Isolation and Characterization of Bacteria from Burn-Injured Patients and Detection of Their Antibiotic Sensitivity Pattern

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Declaration

It is hereby declared that

1. The thesis report submitted is our own original work while completing a degree at BRAC University.
2. The thesis report does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis report does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

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Dedicated To

This research is entirely dedicated to our parents for providing us with spiritual, mental, and financial support throughout our lives.
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Abstract

Burn injuries are one of the most prevalent public health issues and cause more than 26,000 deaths every year all over the world. Burn injuries are skin or tissue damages that are caused by various agents like fire, electricity, and chemical exposures. The study aimed to identify the microbial profile of burn wound infections in burn patients and their resistance pattern against antibiotics. A total of 104 burn-infected bacterial samples were collected and analyzed against 11 antibiotics of different groups. In this study, a total of 104 samples of both male and female patients with an age range of 1-90 were observed. It was found that among the 104 isolates burn infection in males was 67 (64.42%) and females were 37 (35.58%). The percentage of burn-injured patients in males was higher than in females. Out of 104 samples, Gram-positive cocci accounted for 30 (28.85%) and Gram-negative Bacilli accounted for 74 (71.15%) which in comparison is approximately double of total Gram-positive isolates. In the case of gram-negative bacteria, the most prevalent bacteria were *Pseudomonas spp.* 43(41.34%) and in the case of gram-positive bacteria, it was *Staphylococcus spp.* 15(14.42%). Other than *Staphylococcus spp, Staphylococcus saprophyticus* 7(6.73%), *Micrococcus spp.* 7(6.73%) and *Streptococcus spp.* 1(0.96%) were also found. After performing the antibiotic susceptibility test, both gram-positive and gram-negative bacteria showed 100% resistance to the penicillin class of antibiotic. Colistin sulfate and Azithromycin showed the least resistance to *Pseudomonas spp* which was 13.51% and 32.43% respectively. In terms of gram-positive bacteria, meropenem and imipenem showed the least resistance, which was 43.3%. Based on their resistance pattern bacterial isolates were classified into multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug resistant (PDR) pathogens, 33(31.73%) were MDR, 58(55.76%) were XDR and 10(9.61%) were PDR. Lastly, the minimum inhibitory concentration (MIC) test was performed on ceftriaxone, levofloxacin, and vancomycin antibiotics against some of the MDR strains. This data reveals that the MIC value rises beyond the standard when resistant bacteria become less sensitive to these medicines.

This study will help the treatment process by providing proper antibiotics in advance without waiting for culture, thereby reducing infection-related morbidity and mortality.

Key words: Burn Wound Infections, Antimicrobial Activity, Antibiotic resistance, Susceptibility.

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Abbreviation

Abbreviation	Elaboration
NI	Nosocomial Infection.
MDR	Multi Drug Resistant
XDR	Extensively Drug-Resistant
PDR	Pan Drug Resistant
et al	and others
mg	Miligram
µg	Microgram
MIC	Minimum Inhibitory Concentration
WHO	World Health Organization
Fig	Figure
spp	Species
TSI	Triple Sugar Iron
VP	Voges Proskauer
MR	Methyl Red
MIU	Motility Indole Urease

Chapter 1

Introduction and Literature Review

1.1 Overview:

Burn injury is one of the most prevalent public health issues all over the world and is becoming a challenge to treat. Burn injuries are skin or organic tissue damages that are typically caused by different factors such as fire, electricity, radioactivity, and chemical exposures like strong acids or gasoline (Uddin et al., 2018). Skin is known as the largest organ of the body and functions as a protective barrier between the internal body and its surroundings. Since burns damage the skin's outermost layer which allows microorganisms to enter the skin easily and cause infection at the site of the injury (Mesbahi et al., 2021). Burns are more persistent and richer sources of infection and provide favorable conditions for bacterial growth (Aali, 2016). Infection in burn patients is one of the leading reasons for high mortality and morbidity in burn patients around the world (Aali, 2016). According to a study, worldwide every year around 2,65,000 deaths occur due to burning injuries (Alamgir, 2020). Bacterial infection in burn sites is responsible for 75% of death. In Bangladesh every year over 3000 people die because of burn injuries (Alamgir, 2020). According to the World Health Organization, around 1,73,000 Bangladeshi children suffer mild to serious burn injuries every year (World Health Organization, 2018).

1.2 Burn Infection:

An infection is defined as the entry and multiplication of pathogens in the body. According to Davis (2021), infection is the growth and multiplication of microorganisms such as bacteria, viruses, and parasites that are not normally present in the body. A burn site is infected when bacteria invade the site.

In the case of burn patients, at the beginning of the injury, burn surfaces remain aseptic. However, within 48 hours, they become colonized by gram-positive bacteria that are part of the normal skin flora, such as *Staphylococcus spp*. After 48-72 hours, wounds are infected by gram-negative bacteria that come from the patient's intestinal and respiratory tracts, including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae,* and *Escherichia coli.* Moreover, burn patients have a high chance to develop nosocomial infection (NI, known as hospital-acquired infection) due to damaged skin barrier, impaired immune system, poor infection control techniques, and extended

hospitalization period (Azimi et al., 2011). Since the burn site lost its first line of defense against microbial invasion, any pathogen can invade a burn site and cause infection.

1.3 Burn infection-causing organisms:

Based on different studies, the most frequent pathogenic organisms isolated from burn patients are - *Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella spp, E. Coli, Proteus vulgaris* (Saldanha et al., 2022). According to one study, the majority of the organisms that infect burn patients belong to the *Enterobacteriaceae* family. *Proteus* species and *Enterobacter* species are the most prevalent isolates in terms of burn infection. The reasons behind this high prevalence of *Enterobacteriaceae* are the colonization of patients' gut flora, the poor hygienic conditions of burn units, the inappropriate use of infection control techniques, and a lack of expertise (Naqvi et al., 2014). Among all the bacterial species, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most predominant species found at infection sites. The hospital settings are an ideal environment for *Pseudomonas aeruginosa* survival, as soon as it has been established in a ward, it can survive for a long period and cause infection among the patients who are admitted here. Medical personnel may potentially be a source for spreading bacteria to burn patients through their contaminated hands (Naqvi et al., 2014). Another major cause of burn wound infection is still *Staphylococcus aureus*. Due to the extensive use of broad-spectrum antibiotics, most of the bacteria are getting resistant, which is becoming a threat on a global scale (Norbury et al., 2016).

1.4. Antibiotic Resistance in Burn Infection:

In recent years, antibiotic resistance has become one of the biggest concerns for world health. The extensive use of antibiotics is probably the most important factor contributing to the rise in drug resistance. First of all, bacteria have the genetic ability to acquire and transmit antibiotic resistance, transferring it from one bacterial strain to another. Additionally, in many bacteria transposable and other exchangeable genetic elements like plasmids, and transposons contribute to the spread of antibiotic resistance in many bacterial species (Nikokar et al., 2013). Besides, antibiotic resistance might emerge spontaneously as a result of mutation (Ventola, 2015). Moreover, excessive usage of

Antibiotics, a lack of new antibiotics, misleading prescriptions, and extensive agricultural use are the reasons for the increase in antibiotic resistance (Ventola, 2015). In case of burn infection, MDR (multi drugs resistance) strains of *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, methicillin-resistant *Staphylococcus aureus* (MRSA) are difficult to treat (Norbury et al., 2016). *Pseudomonas aeruginosa* is resistant to the majority of antibiotic classes including aminoglycosides (gentamicin, tobramycin, amikacin, and streptomycin), carbapenems (imipenem, meropenem), quinolones (levofloxacin, ciprofloxacin), and beta-lactams (penicillins, cephalosporins) (Pang et al., 2018). Carbapenem-resistant *Pseudomonas aeruginosa* possesses a mobile genetic element that can generate carbapenemase enzymes, which subsequently inhibit the activity of carbapenem antibiotics (CDC). *Pseudomonas aeruginosa* is resistant to aminoglycosides for numerous reasons including decreased cell membrane permeability, ribosomal alterations, and enzyme modification (Pang et al., 2018). Again, a harmful strain of *Staphylococcus spp. has* developed over the years and is called methicillin-resistant *Staphylococcus aureus* (MRSA). This strain became resistant to all antibiotics in the beta-lactam class. This class of antibiotics includes widely prescribed amoxicillin, penicillin, methicillin, and others. *Staphylococcus aureus* becomes resistant due to the presence of penicillinase, which works against penicillin (Fatema et al., 2021).

1.5 Antibiotics used against Gram-Positive bacteria:

Vancomycin: The antibiotic belongs to the glycopeptide class which is effective against gram-positive bacteria. This antibiotic is mainly used in life-threatening conditions. Vancomycin prevents cell wall formation in susceptible bacteria as well as peptidoglycan polymerization. It is used against bacteria that are already resistant to other antibiotics such as the beta-lactams group of antibiotics.

Penicillin: Penicillin acts best against gram-positive bacteria. It prevents the synthesis of the peptidoglycan layer of gram-positive bacteria. As a result, the cell becomes weak and porous. This condition leads to the destruction and burst of the cell which then accelerates the recovery process of patients.

Azithromycin: Azithromycin belongs to the macrolide antibiotic family, which is used in treating a wide range of bacterial infections. It blocks protein synthesis by attaching to the bacterial

ribosome's 50S subunit. Also, it prevents bacterial cell-to-cell communication processes known as quorum sensing and biofilm development.

1.6 Antibiotics used against Gram-Negative bacteria:

Ceftriaxone: Ceftriaxone is a third-generation cephalosporin antibiotic. This antibiotic shows a good effect against most gram-negative bacteria. Ceftriaxone inhibits mucopeptide synthesis in the susceptible bacterial cell wall. The beta-lactam portion of ceftriaxone binds to transpeptidases, carboxypeptidases, and endopeptidases present in bacterial cytoplasmic membranes. These enzymes have a role in cell division and cell wall synthesis. When ceftriaxone binds to these enzymes, they lose their activity, and therefore bacteria produce faulty cell walls, which ultimately cause cell death.

Colistin: Colistin works best on gram-negative bacteria. Colistin is a polycationic peptide that has both hydrophilic and hydrophobic components. Colistin binds with the bacterial outer membrane by replacing magnesium and calcium ions in the polysaccharide. It results in the degradation of the outer cell membrane, also leakage of intracellular components, and ultimately bacterial death.

Trimethoprim- Trimethoprim is one of the recent antibacterial and antiparasitic drugs which belongs to the diaminopyrimidines group. Bacteria need folic acid to survive. Dihydrofolate reductase enzyme catalyzes the final step of bacterial folic acid formation, trimethoprim inhibits this enzyme which will kill bacteria by stopping them from producing folic acid.

1.7 Objectives:

- Isolation and identification of pathogenic bacteria from burn-injured patients.
- Performing Antibiotic Susceptibility Test (AST) to find out the drug-resistant pattern of bacteria.
- Find out the Minimum Inhibitory Concentration (MIC) of some selective antibiotics against resistant bacteria.

Chapter 2

Materials and Methods

2.1 Study Design: All samples were collected from burn patients admitted to the burn and plastic surgery unit of Sheikh Hasina National Institute of Burn and Plastic Surgery ((SHNIBPS), Dhaka, Bangladesh. Samples were collected from both sexes and different age groups.

2.2 Study Period: March 20, 2022, to October 20, 2022.

2.3 Study Site: The present study was conducted in the Microbiology and Biotechnology laboratory of the Department of Mathematics and Natural Sciences at BRAC University.

2.4 Ethical Approval: The Project Director and the National Institute of Burn and Plastic Surgery's ethical review committee gave their approval before this study project's ethical work could begin. The inpatient consent form was completed by the patient consent. Before any patient samples were collected, a questionnaire was completed.

2.5 Types of Specimen: In this study, various types of specimens were collected from different burn sites of the patients (hands, legs, and chest). Samples included pus (from wound infection), urine, and blood.

2.6 Quantity of specimen: A total of 104 clinical isolates were tested from burn patients.

2.7 Samples Collection: The sample was collected from the microbiology lab of the Sheikh Hasina National Institute of Burn and Plastic Surgery. A distinct colony was picked from the aerobic plate count. Then, the samples were transported to the laboratory for further analysis.

Flow chart of study design

Sample collection

Streak on selective and universal Media
(MacConkey Agar, Cetrimide Agar and Nutrient agar)

24 hours' incubation at 37°C

Selection of the represented colonies

2.8 Inoculation of Samples:

Plate culture method: All specimens were streaking on selective media such as Mac-Conkey agar, and Cetrimide agar and universal media (Nutrient Agar) plates under an aseptic condition in a laminar airflow cabinet. After that, culture plates were incubated at 37° C for 24 hours aerobically. Here, Nutrient agar was used for the isolation and identification of all kinds of bacteria as it is a universal media, and MacConkey agar was used for gram-negative bacteria (*E. coli*, *Klebsiella spp*, *Proteus spp*, etc.) and Cetrimide agar for *Pseudomonas spp*. Also, Gram Staining was performed for gram-positive and gram-negative bacteria, and visual observation of bacterial colonies was done by microscopic examination for morphology, color, and shape.

2.9 Media for plate culture method: For this study, we have used different types of media:

- Nutrient Agar (NA) - as universal media.
- T1N1 agar (tryptone salt agar) - for stocking purposes.
- Trypticase soy agar or tryptone soya agar (TSA)- as universal media
- Muller Hinton Agar (MHA)- as universal media
- Cetrimide agar and MaCconkey agar- as selective media.

Preparation of Mueller Hinton Agar (MHA) agar:

- MHA agar is a non-selective, differential media.
- The standard form for preparing MHA agar was 38.0 g for 1000 ml of distilled water, we used it as a standard measurement and later we prepared the amount we needed.
- Then it was boiled to dissolve properly. Finally, the media was autoclaved at 121° C for 15 minutes.
- Then it was poured into the sterile, large sizes of Petri dishes.
- After solidifying the agar, it was stored in the refrigerator for further use.

Preparation of Nutrient Agar:

- Nutrient Agar (NA) is used to subculture the selected pathogen for this experiment.
- The standard form for preparing NA was 28.0g for 1000 ml of distilled water. We have used it as a standard measurement and later we prepared the amount we needed.
- Then it was boiled to dissolve properly.
- Finally, the media was autoclaved at 121° C for 15 minutes.
- Then it was poured into the sterile, different sizes of Petri dishes (according to necessity).
- After solidifying the agar, it was stored in the refrigerator for further use.

Preparation of Trypticase soy agar (TSA):

- It is used as a growth medium for the isolation and cultivation of microorganisms.
- Firstly, we had to mix 45 grams of TSA in 1000 ml of distilled water & boil it to dissolve. We used it as a standard measurement and later we prepared the amount we needed.
- Then for sterilization, we autoclaved it at 121°C for 15 minutes.
- After cooling we poured it into sterile Petri plates & kept it in the refrigerator.

Preparation of T1N1 Agar (Tryptone Salt Agar):

- We used this agar for stocking bacteria for further research purposes.
- It is also used for decimal dilution & preparation of specimens for the purpose of microbial tests.
- For 100 ml of T1N1 agar, 1 g of tryptone, 1 g of NaCl, and 2 g of agar were needed to add in 100 ml of distilled water.
- Later It was boiled to dissolve, poured in sterile vials.

- Then for sterilization we autoclaved it at 121°C for 15 minutes.

Preparation of Brain heart infusion (BHI) broth:

- It is used for serial broth dilution to dictate the minimum inhibitory concentration.
- For this media broth 37.0 g of media was suspended into 1000 ml of distilled water and boiled to dissolve. We have used it as a standard measurement and later we prepared the amount we needed.
- Dispense into bottles or test tubes for sterilization.
- Autoclaved at 121° C for 15 minutes**.**

Preparation of Cetrimide agar:

- It is a selective media for the isolation of *pseudomonas aeruginosa*.
- 45.3 g of cetrimide agar was measured, mixed with 1000 ml of distilled water and then boiled to dissolve properly. We used it as a standard measurement and later we prepared the amount we needed.
- Finally, the media was autoclaved at 121°C for 15 minutes.
- Then it was poured into the sterile, different sizes of Petri dishes (according to necessity)
- After solidifying the agar, it was stored in the refrigerator for further use.

Preparation of MacConkey agar:

- It is a selective and differential media used for the differentiation and isolation of fastidious gram-negative bacteria.
- To prepare this medium, first 49.53 g of McConkey agar was measured and mixed with 1000 ml of distilled water. We used it as a standard measurement and later we prepared the amount we needed.
- Then it was boiled until the agar melted.
- Finally, the media was autoclaved at 121°C for 15 minutes.
- Then it was poured into the sterile, different sizes of Petri dishes (according to necessity).
- After solidifying the agar, it was stored at 40°C inside the refrigerator for further use.

Preparation of physiological saline:

- Physiological saline was used for bacterial dilution for antibiotic susceptibility tests.
- 0. 9 g Sodium Chloride (NaCl) was mixed with 100 ml distilled water in a clean conical flask to make 100 ml of physiological saline.
- After mixing saline was transferred into a 15 ml test tube of around 5 to 6 ml and autoclaved for further use.

2.10. Identification of the bacteria: Microscopic Observation of the bacteria

Gram staining:

- Five milliliters of saline were freshly prepared with the intention of making the smear.
- Smeared a loopful of saline on the slides, then added a small inoculum of the isolate to the saline and heat-fixed it.
- After that, crystal violet was added and kept for about 30 seconds to 1 minute. Then rinse with distilled water.
- This was followed by the addition of mordant which is Gram's Iodine, which remained on the smear for a minute. Then rinse with distilled water.
- After that, we added ethanol or acetone for 15 seconds.
- Finally, safranin is added. After air dry slides were observed under a microscope.
- Under a microscope, any signs of purple-colored cells are gram-positive bacteria, while pink or red-colored cells are gram-negative bacteria.

2.11. Biochemical identification:

Multiple biochemical tests were performed to obtain a presumptive identification of the potential bacteria chosen previously. The majority of the procedures were carried out following the microbiology laboratory manual. Biochemical tests included TSI test, IMViC test (Indole production test, Methyl Red test, Voges Proskauer test, Citrate utilization test), MIU test, Catalase test, Oxidase test, kept in an incubator for bacterial growth**.**

Indole test: The enzyme tryptophanase was used to determine the ability of microorganisms to degrade the amino acid tryptophan.

- For the indole test, each indole broth contained 6 ml of peptone and sodium chloride.
- A small amount of the experimental bacteria from the fresh culture was inoculated into the tubes using a sterile technique using the loop inoculation method with an inoculating loop.
- The tubes were then incubated at 37^{o} C for 24 hours.
- To detect indole production, 10 drops of Kovacs reagent were added to each tube.

Indole test result

Result	Interpretation
Positive	A bright pink color (ring-shaped) on top of the layer indicates the presence of indole.
Negative	The absence of pink color means indole negative.

Methyl red (MR) test: The methyl red (MR) test was performed to determine the bacteria's ability to oxidize glucose with the production and stabilization of high concentrations of acid end products.

- Each MR broth contains 5 ml of di peptone, dextrose, and potassium •
- Using the loop inoculation method each tube was inoculated with a fresh culture of experimental bacteria using a sterile technique.
- The methyl red (MR) test was performed to determine the bacteria's ability to oxidize glucose with the production and stabilization of high concentrations of acid end products.
- The tubes were then incubated for 48 hours at37ºC.
- After 48 hours, 5 drops of methyl red indicator were added to each tube and the color of the tubes was observed.

Methyl red (MR) test result

Result	Interpretation
Positive	If the red color develops then it indicates MR positive
Negative	If orange or yellow color develops then it indicates MR negative result

Voges Proskauer test (VP): The Voges-Proskauer (VP) test was used to determine whether an organism produces acetyl methyl carbinol as a result of glucose fermentation.

- Each VP broth containing di peptone, dextrose, and potassium phosphate was used for the Voges-Proskauer test.
- Each tube was inoculated with a fresh culture of experimental bacteria using a sterile technique using the loop inoculation method.
- The tubes were then incubated at 37^{o}C for 48 hours.
- After 48 hours, each tube received 10 drops of Barritt's reagent A and was shaken. The tubes were shaken immediately after 10 drops of Barritt's reagent B were added.
- The color was observed 15-30 minutes after the reagent was added.

VP test result

Result	Interpretation
Positive	If the red color develops then it indicates VP positive
Negative	If no color develops, this indicates a negative VP result

Citrate Test: The citrate utilization test was performed to distinguish between enteric organisms based on their ability to ferment citrate as a sole source of carbon by the enzyme citrase.

- For the citrate utilization test, each vial contained 3 ml of Simmons citrate agar.
- A small amount of the experimental bacteria from 18 to 24-hour pure culture was inoculated into the vials using a sterile technique and an inoculating needle, and the vials were incubated for 48 hours at 37°C.

Citrate test result

Result	Interpretation
Positive	If Prussian blue develops then it indicates Citrate positive
Negative	If no color develops, this indicates a negative citrate result

Triple Sugar Iron test: The TSI agar test was used to distinguish between Gram-negative enteric bacilli based on their ability to ferment carbohydrates and reduce hydrogen sulfide.

- In the test tubes, triple sugar iron slants were prepared and autoclaved at 15 psi 121°C.
- A small amount of the experimental bacteria from a 24-hour-old pure culture was inoculated into the tubes using a sterile technique using a stab and streak inoculation method.
- The tubes were incubated at 37°C for 24 hours.
- After 24-48 hours the color of both the butt and slant of agar slant cultures were observed.

MIU (Motility Indole Urease) test: The MIU test was used to determine bacterial motility, indole production, and urea degradation using the enzyme urease.

- MIU media was prepared by autoclaving at 15 psi and 121°C, then cooling to 50-55°C and aseptically adding urea to the base medium.
- A small amount of the experimental bacteria from the fresh culture was inoculated into the tubes using a sterile technique using a stab inoculation method with an inoculating needle.
- The tubes were then incubated at 37^{o}C for 24 hours.

MIU test Result

Result	Interpretation
Urease Positive	If the Pink color develops then it indicates Urease positive
Urease Negative	If Yellow color develops, this indicates a negative Urease Result
Motile	The organism's growth would spread throughout the test tube
Non-Motile	The organism's growth would not spread throughout the test tube

Catalase test Test

- This test is used to distinguish bacteria that produce the enzyme catalase from those that do not. Catalase works as a catalyst to convert hydrogen peroxide to oxygen and water.
- In a petri dish, a microscopic slide was placed.
- Some bacteria from a 24-hour pure culture were placed on the microscopic slide using a sterile inoculating loop
- Onto the organism on the microscopic slide One drop of 3% H_2O_2 drop was used and looked for instant bubbles formation.

Catalase test result

Result	Interpretation
Positive	If an instant bubble develops then it indicates catalase positive
Negative	If no bubble develops, this indicates a negative catalase result

Oxidase Test result

- The cytochrome oxidase enzyme was detected in the bacteria using an oxidase test.
- Filter papers were used, and two drops of oxidase reagent (p-Amino dimethyl aniline oxalate) were applied to them (Whatman, 1MM).
- One well-isolated colony from a pure 24-hour culture was picked and rubbed onto filter paper using a toothpick and the color change was observed.

Oxidase test result

Result	Interpretation
Positive	A positive reaction would change the color of the paper from violet to purple
Negative	Delayed reactions should be ignored because they may result in false positives.

2.12. Antibiotic Sensitivity Testing:

An antibiotic sensitivity test or susceptibility test was performed to find out the best treatment when someone has an infection. This test helps us determine the specific antibiotic as well as the recommended dosage. Antibiotic sensitivity testing is done when someone has an infection that may be resistant to antibiotics, which indicates that antibiotics are less effective or don't affect specific bacteria. However, this makes it more difficult to treat bacterial infections and illnesses. Different types of methods can be used for antibiotic sensitivity testing, but among them, the most commonly used disk diffusion method was used in this present study, which is known as the Kirby-Bauer method. Moreover, during this study, to check the authenticity of sensitivity patterns, all the samples were tested twice. The work procedure is given below:

Preparation of inoculum: At first, a fresh nutrient media culture plate of one of the organisms was selected to perform the test. Then, using a sterile loop a colony from the fresh plate was collected and dipped in the test tube containing sterile saline solution and it was mixed gently to ensure that no solid material from the colony is visible in the saline solution. Finally, the test tube was vortexed properly so that the suspension became homogeneous. (Labtronics; ISO 9001: 2008 Certified).

Inoculation on Muller Hinton Agar (MHA) plates: Muller Hinton agar plates were prepared which is a non-selective, non-differential microbiological growth medium. Then, the surface of the Muller-Hinton Agar plate was lawned by a sterile cotton swab with the representative bacterial suspension prepared in physiological saline which was compared with McFarland standard 0.5

solutions. McFarland standard 0.5 solution indicates the density of 1.5×10^{-8} CFU (Colony Forming Unit) per ml. Before starting the lawn, the cotton swab was pressed against the wall of the test tube so that extra fluid can be removed. However, it was made sure that the cotton swab should be swiped multiple times at different angles for the equal distribution of bacterial suspension.

Placing the antibiotic disks on MHA plates: Based on bacteria, antibiotic discs were placed carefully on the solidified lawned agar plate by using sterile forceps. During this study, 10 antibiotics were used for Gram-negative bacteria as well as 9 antibiotics for Gram-positive bacteria. Around 11 antibiotics were used for this study. After placing the disks, the plates were covered by the lid and were not inverted, then the plates were incubated at 37°C for 24 hours.

Measuring zone: After the incubation period, the zone of inhibition was measured in millimeters with a ruler. For this, the ruler was placed against the back of the plate, where the zone was visible enough to measure the diameter easily. Moreover, the antimicrobial pattern was determined by the presence or absence of a clear zone around the antibiotic disc. There are three types of possible results on antibiotic susceptibility tests. Such as:

- **Susceptible or Sensitive (S) -** It means a moderate size of the zone which indicates that this organism can be treated with this antibiotic at the recommended level.
- **Intermediate (I)-** It applies to those organisms that are "**moderately susceptible**" to an antibiotic.
- **Resistant (R)-** It means that there will be no clear zone around the antibiotic disc also it can be said that the organism won't give any response to that specific antibiotic.

Finally, antibiotic resistance patterns were categorized into MDR, XDR, and PDR by the guidelines of Clinical and Laboratory Standards Institute (CLSI).

- **MDR (Multidrug-resistant) Bacteria -** When a single bacterium is resistant to at least three classes of antibiotics then it is known as MDR.
- **XDR (Extensively drug-resistant) Bacteria-** When a bacterium remains sensitive to one or two antibiotics then it is known as XDR
- **PDR (Pan Drug-resistant) Bacteria -** These bacteria are non-susceptible to any drugs which are clinically proven. PDR bacteria are difficult to treat.

Table 01: Antibiotic disks used for Gram-Positive bacteria

Name of antibiotics group	Name of Antibiotic	Code	Zone of Resistance	Zone of Sensitivity	Zone of Intermediate	Company name	Concentration
Penicillin	Penicillin	P	≤28	NA	≤29	Himedia	10 unit
	Amoxicillin	AML	≤18	≥19	NA	Himedia	30 µg
	Ampicillin	AMP	≤28	≥29	NA	Himedia	10 µg
	Amoxyclav	AMC	≤19	≥20	NA	Himedia	30 (20/10) µg
Macrolide	Azithromycin	AZM	≤13	≥18	14-17	Himedia	15 µg
Glycopeptide	Vancomycin	VA	≤14	≥15	NA	Himedia	30 µg
Fluoroquinolones	Levofloxacin	LEV	≤15	≥19	16-18	Himedia	5 µg
	Ciprofloxacin	CIP	≤15	≥21	16-20	Himedia	5 µg
Cephalosporin	Cefixime	CFM	≤15	≥19	16-18	Oxoid	5 µg
Aminoglycoside	Amikacin	AK	≤14	≥17	15-16	Himedia	30 µg
	Streptomycin	S	≤11	≥15	12-14	Oxoid	10 µg
	Kanamycin	K	≤13	≥18	14-17	Himedia	30 µg
Carbanem	Imipenem	IMP	≤13	≥16	14-15	Himedia	10 µg
	Meropenem	MEM	≤13	≥16	14-15	Himedia	10 µg

[NA= Not Available]

Table 02: Antibiotic disks used for Gram-Negative bacteria

Name of antibiotics group	Name of Antibiotic	Code	Zone of Resistance	Zone of Sensitive	Zone of Intermediate	Company name	Concentration
Penicillin	Amoxicillin	AML	≤13	≥18	NA	Himedia	30 µg
	Ampicillin	AMP	≤13	≥17	14-16	Himedia	10 µg
	Amoxyclav	AMC	≤19	≥20	NA	Himedia	30 (20/10) µg
Polymyxin class	Colistin sulfate	CT	≤11	≥11	NA	Himedia	10 µg
Fluoroquinolones	Levofloxacin	LEV	≤13	≥17	14-16	Himedia	5 µg
	Ciprofloxacin	CIP	≤15	≥21	16-20	Oxoid	5 µg
Cephalosporin	Cefixime	CFM	≤15	≥19	16-18	Himedia	5 µg
	Ceftriaxone	CTR	≤21	≥25	22-24	Himedia	30 µg
Aminoglycoside	Amikacin	AK	≤14	≥17	15-16	Himedia	30 µg
	Kanamycin	K	≤13	≥18	14-17	Himedia	30 µg
	Streptomycin	S	≤11	≥15	12-14	Oxoid	10 µg
Carbanem	Meropenem	MEM	≤19	≥23	20-22	Himedia	10 µg
	Imipenem	IMP	≤19	≥23	20-22	Himedia	10 µg
Diaminopyrimidines	Trimethoprim	COT	≤10	≥16	11-15	Himedia	25 µg
Macrolides	Azithromycin	AZM	≤13	≥18	14-17	Himedia	15 µg

[NA= Not Available]

2.13. Minimum inhibitory concentration (MIC):

MIC (minimum inhibitory concentration) is used to observe the lowest concentration of an antibacterial agent. It was done following the tube dilution method. This study was observed to determine the efficiency of several antibiotics like vancomycin, ceftriaxone & levofloxacin. For this experiment Brain heart infusion (BHI) broth was used as a diluent as well as a growth medium and different concentrations of antibiotics were also used. Several ranges of dilution were prepared for each antibiotic. Three controls were used during the test:

1. Only media: This was used to determine if the media was sterile or not
2. Media with bacteria: To understand the bacterial growth in the media.
3. Media with antibiotics: To determine the efficiency of antibiotics in the media.

Antibiotics were inoculated with BHI broth at different concentrations and after 18 to 24 hours it was checked for growth at different concentrations (whether it was turbid or not). The one tube which was clear with the lowest concentration was considered as the expected MIC value.

2.14. Quality Control:

To maintain the authenticity of the data, every sample was handled carefully and cross-checked. During this study, various types of ATCC (American Type Culture Collection) bacterial strains were used as quality control for culture, gram-stain, and biochemical analysis. However, ATCC bacterial strains provide detailed information; like the standard result on different tests, the best conditions for growth and storage, standard reference, cell lines, and other materials for research and development.

Table 03: ATCC bacterial strain used in the present study

Biochemical Tests	Control	Used ATCC Bacterial Strain
Indole test	Positive	*Escherichia coli* (ATCC 25922)
	Negative	*Klebsiella pneumoniae*
Methyl Red (MR)	Positive	*Escherichia coli* (ATCC 25922)
	Negative	*Klebsiella pneumoniae*
Voges Proskauer Test (VP)	Positive	*Klebsiella pneumoniae*
	Negative	*Escherichia coli* (ATCC 25922)
Catalase	Positive	*Pseudomonas aeruginosa* (ATCC:27853, S)
	Negative	*Streptococcus pneumoniae* (ATCC: 49619)
Oxidase	Positive	*Pseudomonas aeruginosa* (ATCC:27853, S)
	Negative	*Escherichia coli* (ATCC: 25922)
Motility Indole Urease (MIU)	Urease Positive	*Klebsiella pneumoniae*
	Urease Negative	*Enterobacter cloacae*
	Motility Positive	*Enterobacter cloacae*
	Motility Negative	*Klebsiella pneumoniae*
Gram Stain	Positive	*Streptococcus pneumoniae* (ATCC: 49619)
	Negative	*Escherichia coli* (ATCC 25922)

2.15 Data analysis: Data was analyzed by Microsoft Excel 2016.

Chapter 3

Results

3.1 Percentage of bacterial isolates based on age and gender:

The percentage of isolates based on age and gender is given below in table 4.

Table 04: Distribution of growth positive cases by age and gender

Total cases	Total males with %	Total females with %	Age group (years)	Number of cases	Number of cases %
104	67 (64.42%)	37 (35.58%)	1-10	27	25.96%
			11-20	19	18.26%
			21-30	19	18.26%
			31-40	12	11.54%
			41-50	13	12.5%
			51-60	7	6.73%
			61-70	6	5.8%
			71-80	0	0
			81-90	1	0.96%

Table 4. shows out of 104 samples, 67 (64.42%) were collected from male patients and 37 (35.58%) from female patients. Among the 104 cases, the highest number of cases 27 (25.96%) were found in patients aged 1–10 years.

3.2 Identification of isolates from biochemical test results: Later, biochemical tests were done for further deduction of the organism's identity.

Table 05: Biochemical test results of Gram-Positive isolates from burn-injured patients and their probable identification

Sample ID	Tests													Name of probable organism
	TSI				Gram Stain	Catalase	Oxidase	Citrate	MR	VP	MIU			
	Slant	Butt	H2S	Gas							Motility	Indole	Urease	
4772	Y	Y	(-)	(+)	Purple, cocci	(+)	(-)	(-)	(-)	(+)	M	(-)	(+)	*Staphylococcus spp*
4773	Y	Y	(-)	(-)	Purple, cocci	(+)	(-)	(-)	(+)	(-)	M	(-)	(+)	*Staphylococcus spp*
4802	R	R	(-)	(-)	Purple, cocci	(+)	(+)	(+)	(-)	(-)	M	(-)	(+)	*Micrococcus spp*
4789	R	R	(-)	(-)	Purple, cocci	(+)	(-)	(-)	(-)	(-)	M	(-)	(+)	*Staphylococcus saprophyticus*
4972	R	R	(-)	(-)	Purple, cocci	(+)	(+)	(-)	(-)	(-)	M	(-)	(+)	*Staphycococcus aureus*
5451	R	R	(-)	(-)	Purple, cocci	(+)	(-)	(-)	(-)	(-)	NM	(-)	(-)	*Micrococcus spp*
5442	Y	Y	(-)	(+)	Purple, cocci	(+)	(-)	(-)	(-)	(+)	M	(-)	(+)	*Staphylococcus spp*
5447	R	R	(-)	(-)	Purple, cocci	(+)	(-)	(-)	(+)	(+)	M	(-)	(+)	*Staphylococcus spp*
5445	R	R	(-)	(-)	Purple, cocci	(+)	(-)	(-)	(-)	(-)	M	(-)	(-)	*Micrococcus spp*
5455	Y	R	(-)	(-)	Purple, cocci	(+)	(-)	(-)	(-)	(-)	M	(-)	(+)	*Staphylococcus saprophyticus*
4375	Y	Y	(-)	(-)	Purple, cocci	(+)	(-)	(-)	(+)	(-)	M	(-)	(+)	*Staphylococcus saprophyticus*
679	Y	Y	(-)	(-)	Purple, cocci	(+)	(-)	(+)	(+)	(-)	M	(-)	(+)	*Staphylococcus spp*
5599	Y	Y	(-)	(+)	Purple, cocci	(+)	(-)	(-)	(-)	(+)	M	(-)	(+)	*Staphylococcus spp*
5661	Y	Y	(-)	(+)	Purple, cocci	(+)	(-)	(-)	(+)	(-)	M	(-)	(-)	*Streptococcus spp*
5935	R	R	(-)	(-)	Purple, cocci	(+)	(-)	(+)	(-)	(-)	M	(-)	(+)	*Staphylococcus spp*
5939	R	R	(-)	(-)	Purple, cocci	(+)	(+)	(-)	(-)	(-)	M	(-)	(+)	*Staphylococcus saprophyticus*
5974	Y	Y	(-)	(+)	Purple, cocci	(+)	(-)	(-)	(-)	(+)	M	(-)	(+)	*Staphylococcus saprophyticus*
4779	Y	Y	(-)	(+)	Purple, cocci	(+)	(+)	(+)	(-)	(-)	M	(-)	(+)	*Micrococcus spp*
272	R	R	(-)	(-)	Purple, cocci	(+)	(+)	(-)	(-)	(-)	M	(-)	(-)	*Micrococcus spp*
284	Y	R	(-)	(+)	Purple, cocci	(+)	(-)	(-)	(-)	(-)	M	(-)	(-)	*Micrococcus spp*
304	Y	R	(-)	(-)	Purple, cocci	(+)	(-)	(-)	(-)	(-)	M	(-)	(-)	*Micrococcus spp*
287	R	R	(-)	(-)	Purple, cocci	(+)	(-)	(+)	(-)	(-)	M	(-)	(+)	*Staphylococcus saprophyticus*
288	R	R	(-)	(-)	Purple, cocci	(+)	(-)	(-)	(-)	(-)	M	(-)	(+)	*Staphylococcus saprophyticus*
595	Y	Y	(-)	(+)	Purple, cocci	(+)	(-)	(-)	(+)	(+)	M	(+)	(+)	*Staphylococcus spp*
605	Y	Y	(-)	(+)	Purple, cocci	(+)	(-)	(-)	(+)	(-)	M	(+)	(-)	*Staphylococcus spp*
288 (II)	R	R	(-)	(+)	Purple, cocci	(+)	(-)	(-)	(+)	(-)	M	(+)	(-)	*Staphylococcus spp*
308 (II)	Y	Y	(-)	(+)	Purple, cocci	(+)	(-)	(-)	(+)	(-)	M	(+)	(-)	*Staphylococcus spp*

													Table 5 continued	
604 (II)	R	R	(-)	(-)	Purple, cocci	(+)	(-)	(-)	(-)	(+)	M	(-)	(-)	*Staphylococcus spp*
589(I)	Y	Y	(-)	(+)	Purple, cocci	(+)	(-)	(-)	(+)	(-)	M	(+)	(-)	*Staphylococcus spp*
589 (II)	Y	Y	(-)	(-)	Purple, cocci	(+)	(-)	(-)	(+)	(-)	M	(-)	(-)	*Staphylococcus spp*

[R= Red, Y= Yellow, (+) = Positive, (-) = Negative, M= Motile, NM= Non-motile]

Table 06: Biochemical test results of Gram-Negative isolates from burn-injured patients and their probable identity

	Tests													
Sample ID	**TSI**				**Gram Stain**	**Catalase**	**Oxidase**	**Citrate**	**MR**	**VP**	**MIU**			**Name of probable organism**
	Slant	**Butt**	**H2S**	**Gas**							**Motility**	**Indole**	**Urease**	
4766	R	R	(-)	(-)	Pink, Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
4367	R	R	(-)	(-)	Pink, Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
4792	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
4373	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
4780	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
4765	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
4784	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(-)	(-)	*Pseudomonas spp.*
4759	R	R	(+)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas putrefaciens*
4793	R	R	(-)	(-)	Pink,Rod	(+)	(-)	(-)	(-)	(-)	M	(-)	(-)	*Pseudomonas spp.*
4762	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
4356	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
4360	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
4370	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
4778	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
4345	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
5437	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
5438	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(+)	(-)	*Pseudomonas spp.*
4354	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
5432	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
4473	R	R	(-)	(-)	Pink,Rod	(+)	(-)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
4372	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
4474	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
5433	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
5452	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
454	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
4472	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
688	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
5598	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*

Table 6 continued

5604	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	NM	(-)	(+)	*Pseudomonas spp.*
5611	R	R	(-)	(-)	Pink,Rod	(+)	(-)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
5621	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
5651	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
5653	R	R	(-)	(-)	Pink,Rod	(+)	(-)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
5655	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
5656	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
5657	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
5658	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
5663	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
5668	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
5670	R	R	(-)	(-)	Pink,Rod	(+)	(-)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
5673	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	NM	(-)	(-)	*Pseudomonas spp.*
5674	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
5937	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(+)	(-)	M	(-)	(+)	*Pseudomonas spp.*
5945	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
5946	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
5961	R	R	(-)	(-)	Pink,Rod	(+)	(-)	(+)	(-)	(-)	NM	(-)	(+)	*Pseudomonas spp.*
5962	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
5964	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(+)	M	(-)	(+)	*Pseudomonas spp.*
5965	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
5968	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
5969	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
5982	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
290	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
266	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
301	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	NM	(-)	(-)	*Pseudomonas spp.*
303	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
296	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	NM	(-)	(-)	*Pseudomonas spp.*
270	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(+)	(-)	M	(-)	(+)	*Pseudomonas spp.*
308	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
291	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
295	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	NM	(-)	(+)	*Pseudomonas spp.*
276	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
588	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(+)	(-)	M	(+)	(+)	*Pseudomonas spp.*
596	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(+)	(-)	M	(+)	(+)	*Pseudomonas spp.*
608	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(+)	(-)	M	(-)	(+)	*Pseudomonas spp.*
611	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(+)	(-)	M	(+)	(+)	*Pseudomonas spp.*
594(i)	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(+)	(-)	*Pseudomonas spp.*
578	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(-)	(-)	M	(+)	(+)	*Pseudomonas spp.*
4804	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas aeruginosa*
575	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(+)	(+)	M	(-)	(+)	*Pseudomonas spp.*
604(I)	R	R	(-)	(-)	Pink,Rod	(+)	(-)	(-)	(-)	(+)	M	(-)	(-)	*Pseudomonas spp.*
274	R	R	(-)	(-)	Pink,Rod	(+)	(-)	(-)	(-)	(-)	M	(-)	(+)	*Pseudomonas spp.*
576	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(+)	(+)	NM	(-)	(+)	*Pseudomonas spp.*
598	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(-)	(+)	(+)	NM	(-)	(+)	*Pseudomonas spp.*

[R= Red, (+) = Positive, (-) = Negative, M= Motile, NM= Non-motile]

Table 07: Biochemical test results of the control group and their probable identity

	Tests													
Sample ID	**TSI**				**Gram Stain**	**Catalase**	**Oxidase**	**Citrate**	**MR**	**VP**	**MIU Test**			**Name of probable organism**
	Slant	**Butt**	**H2S**	**Gas**							**Motility**	**Indole**	**Urease**	
C1	Y	Y	(-)	(+)	Pink,Rod	(+)	(-)	(+)	(-)	(+)	M	(-)	(+)	*Klebsiella pneumoniae*
C2	Y	Y	(-)	(+)	Pink,Rod	(+)	(-)	(-)	(+)	(-)	M	(+)	(-)	*E.coli*
C3	R	Y	(-)	(+)	Pink,Rod	(+)	(-)	(+)	(-)	(+)	M	(-)	(-)	*Enterobacter cloacae*
C4	R	R	(-)	(-)	Pink,Rod	(+)	(+)	(+)	(-)	(-)	M	(-)	(-)	*Pseudomonas spp*

[R= Red, Y= Yellow, (+) = Positive, (-) = Negative, M= Motile, NM= Non-motile]

Table 5,6 showed that different biochemical tests were performed to find out probable organisms using the control group which is shown in table 7. Along with the biochemical tests, a gram stain was done for colony morphological examination.

3.3 Percentage of identified isolates

After performing the biochemical test, the probable organisms were *Pseudomonas spp, Pseudomonas aeruginosa, Staphylococcus spp, Staphylococcus saprophyticus, Micrococcus spp,* and *Streptococcus spp*. The number and percentage of gram-positive isolates and gram-negative isolates are given below in table 5 and table 6.

Table 08: Distribution of Gram-Positive bacterial isolates

Name of the organism	Number of Isolates	% of isolates
Staphylococcus spp	15	14.42%
Staphylococcus saprophyticus	7	6.73%
Micrococcus spp	7	6.73%
Streptococcus spp	1	0.96%

Table 8. shows that among gram-positive bacteria the highest number of isolates were *Staphylococcus spp* 15(14.42%), followed by *Staphylococcus saprophyticus* 7(6.73%), *Micrococcus spp* 7(6.73%). The least number of isolates is found in *Streptococcus spp* 1(0.96%).

Table 09: Distribution of Gram-Negative bacterial isolates

Name of the organism	Number of Isolates	% of isolates
Pseudomonas spp.	43	41.34%
Pseudomonas aeruginosa	31	29.80%

Table 9. shows that *Pseudomonas spp.* and *Pseudomonas aeruginosa* were found as gram-negative.

3.4 Result from antibiotic susceptibility test

The 104 isolates from burn lesions were examined for antibiotic susceptibility tests using 11 different antibiotics which are specific for gram-positive and gram-negative bacteria respectively. Based on gram-positive and gram-negative bacteria, the antibiogram results are shown in two different tables -

Table 10: Antibiotic sensitivity pattern of the isolated Gram-Positive bacteria

	Used Antibiotics									
Sample ID	**P**	**AZM**	**VA**	**LEV/ CIP**	**CFM**	**AK/K/S**	**IPM/MEM**	**AML/AMP**	**AMC**	**Probable organism name**
5661	R	R	R	R (LEV)	R	R	S (IPM)	R (AML)	R	*Streptococcus spp*
4779	R	S	S	S (LEV)	S	S (K)	S (MEM)	R (AML)	R	*Micrococcus spp*
5445	R	S	R	S (LEV)	R	S (AK)	S (IPM)	R (AMP)	S	*Micrococcus spp*
5451	R	S	S	S (CIP)	S	S (K)	S (IPM)	R(AML)	S	*Micrococcus spp*
272	R	S	R	R (CIP)	R	R(K)	S (MEM)	R(AMP)	R	*Micrococcus spp*
284	R	R	S	R (CIP)	R	R(K)	R(MEM)	R(AMP)	R	*Micrococcus spp*
304	R	R	S	R (CIP)	R	R(AK)	R(MEM)	RAMP)	R	*Micrococcus spp*
4802	R	R	R	R (CIP)	R	R(S)	S (IPM)	R(AMP)	R	*Micrococcus spp*
5974	R	R	R	S (LEV)	R	R (K)	S (IPM)	R(AML)	R	*Staphylococcus saprophyticus*
4789	R	R	R	R (LEV)	R	R (K)	R (IPM)	R (AML)	R	*Staphylococcus saprophyticus*
5935	R	R	R	R (LEV)	R	R (K)	R (IPM)	R(AML)	R	*Staphylococcus spp*
5455	R	R	S	S (LEV)	R	R(K)	S (IPM)	S(AML)	S	*Staphylococcus saprophyticus*
4972	R	R	R	R (LEV)	R	R(K)	R (IPM)	R(AMP)	R	*Staphycococcus aureus*
5599	R	R	R	R (LEV)	R	R(K)	R(IPM)	R(AMP)	R	*Staphylococcus spp*
679	R	R	R	R (CIP)	R	R(AK)	S (IPM)	R(AMP)	R	*Staphylococcus spp*
5442	R	R	R	R (CIP)	R	R(AK)	R (IPM)	R(AMP)	R	*Staphylococcus spp*
4375	R	R	R	S (CIP)	R	R(AK)	S (IPM)	R(AMP)	R	*Staphylococcus saprophyticus*

Table 10 continued

4773	R	R	R	R (CIP)	R	R (AK)	S(IPM)	R(AMP)	R	*Staphylococcus spp*
287	R	S	R	R (LEV)	R	R (K)	R (MEM)	R(AML)	R	*Staphylococcus saprophyticus*
595	R	R	R	R (CIP)	R	R(K)	R(MEM)	R(AMP)	R	*Staphylococcus spp*
288	R	R	R	R (CIP)	R	R(K)	R (MEM)	R(AMP)	R	*Staphylococcus saprophyticus*
604(II)	R	S	R	S (LEV)	R	R (K)	S (MEM)	R(AML)	R	*Staphylococcus spp*
308(II)	R	R	S	R (LEV)	R	R(K)	R (MEM)	R(AML)	R	*Staphylococcus spp*
4772	R	S	R	S (CIP)	R	S (K)	S (MEM)	R(AMP)	R	*Staphylococcus spp*
605	R	R	R	R(CIP)	R	R(K)	S (MEM)	R(AMP)	R	*Staphylococcus spp*
589(II)	R	S	R	S (LEV)	S	I(K)	S(MEM)	R(AMP)	S	*Staphylococcus spp*
288(II)	R	R	R	R (CIP)	R	R(K)	R(IMP)	R(AMP)	R	*Staphylococcus spp*
5447	R	S	R	R (CIP)	R	R(AK)	R(MEM)	R(AMP)	R	*Staphylococcus spp*
589(I)	R	S	R	S (CIP)	R	I (AK)	S (MEM)	R(AMP)	R	*Staphylococcus spp*
5939	R	S	R	R (LEV)	R	R (K)	S (IPM)	R(AMP)	R	*Staphylococcus saprophyticus*

[R= Resistant, S= Sensitive, I= Intermediate]

Table 11: Antibiotic sensitivity pattern of the isolated Gram-Negative bacteria

	Used Antibiotics										
Sample ID	**AML/AMP**	**AMC**	**CT/CL**	**LEV/CIP**	**CFM**	**AK/KK/S**	**IPM/MEM**	**COT**	**CTR**	**AZM**	**Probable Organism Name**
4766	R (AMP)	R	S	R(LEV)	R	S (AK)	R (IPM)	R	R	R	*Pseudomonas aeruginosa*
4367	R (AMP)	R	R	R(CIP)	R	R(K)	R (IPM)	R	R	R	*Pseudomonas aeruginosa*
4792	R (AMP)	R	S	R(CIP)	R	R(S)	S (IPM)	R	R	R	*Pseudomonas aeruginosa*
4373	R (AMP)	R	S	R (LEV)	R	R(S)	R (IPM)	R	R	R	*Pseudomonas aeruginosa*
4780	R (AMP)	R	S	R(LEV)	R	R(S)	R (IPM)	R	R	I	*Pseudomonas aeruginosa*
4765	R (AMP)	R	S	R(CIP)	R	R(AK)	R (MEM)	R	R	S	*Pseudomonas aeruginosa*
4784	R (AMP)	R	R	R(LEV)	R	R(S)	R (IPM)	R	R	R	*Pseudomonas spp.*
4759	R (AMP)	R	S	R (LEV)	R	S (S)	R (IPM)	R	R	R	*Pseudomonas putrefaciens*
4793	R (AMP)	R	S	R (LEV)	R	R(S)	R (IPM)	R	R	R	*Pseudomonas spp.*

Table 11 continued

4762	R (AMP)	R	S	R (LEV)	R	R(K)	R (IPM)	R	R	S	*Pseudomonas aeruginosa*
4356	R (AMP)	R	S	I (CIP)	R	R (K)	R (IPM)	R	R	S	*Pseudomonas aeruginosa*
4360	R (AMP)	R	S	R(LEV)	R	R(K)	S (IPM)	R	R	S	*Pseudomonas aeruginosa*
4370	R (AMP)	R	S	R(LEV)	R	R(K)	S (IPM)	R	R	S	*Pseudomonas aeruginosa*
4778	R (AMP)	R	S	R(LEV)	R	R(K)	R (IPM)	R	R	S	*Pseudomonas spp.*
4345	R (AMP)	R	R	R (LEV)	R	R (K)	R (IPM)	R	R	I	*Pseudomonas aeruginosa*
5437	R (AMP)	R	S	R (LEV)	R	R (K)	R (IPM)	R	R	S	*Pseudomonas spp.*
5438	R (AMP)	R	S	R (LEV)	R	S (AK)	R (IMP)	R	R	S	*Pseudomonas spp.*
4354	R (AMP)	R	S	R(LEV)	R	I (AK)	R (MRP)	R	R	S	*Pseudomonas aeruginosa*
5432	R (AMP)	R	S	R(LEV)	R	R(K)	R (IPM)	R	R	S	*Pseudomonas aeruginosa*
4473	R (AMP)	R	S	R(LEV)	R	R(K)	R (IPM)	R	R	R	*Pseudomonas spp.*
4372	R (AMP)	R	S	R(LEV)	R	R(S)	R (IPM)	R	R	R	*Pseudomonas aeruginosa*
4474	R (AMP)	R	S	S (LEV)	R	S (K)	S (IPM)	R	R	R	*Pseudomonas aeruginosa*
5433	R (AMP)	R	S	R(LEV)	R	S (AK)	R (MEM)	R	R	R	*Pseudomonas spp.*
5452	R (AMP)	R	S	R(LEV)	R	R(K)	S (IPM)	R	R	S	*Pseudomonas spp.*
454	R (AMP)	R	S	RLEV)	R	S (AK)	R (MRP)	R	R	S	*Pseudomonas spp.*
4472	R (AMP)	R	S	R(LEV)	R	I (AK)	S (IPM)	R	R	S	*Pseudomonas spp.*
688	R (AMP)	R	R	R(LEV)	R	S (S)	R (IPM)	R	R	R	*Pseudomonas aeruginosa*
5598	R (AMP)	R	S	R(LEV)	R	R(S)	R(IPM)	R	R	S	*Pseudomonas spp.*
5604	R (AMP)	R	S	R (CIP)	R	R(S)	R (MEM)	R	R	I	*Pseudomonas spp.*
5611	R (AML)	R	S	R (LEV)	R	S (S)	S (IPM)	R	R	R	*Pseudomonas spp.*
5621	R (AML)	R	S	S (LEV)	R	S (S)	S (IPM)	R	R	S	*Pseudomonas spp.*
5651	R (AML)	R	S	R(LEV)	R	S (S)	R(IPM)	R	R	R	*Pseudomonas aeruginosa*
5653	R (AML)	R	S	R (LEV)	R	R (S)	S (IPM)	R	R	R	*Pseudomonas spp.*
5655	R (AML)	R	S	R(LEV)	R	R (K)	R(IPM)	R	R	R	*Pseudomonas aeruginosa*
5656	R (AML)	R	S	R(LEV)	R	S (AK)	S (MEM)	R	R	S	*Pseudomonas aeruginosa*
5657	R (AML)	R	S	R(LEV)	R	R (K)	R (IPM)	R	R	I	*Pseudomonas aeruginosa*
5658	R (AML)	R	S	S (CIP)	R	R(S)	S (MEM)	R	R	I	*Pseudomonas aeruginosa*
5663	R (AML)	R	S	R (LEV)	R	R (S)	S (IPM)	R	R	S	*Pseudomonas spp.*
5668	R (AML)	R	S	R (LEV)	R	R(K)	R (IPM)	R	R	R	*Pseudomonas spp.*
5670	R (AML)	R	S	R(LEV)	R	R(K)	I (IPM)	R	R	S	*Pseudomonas spp.*
5673	R (AML)	R	S	R(LEV)	R	I (K)	R (IPM)	R	R	I	*Pseudomonas spp.*
5674	R(AML)	R	S	S (LEV)	R	R(K)	R (IPM)	R	R	R	*Pseudomonas aeruginosa*
5937	R(AML)	R	R	R(LEV)	R	R (S)	S (IPM)	R	R	S	*Pseudomonas spp.*

Table 11 continued

5945	R(AMP)	R	S	R(LEV)	R	R(K)	R (IPM)	R	R	S	*Pseudomonas spp.*
5946	R(AMP)	R	S	R(LEV)	R	S (S)	R (IPM)	R	R	S	*Pseudomonas aeruginosa*
5961	R(AMP)	R	S	R (LEV)	R	R (K)	R (IPM)	R	R	S	*Pseudomonas spp.*
5962	R(AMP)	R	S	R(LEV)	R	R(K)	R (IPM)	R	R	I	*Pseudomonas aeruginosa*
5964	R (AML)	R	S	R(LEV)	R	R(S)	R(IPM)	R	R	R	*Pseudomonas spp.*
5965	R (AMP)	R	R	I(CIP)	R	R (K)	R(IPM)	R	R	S	*Pseudomonas aeruginosa*
5968	R (AMP)	R	S	R(LEV)	R	R(AK)	I (IPM)	R	R	I	*Pseudomonas aeruginosa*
5969	R (AMP)	R	S	R(LEV)	R	R(K)	R(IPM)	R	R	S	*Pseudomonas spp.*
5982	R (AMP)	R	S	R(LEV)	R	R(S)	R(IPM)	R	R	R	*Pseudomonas aeruginosa*
290	R (AMP)	R	S	I (CIP)	R	I (AK)	R(IPM)	R	R	S	*Pseudomonas aeruginosa*
266	R (AMP)	R	S	R (CIP)	R	R(K)	R(MEM)	R	R	S	*Pseudomonas spp.*
301	R (AMP)	R	S	R (CIP)	R	R(AK)	R(IPM)	R	R	S	*Pseudomonas spp.*
303	R (AMP)	R	S	S (CIP)	R	S (AK)	R(IPM)	R	R	S	*Pseudomonas spp.*
296	R (AMP)	R	R	I (CIP)	R	S (AK)	R(IPM)	R	R	I	*Pseudomonas spp.*
270	R (AMP)	R	S	I (CIP)	R	R(S)	R(MEM)	R	R	I	*Pseudomonas spp.*
308	R (AMP)	R	S	R(LEV)	R	R(AK)	R(MEM)	R	R	R	*Pseudomonas aeruginosa*
291	R (AMP)	R	S	S (CIP)	R	S (S)	S (MEM)	R	R	S	*Pseudomonas aeruginosa*
295	R (AMP)	R	S	I (CIP)	R	R(AK)	R(IPM)	R	R	R	*Pseudomonas spp.*
276	R (AMP)	R	R	R (CIP)	R	R(AK)	R(IPM)	R	R	S	*Pseudomonas spp.*
588	R (AMP)	R	S	R(LEV)	R	S (AK)	R(IMP)	R	R	S	*Pseudomonas spp.*
596	R (AMP)	R	S	R(LEV)	R	R (AK)	R(MEM)	R	R	R	*Pseudomonas spp.*
608	R (AMP)	S	S	R(LEV)	R	R(AK)	R(IMP)	S	R	I	*Pseudomonas spp.*
611	R (AMP)	R	S	R (CIP)	R	R(K)	R(MEM)	R	R	S	*Pseudomonas spp.*
594(i)	R (AMP)	R	R	R(LEV)	R	R (AK)	R(MEM)	R	R	S	*Pseudomonas spp.*
578	R (AMP)	R	R	S (LEV)	R	R(AK)	R(IMP)	R	R	S	*Pseudomonas spp.*
4804	R (AMP)	R	S	R(CIP)	R	R(S)	S (MEM)	R	R	S	*Pseudomonas aeruginosa*
575	R (AMP)	R	S	R(LEV)	R	R (AK)	R(MEM)	R	R	S	*Pseudomonas spp.*
604(I)	R (AMP)	R	S	R(LEV)	R	R (AK)	R(IMP)	R	R	S	*Pseudomonas spp.*
274	R (AMP)	R	S	R(LEV)	R	S (AK)	R(MEM)	R	R	R	*Pseudomonas spp.*
576	R (AMP)	R	S	R(LEV)	R	S(AK)	R(IMP)	R	R	S	*Pseudomonas spp.*
598	R (AMP)	R	S	R(LEV)	R	S (AK)	R(IPM)	R	R	I	*Pseudomonas spp.*

[R= Resistant, S= Sensitive, I= Intermediate]

Tables 10 and 11 showed that bacterial isolates from burn-injured patients were categorized as Resistant, Intermediate, and Sensitive according to the Clinical and Laboratory Standard Institute (CLSI) guidelines.

3.5 Antibiotic-resistant pattern: From the antibiogram result it was found that most of the isolates from burn patients showed resistance to used antibiotics.

Table 12: Antibiotic resistance pattern (Gram-Positive isolates)

Name of antibiotics group	Name of Antibiotic	Percentage of resistance
Penicillin	Penicillin	100%
	Amoxicillin	96.6%
	Ampicillin	96.6%
	Amoxiclav	86.6%
Macrolides	Azithromycin	63.3%
Glycopeptide	Vancomycin	80%
Fluoroquinolone	Levofloxacin	66.6%
	Ciprofloxacin	66.6%
Cephalosporin	Cefixime	90%
Aminoglycoside	Amikacin	80%
	Streptomycin	80%
	Kanamycin	80%
Carbanem	Imipenem	43.3%
	Meropenem	43.3%

Table 12 shows that the gram-positive isolates from burn patients showed 100% resistance to penicillin, amoxicillin, and ampicillin. The least resistance was observed against imipenem, meropenem and it was 43.3%.

Table 13: Antibiotic resistance pattern (Gram-Negative isolates)

Name of antibiotics group	Name of antibiotics	Percentage of resistance
Penicillin	Amoxicillin	100%
	Ampicillin	100%
	Amoxyclav	98.64%
Polymyxin class	Colistin sulfate	13.51%
Fluoroquinolones	Levofloxacin	82.43%
	Ciprofloxacin	82.43%
Cephalosporin	Cefixime	100%
	Ceftriaxone	100%
Aminoglycoside	Amikacin	70.27%
	Kanamycin	70.27%
	Streptomycin	70.27%
Carbanem	Meropenem	77.02%
	Imipenem	77.02%
Diaminopyrimidines	Trimethoprim	98.64%
Macrolides	Azithromycin	32.43%

Table 13. shows that the gram-negative isolates from burn patients showed 100% resistance to penicillin, amoxicillin, and ampicillin. The least resistance was observed against colistin sulfate (13.51%), followed by azithromycin and 32.43%.

3.6 Classification of organisms based on MDR, XDR, and PDR:

Almost all isolates from burn patients showed resistance against most of the antibiotics. Based on their resistance pattern, these organisms are classified into MDR, XDR and PDR.

Table 14: Percentage of MDR, XDR, and PDR for isolates

Total isolates	Category	Number of isolates	Percentage
104	MDR	33	31.73%
	XDR	58	55.76%
	PDR	10	9.61%

[MDR: Multidrug-Resistant, XDR: Extensively Drug Resistant, PDR: Pan Drug Resistant]

Table 14. includes the percentage of isolates based on MDR, XDR and PDR where maximum strains were XDR, and it was 55.76%.

3.7: Determination of Minimum Inhibitory Concentration (MIC)

In the next step Minimum Inhibitory Concentrations (MIC) of three selective antibiotics (ceftriaxone, levofloxacin, vancomycin) were investigated against isolates that were resistant to these respective antibiotics.

Table 15: The MIC value of Ceftriaxone and Levofloxacin

Name of antibiotic	Standard MIC value	Antibiotics concentration	Sample ID									
			4472	276	4481	578	4370	5670	598	5438	4483	5452
Ceftriaxone	2 µg/ml	10 mg/ml	C	T	C	C	C	C	C	C	C	C
		1 mg/ml	T	T	T	T	C	T	T	T	T	C
		500 µg/ml	T	T	T	T	C	T	T	T	T	T
		300 µg/ml	T	T	T	T	T	T	T	T	T	T
		180 µg/ml	T	T	T	T	T	T	T	T	T	T
		108 µg/ml	T	T	T	T	T	T	T	T	T	T
		64.8 µg/ml	T	T	T	T	T	T	T	T	T	T
		38.88 µg/ml	T	T	T	T	T	T	T	T	T	T
		23.32 µg/ml	T	T	T	T	T	T	T	T	T	T
		13.9 µg/ml	T	T	T	T	T	T	T	T	T	T
			5962	4804	5965	688	5972	274	5651	4784		
Levofloxacin	0.002-32 µg/mL	2.5 mg/ml	C	C	C	C	C	C	C	C		
		1.25 mg/ml	C	C	C	C	C	C	C	C		
		625 µg/ml	C	C	C	C	C	C	C	C		
		312 µg/ml	C	T	C	C	C	C	C	C		
		156 µg/ml	C	T	C	T	C	C	C	C		
		78 µg/ml	C	T	C	T	C	C	C	C		
		39 µg/ml	C	T	T	T	T	C	T	C		
		19.5 µg/ml	T	T	T	T	T	T	T	T		
		9.75 µg/ml	T	T	T	T	T	T	T	T		

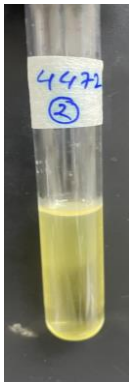
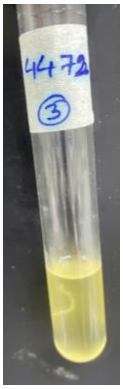
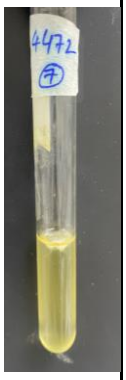
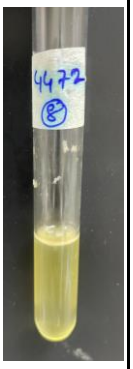
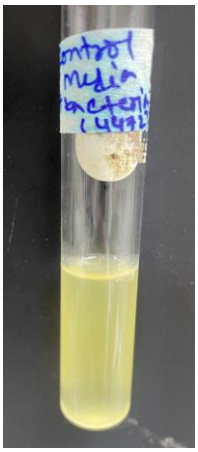
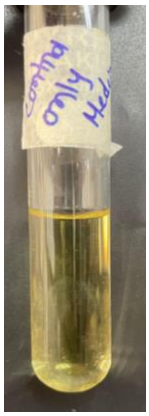
[Key: C = Clear, T = Turbid, C = MIC Value]

Table 16: The MIC value of Vancomycin

Name of antibiotic	Standard MIC value	Antibiotics concentration	Sample ID												
			5455	5974	4772	5661	5442	272	287	288	5599	589(ii)	679	4375	4773
Vancomycin	≤2 µg/m	1 mg/ml	C	C	T	C	C	C	T	C	C	C	T	T	C
		500 µg/ml	C	C	T	T	T	T	T	T	T	C	T	T	T
		250 µg/ml	C	T	T	T	T	T	T	T	T	C	T	T	T
		125 µg/ml	T	T	T	T	T	T	T	T	T	T	T	T	T
		62.5 µg/ml	T	T	T	T	T	T	T	T	T	T	T	T	T
		31.25 µg/ml	T	T	T	T	T	T	T	T	T	T	T	T	T
		15.625µg/ml	T	T	T	T	T	T	T	T	T	T	T	T	T

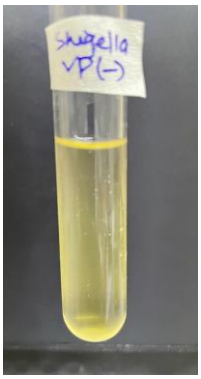
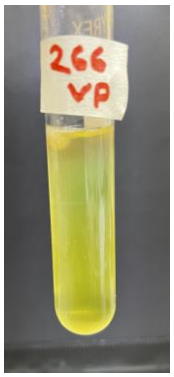
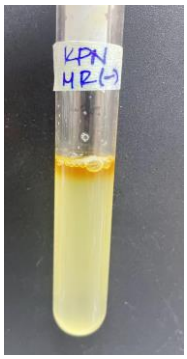
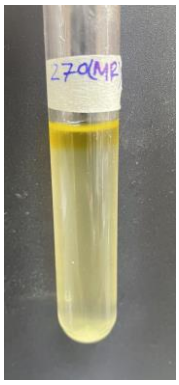
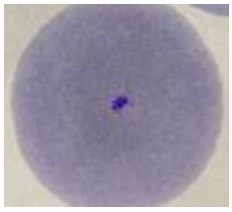
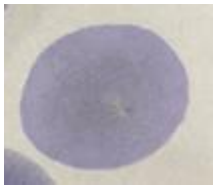
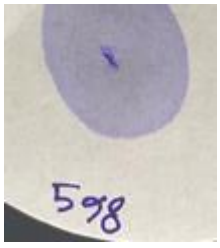
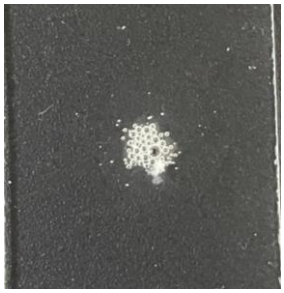
[Key: C = Clear, T = Turbid, C = MIC Value]

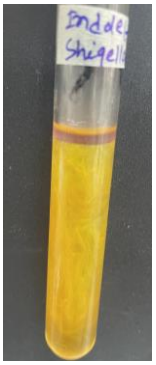
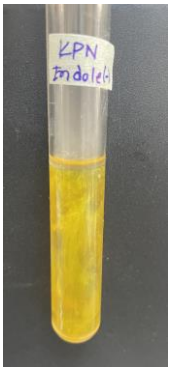
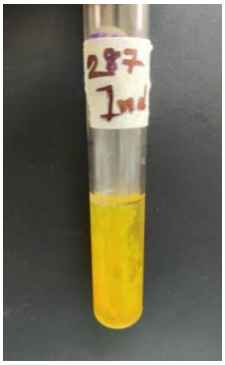
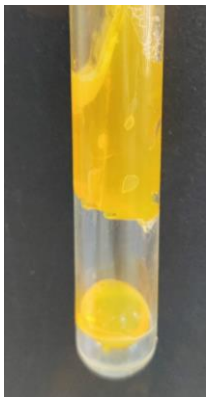
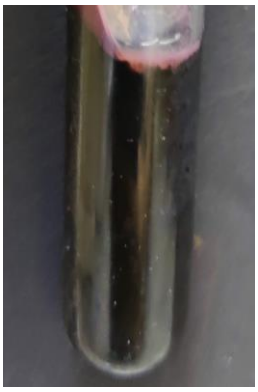
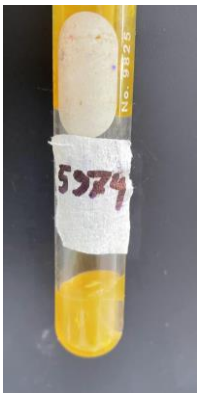
Table17: The MIC result picture

	Antibiotic concentrations (Ceftriaxone)									
	tt:1	tt:2	tt:3	tt:4	tt:5	tt:6	tt:7	tt:8	tt:9	tt:10
	10 mg/ml	**1 mg/ml**	**500 µg/ml**	**300 µg/ml**	**180 µg/ml**	**108 µg/ml**	**64.8 µg/ml**	**38.88 µg/ml**	**23.32 µg/ml**	**23.32 µg/ml**
Sample ID 4472										
MIC value 10 mg/ml	**Controls**	 **Fig 1: C1: Media + bacteria**	 **Fig 2: C2: Media + antibiotic**	 **Fig 3: C3: only media**						

[tt: Test tube, C= Control]

Table 18: Biochemical result picture with control

Test name	Controls		Test organism
VP test	 Fig 4: VP (+)	 Fig 5: VP (-)	 Fig 6: 266 VP (-)
MR test	 Fig 7: MR (+)	 Fig 8: MR(-)	 Fig 9: 270 (-)
Oxidase test	 Fig 10: oxidase (+)	 Fig 11:oxidase (-)	 Fig 12: 598 (+)
Catalase test	 Fig 13: catalase (+)		 Fig 14: 5661 (+)

MIU test	 **Fig 15: Urease (+) and (-), motility (+)**		 **Fig 16: 5442 Urease (+), motility (+)**
Indole test	 **Fig 17: Indole (+)**	 **Fig 18: Indole (-)**	 **Fig 19: 287 (-)**
TSI test	 **Fig 20: Y/Y , gas (+)**  **A/A, gas (-)**  **H2S (+)**		 **Fig 21: 5974 Y/Y, gas (+)**
Citrate test	 **Fig 22: citrate (+)**	 **Fig 23: citrate (-)**	 **Fig 24: 272 citrate (+)**

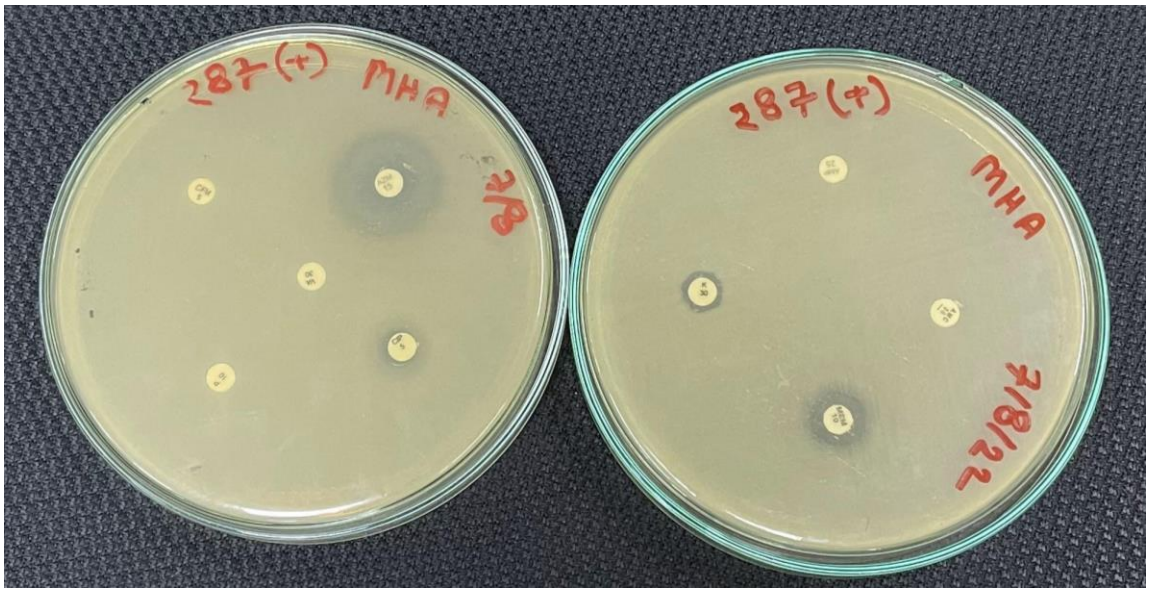


Fig 25: Antibiotic susceptibility test with antibiotic discs

Chapter 4

Discussion

Burn infection can result in chronic illness if the infection-causing agent is not properly identified and treated immediately. Our study aimed to isolate and identify pathogenic bacteria from burn-injured patients and observe their Antibiotic Susceptibility pattern against different classes of antibiotics. Also, Minimum Inhibitory Concentration (MIC) of some selective antibiotics was observed to find out the MIC value for some selected antibiotics against resistant bacteria.

During the study period (March 2022 - October 2022) a total of 104 samples of both sex and age groups between 1-90 were observed. Out of 104 samples, Gram-positive cocci accounted for 30 (28.85%) and Gram-negative Bacilli accounted for 74 (71.15%) which in comparison is approximately double of total Gram-positive isolates. It was also found that the number of male patients 67 (64.42%) was higher than female 37 (35.58%) patients. Another study reported by B et al. 2013 claimed that burn wound infection in males was 1447 (64.4%) but burn wound infection in females was 799 (35.6%) which corresponds to our study. Almost in every case, the percentage of injured patients in males was high. This may be due to males being more exposed to burns and wearing loose-fitting clothes rather than women. Again, the highest number of infection cases was observed between 1-10 age groups which are 27 (25.96%). A similar study reported by Gulati et al. 2014 claimed that the lowest distribution of burn patients belongs to the 5-15 years' age group which is 12.2%. However, it can be said that accidents by flame burns might occur to any person at any time. Safety protocol should be maintained and for children parents need to be more careful. Gram-positive bacteria included *Staphylococcus spp* 15 (14.42%), *Staphylococcus saprophyticus* 7(6.73%), *Micrococcus spp* 7 (6.73%), and *Streptococcus spp* 1(0.96%) while Gram-negative bacteria included *Pseudomonas spp*. 43 (41.34%) and *Pseudomonas aeruginosa* 31(29.80%).

In our study most prevalent bacteria were *Pseudomonas spp.* Patients suffering with severe burns are more prone to infections caused by *Pseudomonas species (*Pruitt, 1974). *Pseudomonas aeruginosa* is known as the most resistant organism of nosocomial infections which causes 10-11% of all nosocomial infections (Labovská, 2020). Out of 104 samples from burn patients 43(41.34%) isolates were found *Pseudomonas spp* and 31(29.80%) were *Pseudomonas aeruginosa*. This result is comparable to a study where *Pseudomonas aeruginosa* had a prevalence of 57% (Fatema et al., 2021). The next most prevalent organism was *Staphylococcus spp* 15 (14.42%). The reason is that *Staphylococcus spp*. is part of human normal flora and starts to

colonize in the burn injury site and cause opportunistic infection. The next higher number of isolates was *Staphylococcus saprophyticus* 7(6.73%) and *Micrococcus spp*. 7 (6.73%). *Micrococci* are not pathogenic bacteria but they cause a variety of infections as opportunistic pathogens in immuno-compromised patients. Moreover, only one *Streptococcus spp* was found in the study which accounted for 0.96%. *Streptococcus spp.* causes infection when patients have a break in the skin which allows the bacteria to enter the tissue.

Antibiotic resistance is now the greatest threat to mankind and it is becoming a challenge to treat infections through antibiotics. Our study also focused on antibiotic susceptibility patterns of isolated bacteria. From the antibiogram results, it was found that all the gram-positive isolates from burn patients showed resistance to Penicillin group antibiotics. This penicillin group includes Penicillin-G, amoxicillin, ampicillin, and amoxiclav. Amongst them Penicillin-G showed 100% resistance and amoxicillin, ampicillin, and amoxiclav showed 96.6%, 96.6%, and 86.6% resistance respectively. The next highest resistance showed cefixime from the cephalosporin group and it was 90%. Both glycopeptide groups of antibiotics including vancomycin and aminoglycoside groups of antibiotics including amikacin, kanamycin, and streptomycin showed 80% resistance. Moreover, fluoroquinolone groups including levofloxacin and ciprofloxacin showed 66.6% resistance and macrolides groups including azithromycin showed 63.3% resistance. However, the least resistance showed the carbapenem group which was 43.3%. A similar study reported by Fatema et.al 2020 claimed that *Staphylococcus spp.* were found to be sensitive to imipenem and meropenem against their isolated bacteria.

From the antibiogram results, gram-negative isolates from burn patients showed 100% resistance to Penicillin (amoxicillin, ampicillin, and amoxiclav) and cephalosporin (cefixime and ceftriaxone) groups of antibiotics. Another study claimed that *Pseudomonas spp* showed 65.7% resistance to ceftriaxone (Islam et al., 2012). The next highest resistance showed the Diaminopyrimidines group of antibiotics including trimethoprim and it was 98.64%. Moreover, fluoroquinolone groups including levofloxacin and ciprofloxacin showed 82.43% resistance. The carbapenem groups including meropenem and imipenem showed 77.02% resistance. Aminoglycoside groups of antibiotics including amikacin, kanamycin, streptomycin showed 70.27% resistance. Furthermore, a similar study reported by Fatema et.al. 2020 claimed that

Pseudomonas spp was found resistant to all antibiotics. But according to our study, it was observed that Colistin Sulfate (13.51%) and Azithromycin (32.43%) were more effective than other antibiotics against *Pseudomonas spp.* A study reported that Amikacin is the most affected drug against gram-negative bacteria (Islam et al., 2012). The reasons behind increasing bacterial resistance patterns could be extensive use of broad-spectrum antibiotics, lack of new antibiotics, misleading prescriptions, less awareness about the consequences, and so on.

From antibiogram results, the majority of the isolates from burn patients showed resistance against used antibiotics. Based on their resistance pattern, these organisms are classified into MDR, XDR, and PDR. Most of the strains were observed as XDR which is 55.76%. Gram-negative bacteria showed resistance against β-lactam drugs including penicillin and cephalosporin drugs because they can produce β-lactamases enzymes widely (Reygaert, 2018). From several experiments, it is found that *staphylococcus spp* acquired resistance towards vancomycin antibiotics through gene transfer.

In this study, ceftriaxone, levofloxacin, and vancomycin antibiotics were used to get MIC values against some of the MDR strains. For ceftriaxone, the MIC value against *Pseudomonas spp*. was mostly 10 mg/ml. The standard MIC value for ceftriaxone is 2 µg/ml. For vancomycin, the MIC value was mostly 1 mg/ml against *Staphylococcus spp., Micrococcus* spp., *Streptococcus spp.*. The standard MIC value for vancomycin is ≤2 µg/ml. In terms of Levofloxacin, the MIC value was observed at 78 µg/ml and 39 µg/ml. The standard MIC value for levofloxacin is 0.002-32 µg/mL. From this result, it is clearly visible that MIC value increases than the standard one as resistant bacteria are less susceptible to these antibiotics.

Recommendation

Nosocomial infections are common in burn patients, where multidrug-resistant bacteria enter the body and cause sepsis or death. Pathogenic bacteria thrive in unhygienic environments. To prevent nosocomial infections through the environment, the hospital environment should be kept clean by disinfecting the bed, walls, bathrooms, and windows. Airborne pathogens can be controlled by using proper air filtration and ventilation. In addition, hospitalized patients' immune systems are

compromised, making them vulnerable to nosocomial infections. To avoid food-borne diseases, a healthy, nutritious diet should be provided, as well as careful food handling. To prevent pathogens from entering the body through direct contact, people should wash their hands immediately. Gloves and mask head covers can also be useful. (Leseva et al., 2013) Antibacterial resistance is the most common cause of nosocomial infection prevalence. To prevent antibiotic resistance, proper antibiotic use, which includes only using them when there is a bacterial infection, finishing the course because if the course isn't completed, the remaining bacteria learn how to function in critical conditions and become resistant, only using antibiotics prescribed by a doctor, and never using leftover antibiotics since the dose and type may not be the same to fight new infections, can also help. As antibacterial resistance is an emerging issue these days, the development of new drugs and other tools is required to combat evolving resistance. Additionally, more antibiotic combination research should be conducted because a single antibiotic may not kill the bacteria but a combination of two or more drugs or herbal extracts can work against resistance.

Conclusion

In the conclusion, we can clearly see that among 104 cases, gram-negative bacteria were the dominating bacteria all over the study, especially *Pseudomonas.spp*. Different types of antibiotics had been used, among them, azithromycin was the most effective for gram-negative bacteria and imipenem was the most effective for gram-positive bacteria. As multidrug-resistant bacteria are highly isolated from burn wounds, it is important to continuously determine their specific resistance pattern. Moreover, we have to select antibiotics carefully for the treatment of burn wound infection to reduce morbidity and mortality which are related to multidrug-resistant bacteria.

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