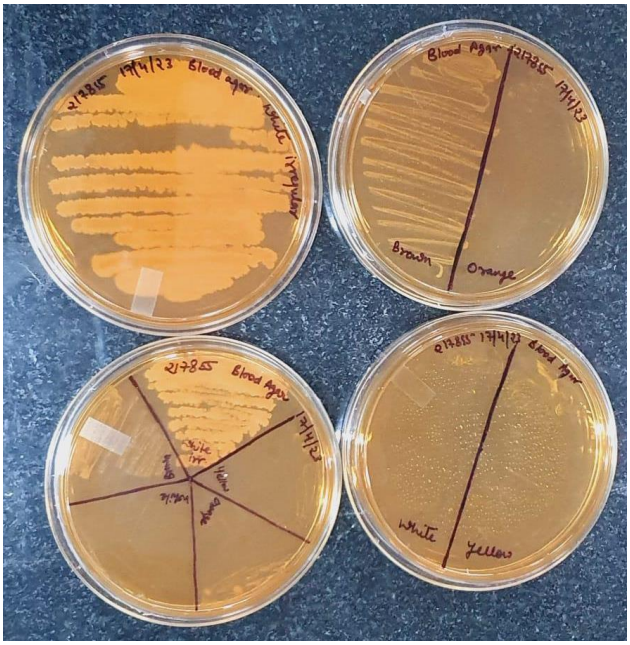

Fig 26 - Different colonies shown on Blood agar.

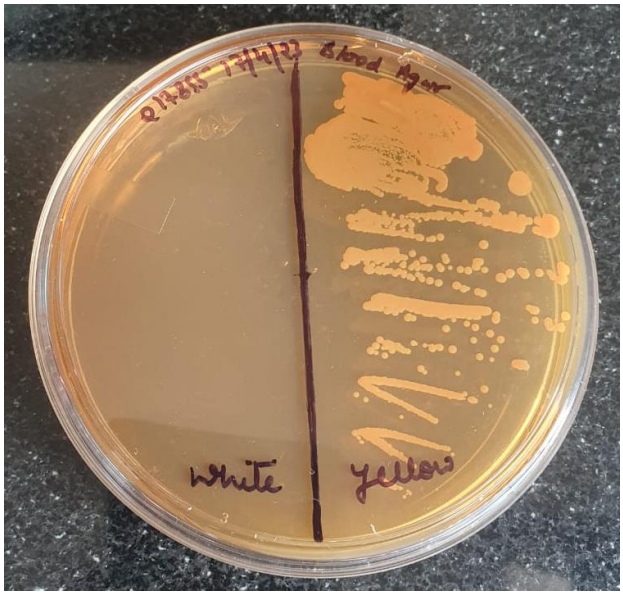

Fig 27 – yellow colony shows result after 7 days of incubation.

4.6 GROWTH RESULT ON MACCONKEY AGAR

The lactose fermentering bacteria forms pink colonies and non-lactose fermentering produce colourless or pale colonies**.**

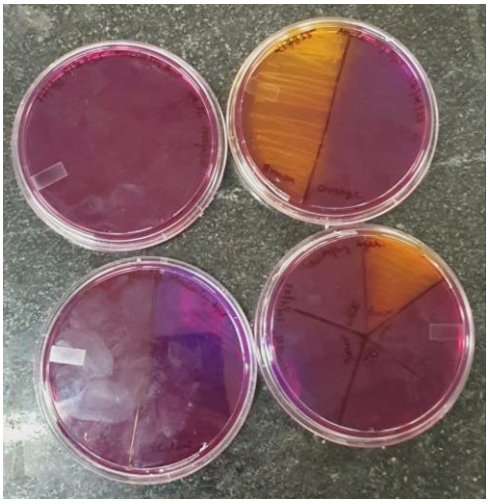

Fig 28 – Different colonies shown on MacConkey agar

4.7 CITRATE UTILIZATION TEST

At first, after 2 days of incubation no results are found. Then incubate these plates for more 7 days. After incubation of 7 days, plates are showing citrate positive result. Only one colony shows positive citrate result.

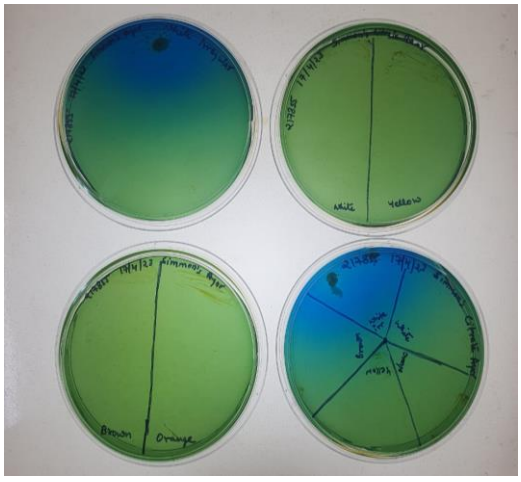

Fig 29 – Result of Citrate utilization test

4.8 METHYL RED TEST

Bacteria that are produced by acid by glucose fermentation and also changes the pH of the media. If bright red colour is shown then it means it is a positive result. Those who are showing yellow colour it means the result are negative. In this fig. three colonies showing positive result and two are showing negative result.

Fig 30- Result of isolates after methyl red test.

4.9 INDOLE PRODUCTION TEST

Pink/red colour in the top layer means positive result. But there is no change seen in this test, thus there is no colony who is showing indole positive result.

Fig 31 – Indole test result of isolate.

Table 11 – Summary information of all cultures .

Colony	Gram staining	Hemolytic Activity	MacConkey Agar	Citrate Test	Methyl red test	Indole Test
Yellow colony (Y)	-	+	-	-	+	-
Orange colony (O)	-	-	-	-	+	-
White irregular colony (R)	-	+	-	+	-	-
Brown colony (B)	-	+	+	-	+	-
White round colony (W)	-	-	-	-	-	-

DISCUSSION

Yeasts, bacteria and molds have been identified for both good and bad reasons, and they have always been crucial to human existence. They are inextricably linked to biotech., food science, medicine, genetic engineering and other areas of life, as they were in the past and are now. Regardless of the technique used to detect microorganisms, immobilizing a biological particle on the surface of a measurement circuit can be difficult. This procedure is essential to enhance the performance of the kit in terms of specificity, sensitivity, and long-term stability as it enables the bio-reporter, which defines the specificity of the kit for a particular target, to adhere properly to its surface. A min. of two to five days, or possibly up to a dozen days in the case of molds, is believed to be necessary for microbial identification based on conventional techniques, including morphology, chemistry, physiology, and biochemical characteristics. In addition, most phenotypic techniques used in microbiological laboratories require a lot of labor & resources. Identification of microbes to the species level, or more often to the strain level, is sometimes diff. using phenotypic approaches. To assess the bacterial flora on fingerprint scanners used in biometric attendance devices, the present investigation was carried out.

In the current study, total 5 samples were collected from different locations of biometric devices. Bacterial are seen. After taking sample and growth also seen we purify the isolates by quadrant streaking and give them specific names and then done staining. In staining all colonies shows negative result which means all bacteria are gram negative bacteria. After this biochemical test are done. In Hemolytic Activity Y, R, B shows positive result. Growth on MacConkey Agar, Only B shows positive result. In citrate test, R colony shows positive result hence axcept this all shows negative result. In methyl red test Y, O, B shows positive result.

CONCLUSION

The research found that numerous bacteria, mosts of which belong to the nature fauna of the person's body, were present in all cell phones, laptops and biometric devices used in work-places and schools. Consequently, it is crucial to clean hands after using phones, laptops and most critical biometric devices. The main way that diseases spread. These results call for regular cleaning of the biometric device and its attachments. Cell phones and laptops should always be cleaned with skin-friendly antibacterial agents. To reduce the spread of infection, always use preventive measures including hand washing, regular user checks, and biometric device decontamination with isopropyl alcohol/chlorhexidine. Bacteria which are pathogenic are destructive to human life and can cause illnesses and disorders, are present in cell phones and laptops. These types of infectious and common flora found in devices are extremely harmful to auto-immune people and are associated with opportunistic infections. Therefore, it is necessary to remove dust from frequently used items like keyboards and mice to prevent the spread of dangerous microbes that can cause very serious diseases in people. This work will benefit from the development of pathogenic bacterial detectionss techniques.

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