ISOLATION, IDENTIFICATION, AND DETERMINATION OF ANTIMICROBIAL RESISTANCE PATTERN OF ISOLATES COLLECTED FROM UTI PATIENTS OF DHAKA, BANGLADESH

Tanjila Hossain
ID: 19126054
Tilottoma Joanna Rodrigues
ID: 19126020

A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of Bachelor of Science in Microbiology

Microbiology Program
Department of Mathematics and Natural Sciences
BRAC University

Declaration

This declaration states that

1. The thesis submitted is the product of our original research conducted while pursuing our degrees at Brac University
2. Only properly cited references are included; no other previously published or written works by others are used
3. No content of the thesis has been submitted or approved for any other degree or certificate at any institution
4. All sources of primary assistance have been appropriately credited.

Student's Full Name & Signature:

Tanjila Hossain
ID: 19126054

Tilottoma Joanna Rodrigues
ID: 19126020

Approval

The thesis/project titled "Isolation, identification, and determination of antimicrobial resistance pattern of isolates collected from UTI patients of Dhaka, Bangladesh" submitted by

Tanjila Hossain (19126054)

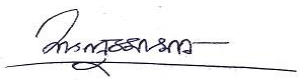
Tilottoma Joanna Rodrigues (19126020)

Has been accepted as satisfactory in partial fulfillment of the requirement for the degree of B.Sc. in Microbiology on July 18, 2023

Examining Committee:

Supervisor:
(Member)
Fahmida Sultana
Senior Lecturer
Department of Mathematics and Natural Sciences
BRAC University

Co-Supervisor:
(Member)
Md. Hasanuzzaman
Senior Lecturer
Department of Mathematics and Natural Sciences
BRAC University



Program Coordinator:
(Member)
Dr. Nadia Sultana Deen
Associate Professor
Department of Mathematics and Natural Sciences
BRAC University

Departmental Head:
(Chair)
Dr. Md. Firoze H. Haque
Department of Mathematics and Natural Sciences
BRAC University

Abstract:

Nearly 150 million individuals all over the world suffer from urinary tract infections, which are among the most prevalent bacterial infections. The uropathogens responsible for such infections are predominantly *Escherichia coli* (UPEC), with Klebsiella spp., Acinetobacter spp, and other bacteria following closely behind. The discovery of antibiotics, namely penicillin, paved the way for modern treatment of UTIs. Antibiotic resistance, however, has risen substantially as the consequence of antibiotic abuse and overuse and now presents a global threat. By isolating, identifying, and analyzing the antimicrobial resistance patterns of uropathogens from UTI patients in Dhaka, Bangladesh, this study aims to provide data that can shape the future clinical practices and contribute to effective treatments. By comparing the results with previously conducted studies, our study also intends to identify trends or changes in resistance patterns as the data acquired may ultimately offer new insights that will be beneficial for clinical practices in the future.

From March 2023 to October 2023, 152 urine samples were collected from UTI patients at Labaid Specialized Hospital and Bangladesh Shishu Hospital and Institute and carried to Brac University microbiology laboratory. Initial isolation was conducted using HiCrome differential medium and NA media was used to derive pure cultures. Identification of the isolates included Gram staining and biochemical tests (Catalase, Oxidase, Indole, Methyl Red, Voges-Proskauer, Citrate Utilization, and TSI test). The tests identified uropathogenic *Escherichia coli* (UPEC) as the most frequent pathogen (51%), followed by *Klebsiella* spp. (16.3%), *Acinetobacter* spp. (12.2%), *Pseudomonas* spp. (8.2%), *Flavobacterium* and *Citrobacter* spp. (4.1% each), and GBS and *Proteus* spp. (2% each). 94.4% of the detected isolates were gram-negative. Of all the patients, 61.2% were women and 51.7% were pediatric cases. Antimicrobial susceptibility testing was performed using the Kirby Bauer disc diffusion method using 13 antibiotics from 10 different groups while maintaining the global CSI guidelines. The test revealed high resistance rates to Ampicillin (AMP25) and Cefixime (CFM5) at nearly 100%. The two most effective antibiotics were Colistin (CL10) and Imipenem (IPM10), with resistance rates of 15% and 25%, respectively.

The study corroborates regional research, suggesting UPEC as the predominant pathogen and significant resistance to commonly used antibiotics such as ampicillin. The study results raised concerns as it showed a high resistance to the last resort antibiotic– Imipenem. Comparative analysis with studies conducted in South Asia emphasizes regional differences in antibiotic resistant patterns. The study also provides a comprehensive analysis of uropathogen prevalence and resistance patterns in Dhaka. The findings in the study underline the urgent necessity for surveillance and regulation of antibiotic drug usage in order to counter the threat that antibiotic resistance poses. The data can guide effective treatment strategies, especially for high-risk individuals such as women and pediatric patients.

Dedication

To our beloved grandparents for always believing in us, our parents for their unwavering support, and our pets whose comfort carried us through the most challenging times. Thank you for the dreams you encouraged us to pursue.

Acknowledgment

We appreciate the thoughtful support provided by our respected program coordinator of Microbiology, Dr. Nadia Sultana Deen, PhD and respected chair, Dr. Md. Firoze H. Haque, PhD.

We would also like to express our sincere gratitude towards our advisor Senior Lecturer Fahmida Sultana for her constant support.

Special thanks to our co-advisor Senior Lecturer Md. Hasanuzzaman Sir, whose dedication and sincerity towards his students was instrumental in shaping our journey.

We are indebted to Zwad Al Saiyan, without whom, this journey would be exceptionally challenging. We also thank Aditya Banik, Tonuka Tunazzina Chowdhury, Shobnom, Subah, Subarna, Farhan and Nazifa.

We owe a great deal to Dewan Tanzin, our teaching assistant, as well as, Fairuz Zahedy for their guidance and assistance throughout the journey.

We thank our lab officers- Mahmudul Hasan and Shamim Akhter for being our one-stop-solution for all our queries. And of course, Ashik bhai, Nadira Apu and Tanzila apu whom we have incessantly agonized regarding any lab related issue.

Lastly, we thank all faculty members of the microbiology program for their contributions throughout our academic journey.

Table of Contents

Tables Index

Figures Index

List of Acronyms

UTI- Urinary Tract Infection
UPEC- Uropathogenic *Escherichia coli*
E. coli- *Escherichia coli*
GBS- *group B Streptococcus*
KPN- *Klebsiella pneumoniae*
NA- Nutrient Agar
MHA- Mueller Hinton Agar
TSB- Tryptic Soy Broth
AST- Antimicrobial Susceptibility Test
DNA- Deoxyribonucleic Acid
RNA- Ribonucleic Acid
MR- Methyl Red
VP- Voges Proskauer
TSI- Triple Sugar Iron
MIC- Minimum Inhibitory Concentration
MAR- Multiple Antibiotic Resistance
CLSI- Clinical and Laboratory Standards Institute
F300- Nitrofurantoin
AK30- Amikacin
K5- Kanamycin
CFM5- Cefixime
CPM30- Cefepime
CTR30- Ceftriaxone
AZM30- Azithromycin
IPM10- Imipenem
LE5- Levofloxacin
AMP25- Ampicillin
DO30- Doxycycline
CL10- Colistin
AMC30- Amoxiclav

Chapter 1

Introduction:

Urinary Tract Infections (UTIs) refer to one of the most commonly occurring bacterial infections in the body that affect nearly 150 million people each year worldwide. UTI occurs in a multitude of ways, typically starting from the perineum where the bacteria enter the bladder, aided by factors such as anatomical abnormalities or a weakened immune system, catheterization, and sexual activities. UTI can be caused by both Gram-positive and negative pathogens; however, uropathogenic *Escherichia coli* continues to be the most predominant culprit followed by *Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis, Group B Streptococcus (*GBS*), Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Candida* spp and so forth (Flores-Mireles et al., 2015). Urinary tract infections have been a common condition before pathogens were identified as the primary cause of the disease and before urology was deemed a separate medical field. The recognition and treatment of UTIs date back to ancient and medieval times. Ancient tests from 1550 BCE from Egypt revealed symptoms of ailment that matched with those of UTIs, recommending the use of herbal remedies. Likewise, medieval Europe also treated UTIs with herbal medicines (Nickel, 2005). Roman medicine also advanced conventional methods, while Greek physicians advanced invasive techniques like surgical lithotomy for stones and catheterization. However, the discovery of penicillin by Sir Alexander Fleming in 1928 started the antibiotic revolution, and in 1942, the first penicillin became available outside the Allied military in 1945, which paved the way for the antibiotic era (Adedeji, 2016). Nowadays, UTIs are treated with courses of antibiotics and in severe cases, with intravenous antibiotics. The technological revolution has also made diagnosis of UTI much simpler through symptom assessment, urinalysis, and imaging.

Despite being generalized as a benign clinical issue, urinary infections can cause a series of diseases ranging from asymptomatic to cases as serious as recurrences, pyelonephritis with sepsis, renal failure in young children, premature birth, any disease or complications associated with frequent use of antimicrobial agents, such as high-level antibiotic resistance. In uncomplicated UTIs, the infections are usually situated in the lower urinary tract, as in the bladder and the urethra, and are somewhat prevalent in healthy individuals. The symptoms can range from frequent urination, burning sensation while urinating, or cloudy urine. Such infections can often be treated with antibiotics. Then comes the complicated UTIs, which occur in patients who have an already existing health condition such as diabetes or pregnancy. These infections occur in the upper urinary tract as in kidneys, resulting in more serious symptoms. Infections of such kind need more intensive treatment than just the administration of antibiotics. Upper UTIs or pyelonephritis pose the highest risks, as these infections need prompt treatment and otherwise can result in kidney damage, sepsis, or spread. The symptoms are more severe as well, particularly high fever, nausea, and vomiting. Upper UTIs are especially concerning due to the life-threatening complications urosepsis poses as the infection can spread into the bloodstream. Women typically are more prone to developing urinary tract infections due to their urethra being shorter, which makes bacterial entry simpler. 50-60% of women contract UTI once in their lifetimes (Al-Badr & Al-Shaikh, 2013). Oftentimes, sexual activity, menopause, and the use of catheter can also lead to developing UTIs. However, as men grow older, UTIs are often associated with underlying prostate conditions.

As mentioned previously, UTIs are treated with antibiotics. Salvarsan was the first ever antibiotic that was introduced in the early 1910s as the first effective drug against syphilis. Within a century, the usage of antibiotics in modern medicine increased the human lifespan significantly by 23 years (Thomas & Nielsen, 2020). Penicillin was discovered in 1928 which then revolutionized the natural product antibiotic discovery. However, after the 1950s, a steady decline in antibiotic discovery and development

has ultimately resulted in the concurrent antimicrobial resistance crisis. During the early 1980s, antibiotics that offered improved efficacy against Gram-negative bacteria were developed. However, the earlier generations of the antibiotics had significant limitations that urged the development of third-generation cephalosporins, which displayed enhanced efficacy against Gram-negative bacteria while providing an increased resistance against beta-lactamase enzymes.

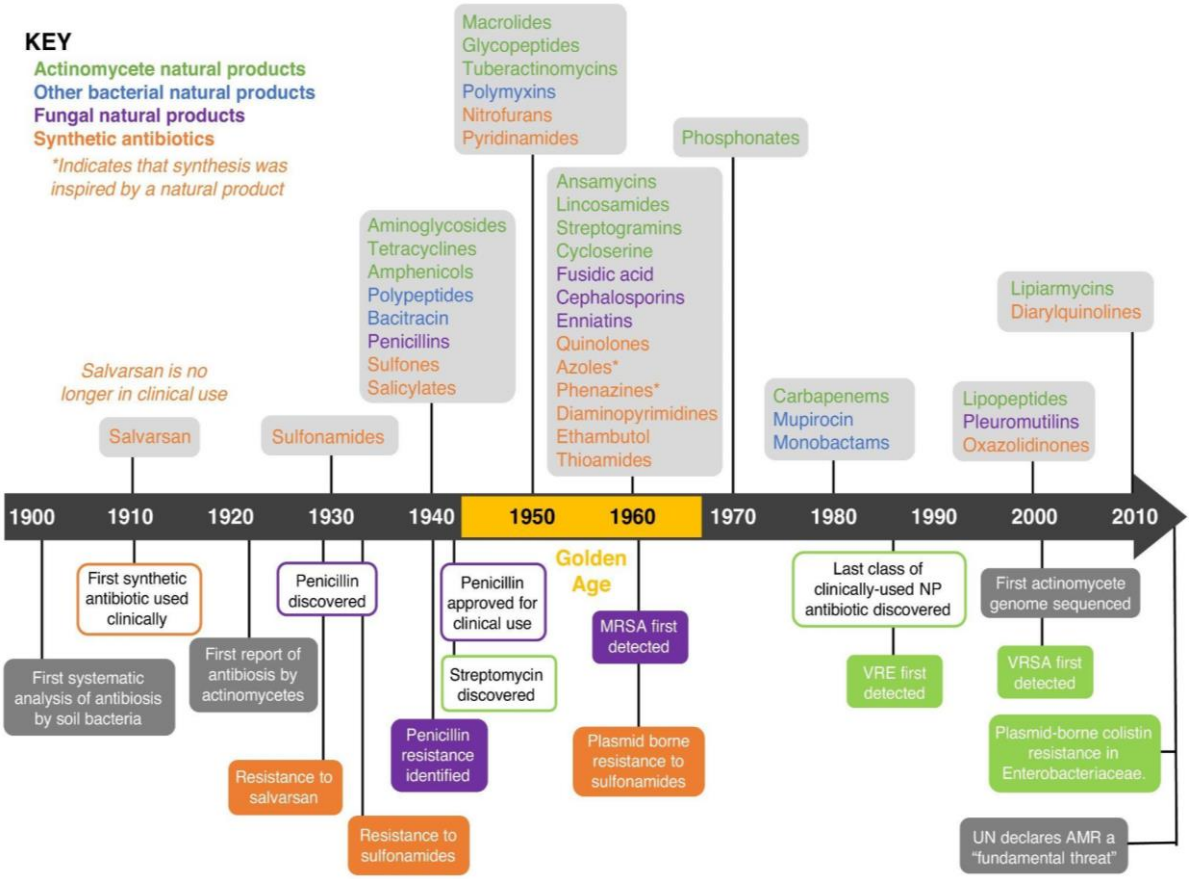


Fig 1: History of antibiotics

On a global scale, antibiotic resistance has grown into an escalating public health concern. Antibiotic resistance refers to the defense mechanisms pathogens have developed against medications that are designed to kill them, rendering conventional therapies useless and contributing to chronic infections. Should this issue persist, we may revert back to conditions similar to those before the discovery of penicillin, when even minor infections were potentially fatal. However, all hope may not be lost, as new technologies such as genome mining and editing are being used in the discovery of new natural products that display different bioactivities. The current status of antibiotic development shows promise as 45 drugs are undergoing clinical trials and a few different classes with novel mechanisms of action are in the phase 3 of clinical trials (Thomas & Nielsen, 2020). However, it should be noted that antibacterial resistance is different for the developing nations since they lack the national guidelines and complete knowledge regarding the patterns of antibiotic resistance due to limited access to the national surveillance data.

The excessive use and abuse of antibiotics in both human health and agriculture is a significant contributing factor to antibiotic resistance. Overprescribing, mishandling, and patients not completing prescribed antibiotic courses can all promote bacterial resistance. Another reason for resistance is the

unrestricted use of antibiotics in cattle to prevent diseases and to promote growth to increase profit. Inadequate hygiene in hospitals also help spread resistant bacteria. Hospital-acquired infections are usually associated with multi-drug resistant superbugs. Without the necessary steps, the O'Neill study, commissioned by the UK government, predicts that by 2050, drug-resistant diseases will be the cause of 10 million annual deaths (O'Neill, 2016).

Antibiotics target specific structures or intracellular processes in bacterial cells. The mechanisms are as followed:

1. **Inhibition of cell wall synthesis:**

Antibiotics such as penicillin and cephalosporin function by inhibiting the peptidoglycan synthesis of the cell wall. The bacteria are unable to preserve their structural integrity and shape which causes cell lysis and death.

2. **Disruption of cell membrane function:**

Polymyxins interact with cell membrane phospholipids and increase permeability which ultimately results in cell death.

3. **Inhibition of nucleic acid synthesis:**

Enzymes that are critical for DNA replication and transcriptions, such as DNA gyrase and topoisomerase IV, are inhibited by quinolones. Likewise, rifamycin interrupts RNA synthesis by inhibiting RNA polymerase. (Hooper, 1999).

4. **Inhibition of protein synthesis:**

Antibiotics bind to the 30S or 50S subunit of the bacterial ribosome, which causes them to inhibit translation, consequently interrupting the protein synthesis process in bacteria. Tetracycline, for example, binds to the 30S subunit, which inhibits cell tRNA from interacting with A site. (Chopra & Roberts, 2001).

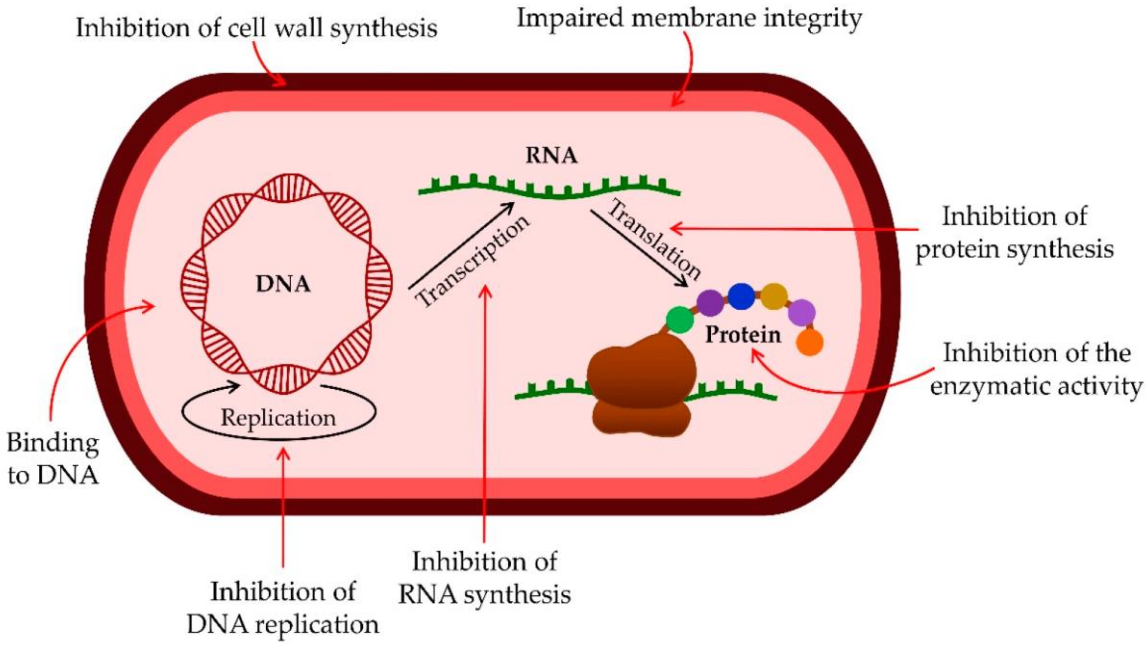


Fig 2: Antibiotic mode of action

There are several mechanisms that bacteria follow in order to develop the ability to survive despite the presence of antimicrobial medicines.

1. **Genetic Mutations:** Mutations or alterations in the bacterial DNA can cause resistance where the mutation may alter the target site of the medicine, making it ineffective.
2. **Gene transfer:** Bacteria are able to acquire resistance genes from other bacteria through a process known as horizontal gene transfer and through mechanisms such as transformation, transduction and conjugation.
3. **Efflux pumps:** Efflux pumps are a type of membrane protein that are responsible for transporting substances out of the cell, thereby serving as a defense mechanism against foreign molecules like detergent, toxic metabolites et cetera. However, in case of antibiotic resistance. These pumps transport even the antibiotics out of the bacterial cell before the effects begin to take place. Efflux pumps enable bacteria to survive by reducing the intracellular concentration of the antibiotics.
4. **Enzymatic degradation or modification:** Bacteria can produce enzymes that destroy or modify the medicine. For instance, beta lactose enzymes can break down beta lactose antibiotics such as penicillin.
5. **Bypass pathways:** The development of alternative metabolic pathways to evade the action of antibiotics.
6. **Limited permeability:** Bacteria are able to alter their cell membrane structure that prohibits antibiotics from entering the cell.
7. **Biofilm formation:** Often bacteria form biofilms as in communities of microorganisms that provide a protective layer and prevent antibiotics from reaching its target.

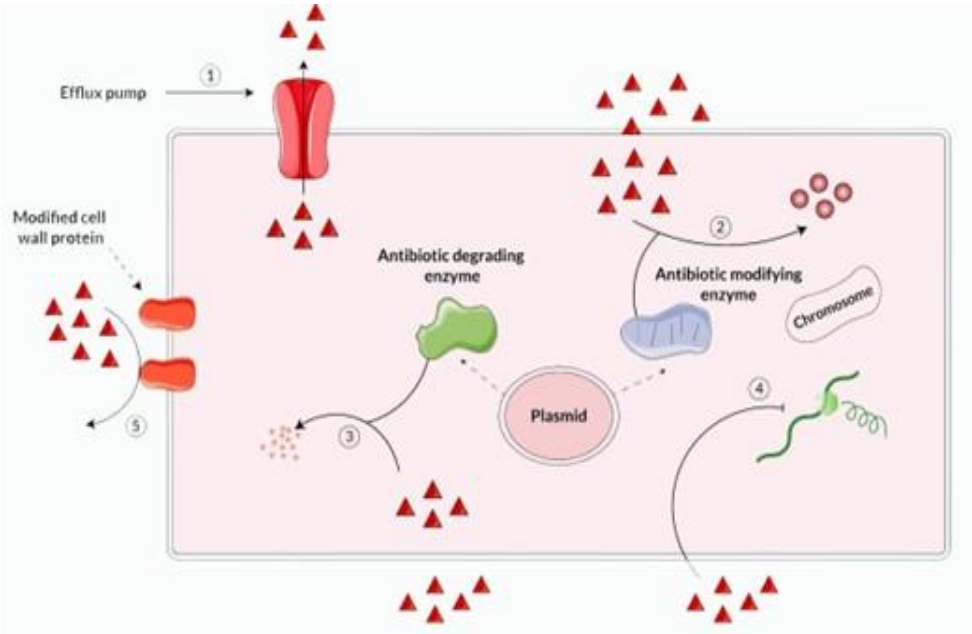


Fig 3: Mechanism of antibiotic resistance

Objectives:

The primary objectives of this study are to

- Isolate uropathogens present in urine samples collected from UTI patients in Dhaka
- Identify pathogens isolated from UTI samples
- Assess the antimicrobial susceptibility pattern of the isolated organisms to regularly used antibiotics.
- Assess the prevalence of antimicrobial resistance
- Compare the results to previous studies, emphasizing any trends or changes

Chapter 2
Methods & Materials

2.1 Study Population & Sample Collection:

We commenced collection of clinical UTI samples from March 2023 and continuing till October 2023 from the microbiology lab of Labaid Specialized Hospital and Bangladesh Shishu Hospital and Institute respectively. The collected specimen was then transported back to BracU microbiology laboratory where further research was conducted. A questionnaire was prepared to facilitate extraction of specific information regarding the topic of interest such as the patient's sex, age, history of illness, dietary habits, recurrence of the issue, and so on. However, due to ethical issues regarding patient confidentiality, we only managed to gather data on age and sex of the patients. In this span of eight months, a total of 152 samples were collected

2.2 Study Area:

The study was carried out in two different tertiary care hospitals of Dhaka. The location is presented through a map below-

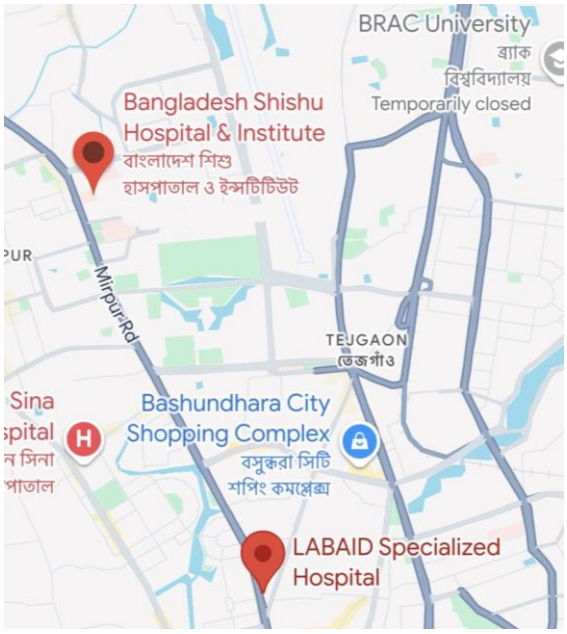
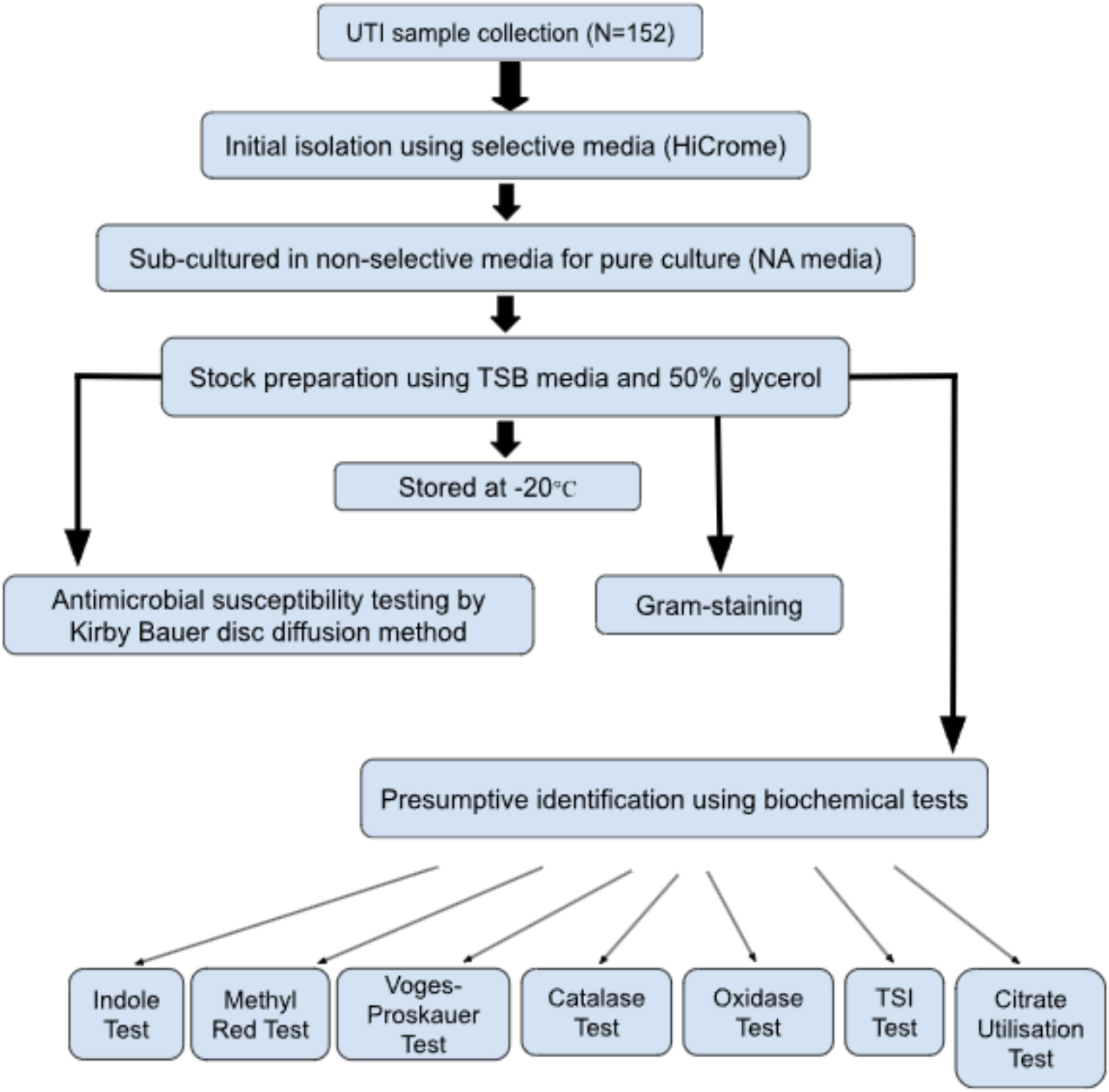


Fig 4: Sample Collection Site

2.3 Experimental Design:



2.4 Collection of UTI samples:

Bacterial isolates were collected from the UTI patients of two reputed private and public hospitals of Dhaka- Labaid Hospital Dhanmondi and Bangladesh Shishu Hospital and Institute. Collected samples were transported inside an icebox from hospital to university laboratory to conduct further research. 152 samples were collected, of which antibiotic resistance pattern has been found in several.

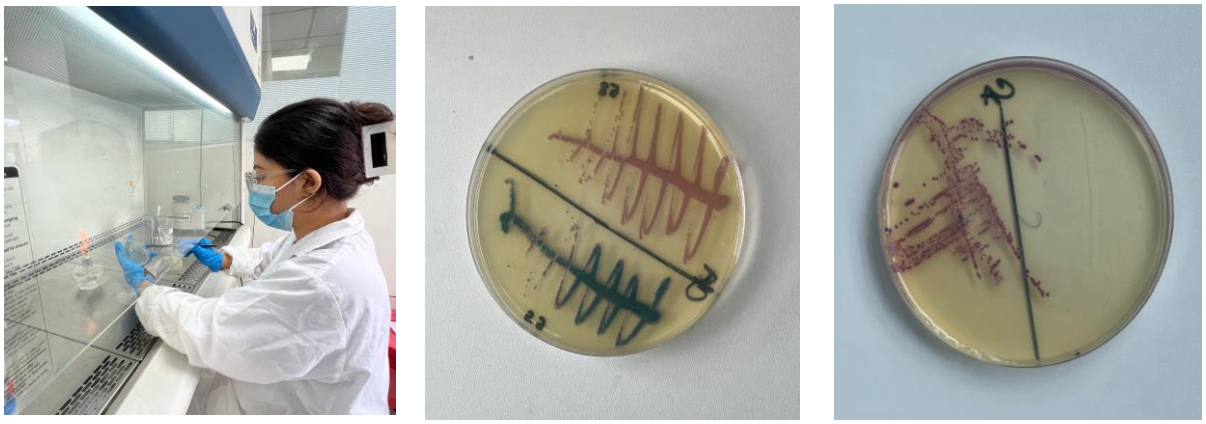


Fig 5: Clinical sample collection from the hospital laboratory

2.5 Isolation of Bacterial Isolates:

In order to obtain a pure culture from a clinical specimen, isolation in the Labaid laboratory was done by laboratory personnel by separating the target bacterium from a mixture of microorganisms present in the sample. Initially, urine samples were collected from UTI patients and streaked onto HiCrome differential medium which facilitates presumptive identification of microorganisms, both gram-positive and gram-negative, found in urinary tract infections. This media mainly detects pathogens of the urinary tract based on the various distinct colony colors generated by the reaction of two chromogenic substrates with either species or genus-specific enzymes. After streaking, the media was incubated at 37°C for 24 hours to obtain pure culture.

Purification of Bacterial Isolates:

Standard procedure has been opted for the sub-culture of collected UTI isolates from the HiCrome plates used previously in the hospital for initial isolation. On the surface of general-purpose solid medium i.e. Nutrient agar, a single isolated colony was streaked and later kept in incubation at 37 °C for 24 hours. This step is necessary in order to ensure the culture consists only of the desired bacterium and is uncontaminated. The culture was conserved as stock for future use.

Stock Preparation:

One loopful of isolate was suspended in 500 microliter TSB (Tryptic Soy Broth) and 500 microliters of 50% glycerol mix. The solution was mixed using a vortex mixer. The prepared stock of bacterial isolates was then preserved at -20°C.

2.6 Identification of Isolated Organisms:

Identification of isolated organisms denote determination of the definite species of the fresh culture through test methods and phenotypic characterization. Through microscopic examination different properties of the bacterium like size, morphology, staining properties, etc. can be discovered. Besides, characterization of the colonies through morphological assessment enables identification by observing physical characteristics like shape, color, etc.

Gram-Staining:

Gram staining is a procedure used to largely distinguish bacterial species into Gram-positive and Gram-negative groups via staining. Smear was prepared on a clean glass slide by dropping a small amount of distilled water where a small amount of bacterial culture was spread using an inoculating loop. After the smear was created it was air dried. Primary stain i.e., crystal violet was applied onto the smear following heat fixation and kept on for 1 minute. The slide was then rinsed gently for the removal of excess dye. Gram's iodine was added for 1 minute and gently rinsed. Gram's iodine solution helps retain crystal violet within the cell. Decolorization was done with ethanol or acetone for 5 seconds then rinsed with distilled water immediately. Counterstaining was done by pouring safranin over the smear and leaving it on for 30-60 seconds. After being rinsed with distilled water, the smear was then air dried. Microscopic examination was done using oil immersion on the smear then observed for results.
Result interpretation for gram-staining:

- Gram-positive bacteria: Violet or purple appearance under the microscope
- Gram-negative bacteria: Pink or red appearance under the microscope

Biochemical Testing:

Several biochemical tests were performed in accordance with the guidelines to identify the uropathogens present in urine samples collected. The tests are as follows:

1. **Indole Test:** The indole test is performed to determine the ability of the pathogen to break down the amino acid drip and produce indole. The samples were cultured in tryptophan broth and then incubated for 48 hours in 37°C. After incubation, Kovac's reagent, which detects the presence of indole, was added and observed for results. Indole reacts with the reagent and produces a red ring, indicating positive result.

2. **Methyl Red Test:** The MR test is conducted to determine the ability of the pathogen to perform mixed acid fermentation when glucose is metabolized. The samples were cultured in a medium called the MR-VP broth and incubated for 48 hours in 37°C. After incubation, a methyl red indicator was added to the culture and observed. If after adding the methyl red indicator the culture turns red, it suggests a positive result. A yellow color, on the other hand, suggests a negative result.

3. **Voges–Proskauer Test:** The VP test is conducted to determine the ability of the pathogen to produce acetoin (acetylmethylcarbinol) from glucose fermentation. In this test, MR-VP broth was used. Samples were cultured and then incubated for 48 hours in 37°C. After the incubation, alpha-naphthol and potassium hydroxide were added to the culture. The cultures were observed for result. A red color indicates a positive result whereas no change in color, or a copper brown result indicates a negative result.

4. **Catalase Test:** The catalase test is performed to detect the presence of the enzyme catalase in bacteria. Catalase decomposes hydrogen peroxide into water and oxygen thereby shielding the cells from oxidative damage. In this test, a single colony from each bacterial culture was placed on a microscope slide and a few drops of hydrogen peroxide were added. The slides were then observed for results. After adding hydrogen peroxide, swift production of bubbles on the slide suggests a positive catalase test, meaning the microorganism is able to produce catalase. No bubbles indicate a negative catalase result.

5. **Oxidase Test:** The oxidase test is a biochemical test that is performed to determine the presence of cytochrome c oxidase, which is an enzyme involved in the electron transport chain of certain species of pathogens. In this test, a single colony from each bacterial culture was added on a piece of filter paper and a few drops of oxidase reagent as in tetramethyl-p-phenylenediamine were added. After waiting for 10 seconds, the results were observed. Within 10 to 30 seconds if there is a dark purple color change, that indicates a positive result. Conversely, no change in color, or if the purple hue appears after 30 seconds have passed, it indicates a negative result.

6. **Citrate Utilization Test:** The citrate utilization test is performed to determine a pathogens ability to utilize citrate as a carbon source and ammonium ions as a nitrogen source. In this test, Simone citrate agar was used as the medium, which contains sodium citrate, ammonium dihydrogen phosphate, and bromothymol blue as the pH indicator. Inoculation was performed by lightly streaking the bacterial culture onto the surface of the agar slant. The test tubes were incubated at 37°C for 24 hours and then observed for results. If the result is positive, a color blue will appear on the slant, indicating that the microorganism is capable of utilizing citrate as its sole carbon source, resulting in the production of alkaline byproducts and an increase in pH level. However, if the color remains green on the slant, it indicates that the microorganism is unable to utilize citrate and thus, is a negative result.

7. **TSI Test:** The Triple Sugar Iron test is used to differentiate and identify bacteria in accordance to their ability to ferment sugar, lactose or produce hydrogen sulfide. After preparing TSI (Triple Sugar Iron) agar with distilled water, it was boiled and poured into test tubes. The media was then autoclaved, and an agar slant was formed. With an inoculating needle, bacterial isolates were stabbed into the slant and then streaked at the surface. The media was incubated at 37°C for 24 hours and color change and gas formation were observed afterwards.

Result interpretation for TSI:
Slant/ Butt colors:

- Red slant/ yellow butt (K/A): Sole fermentation of glucose (alkaline slant/ acid butt)
- Yellow slant/ yellow butt (A/A): Glucose, lactose, sucrose fermentation (Acid slant/ acid butt)
- Red slant/ red butt (K/K): No change or fermentation.

Gas production: If bubbles or cracks in the agar are visible, that suggests gas production.

Hydrogen sulfide production: If there is blackening of the butt, that suggests hydrogen sulfide or H_2S production.

2.7 Antimicrobial susceptibility testing (AST):

Antimicrobial susceptibility testing is a laboratory technique that is used to determine the efficacy of an antimicrobial agent against pathogens. It involves administering a range of antibiotics to clinical samples to ascertain which drugs inhibit bacterial growth effectively. The primary goal of this test is to assist clinicians in choosing the most efficacious antibiotic course of treatment while treating infections. This test helps prevent the use of ineffective antibiotics which can result in treatment failure and furthermore promote antibiotic resistance. In this process, first the clinical samples are isolated and a standardized bacterial suspension prepared. After incubation the results are then measured by zones of inhibition that determine the minimum inhibitory concentration or MIC. There are several methods for this test, including the disc diffusion method, broth dilution method and Etest. In this study, AST was performed using the disk diffusion method also known as the Kirby-Bauer method.

During the disk diffusion method, a bacterial suspension was evenly spread on the surface of each Mueller Hinton agar plate. Antibiotic discs with specific concentrations were carefully placed on the agar surface and the plates were incubated at 37°C for 24 hours for bacterial growth and antibiotic diffusion. After 24 hours, clear zones were observed that are also known as the zones of inhibition. The diameter of the zones was measured in millimeters and compared to the CLSI guideline charts to determine the susceptibility of the antibiotics. 13 antibiotics were administered in total—

Table 1: Antibiotic Zones of Different Antibiotics According to CLSI Standard

Antibiotic Group	Antibiotic Name (Abbreviation)	Concentration	Zone of Inhibition (mm)			Reference
			S	I	R	
Nitrofuran	Nitrofurantoin (F)	300 µg	≥17	15-16	≤14	CLSI, 2021
Aminoglycoside	Amikacin (AK)	30 µg	≥17	15-16^	≤14	CLSI, 2021
	Kanamycin (K)	5 µg	≥18	14-17^	≤13	CLSI, 2021
Cephalosporin	Cefixime (CFM)	5 µg	≥19	16-18^	≤15	CLSI, 2021
	Cefepime (CPM)	30 µg	≥25	19-24 SDD	≤18	CLSI, 2021
	Ceftriaxone (CRO/ CTR)	30 µg	≥23	20-22^	≤19	CLSI, 2021
Macrolide	Azithromycin (AZM)	30 µg	≥13	-	≤12	CLSI, 2021
Carbapenem	Imipenem (IPM)	10 µg	≥23	20-22^	≤19	CLSI, 2021
Fluoroquinolone	Levofloxacin (LE)	5 µg	≥21	17-20^	≤16	CLSI, 2021
Penicillin	Ampicillin (AMP)	25 µg	≥17	14-16^	≤13	CLSI, 2021
Tetracycline	Doxycycline (DO)	30 µg	≥14	11-13	≤10	CLSI, 2021
Polymyxin	Colistin (CL)	10 µg	-	-	-	CLSI, 2021
Penicillin + Beta-lactamase inhibitors	Amoxiclav (AMC)	30 µg	≥18	14-17^	≤13	CLSI, 2021

*S=Sensitive, I=Intermediate, R=Resistant

Chapter 3

Result:

The study focused on determining the microbial profile and the antimicrobial resistance to various groups of antibiotics which were critical in assessing the potential treatment options. The result are as follows-

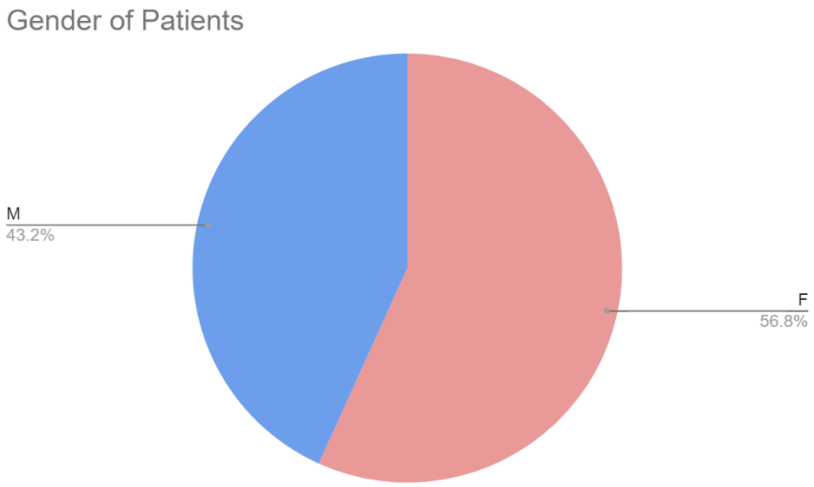


Fig 6: Pie chart showing percentage of male and female patients

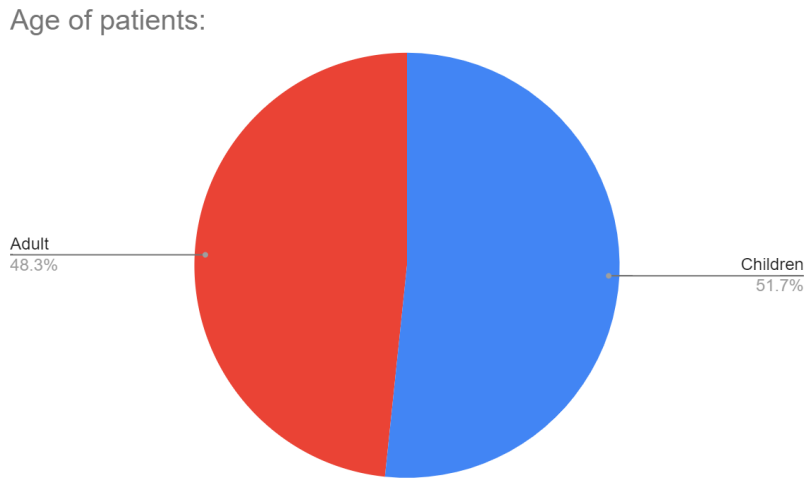


Fig 7: Pie chart showing percentage of adult and non-adult patients in collected samples

Gram-staining:

Gram staining was performed for initial screening. Gram-positive bacteria appear violet or purple under the microscope whereas, gram-negative bacteria appear pink or red under the microscope.

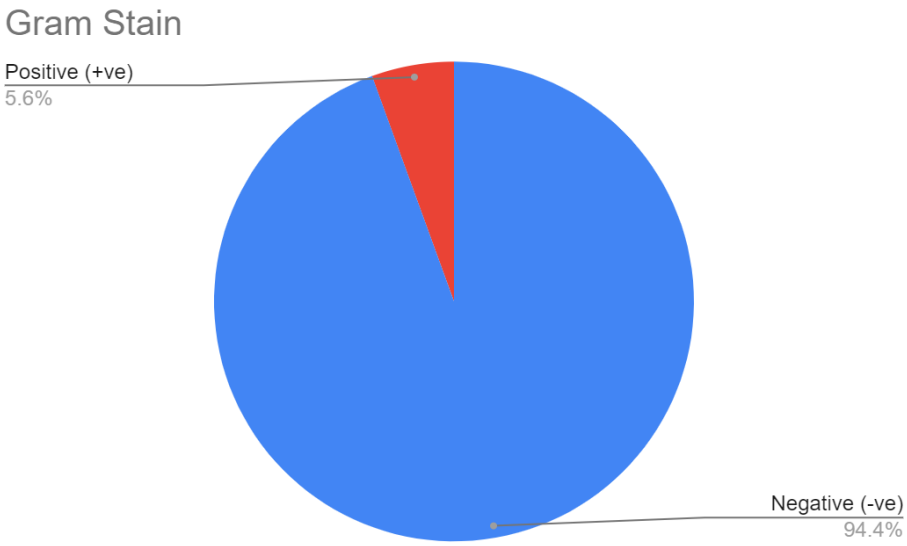


Fig 8: Percentage of Gram positive and Gram-negative pathogens in collected samples

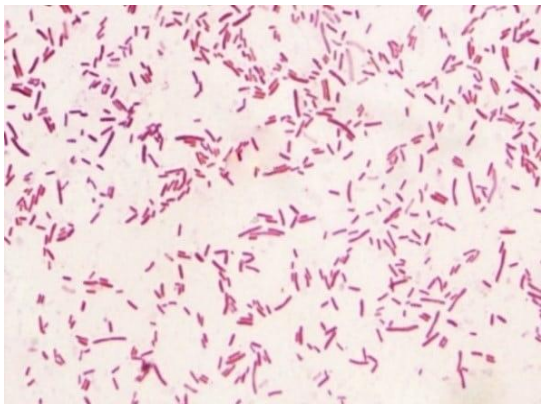
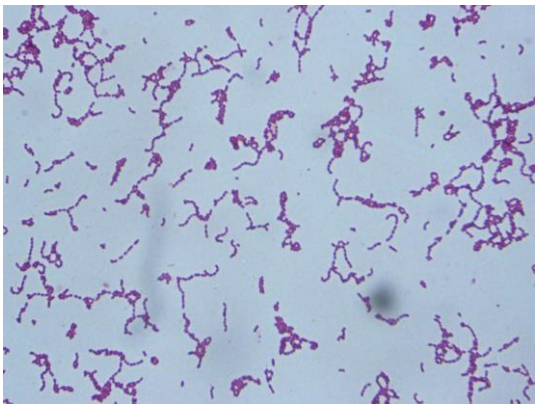


Fig 9 (a): Gram-negative organism



9 (b): Gram-positive organism

Biochemical Tests:

Apart from Gram staining for initial screening, various biochemical tests like- Oxidase test, Catalase test, Triple Sugar Iron test (TSI), Citrate Utilization test, Methyl Red test, Voges-Proskauer test, Indole test etc. were performed on 50 of the 152 isolates to identify the isolated pathogens present in urine samples. The biochemical test results are mentioned here-

Oxidase Test:

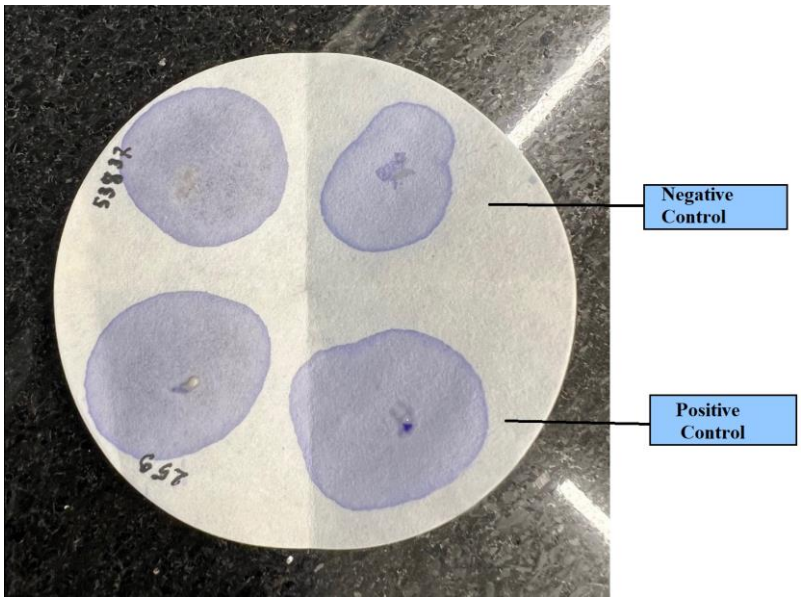


Fig 10: Oxidase test

Catalase Test:

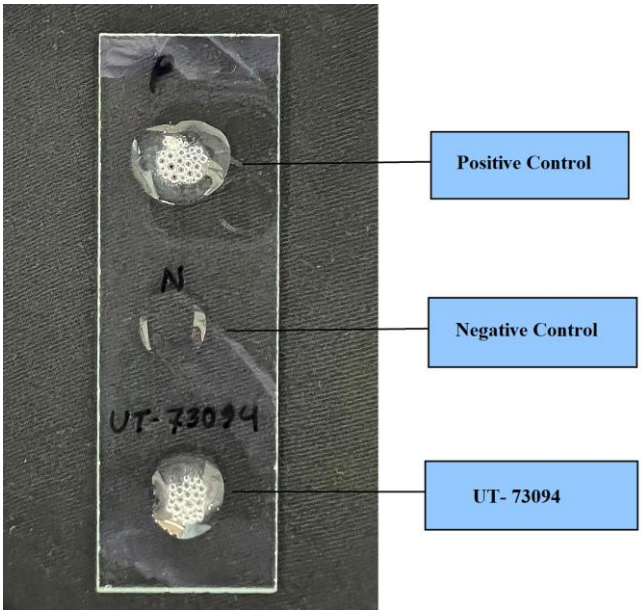


Fig 11: Catalase test

Triple Sugar Iron Test (TSI):

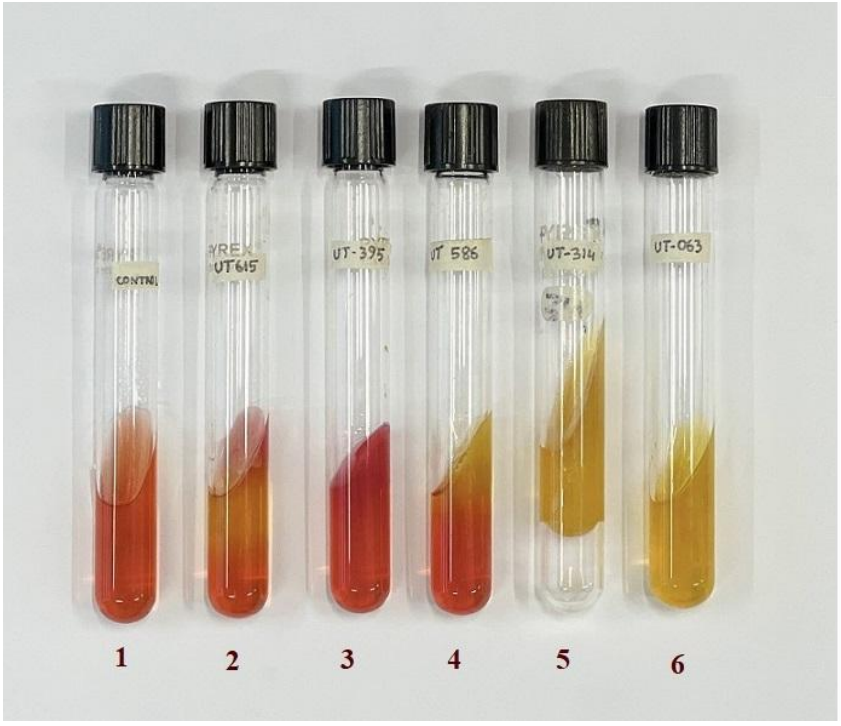


Fig 12 (a): TSI test

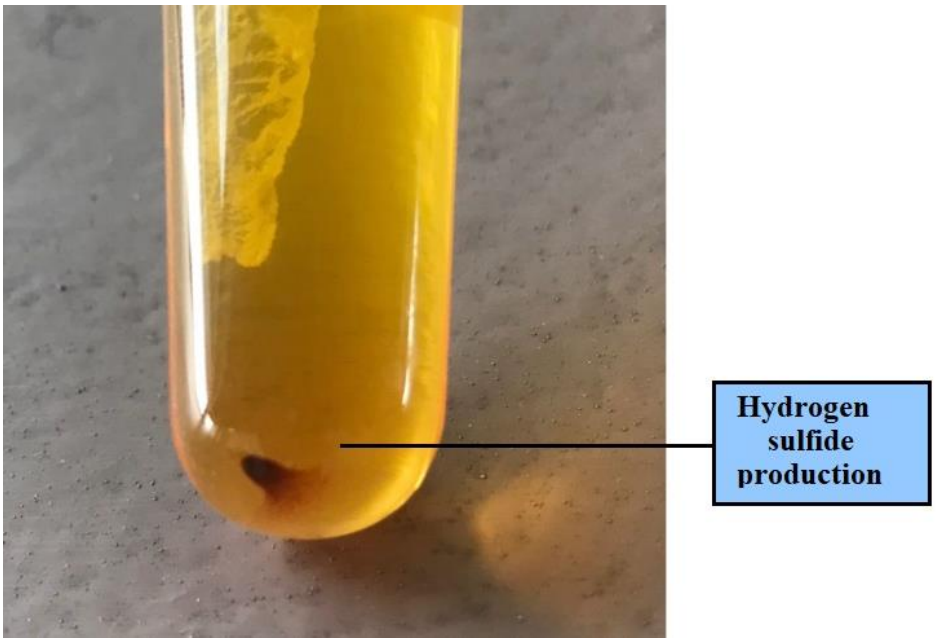


Fig 12 (b): Hydrogen sulfide formation during TSI test

Citrate Utilization Test:

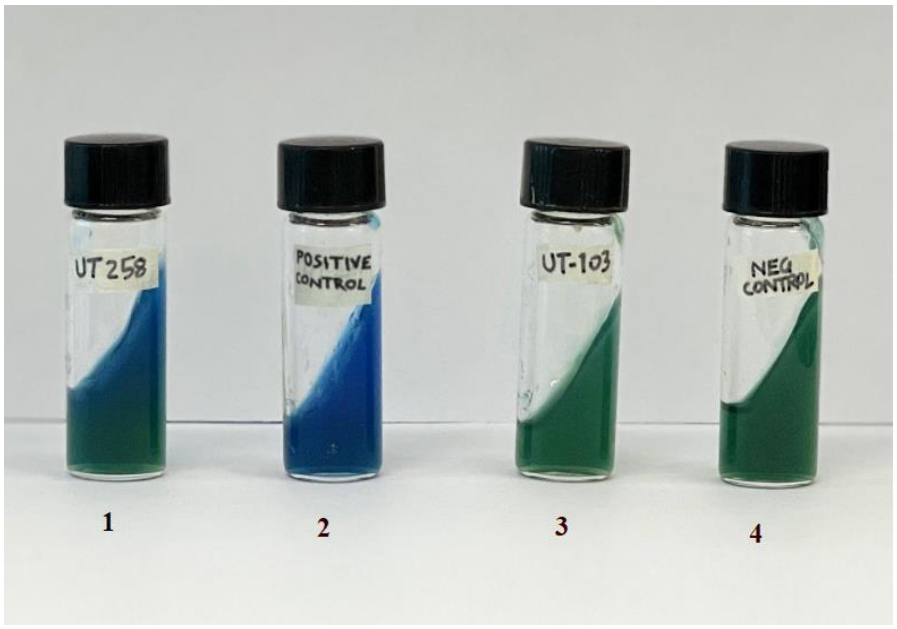


Fig 13: Citrate Utilization test

Methyl Red Test:

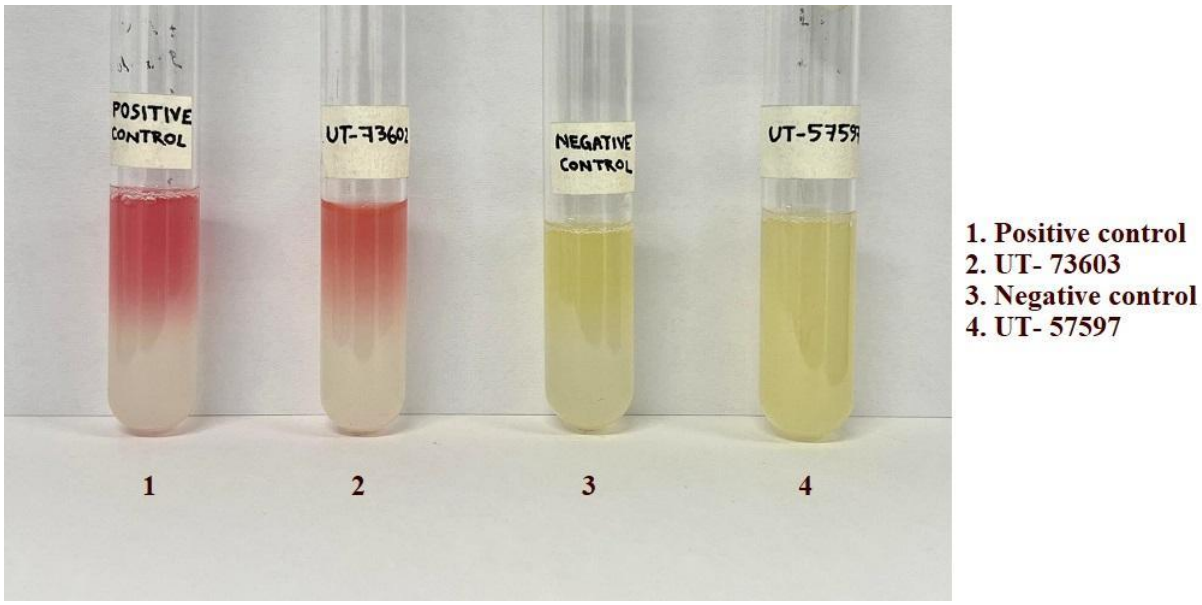


Fig 14: Methyl Red Test

Voges-Proskauer Test:

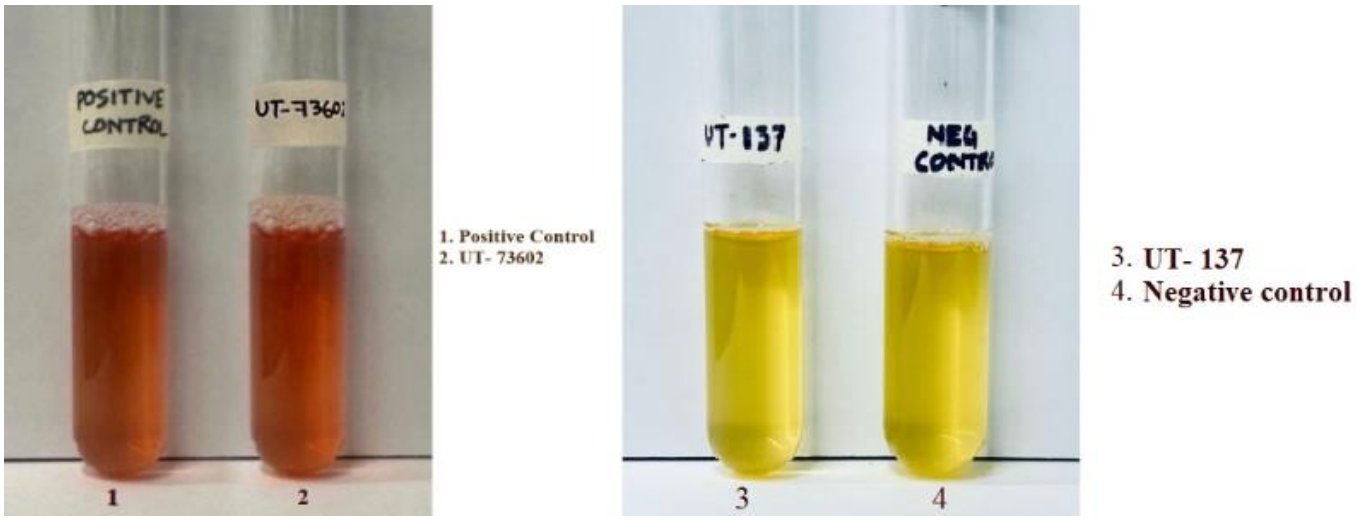


Fig 15: Voges-Proskauer Test

Indole Test:

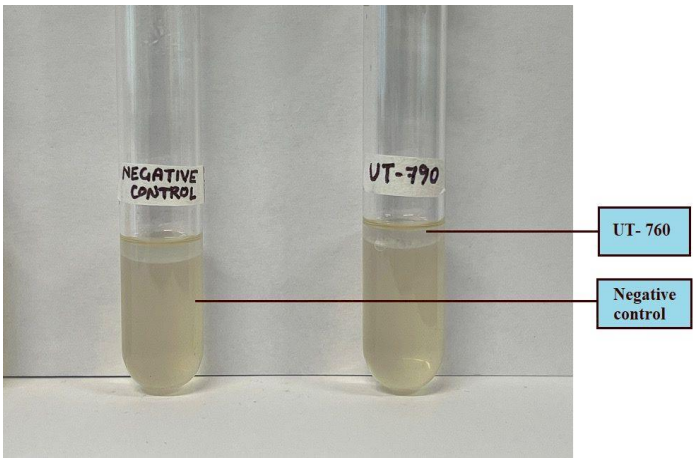


Fig 16 (a): Indole Test Negative Result

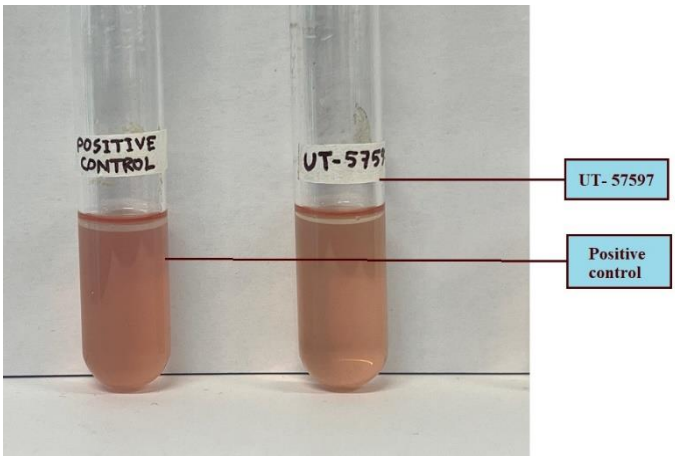


Fig 16 (b): Indole Test Positive Result

Table 2: Biochemical Test Results of the Isolated Organisms

Sample ID	Name	Catalase	Oxidase	Citrate	TSI				Indole	MR	VP
					Slant	Butt	Gas	H2S			
UT-73837	*Klebsiella spp.*	P	N	P	Yellow	Yellow	N	N	N	P	P
UT- 063	*E. coli*	P	N	N	Yellow	Yellow	N	N	P	P	N
UT- 615	*Proteus spp.*	P	N	N	Red	Red	N	P	P	P	N
UT- 079	*Acinetobacter spp.*	P	N	P	Red	Yellow	N	N	N	N	N
UT- 259	*Citrobacter spp.*	P	N	P	Yellow	Yellow	P	N	N	P	P
UT- 258	*Klebsiella spp.*	P	N	P	Yellow	Yellow	N	N	N	P	P
UT- 314	*E. coli*	P	N	N	Yellow	Yellow	N	P	P	P	N
UT-73820	*E. coli*	P	N	N	Yellow	Yellow	N	P	P	P	N
UT- 495	*E. coli*	P	N	N	Yellow	Yellow	N	P	P	P	N
UT- 745	*E. coli*	P	N	N	Yellow	Yellow	N	P	P	P	N
UT- 723	*E. coli*	P	N	N	Yellow	Yellow	N	P	P	P	N
UT- 997	*Klebsiella spp.*	P	N	P	Yellow	Yellow	N	N	N	P	P
UT- 790	*E. coli*	P	N	N	Yellow	Yellow	N	P	P	P	N
UT-71976	*Klebsiella spp.*	P	N	P	Yellow	Yellow	N	N	N	P	P
UT- 993	*E. coli*	P	N	N	Yellow	Yellow	N	P	P	P	N
UT- 257	*Pseudomonas spp.*	P	P	P	Red	Red	N	N	N	N	N
UT- 224	*E. coli*	P	N	N	Yellow	Yellow	N	P	P	P	N
UT- 749	*E. coli*	P	N	N	Yellow	Yellow	N	P	P	P	N
UT-63134	*E. coli*	P	N	N	Yellow	Yellow	N	P	P	P	N
UT-71920	*E. coli*	P	N	N	Yellow	Yellow	N	P	P	P	N
UT- 476	*E. coli*	P	N	N	Yellow	Yellow	N	P	P	P	N
UT- 188	*Acinetobacter spp.*	P	N	P	Red	Yellow	N	N	N	N	N
UT- 400	*E. coli*	P	N	N	Yellow	Yellow	N	P	P	P	N

UT-79668	***Citrobacter spp.***	**P**	**N**	**P**	**Yellow**	**Yellow**	**N**	**N**	**N**	**P**	**P**
UT-79561	***E. coli***	**P**	**N**	**N**	**Yellow**	**Yellow**	**N**	**P**	**P**	**P**	**N**
UT-78141	***Acinetobacter spp.***	**P**	**N**	**P**	**Red**	**Yellow**	**N**	**N**	**N**	**N**	**N**
UT-713713	***Pseudomonas spp.***	**P**	**P**	**P**	**Red**	**Red**	**N**	**N**	**N**	**N**	**N**
UT- 299	***Pseudomonas spp.***	**P**	**P**	**P**	**Red**	**Red**	**N**	**N**	**N**	**N**	**N**
UT-27544	***Pseudomonas spp.***	**P**	**P**	**P**	**Red**	**Red**	**N**	**N**	**N**	**N**	**N**
UT- 826	***Acinetobacter spp.***	**P**	**N**	**P**	**Red**	**Yellow**	**N**	**N**	**N**	**N**	**N**
UT- 103	***Acinetobacter spp.***	**P**	**N**	**N**	**Red**	**Yellow**	**N**	**N**	**N**	**N**	**N**
UT- 138	***Acinetobacter spp.***	**P**	**N**	**P**	**Red**	**Yellow**	**N**	**N**	**N**	**N**	**N**
UT- 297	***Klebsiella spp.***	**P**	**N**	**P**	**Yellow**	**Yellow**	**N**	**N**	**N**	**P**	**P**
UT- 520	***Klebsiella spp.***	**P**	**N**	**P**	**Yellow**	**Yellow**	**N**	**N**	**N**	**P**	**P**
UT-46095	***Klebsiella spp.***	**P**	**N**	**P**	**Yellow**	**Yellow**	**N**	**N**	**N**	**P**	**P**
UT-24826	***Klebsiella spp.***	**P**	**N**	**P**	**Yellow**	**Yellow**	**N**	**N**	**N**	**P**	**P**
UT-63220	***E. coli***	**P**	**N**	**N**	**Yellow**	**Yellow**	**N**	**P**	**P**	**P**	**N**
UT-70751	***E. coli***	**P**	**N**	**N**	**Yellow**	**Yellow**	**N**	**P**	**P**	**P**	**N**
UT-70773	***GBS***	**N**	**N**	**N**	**Yellow**	**Yellow**	**N**	**N**	**N**	**N**	**N**
UT- 485	***Flavobacterium***	**P**	**N**	**N**	**Red**	**Red**	**N**	**N**	**N**	**N**	**N**
UT- 916	***E. coli***	**P**	**N**	**N**	**Yellow**	**Yellow**	**N**	**P**	**P**	**P**	**N**
UT- 495	***E. coli***	**P**	**N**	**N**	**Yellow**	**Yellow**	**N**	**P**	**P**	**P**	**N**
UT- 723	***E. coli***	**P**	**N**	**N**	**Yellow**	**Yellow**	**N**	**P**	**P**	**P**	**N**
UT- 229	***E. coli***	**P**	**N**	**N**	**Yellow**	**Yellow**	**N**	**P**	**P**	**P**	**N**
UT- 911	***E. coli***	**P**	**N**	**N**	**Yellow**	**Yellow**	**N**	**P**	**P**	**P**	**N**
UT- 586	***E. coli***	**P**	**N**	**N**	**Yellow**	**Red**	**N**	**N**	**P**	**P**	**N**

UT-73094	***E. coli***	**P**	**N**	**N**	**Yellow**	**Yellow**	**N**	**P**	**P**	**P**	**N**
UT- 395	***Flavobacterium***	**P**	**N**	**N**	**Red**	**Red**	**N**	**N**	**N**	**N**	**N**
UT-73602	***E. coli***	**P**	**N**	**N**	**Yellow**	**Yellow**	**N**	**P**	**P**	**P**	**P**

P = Positive result
N = Negative Result

Table 3: Biochemical Test Results with Numbers of Each Isolate

Number of Organism	Name	Catalase	Oxidase	Citrate	TSI				Indole	MR	VP
					Slant	Butt	Gas	H2S			
8	***Klebsiella spp.***	P	N	P	Yellow	Yellow	N	N	N	P	P
26	***E. coli***	P	N	N	Yellow	Yellow	N	N	P	P	N
1	***Proteus spp.***	P	N	N	Red	Red	N	P	P	P	N
6	***Acinetobacter spp.***	P	N	P	Red	Yellow	N	N	N	N	N
2	***Citrobacter spp.***	P	N	P	Yellow	Yellow	P	N	N	P	P
4	***Pseudomonas spp.***	P	P	P	Red	Red	N	N	N	N	N
2	***Flavobacterium***	P	N	N	Red	Red	N	N	N	N	N
1	***GBS***	N	N	N	Yellow	Yellow	N	N	N	N	N

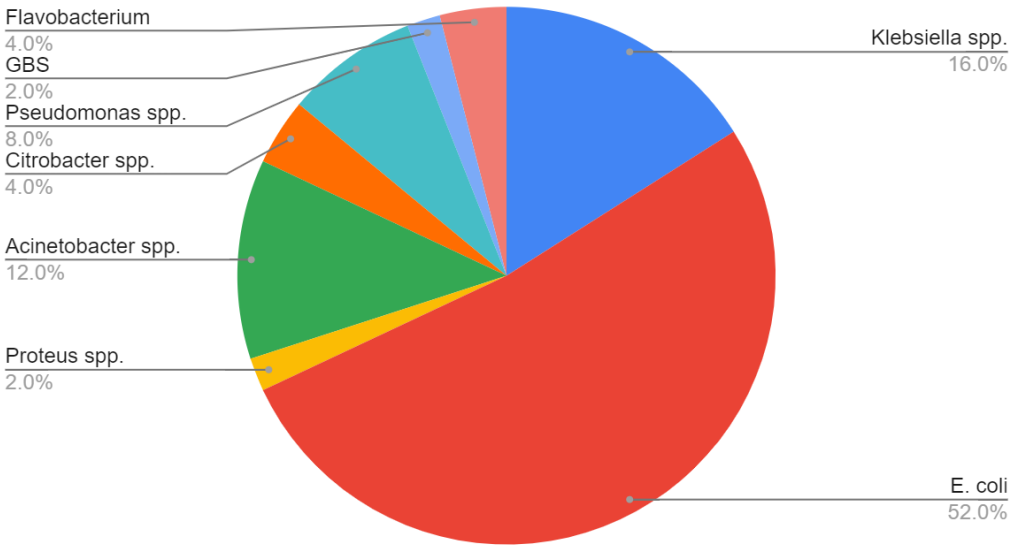


Fig 17: Percentage of Isolated Organisms from Tested Samples

Table 4: Total Number of Presumptive Species after Biochemical Testing

N=50	*E.coli*	*Klebsiella spp.*	*Pseudomonas spp.*	*Proteus spp.*	*Acinetobacter spp.*	*Citrobacter spp.*	*Flavobacterium*	*GBS*
% of Organism	52%	16%	8%	2%	12%	4%	4%	2%
No. of Organism	26	8	4	1	6	2	2	1

Antimicrobial Susceptibility Test:

Antimicrobial Susceptibility testing in this study was carried out using the Kirby-Bauer disc diffusion method on 111 isolates. This is the most widely used method in a clinical setting due to its simplicity and cost effectiveness. The susceptibility of the antibiotics varies according to different antibiotic groups by measuring the zone diameters and comparing it to CLSI guidelines. The test results are mentioned here with susceptibility being interpreted as S (sensitive), I (intermediate) and R (resistant)

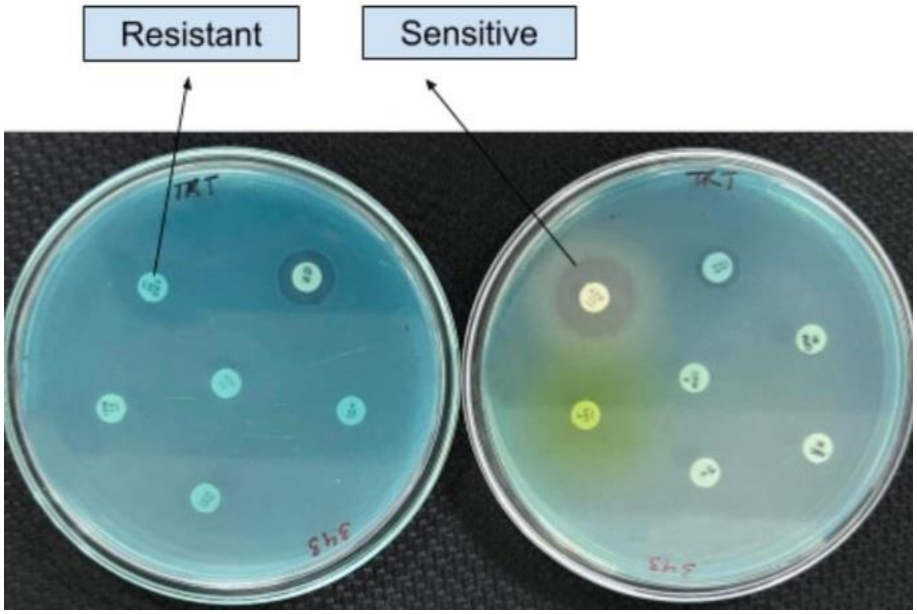


Fig 18: Antimicrobial Susceptibility Test of Different Antibiotics on MHA Agar

Table 4: Antimicrobial Susceptibility Test results of isolated organisms

Sample ID	F	AK	K	CFM	IPM	CPM	AZM	LE	AMP	DO	CL	CTR	AMC
	Nitrofurantoin	Amikacin	Kanamycin	Cefixime	Imipenem	Cefepime	Azithromycin	Levofloxacin	Ampicillin	Doxycycline	Colistin	Ceftriaxone	Amoxicillin + clavulanic acid
UT- 297	R	I	I	R	R	R	R	R	R	S	S	R	R
UT- 520	R	R	R	R	R	R	R	R	R	I	S	R	R
UT- 51503	R	R	I	R	R	R	S	I	R	S	S	S	R
UT- 79703	R	R	I	R	R	R	S	I	R	S	S	S	R
UT- 79440	I	R	I	R	S	I	S	S	R	S	R	R	I
UT- 46095	R	I	R	R	I	R	R	R	R	I	S	R	I
UT- 24826	S	S	I	R	S	R	S	S	R	S	S	S	S
UT- 33231	R	I	I	R	S	R	R	I	R	S	S	R	I
UT- 258	R	R	R	R	S	R	R	R	R	S	S	R	R
UT- 997	S	S	I	R	S	S	S	S	R	S	S	S	S
UT- 79495	R	I	I	R	R	I	S	I	R	S	R	S	R
UT- 54055	R	S	I	R	S	R	S	S	R	S	S	R	S
UT- 99898	S	S	S	R	S	S	S	S	R	S	S	S	S
UT- 71976	S	S	I	R	S	S	R	S	R	S	S	S	S
UT- 73206	S	S	R	R	S	S	R	R	R	S	S	S	R
UT-674672	S	S	S	R	I	R	S	R	R	S	S	R	R
UT- 294	R	R	R	R	R	R	R	R	R	I	S	R	R
UT- 50938	I	I	I	R	R	I	R	S	R	R	S	S	S
UT- 90536	S	S	S	R	S	R	R	R	R	S	S	R	S
UT- 57471	S	S	S	R	S	S	S	S	R	S	S	I	S
UT- 87102	S	S	R	R	S	R	R	R	R	S	S	R	R
UT- 86728	S	S	R	R	S	S	S	I	R	S	S	S	I
UT- 57686	S	S	S	R	S	R	R	R	R	S	S	R	S
UT- 87274	S	S	S	R	S	R	R	S	R	S	R	R	S
UT- 87000	S	S	S	R	S	S	S	R	R	S	S	S	S
UT- 57597	S	S	I	R	S	S	R	S	R	S	S	S	R
UT- 87201	S	S	S	R	S	R	S	R	R	S	S	R	R
UT- 93461	I	R	R	R	I	R	R	R	R	R	S	R	R

UT- 59753	**S**	**I**	**I**	**R**	**S**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**I**
UT- 59704	**S**	**I**	**I**	**R**	**S**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**I**
UT- 59705	**S**	**R**	**R**	**R**	**I**	**R**	**R**	**R**	**R**	**I**	**S**	**R**	**I**
UT- 61240	**S**	**S**	**S**	**R**	**S**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**S**
UT- 61224	**S**	**S**	**I**	**R**	**S**	**R**	**S**	**R**	**R**	**I**	**S**	**R**	**S**
UT-679643	**S**	**S**	**I**	**R**	**S**	**R**	**S**	**S**	**R**	**S**	**S**	**R**	**S**
UT- 97561	**S**	**S**	**I**	**R**	**S**	**S**	**S**	**S**	**R**	**I**	**S**	**S**	**S**
UT- 98003	**S**	**S**	**S**	**R**	**S**	**S**	**R**	**S**	**R**	**S**	**S**	**S**	**S**
UT- 61320	**S**	**R**	**I**	**R**	**S**	**R**	**S**	**R**	**R**	**I**	**S**	**R**	**S**
UT- 81337	**S**	**S**	**S**	**R**	**S**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**S**
UT- 63133	**S**	**S**	**S**	**R**	**S**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**S**
UT- 63180	**S**	**S**	**S**	**R**	**S**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**S**
UT- 63220	**S**	**S**	**S**	**R**	**S**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**S**
UT- 63282	**S**	**S**	**S**	**R**	**S**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**S**
UT- 63301	**S**	**S**	**S**	**R**	**S**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**S**
UT- 11545	**S**	**S**	**S**	**R**	**S**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**S**
UT- 70751	**S**	**S**	**S**	**R**	**S**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**S**
UT- 70777	**S**	**I**	**I**	**R**	**S**	**R**	**R**	**S**	**R**	**S**	**S**	**R**	**I**
UT- 24771	**S**	**S**	**S**	**R**	**S**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**S**
UT- 70865	**S**	**I**	**I**	**R**	**S**	**S**	**R**	**I**	**R**	**R**	**S**	**S**	**I**
UT- 27921	**S**	**R**	**I**	**R**	**S**	**S**	**R**	**S**	**R**	**S**	**S**	**I**	**R**
UT- 71799	**S**	**S**	**I**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**I**
UT- 71925	**S**	**S**	**S**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**S**
UT- 27528	**S**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**R**
UT- 71846	**S**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**R**	**S**	**S**	**S**	**S**
UT- 73096	**S**	**I**	**I**	**R**	**S**	**R**	**S**	**S**	**R**	**S**	**S**	**R**	**S**
UT- 31772	**S**	**S**	**I**	**R**	**S**	**S**	**R**	**S**	**R**	**S**	**S**	**S**	**S**
UT- 73197	**R**	**S**	**S**	**R**	**S**	**R**	**R**	**S**	**R**	**S**	**S**	**R**	**S**
UT- 73155	**S**	**S**	**I**	**R**	**S**	**S**	**S**	**S**	**R**	**S**	**S**	**S**	**S**
UT- 73094	**S**	**S**	**I**	**R**	**S**	**S**	**S**	**S**	**R**	**R**	**S**	**S**	**S**
UT- 72990	**S**	**S**	**I**	**R**	**I**	**S**	**R**	**I**	**R**	**I**	**S**	**S**	**R**
UT- 631620	**S**	**S**	**S**	**R**	**S**	**R**	**S**	**S**	**R**	**S**	**S**	**R**	**R**
UT- 72992	**I**	**R**	**I**	**R**	**I**	**R**	**R**	**I**	**R**	**S**	**S**	**R**	**R**

UT- 659	**R**	**I**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**
UT- 73602	**I**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**R**	**R**	**S**	**R**	**R**
UT- 73631	**I**	**R**	**I**	**R**	**I**	**R**	**R**	**R**	**R**	**I**	**R**	**I**	**R**
UT- 73743	**S**	**S**	**S**	**R**	**S**	**S**	**R**	**S**	**R**	**S**	**S**	**S**	**I**
UT- 73752	**I**	**I**	**I**	**R**	**I**	**R**	**R**	**I**	**R**	**I**	**R**	**R**	**S**
UT- 73821	**S**	**R**	**I**	**R**	**S**	**S**	**R**	**S**	**R**	**R**	**S**	**S**	**S**
UT- 916	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**
UT- 495	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**
UT- 723	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**
UT- 314	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
UT- 229	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**I**	**S**	**R**	**R**
UT- 911	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**
UT- 586	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**
UT- 993	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**
UT- 713	**R**	**S**	**R**	**R**	**S**	**S**	**R**	**S**	**R**	**R**	**S**	**R**	**R**
UT- 299	**R**	**S**	**R**	**R**	**S**	**S**	**R**	**S**	**R**	**R**	**S**	**R**	**R**
UT- 27544	**R**	**S**	**R**	**R**	**S**	**S**	**R**	**S**	**R**	**R**	**S**	**R**	**R**
UT- 310	**R**	**S**	**R**	**R**	**S**	**S**	**R**	**S**	**R**	**R**	**S**	**R**	**R**
UT- 000	**R**	**S**	**R**	**R**	**S**	**S**	**R**	**S**	**R**	**R**	**S**	**R**	**R**
UT- 90109	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**
UT- 382	**R**	**S**	**R**	**R**	**S**	**S**	**R**	**S**	**R**	**R**	**S**	**R**	**R**
UT- 735	**R**	**S**	**R**	**R**	**S**	**S**	**R**	**S**	**R**	**R**	**S**	**R**	**R**
UT- 065	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**
UT- 14272	**R**	**S**	**R**	**R**	**S**	**S**	**R**	**S**	**R**	**R**	**S**	**R**	**R**
UT- 045	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**
UT- 020	**R**	**S**	**R**	**R**	**S**	**S**	**R**	**S**	**R**	**R**	**S**	**R**	**R**
UT- 196	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**
UT- 826	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**R**
UT- 103	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**
UT- 138	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**
UT- 320	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**
UT- 010	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**
UT- 079	**R**	**S**	**I**	**R**	**R**	**R**	**S**	**S**	**R**	**S**	**S**	**R**	**I**
UT- 605	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**

UT- 007	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**
UT- 188	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
UT- 073	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**I**	**R**	**S**	**S**	**R**	**R**
UT- 137	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**R**
UT- 698	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**R**
UT- 329	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**S**	**S**	**R**	**R**
UT- 817	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**
UT- 259	**R**	**S**	**S**	**R**	**R**	**I**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
UT- 73837	**R**	**S**	**S**	**R**	**S**	**R**	**S**	**S**	**R**	**S**	**S**	**R**	**R**
UT- 79668	**R**	**S**	**S**	**R**	**S**	**R**	**S**	**S**	**R**	**S**	**R**	**R**	**S**
UT- 78141	**R**	**S**	**R**	**I**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**S**	**I**
UT- 73598	**R**	**S**	**R**	**I**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**S**	**I**
UT- 70773	**R**	**R**	**R**	**R**	**S**	**R**	**I**	**S**	**R**	**R**	**S**	**S**	**R**
UT- 50985	**R**	**R**	**I**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**
UT- 485	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**S**	**R**	**R**	**R**

The susceptibility profile created from our findings are as follows-

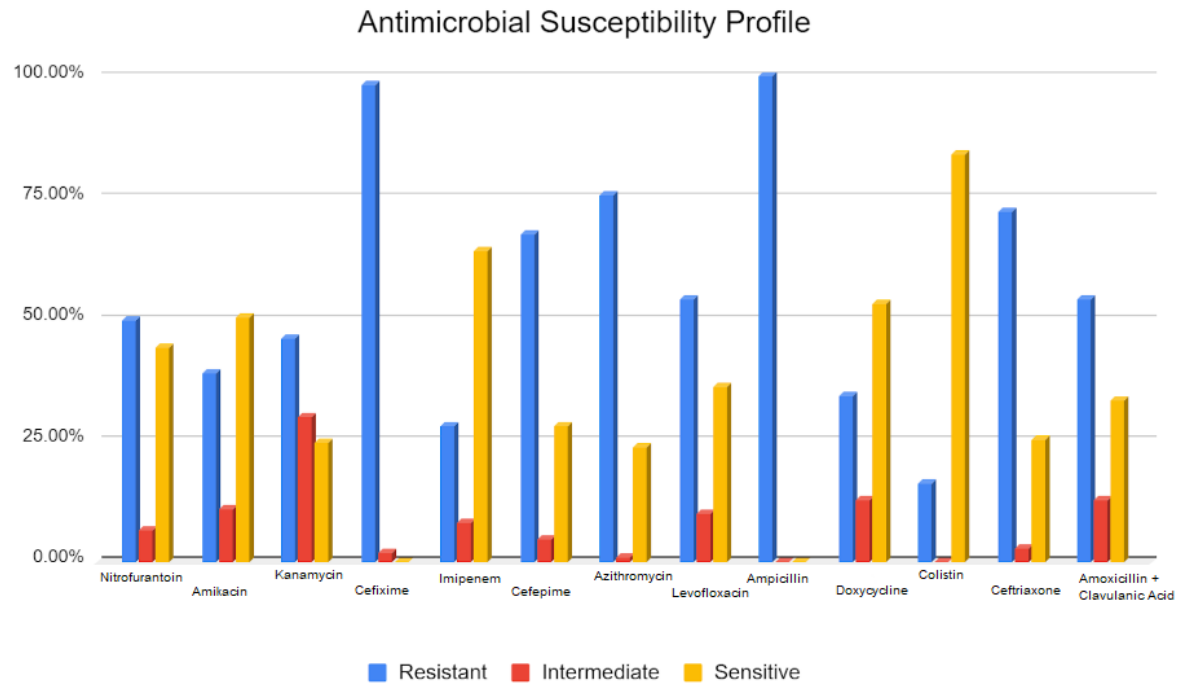


Fig 19: Antimicrobial Susceptibility Profile of All Antibiotic Against Clinical Samples

Table 5: Susceptibility of Total Number of Organism Based on Antibiotic Groups

Antibiotic Group	Antibiotic Name (Abbreviation)	Concentration	Number of Organisms (N= 111)		
			S	I	R
Nitrofuran	Nitrofurantoin (F)	300 µg	49	7	55
Aminoglycoside	Amikacin (AK)	30 µg	56	12	43
	Kanamycin (K)	5 µg	26	33	52
Cephalosporin	Cefixime (CFM)	5 µg	0	2	109
	Cefepime (CPM)	30 µg	31	5	75
	Ceftriaxone (CRO/ CTR)	30 µg	27	3	81
Macrolide	Azithromycin (AZM)	30 µg	26	1	84
Carbapenem	Imipenem (IPM)	10 µg	71	9	31
Fluoroquinolone	Levofloxacin (LE)	5 µg	60	11	40
Penicillin	Ampicillin (AMP)	25 µg	0	0	111
Tetracycline	Doxycycline (DO)	30 µg	60	14	37
Polymyxin	Colistin (CL)	10 µg	93	0	18
Penicillin + Beta Lactamase inhibitors	Amoxiclav (AMC)	30 µg	37	14	60

Multiple Antibiotic Resistance (MAR) Index:

The Multiple Antibiotic Resistance (MAR) index is determined by dividing the number of antibiotics to which individual isolates are resistant by the total number of antibiotics against which the microorganism was tested. MAR index larger than 0.2 indicates that the isolates have been exposed to high levels of antibiotics, whereas a lower index indicates a controlled or regulated use of antibiotics. In our findings, only 7 of the isolates (highlighted in color orange) have the MAR index lower than that of 0.2, pointing towards an unregulated and overexposure of antibiotics.

Table 6: MAR Index of All Isolates

Sample ID	Total Resistant To	Total Number of Antibiotics	MAR Index
UT- 297	9	13	0.6923076923
UT- 520	11	13	0.8461538462
UT- 51503	7	13	0.5384615385
UT- 79703	7	13	0.5384615385
UT- 79440	4	13	0.3076923077
UT- 46095	4	13	0.3076923077
UT- 24826	9	13	0.6923076923
UT- 33231	4	13	0.3076923077
UT- 258	10	13	0.7692307692
UT- 997	10	13	0.7692307692
UT- 79495	6	13	0.4615384615
UT- 54055	7	13	0.5384615385
UT- 99898	11	13	0.8461538462
UT- 71976	9	13	0.6923076923
UT- 73206	6	13	0.4615384615
UT- 674672	6	13	0.4615384615
UT- 294	11	13	0.8461538462
UT- 50938	4	13	0.3076923077
UT- 90536	7	13	0.5384615385
UT- 57471	10	13	0.7692307692
UT- 87102	8	13	0.6153846154
UT- 86728	2	13	0.1538461538
UT- 57686	7	13	0.5384615385
UT- 87274	7	13	0.5384615385
UT- 87000	10	13	0.7692307692
UT- 57597	4	13	0.3076923077
UT- 87201	6	13	0.4615384615
UT- 87384	9	13	0.6923076923
UT- 93461	10	13	0.7692307692
UT- 59753	4	13	0.3076923077
UT- 59704	4	13	0.3076923077
UT- 59705	3	13	0.2307692308

Sample ID	Total Resistant To	Total Number of Antibiotics	MAR Index
UT- 61240	7	13	0.5384615385
UT- 61224	6	13	0.4615384615
UT- 679643	8	13	0.6153846154
UT- 97561	9	13	0.6923076923
UT- 98003	10	13	0.7692307692
UT- 61320	5	13	0.3846153846
UT- 81337	7	13	0.5384615385
UT- 63133	7	13	0.5384615385
UT- 63180	7	13	0.5384615385
UT- 63220	7	13	0.5384615385
UT- 63282	7	13	0.5384615385
UT- 63301	7	13	0.5384615385
UT- 11545	7	13	0.5384615385
UT- 70751	7	13	0.5384615385
UT- 70777	3	13	0.2307692308
UT- 24771	7	13	0.5384615385
UT- 70865	4	13	0.3076923077
UT- 27921	5	13	0.3846153846
UT- 71799	2	13	0.1538461538
UT- 71925	6	13	0.4615384615
UT- 27528	9	13	0.6923076923
UT- 71846	10	13	0.7692307692
UT- 73096	7	13	0.5384615385
UT- 31772	9	13	0.6923076923
UT- 73197	7	13	0.5384615385
UT- 73155	10	13	0.7692307692
UT- 73094	9	13	0.6923076923
UT- 72990	4	13	0.3076923077
UT- 631620	5	13	0.3846153846
UT- 72992	7	13	0.5384615385
UT- 659	10	13	0.7692307692
UT- 73602	10	13	0.7692307692

Sample ID	Total Resistant To	Total Number of Antibiotics	MAR Index
UT- 73631	8	13	0.6153846154
UT- 73743	1	13	0.07692307692
UT- 73752	1	13	0.07692307692
UT- 73821	7	13	0.5384615385
UT- 916	11	13	0.8461538462
UT- 495	12	13	0.9230769231
UT- 723	12	13	0.9230769231
UT- 314	12	13	0.9230769231
UT- 229	10	13	0.7692307692
UT- 911	11	13	0.8461538462
UT- 586	11	13	0.8461538462
UT- 993	11	13	0.8461538462
UT- 713	8	13	0.6153846154
UT- 299	8	13	0.6153846154
UT- 27544	8	13	0.6153846154
UT- 310	8	13	0.6153846154
UT- 000	8	13	0.6153846154
UT- 90109	12	13	0.9230769231
UT- 382	8	13	0.6153846154
UT- 735	8	13	0.6153846154
UT- 065	12	13	0.9230769231
UT- 14272	8	13	0.6153846154
UT- 045	12	13	0.9230769231
UT- 020	8	13	0.6153846154
UT- 196	12	13	0.9230769231
UT- 826	10	13	0.7692307692
UT- 103	12	13	0.9230769231
UT- 138	12	13	0.9230769231
UT- 320	12	13	0.9230769231
UT- 010	12	13	0.9230769231
UT- 079	2	13	0.1538461538
UT- 605	12	13	0.9230769231

Sample ID	Total Resistant To	Total Number of Antibiotics	MAR Index
UT- 007	12	13	0.9230769231
UT- 188	13	13	1
UT- 073	9	13	0.6923076923
UT- 137	11	13	0.8461538462
UT- 698	11	13	0.8461538462
UT- 329	10	13	0.7692307692
UT- 817	12	13	0.9230769231
UT- 259	7	13	0.5384615385
UT- 73837	6	13	0.4615384615
UT- 79668	7	13	0.5384615385
UT- 78141	2	13	0.1538461538
UT- 73598	2	13	0.1538461538
UT- 70773	8	13	0.6153846154
UT- 50985	10	13	0.7692307692
UT- 485	11	13	0.8461538462

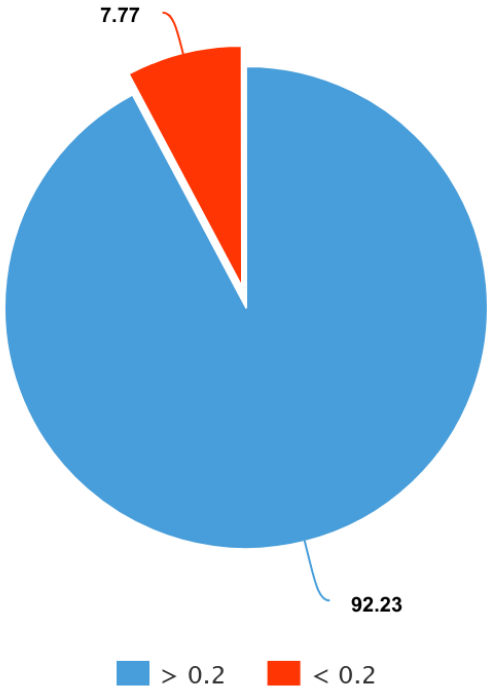


Figure 20: Distribution of MAR Index values among isolates

Chapter 4
Discussion:

One of the most common infections that affect hundreds of millions of people worldwide is urinary tract infections. UTI can affect the kidneys, bladder, urethra and can lead to severe complications if left untreated. To battle UTIs, antibiotics are the most common treatment because they effectively destroy the bacteria causing the infection. These medications are prescribed by physicians. However, the misuse of antibiotic medications has resulted in a significant global crisis— antibiotic resistance. Antibiotic resistance refers to the mechanism that pathogens evolve to fight the effects of antibiotics which makes the treatment unsuccessful. Antibiotic resistance is the cause of recurring illnesses and higher mortality rates.

The identification process performed in the study demonstrates the wide range of microorganisms detected in the UTI patients of Bangladesh that contribute to the already available body of research on the infection and antimicrobial resistance in the area. The pathogens that were detected via biochemical tests revealed that 52% of the pathogens found were UPEC as in uropathogenic *E.coli*. The next highest prevalent pathogens found were *Klebsiella* spp. at 16%, followed by *Acinetobacter* spp. at 12%, *Pseudomonas* spp. at 8 %, *Flavobacterium* and *Citrobacter* spp. both at 4% and finally, GBS and Proteus spp. at 2% each. The study also illustrated that Gram negative bacteria was predominant in the detected bacterial population, standing at approximately 94.4%. A higher incidence of infections was noted in female patients at 61.2%. A significant portion of the cases were pediatric, standing at 51.7%.

The antimicrobial susceptibility test was conducted using 13 antimicrobial discs in total - Nitrofurantoin (F300), Amikacin (AK30), Kanamycin (K5), Cefixime (CFM5), Cefepime (CPM30), Ceftriaxone (CTR30), Azithromycin (AZM30), Imipenem (IPM10), Levofloxacin. (LE5), Ampicillin (AMP25), Doxycycline (DO30) and Colistin (CL10) and finally Amoxiclav (AMC30) against all the samples collected. The result showed the antimicrobial pattern of the isolates. The graph illustrated that, among the 13 antibiotics, AMP25 and CFM5 were resistant to 100% and 98.2% of the samples respectively. AZM30, CTR30 and CPM30 followed closely, showing a resistant rate of 75.68%, 72.08%, and 67.57%. LE5 and AMC30 exhibited resistance against 54.05% of the isolates whereas F300 was resistant against 49.55% of the samples. K5 and AK30 were resistant against 45.95% and 38.74% of the total samples. And DO30, IPM10 and finally CL10 were the least resistant of them all standing at 34.23%, 27.93%, and 25% respectively. Imipenem resistance is concerning as carbapenems are often regarded as the most foolproof last-resort antibiotics that too possessing lesser side effects. Hence, the emergence of carbapenem resistance, especially in gram-negative bacteria is a global public-healthcare concern. On the other hand, among the cephalosporin group CFM5 is almost completely resistant while CPM30 and CTR30 show mixed responses with the resistant rate still being higher. For example, UT-24826 shows sensitivity to CPM30 but is resistant to CTR30 which emphasizes the significance of antimicrobial susceptibility testing for efficient treatment. Similarly, for fluoroquinolones (AZM30, LE5), antimicrobial susceptibility testing should be done first and the more effective antibiotic should be prescribed. Although AMP from the penicillin group are totally resistant, the incorporation of penicillin and beta-lactamase inhibitor (Amoxicillin Clavulanic acid) i.e. AMC30 shows promising results in terms of efficacy. Lesser used Nitrofurantoin (F300) shows variability in susceptibility patterns, indicating moderate effectiveness can be expected. Lastly, CL10 from the polymyxin group being sensitive against 90% of the samples proved to be the most potent against resistant strains.

MAR index greater than 0.2 is indicative of bacterial isolates originating from sources where high use of antibiotics is practiced whereas a lower index denotes usage of antibiotics in a controlled environment. Upon observing the Multiple Antibiotic Resistance (MAR) index of all isolates in table-4, it is evident that most of the UTI isolates bear high MAR indices. Except 7 isolates, all the other isolates have MAR index higher than 0.2. The extremely high MAR index possessing isolates range as

high as 0.8 to 0.9 such as- UT-520, UT-320 etc. Isolates as such are particularly challenging to treat due to their developed resistance against multiple antibiotics. This high MAR index is suggestive of exposure to excessive antibiotics and its heavy usage in a clinical setting. The MAR index of UT-188 is 1 which means this isolate was resistant against all 13 antibiotics and warrants a more complex alternate form of treatment that involves using a less commonly used antibiotic or combination therapy. Some isolates with the MAR index being around 0.6 are regarded as moderate such as UT-63220, UT-27543 etc. which shows partial resistance to the antibiotics used and treatment for such cases should be carried out carefully and efficiently. The number of isolates with low MAR index i.e. less than 0.2 is very few e.g.- UT-78141, UT-73598 etc. which means these isolates are resistant against very few antibiotics. Standard antibiotic practice must be maintained for such isolates in order to prevent future multidrug resistance.

Our study aligns with several similar studies conducted within Asia while also demonstrating unique patterns specific to that of Dhaka region. A 2011 study conducted in India reported finding UPEC to be the predominant pathogen found in the locality that aligned with our finding. Nevertheless, the study also reported finding a lower prevalence of Klebsiella spp. (12%). (Manikandan et al. 2011). Another south Asian nation Nepal published a similar paper where *E.coli* was also a predominant culprit. The same study also reported finding a high resistance rate to Ampicillin which also aligns with our finding where 100% of the isolates were resistant to AMP25 (Gupta et al. (2017). In Japan, a retrospective study was conducted between April 2010 to March 2015 from a national database of 31 million people that concluded that 64.9% of the patients were female; another research that is in alignment with our study. The same study, however, was only conducted on patients above the age of 15 (Sako, et al., 2021) Another research conducted in Thailand in 2018 reported that the resistance rate to CTR30 that they found (60%) showed lower than the percentage we observed in our study. This indicates that Thailand has a more effective use of Ceftriaxone as a treatment option. If we explore the European region, a 2011 study reported a high level of Ampicillin resistance found in *E.coli* strains isolated from UTI patients across several European countries, pointing to the fact that Ampicillin resistance indeed is a matter of growing concern (Journal of Antimicrobial Chemotherapy, 2011).

4.1 Conclusion:

Every year, nearly 150 million people contract UTI worldwide, and although it is characterized as a benign medical issue, urinary tract infection can cause acute complications pertaining to the frequent use of antibiotics, thus giving rise to antibiotic resistance- a concerning global threat. This study highlights the prevalence of bacteria within the study area and shows demographic analysis. Additionally, antimicrobial resistance patterns of uropathogens collected from UTI patients in Dhaka, Bangladesh were successfully determined and further dissected for future study.

In this study, 94.4 % of the total isolates comprise gram-negative bacteria, indicating a high- prevalence of the said bacterial group. Demographic analysis shows a higher incidence in female patients and pediatric cases, standing at 61.2% and 51.7%, respectively. Women, due to their anatomical features, are more prone to UTI, while in young children, it poses significant health hazards like renal failure, premature births, etc. For pregnant women regular health checkup and diagnosis should be done to not pass on the infection to their offspring and intake of antibiotics should be in regulated doses in order to prevent antibiotic resistance. Selective confirmation of the isolates was done by conducting biochemical tests, and observing the characteristics. After biochemical confirmation, the bacteria were subsequently tested for antimicrobial susceptibility using a wide range of antibiotic groups. In this study, the alarming rate of Imipenem resistance is noted as carbapenems are regarded as the last-resort antibiotics with

lesser adverse effects than others and is a healthcare concern worldwide. This resistance, especially noticeable in gram-negative bacteria necessitates immediate public health initiatives and proper clinical practice. Besides, antibiotics of all groups should undergo susceptibility testing for effective treatment as variability in susceptibility patterns is noticed. High sensitivity of Colistin suggests it has the most potential to combat resistant strains and therefore, its use should be fortified in treating resistant infections. The alarming rate of high MAR indices highlight the rise in multidrug resistance. This is a global healthcare concern that disrupts conventional treatment methods and warrants a more complex treatment regimen. To combat this widespread public health issue, mass awareness is necessary and practicing regulated antibiotic use is of utmost importance.

4.2 Future Perspectives of the Study:

All bacteria that were confirmed biochemically should undergo identification on a molecular level for accurate and strain-specific identification. Gene-based PCR would help identify the specific genes responsible for causing antibiotic resistance. A larger-scale surveillance study that expands beyond Dhaka to different regions of Bangladesh would help recognize the variations in prevalence of pathogens and antimicrobial resistance patterns on a geographical level. Lastly, preventative strategies to minimize the incidence of UTIs, including public health education and vaccine development, should be adapted to eliminate risks in vulnerable groups such as women and children.

References:

1. Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature Reviews. Microbiology, 13(5), 269–284. https://doi.org/10.1038/nrmicro3432
2. Chopra, I., & Roberts, M. (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and molecular biology reviews : MMBR*, *65*(2), 232–260. https://doi.org/10.1128/MMBR.65.2.232-260.2001
3. David C. Hooper, Mechanisms of Action and Resistance of Older and Newer Fluoroquinolones, *Clinical Infectious Diseases*, Volume 31, Issue Supplement_2, August 2000, Pages S24–S28, https://doi.org/10.1086/314056
4. Nickel, J. C. (2005). Management of Urinary Tract Infections: Historical Perspective and Current Strategies: Part 1—Before antibiotics. Journal of Urology, 173(1), 21–26. https://doi.org/10.1097/01.ju.0000141496.59533.b2 (Original work published January 1, 2005)
5. Adedeji W. A. (2016). The Treasure Called Antibiotics. *Annals of Ibadan postgraduate medicine*, *14*(2), 56–57
6. Cappuccino, J.G., & Sherman, N. (2008). Microbiology: A Laboratory Manual (9th Edition). Pearson Benjamin Cummings
7. *HicromeTM uti agar*. HiMedia Leading BioSciences Company. (n.d.). https://www.himedialabs.com/us/m1353-hicrome-uti-agar.html
8. CLSI. (2021). Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

9. Al-Badr, A., & Al-Shaikh, G. (2013). Recurrent Urinary Tract Infections Management in Women: A review. *Sultan Qaboos University medical journal*, *13*(3), 359–367. https://doi.org/10.12816/0003256
10. Thomas, C. M., & Nielsen, K. M. (2020). Mechanisms of, and barriers to, horizontal gene transfer between bacteria. *Current Opinion in Microbiology, 53*, 1-9. https://doi.org/10.1016/j.mib.2019.10.008
11. O'Neill, J. (2016). Tackling drug-resistant infections globally: Final report and recommendations. The Review on Antimicrobial Resistance. Retrieved from https://amr-review.org
12. Alav, Ilyas & Sutton, J. Mark & Rahman, Khondaker. (2018). Role of bacterial efflux pumps in biofilm formation. The Journal of antimicrobial chemotherapy. 73. 10.1093/jac/dky042
13. Md. Anowar Khasru Parvez, Nurnabi Sheikh, Asif Iqbal, Rashed Al Mahmud, Md. Akteruzzaman, & Md. Mahmud Hossain. (2022). Epidemiology and Antimicrobial Resistance Pattern of Urinary Tract Infection (UTI) in Bangladesh. *Antibiotics*, 11(10), 1417. https://doi.org/10.3390/antibiotics11101417
14. Talapko J, Meštrović T, Juzbašić M, Tomas M, Erić S, Horvat Aleksijević L, Bekić S, Schwarz D, Matić S, Neuberg M, et al. Antimicrobial Peptides—Mechanisms of Action, Antimicrobial Effects and Clinical Applications. *Antibiotics*. 2022; 11(10):1417. https://doi.org/10.3390/antibiotics11101417
15. Microbe Notes. (n.d.). Gram stain: Principle, reagents, procedure, and result interpretation. Retrieved July 2, 2024, from https://microbenotes.com/gram-stain-principle-reagents-procedure-and-result-interpretation/
16. Sher, E. K., Džidić-Krivić, A., Sesar, A., Farhat, E. K., Čeliković, A., Beća-Zećo, M., Pinjic, E., & Sher, F. (2024). Current State and novel outlook on prevention and treatment of rising antibiotic resistance in urinary tract infections. *Pharmacology & Therapeutics*, 108688. https://doi.org/10.1016/j.pharmthera.2024.108688
17. Manikandan, S., & Amsath, A. (2011). Antibiotic susceptibility pattern of Escherichia coli isolated from urine samples in Namakkal, Tamil Nadu, India. Journal of Bacteriology Research, 3(2), 25-27
18. Gupta, K., Hooton, T. M., & Naber, K. G. (2017). International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2017 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clinical Infectious Diseases, 64(6), e1-e43
19. Hongsuwan, M., Srisamang, P., & Suksakulchai, P. (2018). Antimicrobial resistance patterns of uropathogens causing community-acquired urinary tract infections in Thailand. *Journal of Infection and Public Health*, 11(6), 852-857
20. Gajdács, M., & Urbán, E. (2019). Resistance trends and epidemiology of Citrobacter species in the context of human diseases: A 10-year retrospective study. *International Journal of Medical Microbiology*, 309(1), 76-82. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4735501/
21. Jain, P., Bepari, A.K., Sen, P.K. *et al.* High prevalence of multiple antibiotic resistance in clinical *E. coli* isolates from Bangladesh and prediction of molecular resistance determinants using WGS of an XDR isolate. *Sci Rep* 11, 22859 (2021). https://doi.org/10.1038/s41598-021-02251-w
22. Sako A, Yasunaga H, Matsui H, Fushimi K, Yanai H, Gu Y, Ohmagari N. Hospitalization for urinary tract infections in Japan, 2010-2015: a retrospective study using a national inpatient database. BMC Infect Dis. 2021 Oct 9;21(1):1048. doi: 10.1186/s12879-021-06735-y. PMID: 34627171; PMCID: PMC8502312.

23. Livermore, D. M., et al. (2011). "Antimicrobial Resistance among Escherichia coli Isolates from Community-Acquired Urinary Tract Infections in Europe: The ECO.SENS Study Revisited." Journal of Antimicrobial Chemotherapy, 66(2), 203-208. DOI: 10.1093/jac/dkq431