Identification of Uropathogens and Antimicrobial Resistance of Urinary Tract Infections at Tertiary Medical College in Bangladesh

By

Noshin Tabassum Orchee

ID: 19126069

A Thesis Submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology

Department of Mathematics and Natural Sciences Brac University

2024

Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at BRAC University.
2. The 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 report does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

_____

Noshin Tabassum Orchee
ID: 19126069

Approval

The thesis titled "[Identification of Uropathogens and Antimicrobial Resistance of Urinary Tract Infections at Tertiary Medical College in Bangladesh]" submitted by [Noshin Tabassum Orchee, (19126069)] has been accepted as satisfactory in partial fulfillment of the requirement for the degree of 'Bachelor of Science in Microbiology' on 14th March.

Examining committee

Supervisor: (Member) _____

Fahim Kabir Monjurul Haque, PhD

Associate Professor, Department of Mathematics and Natural Sciences

BRAC University

External Supervisor: _____

Professor Dr. Sabera Gul Nahar

Department Head of Microbiology,

Rajshahi Medical College

Program Director: _____

Dr. Nadia Sultana Deen

Associate Professor, Department of Mathematics and Natural Sciences

BRAC University

Chairperson: _____

A F M Yusuf Haider, PhD

Professor, Department of Mathematics and Natural Sciences

BRAC University

Ethics statement

The departmental review board of BRAC University in Dhaka, Bangladesh, provided ethical clearance. Rajshahi Medical College (RMC) gave permission to complete my thesis in their microbiology laboratory. The respondents' privacy and the data's confidentiality were strictly protected during data collection.

Abstract

The goal of this study was to determine the frequency of different bacterial microorganisms and their antibiotic sensitivity in urine infections.

Antimicrobial resistance has been steadily increasing for a number of years, reducing the effectiveness of antibiotics. Numerous factors, such as human behavior, can hasten the development of antibiotic resistance, drug impermeability, drug tolerance, drug destruction, and practices.

The frequency, kinds, and antibiotic susceptibility of bacteria found in this investigation were urine sample investigations were conducted. 200 urine samples altogether, including both sexes and ages 0-90 years were examined in this investigation. These samples underwent analysis to look for the antibiotic vulnerability to pyogenic bacteria growing significantly. Organisms that are gram-positive accounted for 14% of the total, while Gram-negative microbes made for 86% which means gram-negative bacteria have a huge impact than Gram-positive bacteria in comparison. *Enterococci spp, Staphylococcus aureus, Staphylococcus saprophyticus* etc are examples of Gram-positive bacteria. On the other hand, *Escherichia coli*, *Klebsiella spp, Acinetobacter spp, Pseudomonas spp* etc are example of Gram-negative bacteria. The majority of the resistant Gram-positive bacteria susceptible to Nitrofurantoin but resistant in opposition to Ciprofloxacin. The majority of the resistant Gram-negative bacteria susceptible to Amikacin and Colistin but resistant in opposition to Azithromycin and Cefepime.

Acknowledgement

At first, I would want to thank Allah for giving me the perseverance and patience I needed to finish the Thesis paper—which is a requirement for completing the degree safely and successfully.

My sincere gratitude is extended to my supervisor, Fahim Kabir Monjurul Haque, Associate Professor of Mathematics and Natural Sciences at BRAC University, for his unwavering support and supervision throughout my paper-writing process. My sincere appreciation also goes out to him for always making time, politely and patiently, for significant recommendations.

I would especially want to thank Professor Dr. Sabera Gul Nahar, Departmental head of Pathology, Rajshahi Medical College for her time and supervision of my work in her lab. Also I want to thank microbiologist Golam Mostafa for his ongoing instruction during the course of my lab work practice in the microbiology lab.

This was a great experience for me and I am lucky that I had worked in a prestigious medical college of Bangladesh.

Table of contents:

List of Figures:

List of Tables:

Chapter 1

Introduction

1.1 INTRODUCTION OF UTI AND UROPATHOGENS

A UTI (Urinary Tract Infection) is an infection that can occur in any part of the urinary system, which includes the kidneys, bladder, urethra, and ureters. UTIs are most commonly caused by bacteria, but they can also be caused by viruses or fungi. The majority of UTIs are caused by bacteria, with *Escherichia coli (E. coli)* being the most common culprit (Flores-Moreles & Walker et, al., 2015)

Uropathogens are microorganisms, typically bacteria that have the potential to cause urinary tract infections. These bacteria enter the urinary tract and can lead to infection if they are not adequately flushed out by the body's natural defenses, such as urination and the immune system. Some of the common uropathogens include *E. coli, Staphylococcus saprophyticus, Klebsiella, Enterococcus*, and *Proteus* species. Upper tract infection that involve the kidneys called pyelonephritis. Lower tract infection that involve the bladder called cystitis, involve the urethra called urethritis, involve the prostate called Prostatitis (Shaifali & Gupta et, al,. 2012).

At first, the periurethral region is contaminated by gut-resident uropathogens which then have the ability to colonize the urethra and cause simple UTIs. After that, colonization and invasion of the superficial umbrella cells occur as a result of subsequent migration to the bladder and pili and adhesin being expressed. Then extracellular bacteria are eliminated by the host's inflammatory reactions, which include neutrophil infiltration. Some bacteria multiply and build biofilms in order to elude the immune system. This can happen through host cell invasion or morphological changes that make the bacteria resistant to neutrophils. In order to cause harm to host cells, these bacteria release toxins and proteases, which release vital nutrients that aid in the survival of the bacteria and their ascent to the kidneys. Kidney colonization leads to step 10 host tissue damage and bacterial toxin production. Finally if the infection penetrates the kidneys' tubular epithelial barrier, untreated UTIs may eventually lead to bacteraemia.

On the other hand, uropathogens that cause complex UTIs proceed through the same initial stages—periurethral colonization, urethral progression, and bladder migration —as those that are described for simple infections. But the bladder needs to be compromised for the germs to invade it. Catheterization is the most frequent cause of a compromised bladder. Because catheterization triggers a strong immune response, fibrinogen builds up on the catheter, creating a perfect surface

for uropathogens that express fibrinogen-binding proteins to attach to. Neutrophils infiltrate the body as a result of infection. However, following their initial attachment to the fibrinogen-coated catheters, the bacteria multiply form biofilms to encourage damage to the epithelium and can also cause kidney infection, which can lead to tissue damage. In the end uropathogens that cause complex UTIs have the ability to breach the tubular epithelial cell barrier and generate bacteraemia if left untreated (Flores-Moreles & Walker et, al., 2015)

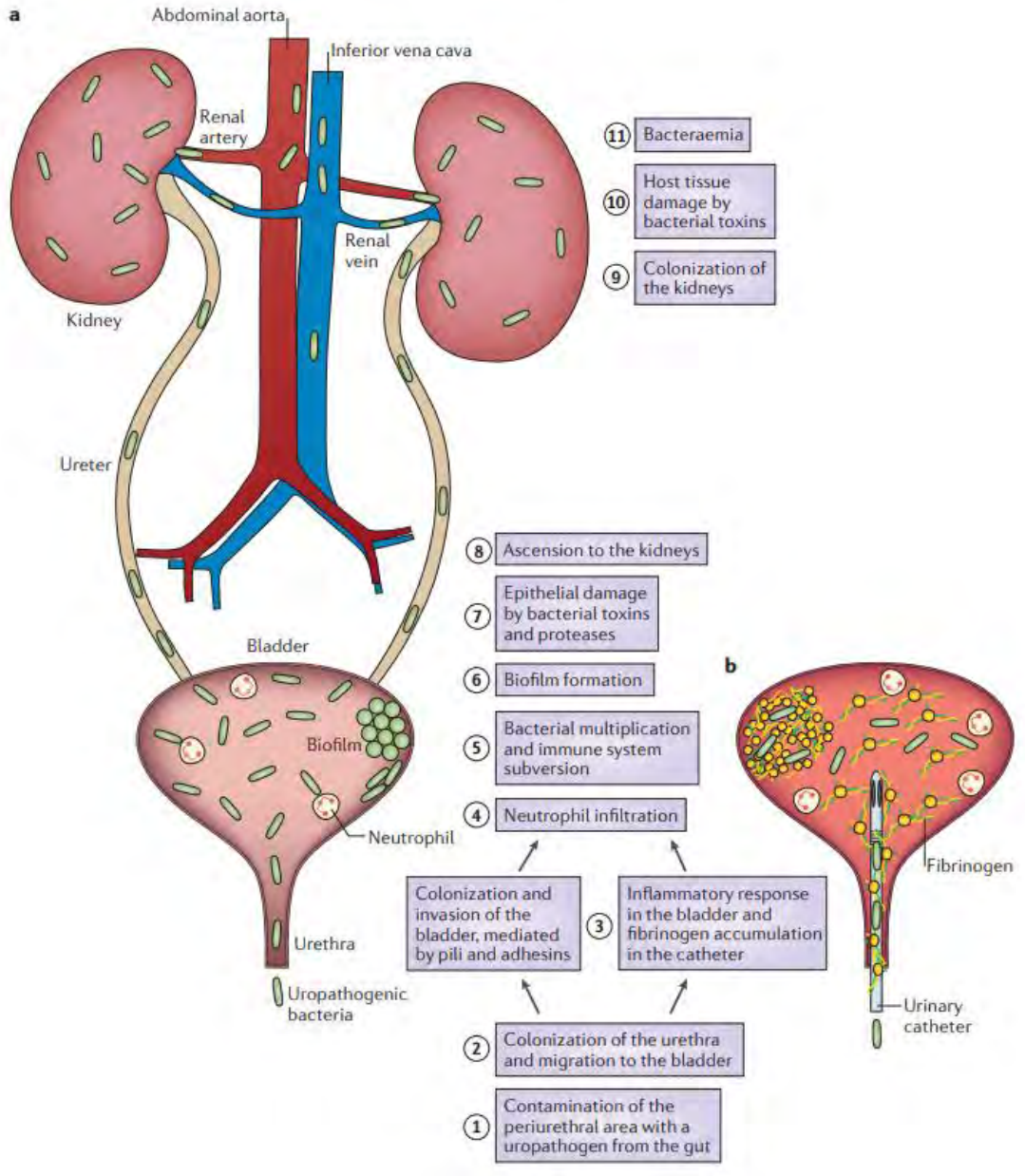


Figure 1: Pathogenesis of urinary tract infections (Flores-Moreles & Walker et, al., 2015)

1.2 Transmission of uropathogens:

Uropathogens are microorganisms that can cause infections in the urinary tract, which includes the bladder, urethra, ureters, and kidneys. These pathogens can enter the body through various routes, and the most common entry points are:

I. Ascending Infection: This is the most common route for uropathogens. Bacteria from the perineum (the area between the anus and genitals) can enter the urethra and travel up into the urinary tract. In women, the urethra is relatively short and close to the anus, making it easier for bacteria to ascend into the bladder. In men, the longer urethra offers some protection, but it can still happen.

II. Hematogenous Spread: In some cases, uropathogens can enter the urinary tract through the bloodstream. This is relatively rare, but it can occur when bacteria from infections in other parts of the body are carried through the bloodstream and reach the kidneys or other parts of the urinary tract. (Behzadi & Garcia et, al., 2023)

III. Lymphatic Spread: Similar to hematogenous spread, bacteria can also enter the urinary tract through the lymphatic system. This is less common but can happen when bacteria travel through the lymphatic vessels and reach the urinary organs.

IV. Direct Extension: In some situations, infections from nearby structures or organs can spread directly into the urinary tract. For example, if a person has an abscess or infection in the pelvic area, it can extend into the bladder or other parts of the urinary tract.

V. Instrumentation or Medical Procedures: The use of medical instruments, such as urinary catheters or scopes, can introduce uropathogens into the urinary tract. This is more common in healthcare settings, where these procedures are performed. (Kunin et, al., 1994)

VI. Sexual Activity: Sexual activity can introduce uropathogens into the urinary tract. In women, sexual intercourse can potentially push bacteria into the urethra. Proper hygiene and urination after sexual activity can help reduce this risk.

VII. Contaminated Water: In rare cases, exposure to contaminated water, such as in swimming pools or hot tubs, can introduce uropathogens into the urinary tract. (Minardi & Catoro et, al., 2011)

Chapter 2
Causes and Symptoms

2.1 Causes of urinary tract infection:

Urinary tract infections (UTIs) are commonly caused by bacteria that enter the urinary tract. The urinary tract includes the kidneys, ureters, bladder, and urethra. Here are some of the common causes and risk factors for urinary tract infections:

- Bacterial Entry: The most common cause of UTIs is the entry of bacteria, usually Escherichia coli (E. coli), into the urinary tract through the urethra. This can happen for various reasons, including improper hygiene practices.
- Sexual Activity: Sexual activity can introduce bacteria into the urinary tract. In women, this is sometimes called "honeymoon cystitis" and is more common after sexual intercourse.
- Urinary Retention: Incomplete emptying of the bladder can lead to stagnant urine, creating a breeding ground for bacteria. Conditions that hinder the complete emptying of the bladder, like an enlarged prostate in men, can be a risk factor.
- Obstruction: Any obstruction in the urinary tract, such as kidney stones or tumors, can cause stagnant urine and increase the risk of infection.
- Catheter Use: People with urinary catheters are at a higher risk of UTIs because the catheter can introduce bacteria into the bladder.
- Pregnancy: Changes in the urinary tract during pregnancy can increase the risk of UTIs.
- Menopause: A drop in estrogen levels in menopausal women can thin the lining of the urinary tract, making it more susceptible to infection.
- Diabetes: People with diabetes may have reduced immune function, which can increase the risk of infections, including UTIs (NHS et, al., 2022).
- Weakened Immune System: Any condition or medication that weakens the immune system, such as HIV/AIDS or chemotherapy, can increase susceptibility to UTIs.
- Dehydration: Not drinking enough fluids can reduce the body's ability to flush out bacteria from the urinary tract.((Minardi & Catoro et, al., 2011).
- Holding Urine: Delaying urination for extended periods can allow bacteria to multiply in the bladder.
- Use of Spermicides or Diaphragms: Certain forms of birth control, such as spermicides or diaphragms, can increase the risk of UTIs in women.

- Sexually Transmitted Infections (STIs): Some sexually transmitted infections, such as chlamydia and gonorrhea, can cause symptoms similar to UTIs (Cleveland et, al., 2023).

2.2 Symptoms :

A urinary tract infection (UTI), often referred to as a urine infection, can have a range of symptoms. The symptoms may vary depending on which part of the urinary tract is infected (bladder, urethra, or kidneys), and the severity of the infection. Common symptoms of a UTI can include:

- Frequent and Urgent Urination: You may feel a strong need to urinate more frequently than usual, and when you do, only small amounts of urine come out.
- Burning Sensation: A burning or painful sensation during urination is a classic symptom of a UTI. This is often described as a "burning" feeling.
- Cloudy, Bloody, or Strong-Smelling Urine: Changes in the appearance or smell of urine can be a sign of infection. Urine may appear cloudy, contain traces of blood, or have a particularly strong or foul odor (NHS et, al., 2022).
- Pelvic Pain: Some individuals experience pain or discomfort in the lower abdomen or pelvic region. This discomfort may range from a mild ache to more severe pain.
- Feeling of Incomplete Emptying: You may feel as if you still need to urinate even after doing so.
- Fatigue: General tiredness and fatigue can be associated with a UTI, especially if the infection has spread to the kidneys.
- Fever and Chills: In more severe cases, a UTI can lead to fever and chills. These symptoms may indicate that the infection has reached the kidneys and is a more serious condition.
- Nausea and Vomiting: Kidney infections, which are a more severe form of UTI, can lead to nausea and vomiting (Cleveland et, al., 2023).

It is important to note that not everyone with a UTI will experience all of these symptoms, and some people may have subtle or atypical symptoms. If someone suspect to have a UTI, it's crucial to see a healthcare provider for a proper diagnosis and treatment. UTIs are typically treated with antibiotics, and it's important to get timely treatment to prevent the infection from spreading and causing more serious complications.

Chapter 3

Methodology

3.1 Study Design

All samples were collected from patients of Rajshahi Medical College.

Samples were collected from both sexes and different age groups.

3.1.1 Sample collection Period

March 5th – September 15th , 2023

3.1.2 Types of specimen

The specimen type that included in this study was urine sample.

3.1.3 Quantity of specimen

A total of 200 clinical isolates were tested from patients.

3.1.4 Sample collection technique:

People came to the medical college after collecting their mid-stream urine in a container or tube. This tube is used to protect the sample from light exposure. Also some samples came from hospital sector such as ICU or word sector. The sample tubes were covered with cotton to avoid contamination.

3.2 Media

- MacConkey agar media
- Nutrient agar media
- TSI slants
- MIU media
- Muller-Hinton broth

3.3 Bacterial culture

Usually two types of media are mostly used in Rajshahi Medical College which are nutrient media and MacConkey media.

I. At first after collecting the sample, the loop was sterilized by Bunsen burner.
II. Then by the loop, a little portion was collected from the sample tube and then the isolated bacteria were spreaded over the surface into 90 degree angle throughout the sterile agar plate (Petri dish) using a technique called streaking.
III. Then the plate was incubated for around 24 hours at 35-37 degree Celcius.
IV. After incubation, the plates were examined to see the growth. If there was any microorganism present in the plate, then there would be a growth in the plate.
V. Then finally using various biochemical methods, the pathogen was identified.

3.4 Biochemical tests & Reagents

- ✓ Catalase
- ✓ Oxidase
- ✓ TSI
- ✓ MIU
- ✓ Citrate

3.5 Biochemical test to identify micro-organisms :

Catalase –

- The catalase test is used in to determine the presence of the enzyme catalase in a bacterial sample.
- Catalase is an enzyme that catalyzes the breakdown of hydrogen peroxide (H2O2) into water (H2O) and oxygen (O2).
- This reaction is crucial for some bacteria to protect themselves from oxidative damage caused by hydrogen peroxide, a byproduct of metabolism.
- Result : Positive – Bubble formation
  Negative – No bubble formation

Oxidase –

- The oxidase test is a biochemical test used in microbiology to determine the presence of cytochrome c oxidase, an enzyme involved in the electron transport chain of aerobic organisms by measuring the enzyme's ability to oxidize a specific reagent.
- Cytochrome c oxidase is an enzyme found in the electron transport chain of aerobic bacteria and it is responsible for the final transfer of electrons to oxygen during respiration.
- Result : Positive – Color change to purple or blue
  Negative – No color

Triple Sugar Iron (TSI) –

- This test helps in identifying bacteria among *Enterobacteriaceae* based on their ability to ferment sugars and produce gas.
- To differentiate members of the *Enterobacteriaceae* family from other gram-negative rods, this method is also used.
  - Lactose fermenting bacteria – *E.coli, Klebsiella, Enterobacter*
  - Non-lactose fermenting bacteria – *Proteus spp , Pseudomonas spp*.
- Results –
  - Yellow slant/yellow butt: Fermentation of both glucose and lactose/sucrose.
  - Yellow slant/red butt: Fermentation of glucose only.

- Red slant/yellow butt: No fermentation of any sugars.
- Blackening of the medium: Production of hydrogen sulfide (H2S).
- Gas production: Bubbles in the tube indicates gas production.

MIU (motile indole urease test) –

- The Motility Indole Urea (MIU) test is a combination of three tests used to differentiate bacteria based on their motility ability to produce indole, and their ability to hydrolyze urea.
- It is commonly used to identify and classify *Enterobacteriaceae*, a family of bacteria that includes *Escherichia coli (E. coli).*
- Result : If the tube shows motility, is indole-positive, and urease-negative
  If the tube shows motility, is indole-negative, and urease-positive
  If the tube shows no motility and is indole-negative.

Citrate test –

- The only carbon source in the media is citrate. The ability of an organism to use citrate as a source of energy is tested using citrate agar.
- Result
  - Positive Reaction: Growth with color change from green to intense blue along the slant.
  - Negative Reaction: No growth and No color change; Slant remains green

3.6 Biochemical test interpretation for different UTI organisms –

Table 1-

- Lactose fermenting organism:

Organisms	Mobility test	Indole test	Urease test	Citrate utilization test	Oxidase test	VP	TSI
E.coli	motile	positive	negative	negative	negative	negative	S=yellow B=Yellow H2S=No

							Gas=Yes
Klebsiella spp	Non-motile	negative	positive	positive	negative	positive	S=Yellow B=Yellow H2S=No Gas=Yes
Enterobacter	Motile	negative	negative	positive	negative	positive	S=Yellow B=Yellow H2S=No Gas=Yes

- Non-lactose fermenting organism:

Organisms	Motility test	Indole test	Urease test	Citrate utilization test	Oxidase test	Catalase test	TSI
Proteus spp	Motile	+ve/-ve	positive	Negative(vulgaris) /Positive	negative	-	S=Red B=Yellow H2S=Yes Gas=+ve(Mira) -ve(Vulgaris)
Pseudomonas spp	Motile	Positive	Negative	negative	positive	positive	S=Yellow B=Yellow H2S=No Gas=No
Acinetobacter	Non-motile	negative	negative	positive	negative	positive	S=Red B=Red H2S=No Gas=No

- Gram-positive Cocci :

Organisms	Catalase	Coagulase	PVR
Staph. aureus	positive	positive	negative
S. saprophyticuss	positive	negative	negative
Enterococci spp	negative	negative	positive

[Note : yellow = Acid, Red = alkaline, S = slant, B = butt]

3.7 Flow-chart:

Flowchart to describe the examination of urine sample to determine organisms

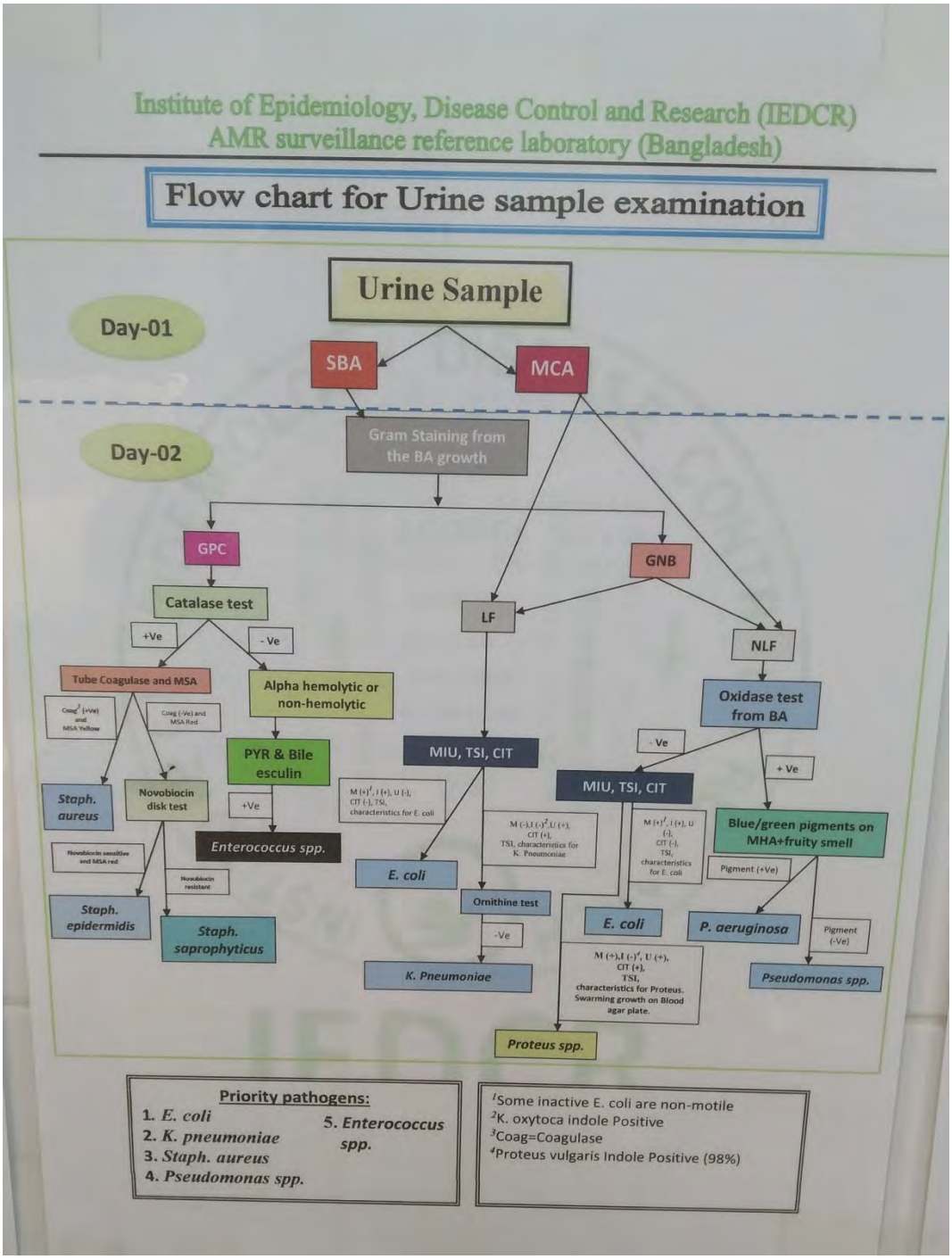


Figure 2 : Flowchart for urine sample examination

3.8 Antibiotic Susceptibility Test :

Antibiotic susceptibility testing, also known as antibiotic sensitivity testing or antimicrobial susceptibility testing, is a laboratory technique used to determine which antibiotics are effective in treating urine bacterial infections. This test helps healthcare providers select the most appropriate antibiotic for a patient's infection, ensuring effective treatment and minimizing the development of antibiotic resistance. Larger scale evaluation of hospital, clinic, and national programs for the control and prevention of infectious illnesses is made easier with its assistance. Due to the alterations in bacterial DNA, researchers have recently had to undertake ongoing surveillance operations for resistance patterns. Currently, clinical laboratories use a variety of techniques based on the laboratory test menu they offer.

Here's how the antibiotic susceptibility test typically works:

1. After completing the identifying of bacteria in the sample, the antibiotic discs were used and placed evenly on the agar surface.
2. The agar plates are incubated for around 24 hours at 35-37°C (95-98.6°F).
3. After incubation (usually 24-48 hours), the plates are examined. If the antibiotic is effective against the bacteria, there will be a clear zone (called a "zone of inhibition") around the disc or strip where bacterial growth is inhibited. The size of this zone is measured.
4. The diameter of the zone of inhibition is compared to standardized tables or guidelines provided by organizations like the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST). These guidelines classify bacteria as either susceptible, intermediate, or resistant to a particular antibiotic.

- Susceptible: The antibiotic is effective against the bacteria.
- Intermediate: The antibiotic may work at higher doses or in specific circumstances.
- Resistant: The antibiotic is not effective against the bacteria.

3.8.1 Antibiotic Panel for C/S (on the basis of CLSI guideline) :

CLSI guideline means clinical and laboratory standard institute guideline which is the most widely recognized for improving quality, safety and efficiency. After recognition of the organisms, the antibiotic discs are placed in the media.

For every specific organisms there are some certain antibiotics which designated. Here they are –

(Table 2) -

Urine				
E.coli/ kleb/proteus	***S. aureus***	***Enterococcus***	***Pseudo. Spp***	***Acinetobactor***
Amikacin	Amikacin	Ampicilin	Amikacin	Amikacin
AMC	AMC	Pencilin	Aztreonem	Ceftazidime
Aztreonem	Azithromycin	Linezolid	Ceftazidime	Colistin
Ciprofloxacin	Cefoxitin	Vancomycin	Cefepime	Meropenem
Ceftriaxone	Ciprofloxacin	Ciprofloxacin	Colistin	Ciprofloxacin
Cefepime	Clindamycin	Nitrofurantoin	Meropenem	Piperacilin+Tazo
Cefixime	Doxycycline	Tetracycline	Ciprofloxacin	Gentamycin
Cefuroxime	Gentamycin	Teicoplanin	Piperacilin+Tazo	Cefepime
Gentamycin	Linezolid		Gentamycin	Ceftriaxone
Meropenem	Nitrofurantoin		Netilimycin	Doxycycline
Nitrofurantoin	Cotrimoxazole			
Piperacillin+Tazo	Vancomycin			
Cotrimoxazole	Penicillin			
Tetracycline				

3.8.2 Antibiotic Susceptibility criteria :

By measuring the zone of inhibition, we can determine it is either susceptible or intermediate or resistant. (Table 3) -

SI No	Antibiotic Disc name	Code	Disk strength	Susceptibility criteria		
				Susceptible	Intermediate	Resistant
01	Amoxicillin	AMC	20/10µg	≥18	14-17	≤13
02	Amikacin	AK/AN	30 µg	≥17	15-16	≤14
03	Ampicillin	AMP	10 µg	≥17	14-16	≤13
04	Aztreonam	ATM	30 µg	≥21	18-20	≤17
05	Azithromycin	AZM	30 µg	≥18	14-17	≤13
06	Ceftazidime	CAZ	30 µg	≥21	18-20	≤17
07	Cephazolin	KZ	30 µg	≥15		≤14
08	Cefoxitin	FOX	30 µg	≥22		≤21
09	Ciprofloxacin	CIP	5 µg	≥26	22-26	≤21
10	Cefepime	FEP/CPM	30 µg	≥25	19-24	≤18
11	Colistin	CT/CL	10 µg	≥11		≤10
12	Cefixime	CFM	5 µg	≥19	16-18	≤15
13	Ceftriaxone	CRO/CTR	30 µg	≥23	20-22	≤19
14	Cefuroxime	CXM	30 µg	≥23	15-22	≤14
15	Clindamycin	CD	2 µg	≥21	15-20	≤14
16	Doxycycline	DOX/D	30 µg	≥16	12-15	≤12
17	Fosfomycin	FOS	200 µg	≥16	13-15	≤12
18	Gentamycin	GEN/CN	10 µg	≥15	13-14	≤12

19	Imipenem	IPM	10 µg	≥23	20-22	≤19
20	Linezolid	LZD	30 µg	≥21		≤20
21	Meropenem	MEM	10 µg	≥23	20-22	≤19
22	Nitrofurantoin	NIT/F	300 µg	≥17	15-16	≤14
23	Netilmycin	NET	30 µg	≥15	13-14	≤12
24	Pipercillin-Tazo	TZP/PIT	100/10 µg	≥21	18-20	≤17
25	Penicillin-G4	P	10 µg	≥29		≤28
26	Sulfamethoxazole-Trimethoprim	SXT	25 µg	≥16	11-15	≤10
27	Tetracycline	TET/TE	30 µg	≥15	12-14	≤11
28	Teciplanin	TEC	30 µg	≥14	11-13	≤10
29	Tegecycline	TGC	15 µg	≥19	15-18	≤14
30	Vancomycin	VAN	30 µg	≥15		

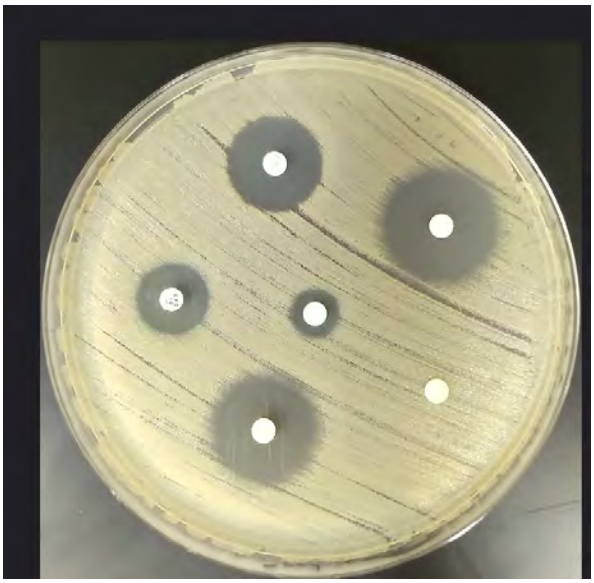


Figure 3: Antibiotic susceptibility test

Chapter 4

Result

4.1 Isolation of Bacteria :

4.1.1 Percetage of gram positive & gram negative bacteria of total positive samples

Among all the positive samples, I got 28 gram-positive bacteria and 172 gram-negative bacteria which means gram-negative bacteria has a huge impact on UTI infection.

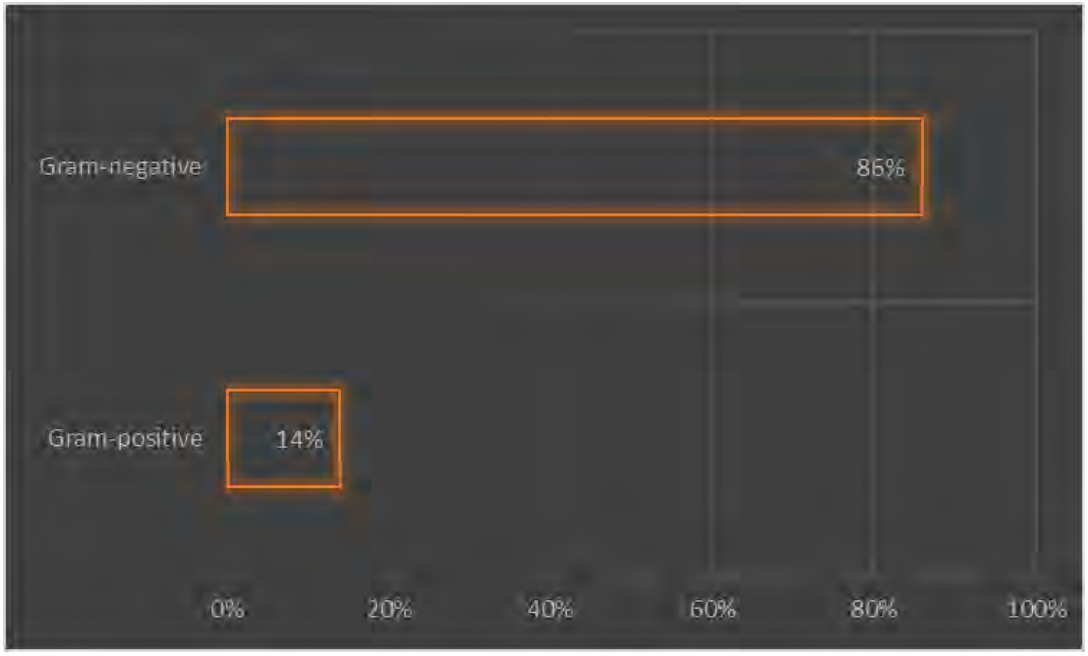


Figure 4: Percentages of gram-positive and gram –negative organisms

4.1.2 - Table 4: Ratio of gram-positive organisms in samples -

Category	Organism name	Number (samples)	Percentage (%)
Gram-positive	*CONS*	3	1.50%
	S. aureus	4	2.00%
	S. saprophyticus	2	1.00%
	Enterococci	19	9.50%
Total		=28	=14.00%

Among all the gram-positive bacteria, *Enterococci* spp has the highest number of sample in the urine (19 sample numbers).

4.1.3 - Table 5: Ratio of gram-negative organisms in samples -

Category	Organism name	Number (samples)	Percentage (%)
Gram-negative	*E.coli*	106	53.00%
	Klebsiella	42	21.00%
	Proteus	1	0.50%
	Pseudomonas	21	10.50%
	Acinetobacter	1	0.50%
	Enterobacter	1	0.50%
Total		=172	=86.00%

Among all the bacteria *E.coli* and *klebsiella spp* had the most number of samples which 106 and 42 sample numbers.

4.2 - Percentages of etiological agents of bacterial infections:

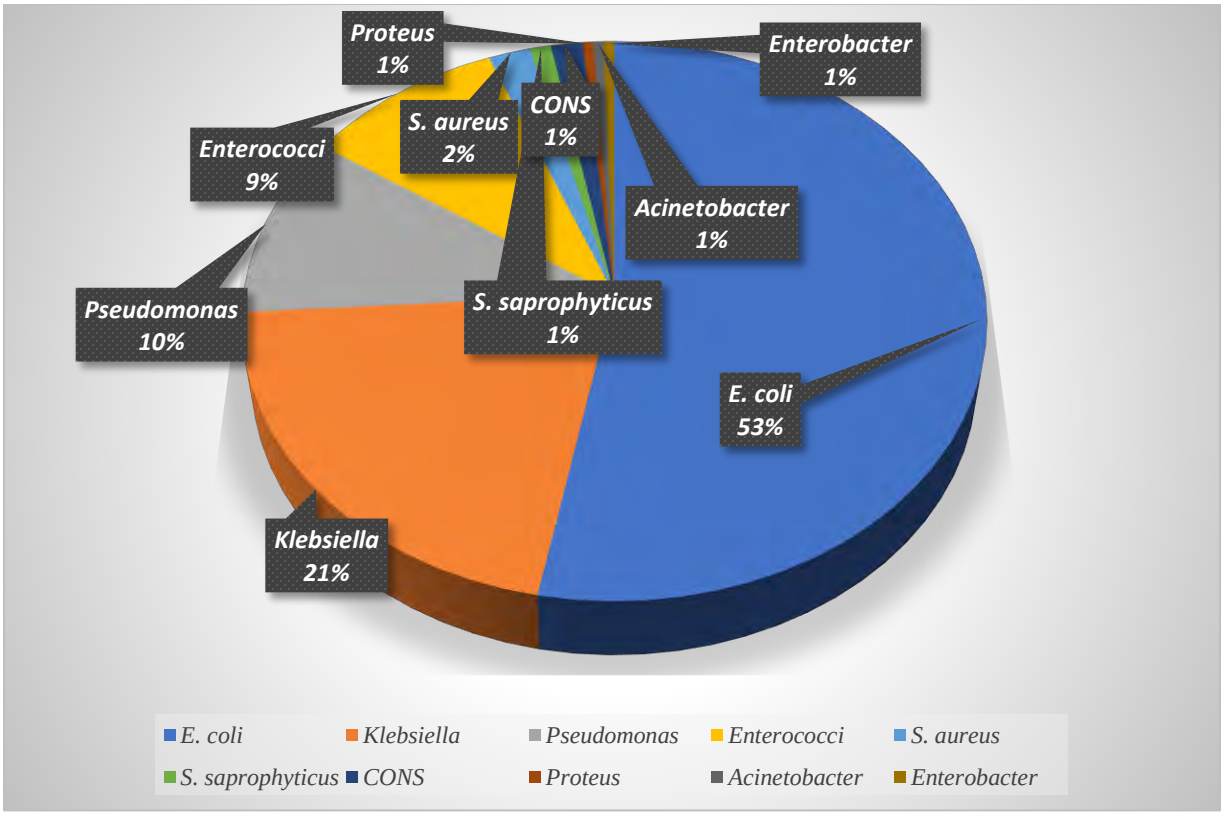


Figure 5: Percentages of etiological agents of bacterial infection

4.3 - Percentages of positive patients according to sex

Female patients are usually more affected by urinary tract infection. Among the 200 positive samples, 131 patients were female (66%) and 69 patients were male (34%) which means the number of female sample were almost doubled then male patients.

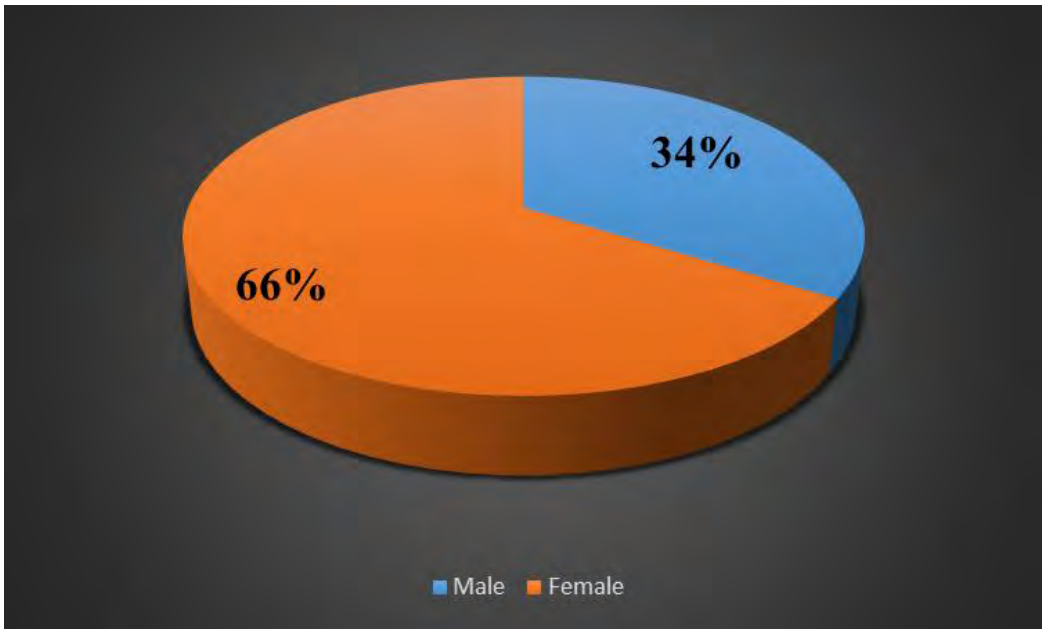


Figure 6: Percentages of positive patients according to sex

4.3.1- Table 6: Ratio of positive sample in male according to age range

	Age range	Number of positive sample
Male	<10 years	3
	>=10 years to <=30 years	22
	>30 years to <50 years	19
	>=50 years	25
Total =69		

4.3.2- Table 7: Ratio of positive sample in female according to age range

	Age range	Number of positive sample
Female	<10 years	3
	>=10 years to <=30 years	53
	>30 years to <50 years	33
	>=50 years	42
Total = 131		

4.3.3 - Percentages of positive patients according to their age range:

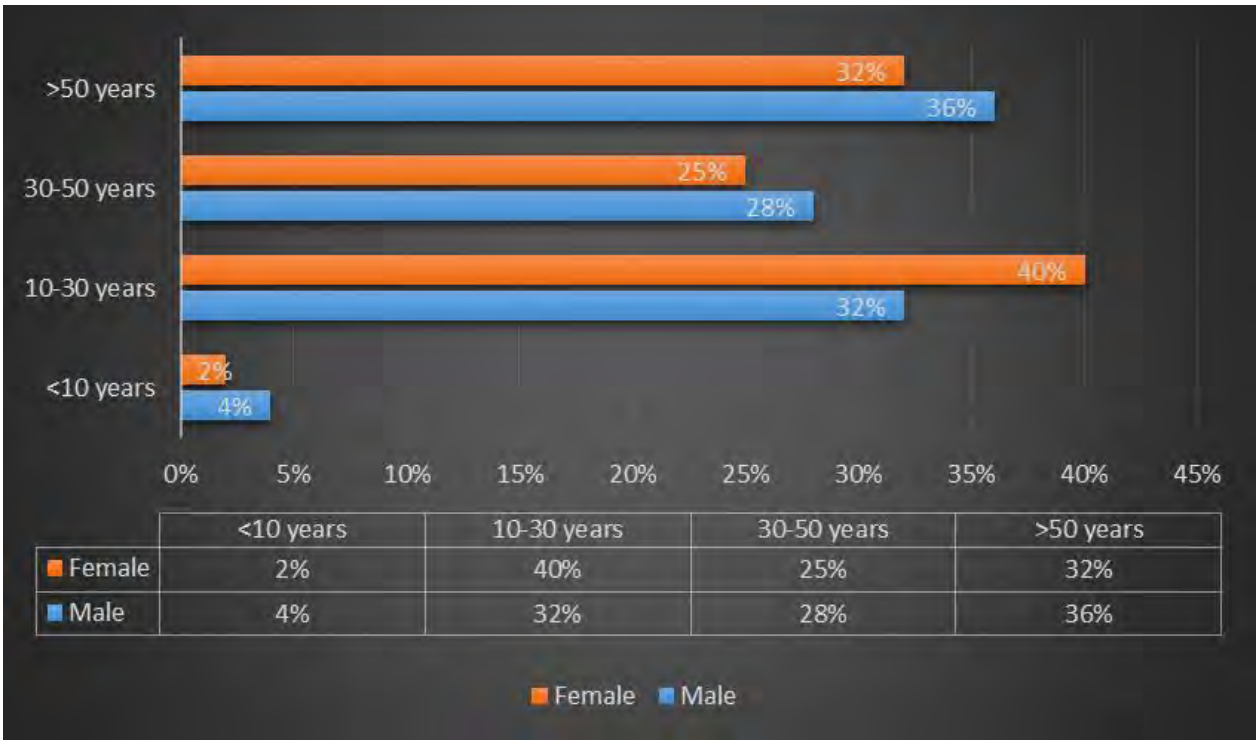


Figure 7: Percentages of positive patients according to their age range

Among Female patients, 10 to 30 years people are most likely to be affected by UTI which was almost 40%. But in male patients, the percentages of affected people are almost same for 10-30 years (32%) and older people then 50 years (36%).

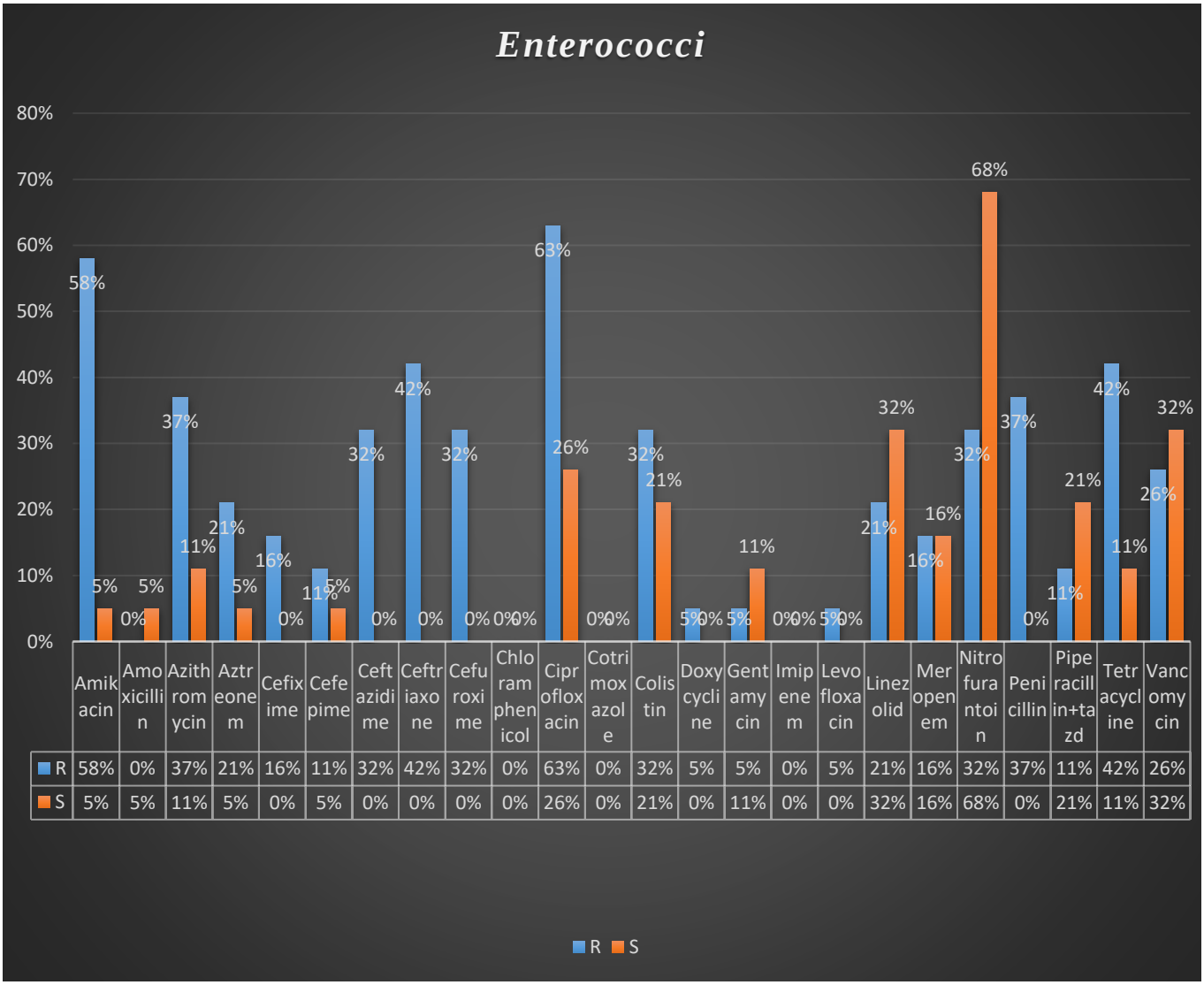
4.4 Antibiotic susceptibility and resistance pattern of different organisms:

Antibiotics name	Gram -positive				Gram-negative							
	Enterococci		Others (*S. aureus, S. saprophyticus, CONS*)		*E.coli*		*Klebsiella*		*Pseudomonas*		Others (*Proteus, Acinetobacter, Enterobacter*)	
	R	S	R	S	R	S	R	S	R	S	R	S
Amikacin	58%	5%	56%	33%	23%	67%	40%	55%	43%	43%	33%	67%
Amoxicillin	0%	5%	0%	0%	14%	5%	14%	5%	0%	0%	33%	33%
Azithromycin	37%	11%	56%	0%	51%	10%	60%	7%	29%	5%	67%	0%
Aztreonem	21%	5%	11%	11%	12%	7%	0%	10%	29%	10%	33%	0%
Cefixime	16%	0%	22%	0%	63%	4%	45%	10%	19%	5%	0%	0%
Cefepime	11%	5%	11%	11%	27%	13%	29%	21%	57%	5%	33%	33%
Ceftazidime	32%	0%	33%	0%	24%	7%	31%	5%	48%	10%	67%	0%
Ceftriaxone	42%	0%	56%	11%	48%	19%	52%	19%	38%	10%	100%	0%
Cefuroxime	32%	0%	33%	0%	44%	5%	48%	7%	14%	0%	67%	0%
Chloramphenicol	0%	0%	0	0	0%	0%	0%	0%	0%	0%	0%	0%
Ciprofloxacin	63%	26%	56%	22%	51%	28%	29%	48%	43%	29%	33%	67%
Cotrimoxazole	0%	0%	22%	11%	7%	8%	12%	10%	10%	5%	33%	0%
Colistin	32%	21%	0%	44%	28%	43%	7%	36%	10%	76%	0%	67%
Doxycycline	5%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Gentamycin	5%	11%	44%	33%	11%	24%	21%	24%	33%	43%	33%	33%
Imipenem	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Levofloxacin	5%	0%	22%	0%	5%	8%	7%	2%	5%	10%	0%	0%
Linezolid	21%	32%	56%	11%	2%	1%	0%	0%	0%	0%	0%	0%
Meropenem	16%	16%	22%	0%	21%	43%	36%	38%	48%	19%	33%	33%
Nitrofurantoin	32%	68%	67%	11%	19%	71%	55%	26%	38%	14%	67%	0%
Penicillin	37%	0%	0%	0%	2%	2%	2%	0%	0%	0%	0%	0%
Piperacillin+tazd	11%	21%	22%	22%	28%	35%	29%	33%	43%	24%	33%	33%
Tetracycline	42%	11%	11%	22%	5%	8%	14%	10%	24%	0%	33%	0%
Vancomycin	26%	32%	33%	22%	0%	0%	0%	0%	0%	0%	33%	0%

4.5 Illustration of antibiotics on gram-positive pathogens

➢ Gram-positive pathogens

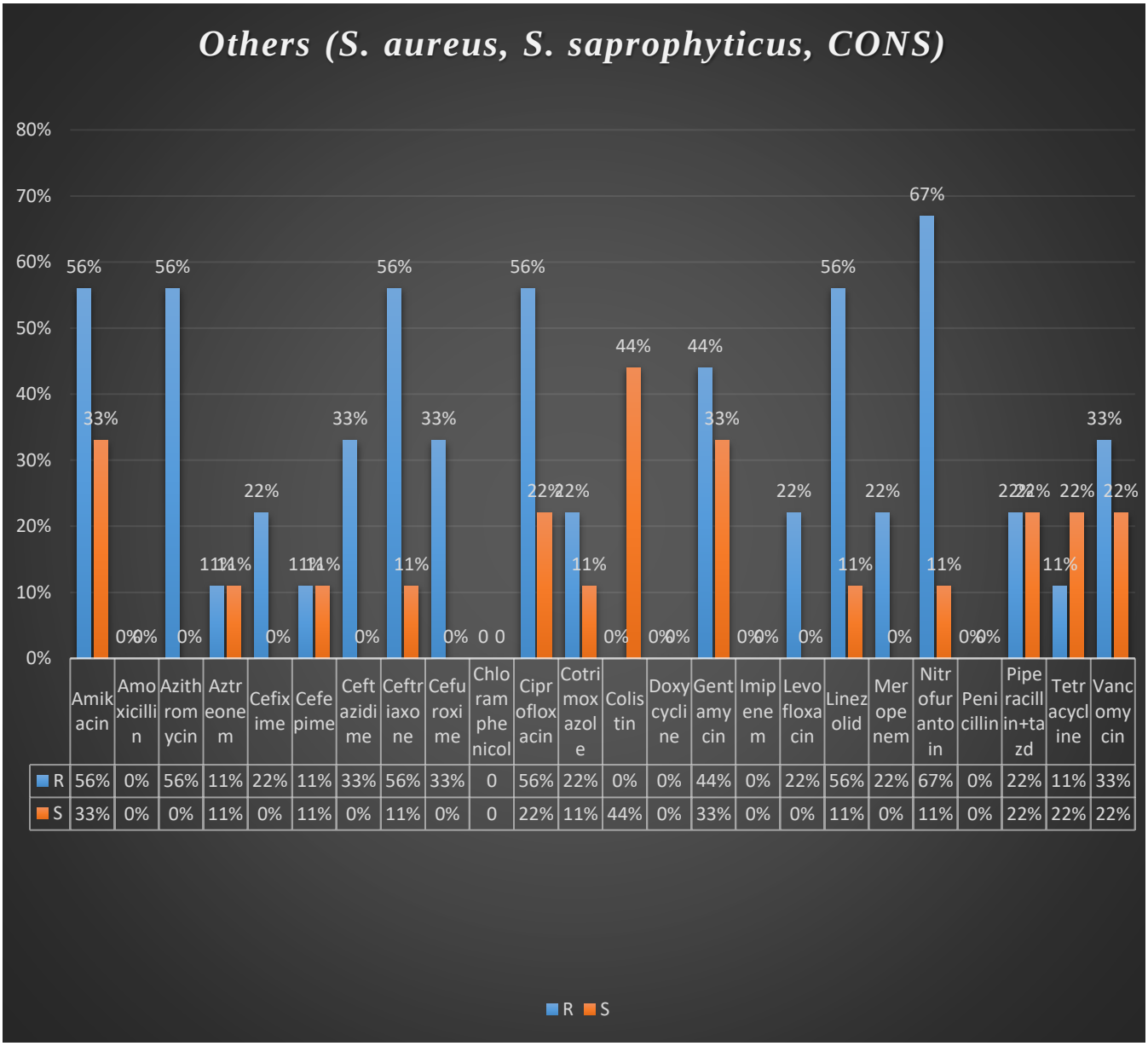
***Enterococci spp*:** Among all the antibiotics, Nitrofurantoin (68%), Vancomycin (32%), Linezolid (32%) and Gentamycin (11%) are sensitive for *Enterococci spp*. Amikacin (58%), Ceftriaxone (42%), Ciprofloxacin (63%), Azithromycin (37%) and tetracycline (42%) are highly resistant to *Enterococci.*



	Amikacin	Amoxicillin	Azithromycin	Aztreonem	Cefixime	Cefepime	Ceftazidime	Ceftriaxone	Cefuroxime	Chloramphenicol	Ciprofloxacin	Cotrimoxazole	Colistin	Doxycycline	Gentamycin	Imipenem	Levofloxacin	Linezolid	Meropenem	Nitrofurantoin	Penicillin	Piperacillin+tazd	Tetracycline	Vancomycin
R	58%	0%	37%	21%	16%	11%	32%	42%	32%	0%	63%	0%	32%	5%	5%	0%	5%	21%	16%	32%	37%	11%	42%	26%
S	5%	5%	11%	5%	0%	5%	0%	0%	0%	0%	26%	0%	21%	0%	11%	0%	0%	32%	16%	68%	0%	21%	11%	32%

Figure 8: Antibiotic resistance pattern of *Enterococci spp.*

Gram-positive others (*S. aureus, S. saprophyticus, CONS*): Antibiotics are more resistance for these organisms. Colistin (44%), Aztreonem (11%) are still an alternative antibiotics for these pathogens. Azithromycin (56%), Ceftriaxone (56%), Ciprofloxacin (56%), Linezolid (56%) and Nitrofurantoin (67%) are highly resistant towards these pathogens.



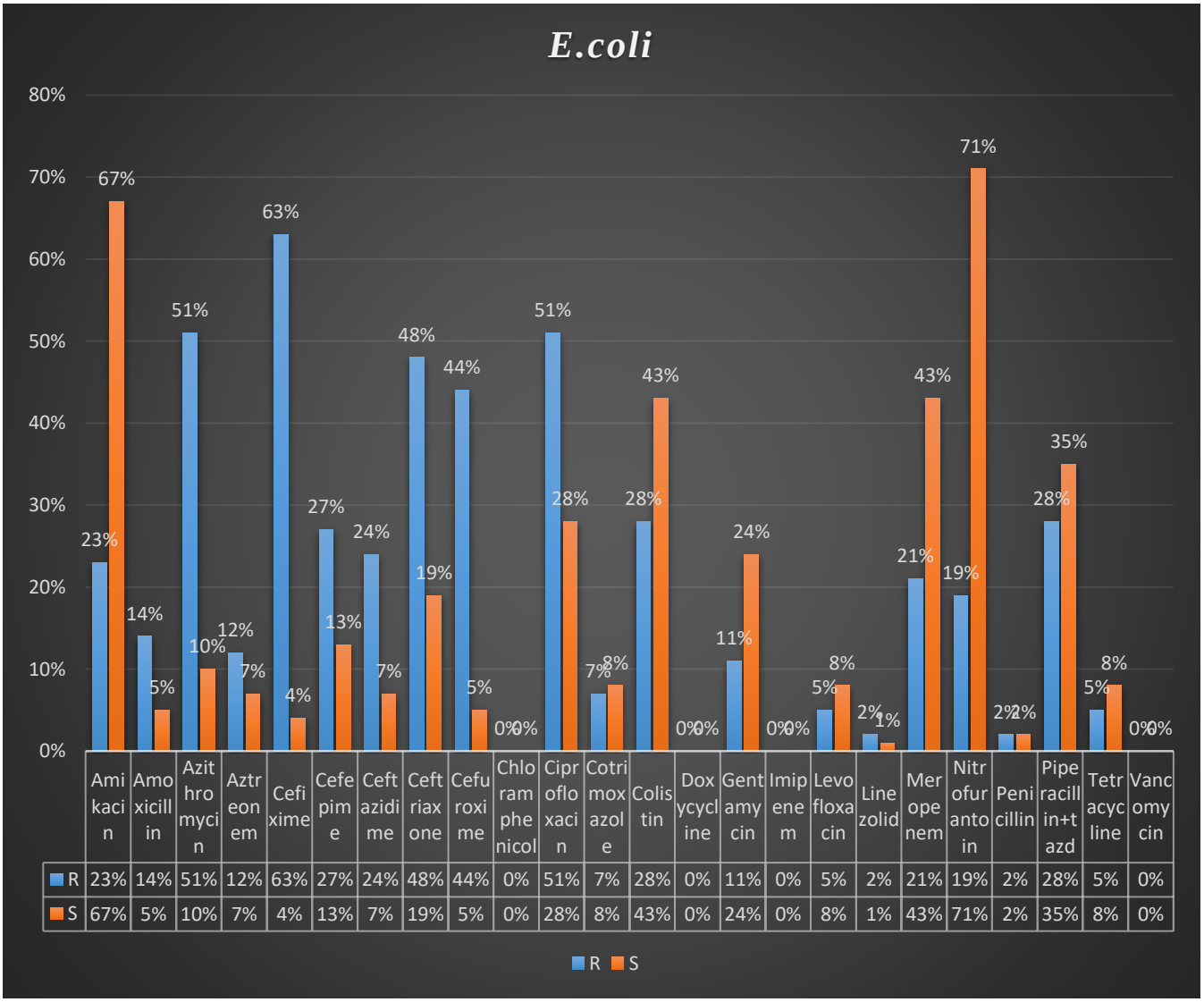
	Amikacin	Amoxicillin	Azithromycin	Aztreonem	Cefixime	Cefepime	Ceftazidime	Ceftriaxone	Cefuroxime	Chloramphenicol	Ciprofloxacin	Cotrimoxazole	Colistin	Doxycycline	Gentamycin	Imipenem	Levofloxacin	Linezolid	Meropenem	Nitrofurantoin	Penicillin	Pipracillin+tazd	Tetracycline	Vancomycin
R	56%	0%	56%	11%	22%	11%	33%	56%	33%	0	56%	22%	0%	0%	44%	0%	22%	56%	22%	67%	0%	22%	11%	33%
S	33%	0%	0%	11%	0%	11%	0%	11%	0%	0	22%	11%	44%	0%	33%	0%	0%	11%	0%	11%	0%	22%	22%	22%

Figure 9: Antibiotic resistance pattern of *S. aureus, S. saprophyticus and CONS*

4.6 Illustration of antibiotics on Gram-negative pathogens

➢ Gram-negative pathogens

***E.coli*:** Most of the antibiotics are resistant to *E.coli* pathogens. Cefixime (63%), Ceftriaxone (48%), Azithromycin (51%), Cefuroxime (44%), Ciprofloxacin (51%) are highly resistant towards E.coli. But still now Amikacin (67%), Colistin (43%), Gentamycin (24%), Meropenem (43%) and Nitrofurantoin (71%) can be a good option to treat patients.



	Amikacin	Amoxicillin	Azithromycin	Aztreonem	Cefixime	Cefepime	Ceftazidime	Ceftriaxone	Cefuroxime	Chloramphenicol	Ciprofloxacin	Cotrimoxazole	Colistin	Doxycycline	Gentamycin	Imipenem	Levofloxacin	Linezolid	Meropenem	Nitrofurantoin	Penicillin	Piperacillin+tazd	Tetracycline	Vancomycin
R	23%	14%	51%	12%	63%	27%	24%	48%	44%	0%	51%	7%	28%	0%	11%	0%	5%	2%	21%	19%	2%	28%	5%	0%
S	67%	5%	10%	7%	4%	13%	7%	19%	5%	0%	28%	8%	43%	0%	24%	0%	8%	1%	43%	71%	2%	35%	8%	0%

Figure 10: Antibiotic resistance pattern of *E.coli*

Klebsiella spp: *Klebsiella spp* is also resistant to most of the antibiotics. But Amikacin (55%), Ciprofloxacin (48%), Colistin (36%), Gentamycin (24%) and Meropenem (38%) are sensitive to *Klebsiella spp.*

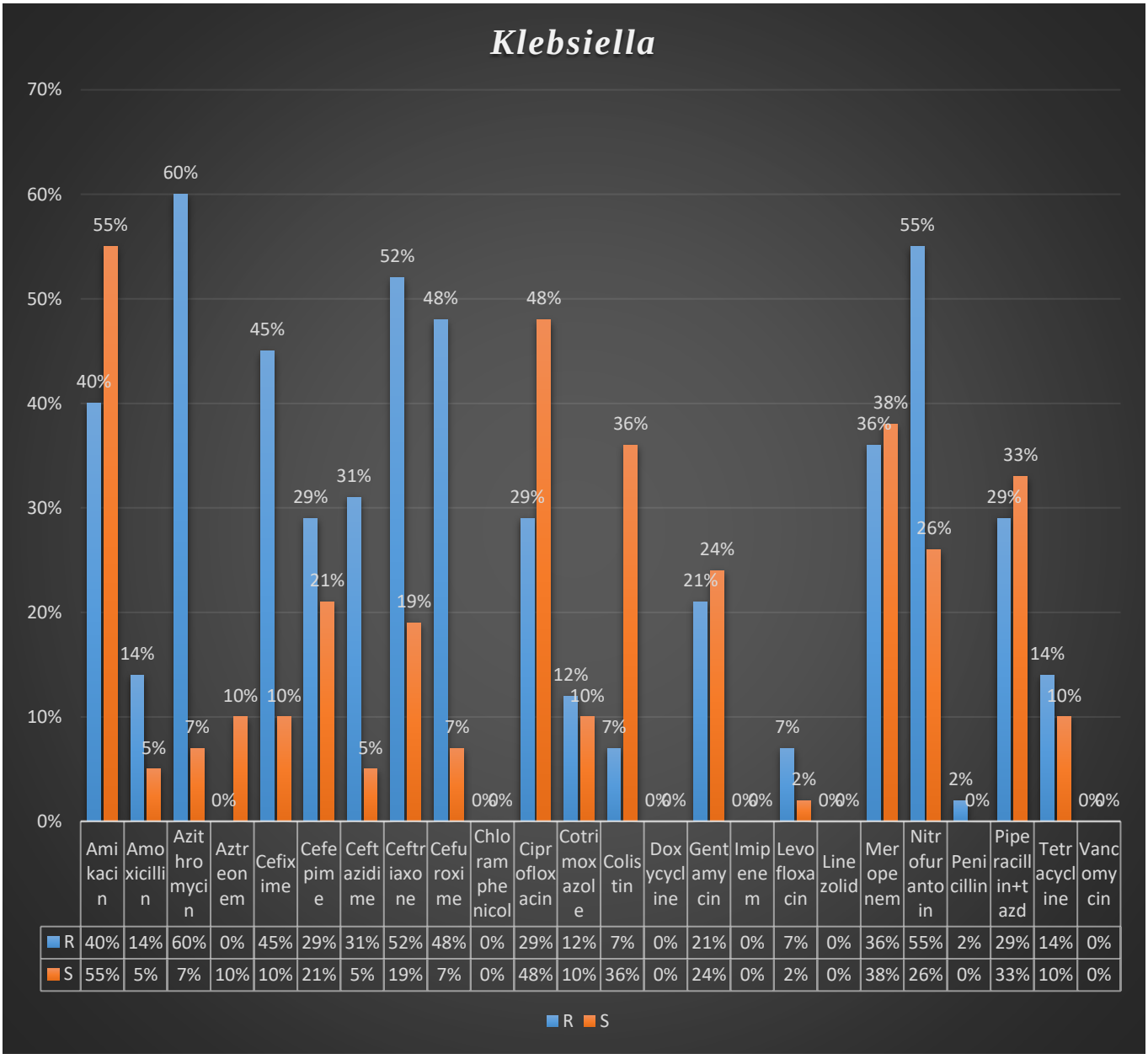
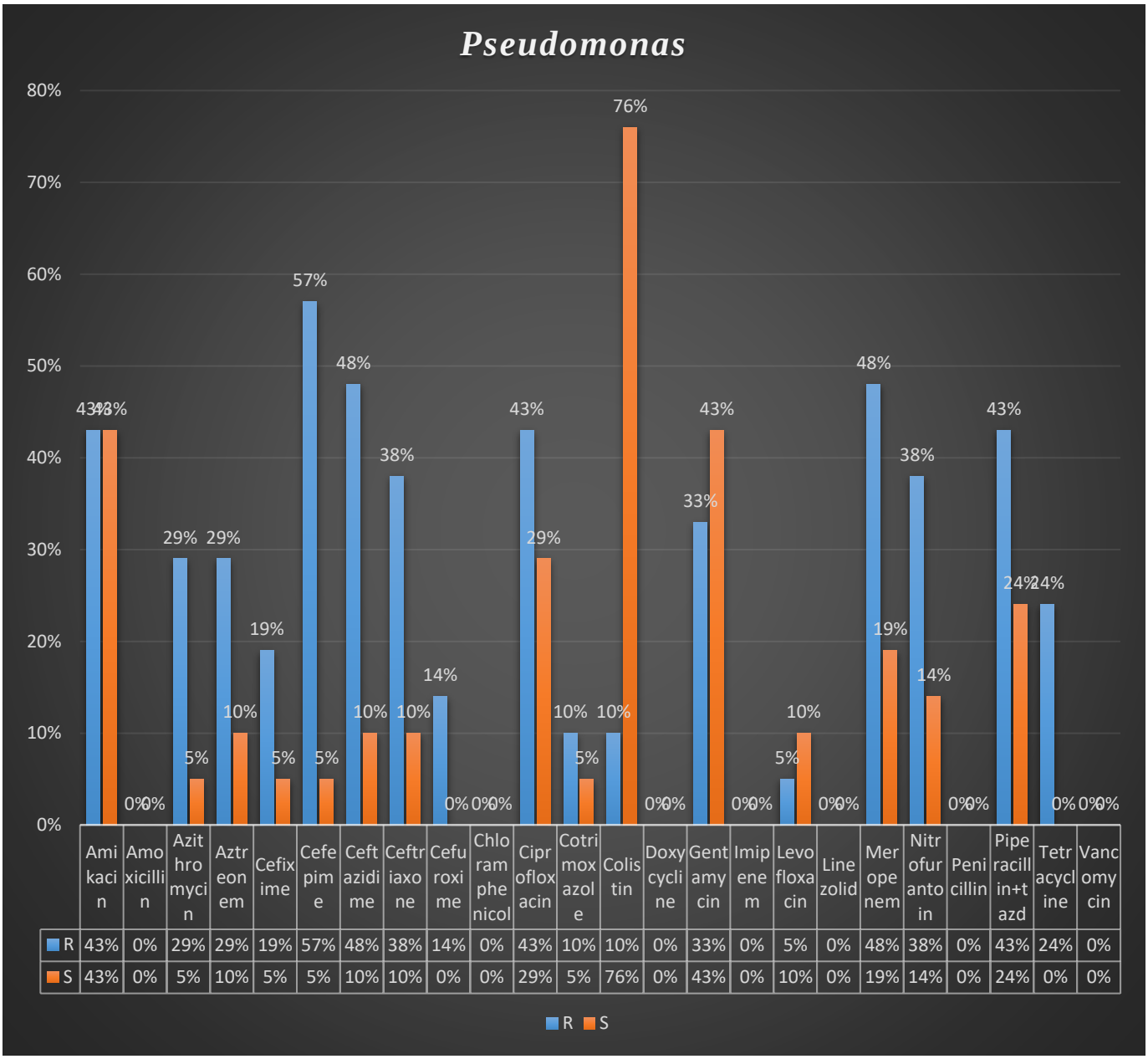


Figure 11: Antibiotic resistance pattern of *Klebsiella spp.*

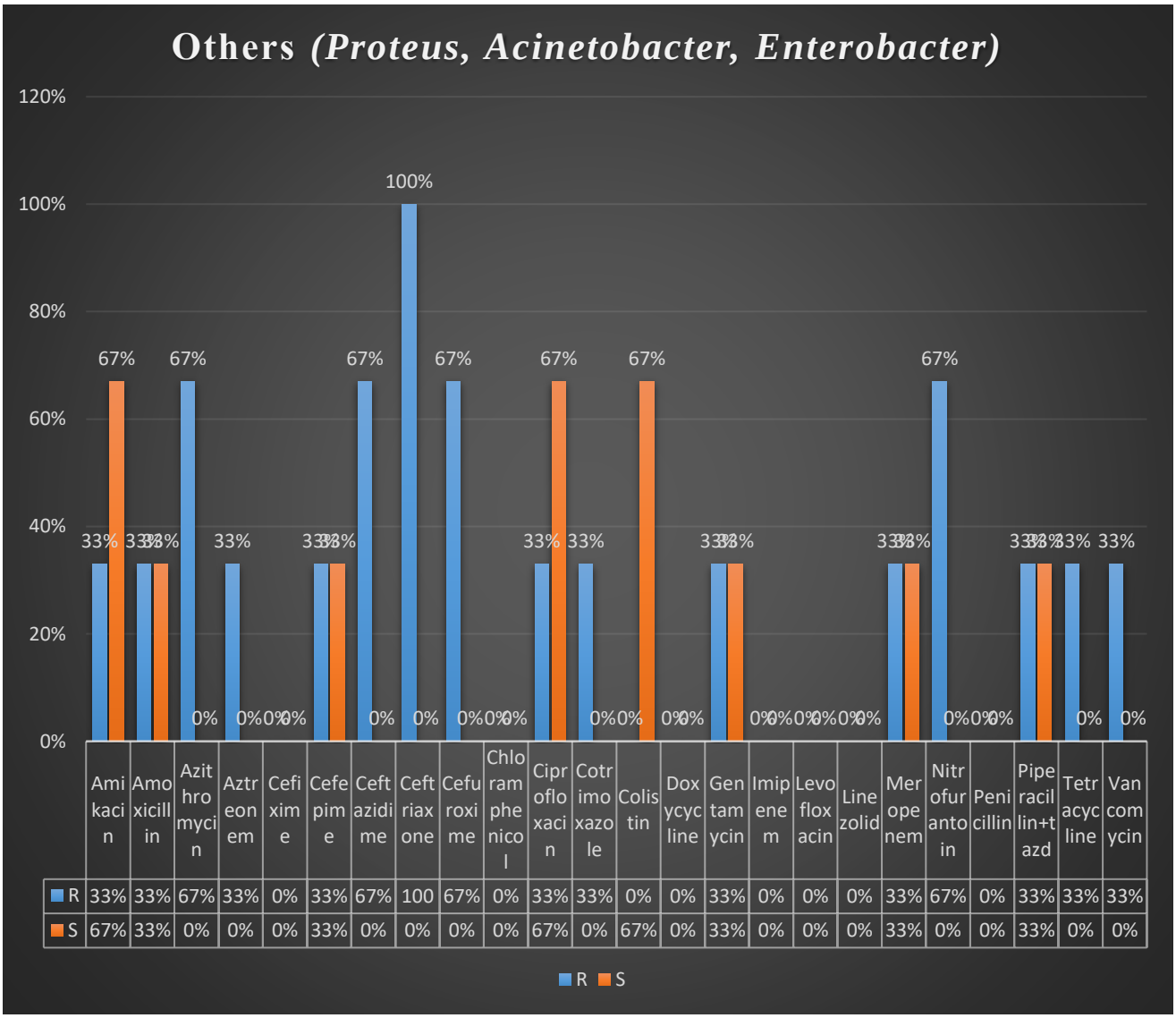
***Pseudomonas spp*:** Colistin (76%) is the most sensitive antibiotic towards *Pseudomonas spp*. Along with Colistin, Gentamycin (43%) and Amikacin (43%) are also useful for patients as sensitive.



	Amikacin	Amoxicillin	Azithromycin	Aztreonem	Cefixime	Cefepime	Ceftazidime	Ceftriaxone	Cefuroxime	Chloramphenicol	Ciprofloxacin	Cotrimoxazole	Colistin	Doxycycline	Gentamycin	Imipenem	Levofloxacin	Linezolid	Meropenem	Nitrofurantoin	Penicillin	Piperacillin+tazd	Tetracycline	Vancomycin
R	43%	0%	29%	29%	19%	57%	48%	38%	14%	0%	43%	10%	10%	0%	33%	0%	5%	0%	48%	38%	0%	43%	24%	0%
S	43%	0%	5%	10%	5%	5%	10%	10%	0%	0%	29%	5%	76%	0%	43%	0%	10%	0%	19%	14%	0%	24%	0%	0%

Figure 12: Antibiotic resistance pattern of *Pseudomnas spp.*

Gram-negative others (*Proteus, Acinetobacter, Enterobacter*) : I got only 3 samples fot these pathogen.



	Amikacin	Amoxicillin	Azithromycin	Aztreonem	Cefixime	Cefepime	Ceftazidime	Ceftriaxone	Cefuroxime	Chloramphenicol	Ciprofloxacin	Cotrimoxazole	Colistin	Doxycycline	Gentamycin	Imipenem	Levofloxacin	Linezolid	Meropenem	Nitrofurantoin	Penicillin	Piperacillin+tazd	Tetracycline	Vancomycin
R	33%	33%	67%	33%	0%	33%	67%	100	67%	0%	33%	33%	0%	0%	33%	0%	0%	0%	33%	67%	0%	33%	33%	33%
S	67%	33%	0%	0%	0%	33%	0%	0%	0%	0%	67%	0%	67%	0%	33%	0%	0%	0%	33%	0%	0%	33%	0%	0%

Figure 13: Antibiotic resistance pattern of *Proteus, Acinetobacter, Enterobacter*

Chapter 5

Discussion

Discussion

The outcome of this thesis is to determine the antibiotic resistance of urine culture patients in Bangladesh. Gram-negative (86%) pathogens have an upper hand in this culture compare to gram-positive (14%) pathogens. In this study, I isolated 200 positive samples from outpatients and inpatients of Rajshahi Medical College (both sex). Few research were found like ours but some information was collected from a study conducted in Multan where 120 samples were collected. Among the positive samples, 67% female and 33% male were found (Muzammil and Adnan et al., 2020). In my study, I found similar result as 66 female and 34 male patients in the positive samples. UTIs are more common in women than in men, and our data support this generalization and line up with findings from a previous study conducted by Deshpande et al., (2011).

According to a study published in the United States, women are estimated to have a lifetime risk of around 50% for developing a UTI, while the lifetime risk for men is significantly lower at around 12% (Medina et al., 2019). In the United Kingdom, research has also indicated a higher prevalence of UTIs among female patients, with approximately 40-50% of women experiencing at least one UTI in their lifetime (Fanshawe & Stoesser et al., 2014). Additionally, studies from countries like Canada, Australia and European nations have reported similar trends, with female patients being more susceptible to UTIs compared to males. Factors such as hormonal changes, sexual activity, and hygiene practices may also contribute to the higher prevalence of UTIs in women (Cuningham & Perera et al., 2021).

One of the main concerns with common bacterial illnesses, such as UTIs, is antibiotic resistance. In many underdeveloped nations, including Bangladesh, antibiotics including azithromycin, ciprofloxacin, cephradin, nalidixic acid, amoxicillin, and cotrimoxazole are still used to treat gram-positive and gram-negative bacterial infections, including UTIs. Regretfully, our investigation revealed that the antibacterial activity of all these medicines against the uropathogens was insufficiently broad. In this thesis study, *E.coli* has shown the most sensitivity towards the antibiotics among all the isolated gram-positive pathogens. On the other hand, Gram-negative bacteria *Klebsiella spp* was highly resistant against to some of the most prescribed antibiotics.

Gram- negative bacteria were mostly resistant against Azithromycin, cefuroxime and cefixime. nitrofurantoin and colistin are highly sensitive for *E.coli, Klebsiella spp, pseudomonas spp* and *Enterococci spp*.

A study conducted in Puducherry, south India from August 2011 to July 2012 involved 547 positive patients of UTI. In their study, *E.coli* was sensitive to amikacin (82.6%), piperacillin-tazobactum (78.2%), nitrofurantoin (82.1%) and imipenem (98.9%) (Niranjan & Malini et al., 2014).). Results from our study showed that *E.coli* was the most prevalent organism (53%). Highly resistant to cefixime (63%), Azithromycin (51%), ciprofloxacin (51%). Studies from European countries, such as the United Kingdom, have also documented high levels of antibiotic resistance in UTI pathogens (Paul et al., 2018). For instance, resistance rates to key antibiotics like ciprofloxacin and nitrofurantoin have been reported to be on the rise, posing a threat to the effectiveness of standard UTI treatments.

On the other hand, study from asian countries such as Pakistan, India, Srilanka reported highly sensitive towards these antibiotics for *Klebsiella spp* (Saha et, al., 2019; Sharma & Thakur et, al., 2023; Saleem & Haseeb et, al., 2023). Our study showed that Nitrofurantoin to be the most sensitive against *E. coli* but a study from Namibia reported moderately resistant against this pathogen. But for *Klebsiella* it is highly sensitive towards nitrofurantoin same as my study (Haindongo & Funtua et, al., 2022). After *E. coli*, *Klebsiella spp.* showed the most multidrug resistance against antibiotics. Several drugs including Azithromycin, Nitrofurantoin were highly resistant for *Klebsiella spp*. In countries like India (Mohapatra & Panigraphy et al., 2022) and China (Yuan & Shi et al., 2021), where antibiotic misuse and overuse are common, UTI pathogens have shown alarming levels of resistance to multiple antibiotics. This has raised concerns about the spread of multidrug-resistant UTI strains and the limited treatment options available for patients.

Conclusion

More than 92 percent of bacteria that cause UTIs are resistant to at least one common antibiotic, and almost 80 percent are resistant to at least two in 2019. At this point, we can say that in the near future people may die from secondary bacterial infections. The rate of antibiotic resistance is almost doubled in past 10 years. If this continues in future, then in no time there will be no antibiotics for us. Developed new antibiotics are a difficult challenge every time. There are several reasons for the rising trend of antibiotic resistance. One major reason is over-prescribing of antibiotics. We have a tendency to take medicines for simple medical issues which is not good for our future medication. Resistance to antibiotics is the best example of it. Because of overuse of antibiotics, the antibiotics are becoming resistant very quickly. Other reason cause could be patients not completing their treatment or doses. Sometimes patients do not finish their course when they feel good after taking initial doses. It is the bad thing for patients. Also misuse of antibiotics is very common mistake. Taking antibiotics without proper culture test and without doctor consultation. For controlling the resistance factor growing awareness is important. For that, government should take necessary steps such as campaign or seminars. WHO (World health organization in November, 2023) showed that one patient can became resistant in just 22 days which means resistance to antibiotics is developing faster than ever. First, second and third stage antibiotics are not fully working on human body. 60-70% UTI bacteria are now resistant to most of the antibiotics. In Bangladesh, from 2017 to 2023, in past 5 years the rate of antibiotic resistance has increased by 11%.

Reference:

- Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. (2022 January 19). 399(605-694).

  https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(21)02724-0/fulltext

- Antimicrobial resistance. (2023 November 21). Retrieved from

  https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

- Antibiotic resistance in Bangladesh rises 11% in 5 years: IEDCR. (2023 November 22). Retrieved from

  https://www.tbsnews.net/bangladesh/health/antibiotic-resistance-bangladesh-rises-11-5-years-iedcr-744522

- 2019 AR Threats Report in the United States. (2023 November 23). Retrieved from https://www.cdc.gov/drugresistance/biggest-threats.html
- National Infection & Death Estimates for Antimicrobial Resistance. (n.d.). Retrieved from

https://www.cdc.gov/drugresistance/national-estimates.html#:~:text=Antimicrobial%20resistance%20is%20an%20urgent,resistant%20infections%20occur%20each%20year

- Urinary Tract Infection. (2021, October 6). Retrieved from https://www.cdc.gov/antibiotic-use/uti.html
- Urinary Tract Infections. (2023, June 4). Retrieved from https://my.clevelandclinic.org/health/diseases/9135-urinary-tract-infections
- Ahmed, I., Rabbi, M. B., Sultana, S. (2019 March). Antibiotic resistance in Bangladesh: A systematic review. 80 (54-61). https://doi.org/10.1016/j.ijid.2018.12.017
- Behzadi, P., Garcia-perdomo, H. A., Gomez, A. M. A., Pinheiro, M., Sarshar, M. (2023 march 23). Editorial: Uropathogens, urinary tract infections, the host-pathogen interactions and treatment. https://doi.org/10.3389%2Ffmicb.2023.1183236

- Cuningham, W., Parera, S., Coulter, S., Nimmo, G. R., Yarwood, T., Tong, S. Y. C., Wozniak, T. M. (2021 Aug 14). Antibiotic resistance in uropathogens across northern Australia 2007–20 and impact on treatment guidelines. 3(3). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8364662/
- Medina, M., Castillo-Pino, E. (2019 May 2). An introduction to the epidemiology and burden of urinary tract infections. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6502976/
- Flores-Moreles, A. L., Walker, J. N., Caparon, M., (2015 May) Urinary tract infections: epidemiology, mechanisms of infection and treatment options. 13(5):269-84. https://doi.org/10.1038/nrmicro3432
- Khan, Z. A., Siddiqui, M. F., Park, S. (2019 June). Current and Emerging Methods of Antibiotic Susceptibility Testing. 9(2): 49. https://doi.org/10.3390%2Fdiagnostics9020049
- Mohapatra, P., Panigraphy, R., Tak, V., J, V, S., Chaudhuri, S., Pundir, S., Kocher, D., Gautam, H., Sood, S., Das, B. K., Kapil, A., Hari, P., Kumar, A., Kumari, M., R, A., Salve, H. R., Malhotra, S., Kant, S. (2022 Feb 9). Prevalence and resistance pattern of uropathogens from community settings of different regions: an experience from India. 4(2). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8941965/
- Minardi, D., d'Anzeo, G., Cantoro, D., Conti, A., Muzzonjgro, G. (2011 April 19). Urinary tract infections in women: etiology and treatment options. 4(333-343). https://doi.org/10.2147%2FIJGM.S11767
- Muzammil, M., Adnan, M., Sikandar, S. M., Waheed, M. U., Javed , N., Rehman, M. F. U. (2020 Feb 16). Study of Culture and Sensitivity Patterns of Urinary Tract Infections in Patients Presenting with Urinary Symptoms in a Tertiary Care Hospital. 12(2). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7081730/
- Paul, R. (2018 Jul-Sep). State of the Globe: Rising Antimicrobial Resistance of Pathogens in Urinary Tract Infection. 10(3): 117–118 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6100332/

- Reller, L. B., Weinstein, M., Jorgensen, J. H., Ferraro, M. J., (2009 December, 1). Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices. 49(11). 1749–1755.

  https://academic.oup.com/cid/article/49/11/1749/344384

- Rizvi, Z. A., Jamal, A. M., Malik, A. H., Zaidi, S. M. J., Rahim, N. U. A., Arshad, D. (2020 Aug 14). Exploring Antimicrobial Resistance in Agents Causing Urinary Tract Infections at a Tertiary Care Hospital in a Developing Country. 12(8): e9735.

  https://doi.org/10.7759%2Fcureus.9735

- Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., Alqumber, M. A. A. (2023 July). Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. 11(13), 1946.

  https://doi.org/10.3390%2Fhealthcare11131946

- Shaifali, I., Gupta, U., Mahmood, S. E., Ahmed, J., (2012 April). Antibiotic Susceptibility Patterns of Urinary Pathogens in Female Outpatients. 4(4): 163–169. https://doi.org/10.4103%2F1947-2714.94940
- Vazquez-Montes, M. D. L. A., Fanshawe, T., Stoesser, N., Walker, A. S., Butler, C., Hayward, G. (2024 Jan 10). Epidemiology and microbiology of recurrent UTI in women in the community in Oxfordshire, UK. 6 (1). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10781434/
- Yuan, S., Shi, Y., Li, M., Hu, X., Bai, R. Trends in Incidence of Urinary Tract Infection in Mainland China from 1990 to 2019 (2021 Apr 20). (14) 1413-1420. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8068484/

Appendix I

Instruments & Reagent kits

The important equipments used through the thesis are listed below:

1. Petri dish
2. Incubator
3. Sterilizer
4. Sterile loop
5. Refrigerator
6. Microscope
7. Antibiotic disk
8. Bunsen burner
9. Hot air oven
10. Incinetor
11. Poly-methylvinyl ether/maleic acid,
12. bromothymol blue, and
13. buffers

Appendix II

Media Preparation:

Media compositions have to be appropriate for a media preparation. Standard methods of compositions are very important in each steps of media preparation. The compositions used for different media given below –

A. Composition of blood agar :

Ingredients	Gram/liter
Peptone	10.0
Tryptose	10.0
Sodium chloride	5.0
Agar	15.0

B. Composition of MacConkey Agar :

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

C. Composition of MHA :

Ingredients	In Gram/Litre
Beef Extract	2.0 gm
Acid Hydrolysate of Casein	17.50 gm
Starch	1.50 gm
Agar	17.0 gm
Distilled Water	1000 ml

D. Composition of Hi-Chrome UTI Agar :

Ingredients	Amount (Gms/litre)
Peptone	18000
Casein enzymic hydrolysate	4000
Meat extract	6000
Bile salts	1500
Chromogenic mixture	12440
Agar	15000
Final pH (at 25°C)	7.2

E. Composition of MIU media :

Ingredients	Amount (g/L)
Tryptone	30.0
Sodium Chloride	5.0
Potassium Dihydrogen Phosphate	5.0
Phenol Red	0.004
Agar	3.0
Final pH	6.9 ± 0.2

F. Composition of Triple Sugar Iron (TSI) Agar

Ingredients	Gms/liter
Pancreatic Digest of Casein	15.0
Lactose	10.0
Sucrose	10.0
Sodium Chloride	5.0
Peptic Digest of Animal Tissue	5.0
Yeast Extract	3.0
Beef Extract	3.0
Dextrose	1.0
Ferric Ammonium Citrate	0.5
Sodium Thiosulfate	0.3
Phenol Red	0.024
Agar	12.0