ANTIBIOTIC RESISTANCE PATTERN OF *ESCHERICHIA COLI* ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTION

BY

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A thesis is submitted to the Department of Mathematics and Natural Sciences

in partial fulfillment of the requirements for the degree of Bachelor of Science in Microbiology

Department of Mathematics and Natural Sciences

Brac University

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis 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.

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Approval

This to announce that the thesis entitled "Antibiotic Resistance Pattern of *Escherichia coli* Isolated from Patients with Urinary Tract Infection" put together by Arifa Taseen, has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Microbiology on 5th September 2019.

It is additionally announced that the exploration work exhibited here is unique, has not been submitted anyplace else for any degree or certificate. Any reference to work done by some other individual or organization has been properly referred to and referenced.

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Abstract

Urinary tract infections are considered as the second most common type of infection occurred in body. *Escherichia coli* is the most common cause of urinary tract of infection for both male and female, higher percentage found in female. This study "Antibiotic Resistance Pattern of *Escherichia coli* Isolated from Patients with Urinary Tract Infection" was done with aim of surveying the amount of resistance among strain of *Escherichia coli*. In this present study, 300 *E. coli* samples were collected from patients with urinary tract infections from which 70% were female and 30% were male. These positive 300 samples of *Escherichia coli* were then taken for the antibiotic susceptibility testing which was performed using Kirby-Bauer disk diffusion method. Macrolides, beta lactam and quinolone group showed most of resistance with Azithromycin 78%, Cetfazidime 63%, ceftriaxone and cefuroxime 59%, Nalidixic acid 69%, Ciprofloxacin 55% and Levofloxacin 52%. From other groups, Cotrimoxazole showed 67% resistance. Aminoglycoside group showed less resistance with Amikacin 6%, Netilmicin 9%, tobramycin 15% and gentamicin 12%.From other groups less resistance showed with Meropenem 6%, Nitrofurantoin 17% and Colistin 20%.Besides that, patients resistant to multi-drug was 89.33% .This study showed increased resistance to antibiotics in *Escherichia coli* that is a serious warning to the treatment of infections caused by this bacterium in the region. Therefore, in order to prevent increased resistance to other antibiotics, it is essential to withhold prescriptions and unessential use of available antibiotics.

Dedicated

To

Almighty, My Beloved

Parents and Husband

Acknowledgement

I am incredibly thankful to Allah, who presented me the comprehension and diligence to make this achievement conceivable.

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Arifa Taseen

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List of abbreviation:

MDR	**Multi-drug resistant**
UTI	**Urinary Tract Infection**
AST	**Antibiotic Susceptibility Test**
MHA	**Muller Hinton Agar**
CFU	**Colony Forming Unit**
Ml	**Mililitre**
Spp.	**Species**

Chapter 1

Introduction

1 Introduction

Antibiotics are dominant medicines that fight infections also can save lives when used properly. They either stop bacteria from reproducing or destroy them (Nordqvist C., 2019). The immune system can typically kill them before bacteria can multiply and cause symptoms. White blood cells (WBCs) can kill harmful bacteria and, the immune system can usually survive and fight off the infection caused by bacteria.

Penicillin was the first antibiotic. Antibiotics that are penicillin based for example amoxicillin, ampicillin and penicillin G, are quiet available to treat a variation of infections and have been there for a long time. From time to time, harmful bacteria are extreme in number, and the immune system cannot battle them all. Antibiotics are very much useful in this situation.

Various kinds of present day anti-toxins are normally just accessible with a solution in greatest nations. Contemporary anti-infection agents are accessible in over-the-counter (OTC) emulsions and treatments. Centers for Disease Control (CDC), said that antibiotic overuse of outpatient is a particular problem .Use of, a noteworthy class of last-line antimicrobials which is carbapenem. They expanded expressively from 2007 to 2010.Alexander Fleming, talking in his Nobel Prize acknowledgment discourse in 1945, stated: "At that point there is the peril that the oblivious man may effectively under dose himself and by presenting his organisms to non-deadly amounts of the medication, make them safe."

There are two principle manners by which anti-infection agents mark microscopic organisms. They either kill the microorganisms or it blocks the multiplication of microscopic organisms, for instance by stopping the instrument in charge of structure their cell dividers. Anti-toxins act by disrupting different sub-atomic focuses inside microorganisms and cell surface, anticipating development or starting executing. 3 expansive instruments:

• Interrupt cell envelope of bacteria

• Block fabricating new proteins

• Inhibit DNA replication (Karanja-Chege C., 2016)

1.1 Literature review

1.2 Antibiotic discovery and resistance timeline

Decades without antibiotic that go on to be used for the treatment of patients have put our defense against bacteria at risk. This timeline pinpoints the year antibiotics were first discovered.

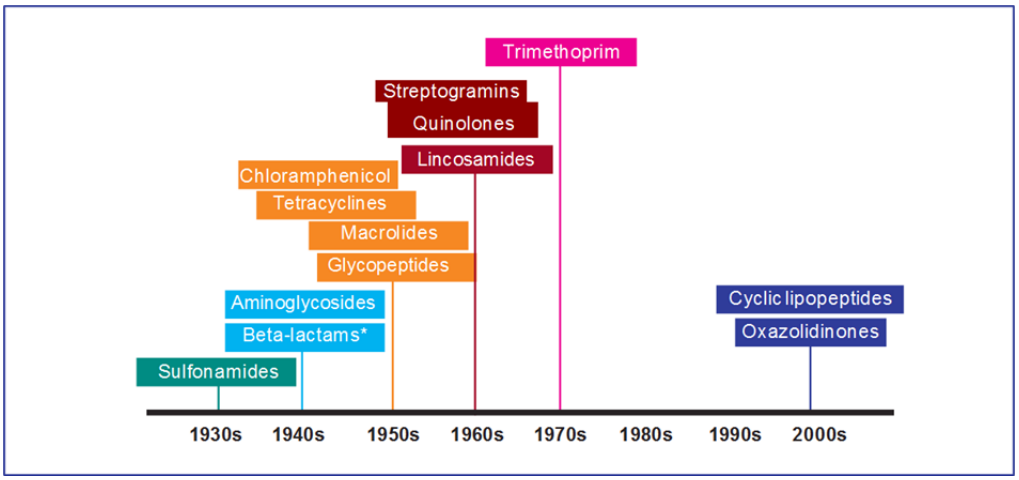

Figure 1.1 : Antibiotic discovery over the years

This timeline shows the year of first resistance and introduction year of some antibiotics.

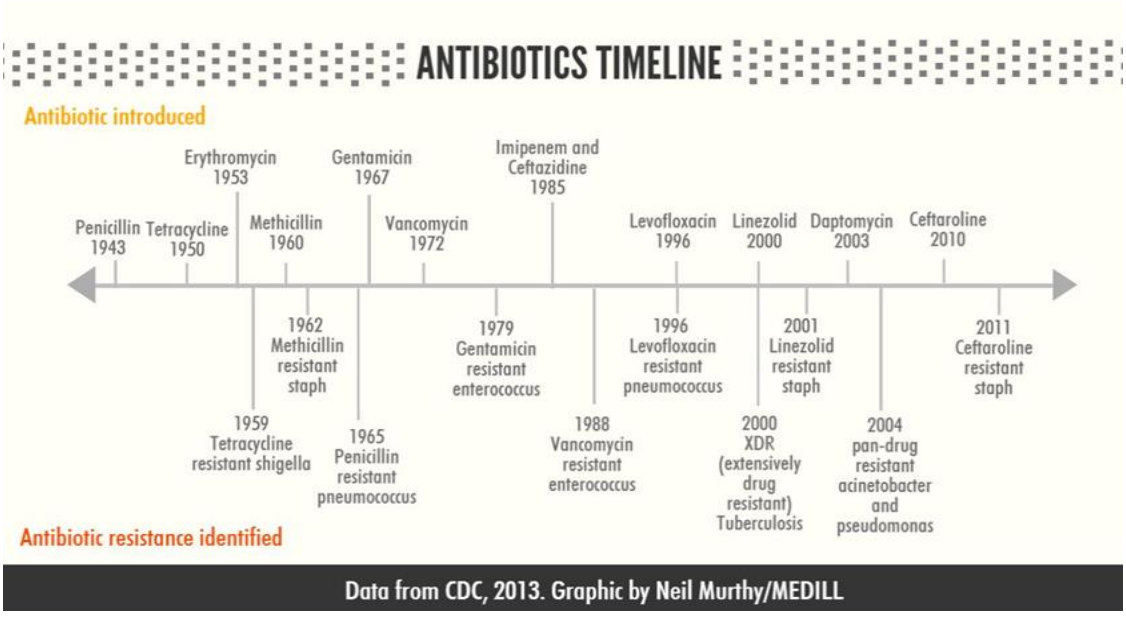

Figure 1.2: Antibiotic resistance identified years

1.3 Classification of antibiotics

INHIBIT		CLASIFICATION		ANTIBIOTICS			
		Beta-Lactamase inhib.	Sulbactam	Clavulanic Acid	Tazobactam	Avibactam	
		Penicillins	***Penicinillase – Sensible***				
			Aminopenicillins (broad spectrum)	Ampicillin Amoxicillin			
			Natural Penicillins (narrow spectrum)	Penicillin G: Na, K, Procainic, Benzathine (IV, IM) Penicillin VK: VO			
			Penicinillase – Resistant *(very narrow spectrum)*				
			Nafcillin	Oxacillin	Dicloxacillin		
			Antipseudomonal *(extended spectrum)*				
			Carboxipenicillins	Ticarcillin	Carbenicillin		
			Ureidopenicillins	Piperacillin	Azlocillin	Mezlocillin	
		Cephalosporins	1° Generation	Cephalexine	Cefazolin	Cefadroxil	
				Cephradrine			
			2° Generation	Cefuroxime	Cefprozil	Cefaclor	
				Cefoxitin	Cefotetan	Loracarbef	
			3° Generation	Cefoperazone	Ceftriaxone	Cefixime	
				Cefpodoxime	Ceftizoxime	Cefotaxime	
				Cefdinir	Ceftibuten	Ceftazidime	
				Cefditoren			
			4° Generation	Cefepime	Cefpirome *		
			5° Generation	Ceftaroline	Ceftolozane		
		Carbapenems	Meropenem	Ertapenem	Doripenem	Imipenem + Cylastatine	
		Monobactams	Aztreonam				
	No lactam	***Glycopeptides***	Vancomycin	Telavancin	Dalbavancin	Oritavancin	
		Other	Colistin	Polymyxin B	Daptomycin	Isoniazid	
Protein Synthesis	***30S***	***Amino-glycosides***	Amikacin	Gentamicin	Tobramycin		
			Streptomycin	Neomycin			
		Tetracyclins	Doxycycline	Minocycline	Tigecyclin		
			Tetracyclin	Demeclocylin*			
	50S	***Oxazolidonones***	Linezolid	Tidezolid			
		Streptogramins	Quinupristin/Dalfopristin				
		Cloramphenicol					
		Macrolides	Erythromycin	Azithromycin	Clarithromycin		
		Lincosamides	Clindamycin	Lincomycin			
DNA topoisomerases		***Fluorquinolones***	Ciprofloxacin	Moxifloxacin	Levofloxacin	Gemifloxacin	
			Norfloxacin	Ofloxacin	Enofloxacin	Sparfloxacin	
		Quinolones	Nalidixic Acid				
Folic Acid Synthesis		***Sulfonamides***	Sulfamethoxazole (SMX)	Ag Sulfadiazine	Sulfasalazine	Sulfisoxazole	
		DHFR inhibitors	Trimethoprim (TMP)	Pyrimethamine			
DNA (damage)		***Nitroimidazoles***	Metronidazole	Tinidazole			
mRNA synth.		Rifampim					

Figure 1.3: Classification of antibiotics

Among all the groups of antibiotics some of them are given below:

1.4 Aminoglycosides and macrolides

Aminoglycosides are wide range(broad spectrum) antibacterials detached from *Streptomyces* and *Micromonospora* microorganisms, and they are portrayed by at least two amino sugars connected by glycosidic bonds to an aminocyclitol segment (Bruce et al., 2011). Aminoglycosides are controlled both remedially and prophylactically to treat dairy cattle, swine, and poultry. Inappropriate utilization of aminoglycosides may create deposits in creature tissues that are conceivably hurtful because of their oto-, neuro-, and nephrotoxicity. Despite the fact that objective modification is a typical component of bacterial medication opposition, clinical aminoglycoside obstruction is commonly not showed by changes of the ribosome. Aminoglycoside-creating life forms have a scope of protective choices accessible to keep away from self-inactivation, including target modification and enzymatic inactivation of the medication. Notwithstanding, to further oppose the discharged dynamic mixes, numerous aminoglycoside-creating living beings additionally express rRNA methylases fit for changing the 16S rRNA atom at specific positions ,in this way averting further authoritative of the medication (Blanchard J.,2009). Inherent obstruction is portrayed by the constitutive articulation of efflux siphons making a characteristic low-level opposition different anti-toxins .They demonstrates great action against high-impact G+ ves, no movement on anaerobes.

Macrolides have a place with the polyketide class of regular items. Some macrolides have anti-toxin or antifungal movement and are utilized as pharmaceutical drugs. They tie firmly to the 50s subunit anticipating way out of the recently orchestrated peptide and consequently blocking protein production. Macrolides are protein amalgamation inhibitors. The instrument of activity of macrolides is hindrance of bacterial protein biosynthesis, and they are thought to do this by counteracting peptidyltransferase from adding the developing peptide appended to tRNA to the following amino corrosive. Azithromycin has been utilized to treat strep throat (Group A streptococcal (GAS) contamination brought about by Streptococcus pyogenes) in penicillin-touchy patients, anyway macrolide-safe strains of GAS are normal. Cephalosporin is another alternative for these patients (Klein J., 1997).

1.5 Beta lactam antibiotics

β- lactam antibiotics (beta-lactam antibiotics) are a class of antibiotic consisting of all antibiotic agents that contain a beta-lactam ring in their molecular structures. This includes penicillin derivatives (penams), cephalosporins (cephems), monobactams, carbapenems and carbacephems. Most β-lactam antibiotics work by inhibiting cell wall biosynthesis in the bacterial organism and are the most widely used group of antibiotics. Until 2003, when measured by sales, more than half of all commercially available antibiotics in use were β-lactam compounds (Elander R.P., 2003). Interest quickly developed, after the introduction of penicillin, as to the mechanism of penicillin action on bacteria. Investigations of the mode of action of β-lactam antibiotics had progressed through several seemingly independent routes. The beta-lactam antibiotics inhibit bacterial cell wall synthesis by targeting bacterial transpeptidase, the enzyme responsible for cross-linking of peptidoglycan subunits within the bacterial cell wall. Without the structural support of cross-linked peptidoglycan, bacteria are sensitive to a variety of environmental stresses that result in lysis of the bacteria; consequently, beta-lactam antibiotics are bactericidal. Several basic mechanisms have evolved among bacteria which can render microorganisms impervious to the actions of beta-lactam antibiotics. Depending on their individual structure, certain beta-lactams may or may not be prone to these resistance mechanisms. Certain bacteria have developed enzymes, known as "beta-lactamases", which can cleave the beta-lactam ring and thus inactivate the drug. Different beta-lactams are more or less susceptible to these beta-lactamases and relatively resistant beta-lactams are used during therapy if beta-lactamases are of concern. Beta-lactam antibiotics normally reach the peptidogycan layer of gram negative organisms by passing through pores within the bacterial outer membrane created by "Porin" molecules. Certain gram negative bacteria possess mutated porin molecules through which certain beta-lactams cannot pass, thus rendering these organisms impervious to the antibiotic.

1.6 Fluoroquinolones

Due to their activity against Gram-negative pathogens, including uropathogens, the most representative quinolones (e.g. ciprofloxacin, ofloxacin and levofloxacin) are indicated for the short-term treatment (3 days) of UTIs (Appelbaum et al.,2000) .Quinolone molecular structure-activity relationships: what we have learned about improving antimicrobial activity.However, an excessive use of both oral and parenteral fluoroquinolones for UTIs or other infections has increased the fluoroquinolone resistance rate among the most common uropathogens.According

to the ECDC 2015 report, resistance to fluoroquinolones among *Enterobacteriaceae* has reached a very high level (22.8% in *E. coli*, 29.7% in *K. pneumoniae*).European center for disease prevention and control. As a result, fluoroquinolones are no longer appropriate for empiric treatment of uncomplicated UTIs, as stated by the main national and international guidelines. Instead, fluoroquinolones are now usually recommended for the empiric treatment of UTIs only when local resistance rates are lower than 10%. A 2016 FDA Drug Safety Communication stated that oral and injectable fluoroquinolone antibiotics are associated with disabling and potentially permanent side effects. For this reason, the FDA stated that fluoroquinolones should be reserved for use in patients who have no other treatment options for acute bacterial sinusitis, acute bacterial exacerbation of chronic bronchitis and uncomplicated UTIs because the risk of serious side effects generally outweighs the benefits in these patients.

1.7 Antibiotics from other groups

1.7.1 Nitrofurantoin

Nitrofurantoin was approved by the U.S. Food and Drug Administration (FDA) in 1953 and has been used for more than 50 years (Lindgren P.K., 2015). Many *E. coli* and *Citrobacter* spp. strains are susceptible to nitrofurantoin but activity against *Enterobacter* spp. and *Klebsiella pneumoniae* is moderate. However, *Proteus* spp., *Providencia* spp., *Morganella* spp., *Serratia* spp., *Acinetobacter* spp. and *Pseudomonas* spp. are generally resistant to nitrofurantoin. Nitrofurantoin has low activity against ESBL-producing *E. coli* (70% of susceptible strains)(Lindgren P.K.,2015). Nitrofurantoin is mainly indicated for the treatment of UTIs in females because concomitant prostatitis cannot be ruled out in males and nitrofurantoin does not reach therapeutic levels in prostate tissue. Clinical trials have shown that nitrofurantoin is equivalent to trimethoprim/sulphamethoxazole, ciprofloxacin and amoxicillin/clavulanate (all administered for 3 days) when given for 5 or 7 days in the treatment of uncomplicated UTIs but less effective than comparators when administered for only 3 days. It should be noted that the four-times daily dosing required with nitrofurantoin is not ideal for achieving good patient compliance. Nitrofurantoin must not be used in patients with renal failure or glucose-6-phosphate dehydrogenase deficiency (Huttner A.,2015).

Table 1.1 : Different groups of antibiotics (Karanja-Chege C.,2016)

Group	Example	Mode of Action	Properties
Aminoglycosides	Gentamicin Amikacin Tobramycin Netilmicin streptomycin	Inhibit protein synthesis	Bactericidal Concentration dependent PAE(post antibiotic effect)
Macrolides and ketolides	Azithromycin Telithromycin Erythromycin clarithromycin	Inhibit protein synthesis	Bacteriostatic Time and concentration dependent Long PAE
Beta-lactams	Penicillins Cephalosporins Carbapenems monobactams	Inhibit cell wall synthesis	Bactericidal Time dependent Long PAE on g+ve (carbapenems also g -ve)
Quinolones	Ciprofloxacin Norfloxacin Levofloxacin moxifloxacin	Inhibit DNA gyrase	Bactericidal Conc. dependent Long PAE

1.8 Antibiotic Resistance

The development of antimicrobial agents is one of the most significant achievements in the medical history. The word 'antibiotic' is derived from the Greek words, anti- (against) and bios (life) and it was used before it was established that infections were caused by bacteria, viruses, fungi or prions. Therefore, an antibiotic drug was considered to be a substance which could kill any living organism. However, the word today is used in medical and veterinary sciences to denote a substance, either a chemical agent or a product from a living organism, which kills or inhibits bacteria. Sulfa is–for example–a chemical agent, whereas penicillin is produced naturally by Penicillium notatum. Some antibiotic drugs are naturally derived products with chemical modifications, i.e. fluoroquinolones. Since sulfa and penicillin were first introduced during the 1930s and 1940s, bacteria have revealed a remarkable ability to develop different types of resistance mechanisms which mediate resistance to antimicrobials that are, from the beginning, quite toxic to them. Antibiotic resistance is today one of the most serious threats to public health, and it is a global problem. The evolutionary consequence of a high selective pressure during the years is that there is no longer any antimicrobial drug for which resistance has not been documented (Hasan B., 2013).

A UTI can occur in any part of the urinary tract, such as in the urethra, bladder, ureters, and kidneys. Symptoms include a burning feeling when you urinate, as well as a frequent need to urinate, even when your bladder isn't very full. UTIs can lead to dangerous conditions if not promptly treated.

It is vital to see a doctor as soon as possible, because early treatment with antibiotics can clear up a UTI before it travels to the kidneys. Though antibiotics are the first line of defense against UTIs, there is a reason why they may not always work — namely, antibiotic resistance (Beyer M., 2018). Antimicrobial resistance is a global health problem with far reaching consequences. Antibiotic resistance among commonly pathogenic bacteria is becoming widespread and this is a threat to effective infection control in near future. This, along with similar drug resistance in virus, parasites, and mycobacteria, threatens to pose a serious challenge to infectious disease management as a whole. There are various causes of increasing antibiotic resistance among bacteria. Indiscriminate use of antibiotics, inappropriate dosing, and incomplete treatment in both

humans and also some animals are some of the factors leading to development of resistance in common bacteria (Ray J., 2015).

Factors favoring antimicrobial resistance are mutations, acquiring new genetic material, exposure to cells with new genetic material and use of antimicrobial agents as growth promoters in animal feeds destined for human consumption give rise to multidrug resistance. However, misuse of antimicrobial agents has led to a post antibiotic era which is a current situation worldwide.

The worldwide drug resistance is increasing for both developing and developed countries but in developing countries is multiplied and increased (Gachuhi G.,2010) There is little or no infection control due to financial constrains to implement accepted guidelines related to susceptibility testing in many countries. This compounded by low general education level of the population and their lower standards of living has led to misuse of antibiotics and therefore leading to drug resistance.

1.9 Multi-drug resistance:

Multidrug-resistant organisms (MDRO) are predominantly bacteria, that are resistant to one or more classes of antimicrobial agents (Tenney J., 2018). Multidrug resistant (MDR) bacterial infections are now becoming quite common, not only in hospital settings but also in the community (Ray J, 2015). *Escherichia coli (E. coli),* a common human pathogen of the urinary tract, is well known for being MDR, including resistance to the fluoroquinolones. Similarly, another recent study also depicted high levels of resistance to β-lactam and fluoroquinolones antibiotics in *E. coli* specimens isolated from urinary samples (Ray J., 2015). One of the main risk factors associated with the spread of multidrug-resistant (MDR) strains is the selective pressure of antibiotics, amplified by their overuse or misuse(Concia et al.,2017).Since E. coli is the most common uropathogen in Bangladesh, its drug resistance patterns will have the greatest impact on clinical management and the healthcare costs (Ray J.,2015).

1.9.1 Mechanisms of multi-drug resistance

Antibiotic resistance genes might be transferred to pathogenic bacteria infecting humans, particularly under the selection pressure of antibiotics as well as via the "SOS" response which is a global response to DNA damage .Besides long term exposure of microorganisms to high concentration of antibiotics also giving rise to the multi-drug resistant organisms. Researchers have observed that there has been a "sigmoidal rise in resistance over time in the presence of a constant rate of antibiotic consumption" and a threshold level of antibiotic usage needed to "trigger the emergence of resistance to significant levels. Multidrug resistance in bacteria occurs by the accumulation, on resistance (R) plasmids or transposons, of genes, with each coding for resistance to a specific agent, and/or by the action of multidrug efflux pumps, each of which can pump out more than one drug type.

Global data shows an increasing multidrug resistance among uropathogens to conventional drugs. Drug resistance has emerged even to newer and more potent antimicrobial agents. The resistant pattern has a regional variability and has been changing continuously due to excessive use of antibiotics. However, many studies have indicated the presence of multidrug resistance in organisms causing UTIs is increasing. High multidrug resistance is due to the mal-administration of antibiotics, incorrect use of antibiotics for the prophylaxis of recurrent UTIs and use of drugs over the counter without prescription of the clinicians. However, many studies have indicated the presence of multidrug resistance in organisms causing UTIs.

Multidrug resistance should be monitored worldwide and surveillance systems should be used to determine the etiology for UTIs (Gachuhi G., 2017). There is a worldwide setback in management of many bacterial infectious diseases due to antibiotic resistance. It is estimated that globally 26 % of deaths are due to infectious diseases such as UTIs of which 98 % occur in low income countries.

1.10 The organism: *Escherichia coli*

1.10.1 General characteristics

The bacterium *Escherichia coli* is a Gram-negative, rod shaped, facultatively anaerobic creature that occupies the gut of warm-blooded creatures. Most strains of *E. coli* are kind, however a few strains are pathogenic to people and creatures. In people, they cause gastrointestinal diseases, for example, looseness of the bowels, hemorrhagic colitis, and hemolytic uremic disorder, and extraintestinal contaminations, for example, urinary tract disease and neonatal meningitis (Yang X.,2014). *Escherichia coli* strains are an exceptionally various types of microscopic organisms found normally in the intestinal tract all things considered and numerous other creature species (Hadifar S, 2017).

Escherichia coli are almost universal in the human gastrointestinal tract, in spite of the fact that they represent just a little extent of the general gut vegetation and frequently exist in this setting without trading off host wellbeing. However, *E. coli* communicating destructiveness qualities are equipped for causing an assortment of malady disorders by means of various components and show immense hereditary contrasts inside and between pathotypes (Poolman J.T., 2017)

E. coli can react to natural signal, for example, synthetic compounds, pH, temperature, osmolarity in various truly exceptional ways. For instance, it can detect the nearness or nonappearance of synthetics and gases in its condition and swim towards or far from them. Or on the other hand it can quit swimming and develop fimbriae that will explicitly connect it to a cell or surface receptor. Because of progress in temperature and osmolarity, it can differ with the pore width of its external film porins to oblige bigger particles (supplements) or to bar inhibitory substances.

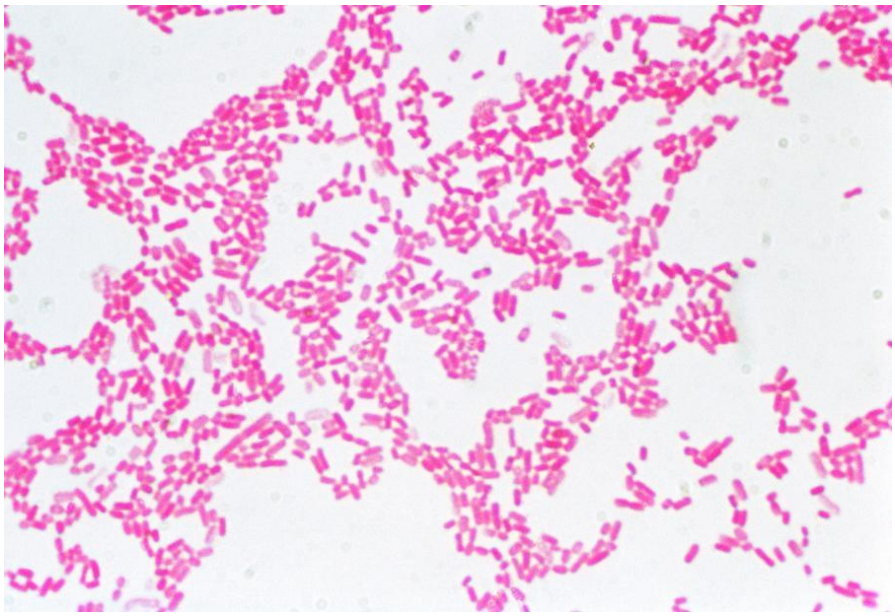

Figure 1.4: Light microscopy of *E. Coli*, the gram negative bacteria is a photograph by Dr. Rosalind uploaded on September 20th, 2018.

There are at least 700 strains of *E. coli*. Most of which don't cause illness on the off chance that they stay in the intestinal lumen. *E. coli* are pathogenic for a few strains and all strains will cause ailment in the event that they contaminate areas that are ordinarily sterile (for example the urinary tract). Truth be told, with people many live commensally smothering the development of pathogenic types of microscopic organisms and delivering vitamin B complex, vitamin K nutrients that are vital for wellbeing.

1.10.2 History

Theodor Escherichia previously portrayed E. coli in 1885, as Bacterium coli collective, which he disconnected from the defecation of babies. Later it was renamed *Escherichia coli* in 1958 out of appreciation for its pioneer. *E.coli* was essentially viewed as a commensal living being of the internal organ for a long time. Until 1935 a strain of *E. coli* was demonstrated to be the reason for an episode of the runs among babies.

E. coli and its relatives are referred to microbiologists as "enteric microscopic organisms". They live in the intestinal tract of people and different creatures. Other enteric microorganisms are *Salmonella*, which incorporates the specialist of typhoid fever, and *Shigella*, which is the bacterial reason for looseness of the bowels.

1.10.3 Role as normal flora

E. coli ordinarily colonizes a baby's gastrointestinal tract inside 40 hours of birth, landing with sustenance or water or with the people taking care of the youngster. In the inside, it holds fast to the bodily fluid of the digestive organ. It is the essential facultative living being of the human gastrointestinal tract. For whatever length of time that these microorganisms don't obtain hereditary components encoding for harmfulness factors, they stay kindhearted commensals.

An extraordinary decent variety of commensal non-pathogenic *E. coli* strains having a place with a wide range of serotypes can be disconnected from the excrement of sound people. These strains are hugely shed in the earth and may taint nourishment of creature birthplace or different sustenance's like vegetables, products of the soil subordinates. They may likewise sully surface and underground water, for the most part with no antagonistic impacts on human wellbeing.

1.10.4 *E. coli* as a pathogen

E. coli from the normal intestinal flora are usually harmless to the host and rather represent opportunistic pathogens. Only in very rare instances can they become a threat to normal individuals. This is mainly the case in patients with impaired immune defenses not able to contain these commensals in their natural habitat or after a traumatic break in the natural barriers between the gut and other normally sterile sites of the body or after surgical interventions. They can also be part of mixed infections when primary pathogens break down the local defenses of a host.

The pathogenic strains of *E. coli* can be classified into different pathotypes, where each pathotype causes a distinct disease (Kaper JB et al., 2004). Strains of *E. coli* can be classified as (i) commensal, (ii) intestinal pathogenic (enteric/diarrheagenic), or (iii) extraintestinal pathogenic *E. coli* (ExPEC) . The intestinal pathogenic E. coli not only colonize the human intestine they also cause diseases ranging from mild diarrhea to severe colitis and dysentery. The extraintestinal pathogenic E. coli reside in the intestine asymptomatically but can cause severe infection upon reaching extraintestinal niches such as the urinary tract or blood stream.

1.10.5 Transmission of *E. coli*

Escherichia coli are basic in all warm-blooded creatures implying that people, residential domesticated animals, pets, wild creatures, and fowls are for the most part supplies. *Escherichia coli* are spread by the fecal-oral course. Transmission to people from these supplies may happen in an assortment of ways incorporating contact with: different people or creatures, debased surfaces (for example entryway handles), excrement or sewage, or debased meat/poultry items, vegetables, crude milk, or untreated water (Kümmerer K, 2004)

Dairy and hamburger steers are essential supplies of *E. coli* O157:H7, (Allen et al., 1999) and they can convey it asymptomatically and shed it in their dung (Allen et al., 1999). Nourishment items related with E. coli episodes incorporate crude ground hamburger, (Institute of Medicine of the National Academies, 2002), crude seed sprouts or spinach, (Barnich N., 2007) crude milk, unpasteurized juice, and sustenance degraded by contaminated nourishment specialists by means of fecal-oral course. (U.S. Branch of Health and Human Services Food and Drug Administration Center for Food Safety and Applied Nutrition, 2006).

Water is a known vehicle for the transmission of microscopic organisms, including *E. coli*, in fixations huge enough to cause ailment in people (Allen et al., 1999). In Canada and the United States, a few huge episodes of *E. coli* O157:H7 have embroiled drinking water as the wellspring of contamination, incorporating a huge flare-up in Walkerton, Ontario in 2000 according to Bruce gray.

As indicated by the U.S. Sustenance and Drug Administration, the fecal-oral cycle of transmission can be upset by preparing nourishment appropriately, avoiding cross-sullying, establishing hindrances, for example, gloves for nourishment specialists, initiating human services arrangements so sustenance industry workers look for treatment when they are sick, purification of juice or dairy items and legitimate hand washing necessities. (U.S. Branch of Health and Human Services Food and Drug Administration Center for Food Safety and Applied Nutrition, 2006) Shiga poison delivering E. coli (STEC), explicitly serotype O157:H7, have additionally been transmitted by flies, (Carl B., 2014) just as immediate contact with homestead creatures, petting zoo creatures, and airborne particles found in creature raising situations.

According to the U.S. Food and Drug Administration, the fecal-oral cycle of transmission can be disrupted by cooking food properly, preventing cross-contamination, instituting barriers such as gloves for food workers, instituting health care policies so food industry employees seek treatment when they are ill, pasteurization of juice or dairy products and proper hand washing requirements. (U.S. Department of Health and Human Services Food and Drug Administration Center for Food Safety and Applied Nutrition, 2006) Shiga toxin-producing *E. coli* (STEC), specifically serotype O157:H7, have also been transmitted by flies, (Carl B., 2014) as well as direct contact with farm animals, petting zoo animals, and airborne particles found in animal-rearing environments.

1.10.6 Infections Caused by Pathogenic *E. coli*

E. coli is responsible primarily for three types of infections in humans: urinary tract infections, neonatal meningitis, and intestinal diseases. These conditions depend on a specific array of pathogenic (virulence) determinants possessed by the organism.

1.11 Urinary Tract Infections

Urinary tract contaminations (UTIs) are the most much of the time announced diseases and drive anti-infection use the world over (Allen et al., 1999). UTIs are the fourth most regular sort of social insurance related disease (Magill et al., 2014). Urinary tract infection (UTIs) are a noteworthy general medical issue as far as bleakness and money related expense, and acquire the most noteworthy complete medicinal services cost among urological ailments, surpassing that of ceaseless renal disappointment notwithstanding when renal dialysis and renal transplantation are included. UTI speaks to one of the most widely recognized infections experienced in restorative practice today with an expected 150 million UTIs every year around the world (Biswas R.,2014) .UTIs are primarily started by the bacterial species. *Escherichia coli* have a place with the particular serogroups of uropathogenic and considered as every now and again distinguished living being. Serogroups have numerous harmfulness factors that are explicit for intrusion of urinary epithelium. *E. coli* is the most widely recognized reason for the uncomplicated UTI and record for about 95% all things considered. *E.coli* is the primary causative factor for the acceptance of UTIs in ladies and furthermore improves the probability of persevering UTIs (Zahra N., 2016).

It is evaluated that 35 % of women who are healthy experience the ill effects of side effects of urinary tract disease sooner or later in their life. The rate of UTI is more prominent in ladies when contrasted with men, which might be either because of anatomical inclination or other host factors (Siddiqua M., 2017). UTI is ordinarily brought about by *Escherichia coli, Proteus, Klebsiella, Enterococcus, and Enterobacter spp*.Most every now and again *Escherichia coli* are secluded in confounded or uncomplicated, nosocomial or network obtained urinary tract diseases. Nearness of in excess of 105 creatures for each ml in a midstream test of pee alludes to huge bacteriuria and caused for the most part by typical inside greenery, *Escherichia coli*, which is in charge of more than 75 % of cases (Haque R., 2015).

1.12 The prevalence of UTI

The predominance of UTI is normally higher in females. The higher pervasiveness is UTI in females is credited to the idea of their urinogental tract; the urethra of the female is a lot shorter and closer to the end than in guys and it additionally does not have the bacteriostatic properties of prostratic emissions. The UTI happen most noteworthy in the explicitly dynamic age gathering. This might be a consequence of expanded sexual action with explicitly dynamic gathering which inclines them to UTI. Sex related pervasiveness of uropathogens among the patients gram negative bars are primary driver of UTIs in both genders. They are generally found in the perineum of the digestive organs as commensals (Ohieku et al., 2013).

Patients were doled out into two classifications: one gathering including pee tests from patients conceded in the emergency clinic for in any event 48 h (medical clinic gained urinary tract contamination or HA-UTI), and another gathering containing pee tests from patients visiting the clinic on an outpatient premise or conceded for lesser than 48 h (network obtained urinary tract disease or CA-UTI) (Ahmed N.H., 2014). Network obtained uncomplicated urinary tract contaminations (UTIs) represent a huge extent of irresistible sicknesses in females , and a considerable measure of oral anti-toxins is endorsed consistently to treat UTIs among females in network based outpatient facilities. Since *E. coli* represents up to 80% of network gained uncomplicated UTIs, these microorganisms ought to be focused on when picking observational anti-infection agents (Lee D.S. , 2018).

1.13 The therapeutic management of urinary tract infections

An increase in resistance has been seen from the most every now and again utilized foundational anti-microbials (for example beta-lactams in addition to β-lactamase inhibitors, trimethoprim/sulphamethoxazole and fluoroquinolones) in both the network and emergency clinic settings over late years, implying that the majority of them are never again reasonable for the empiric treatment of UTIs. What's more, there is an absence of new anti-infection agents. Be that as it may, some old anti-infection agents keep up high microbiological movement against uropathogens. In this way, doctors need to execute explicit remedial systems for every patient, in view of nearby the study of disease transmission of obstruction, accessibility of dynamic anti-infection agents and national and global rule proposals (Concia E., 2017).

1.13.1 Asymptomatic bacteriuria

Asymptomatic bacteriuria is portrayed by the nearness of microbes in urine (≥105 settlement shaping units [CFU)/mL in pee culture], with no particular UTI side effects (dysuria, pollachiuria, fever, and so on.). Bacteriuria must be affirmed by a microbiological trial of a second continuous example in female patients (Nicolle LE., 2005). The pervasiveness of asymptomatic bacteruria shifts between populaces, from 1% in kids to 100% in long haul siphoned patients and it is higher within the sight of anatomic or practical changes of urinary tract, in older patients and in female patients. Because of a bacterial colonization without mucosa attack and fiery reaction, asymptomatic bacteriuria does not speak to a genuine illness.

In this manner, just chosen cases ought to be screened and, in the end, treated. In light of clinical preliminaries demonstrating the viability of treating asymptomatic bacteriuria in decreasing confusions in explicit populaces, screening is shown in pregnant ladies, neutropenic patients and those experiencing surgeries including the urinary tract (situating or substitution of nephrostomy or stents or endoscopic strategies with a mucosal harm).

1.13.2 Uncomplicated cystitis

Uncomplicated cystitis is characterized as an UTI in a generally sound person who does not have any hazard factors for confusions. UTIs in male patients are normally viewed as convoluted in light of the fact that most happen correspondingly with anatomic or useful changes of urinary tract. The danger of creating cystitis in females is similarly high, chiefly because of the short urethra, and is additionally expanded by sexual movement, deferred post-coital micturition,

utilization of stomach or spermicidal gels or a past filled with repetitive UTIs(Hertting O.,2011) .Uncomplicated cystitis is the most continuous UTI (40%) and it is the second most normal contamination in the network setting in Europe, after respiratory tract diseases. *E. coli* is the major causative pathogen (70–95%), trailed by *Staphylococcus saprophyticus* (5–10%), Enterobacteriaceae (*Proteus mirabilis* and *Klebsiella spp.)* and *Enterococcus faecalis*(Lee et al., 2018) .

1.13.3 Catheter-related urinary tract contaminations

A catheter-related UTI is portrayed by in any event a positive urine culture with bacterial burden ≥103 CFU/mL in a siphoned patient with signs or side effects of UTIs, subsequent to barring other potential irresistible destinations (manifestations are frequently non-explicit in a siphoned patient). Another significant symptomatic parameter is the nearness of pyuria, which has a high negative prescient worth. UTIs are the principle nosocomial diseases, for the most part connected with the urinary catheter. A few epidemiologic reviews demonstrated that the urinary catheter is the most utilized nosocomial gadget, situated in 15–25% of hospitalized patients and in at any rate 10% of subjects in LTCFs. The primary hazard factor for catheter-related UTIs is the length of catheterization: it has been evaluated that the danger of bacterial colonization increments by at any rate 3% for every day of catheterization, achieving 100% following multi month. Bacterial colonization as a rule settle after catheter evacuation yet can cause contamination in around 30% of cases(Hertting O., 2011).A monomicrobial greenery (dominatingly *E. coli*, trailed by *K. pneumoniae, P. mirabilis, P. aeruginosa, Enterococcus spp., Staphylococcus spp.*) is normally seen during momentary catheterization (<1 month), while a polymicrobial etiology with regularly non-urological pathogens, for example, Providencia stuartii or Morganella morganii, is commonplace during long haul catheterization (>1 month). A urine culture is unequivocally suggested. On the off chance that a catheter has been set up for about fourteen days or more, it is suggested that it is evacuated before accumulation of pee tests. Table 5 demonstrates the most appropriate remedial alternatives for the empiric treatment of catheter-related UTIs. Expulsion or change of the urinary catheter before beginning anti-infection treatment is suggested (Concia E., 2017).

1.13.4 Urinary tract infections in males

Urinary infections in males generally occur in the presence of anatomic or functional changes of the urinary tract, obstruction (mainly due to benign prostatic hypertrophy) or instrumental procedures. About 20% of communityUTIs affect male patients. The incidence of uncomplicated UTIs, without prostatic involvement or predisposing risk factors, is very low (<1/100 male patients aged 15–50 years) (Hertting O., 2011) .Before starting empiric treatment, a urine culture is strongly recommended. *E. coli* is the main causative pathogen of UTIs in male patients, although the frequency of this pathogen is lower in males versus females, followed by other *Enterobacteriaceae* (*Proteus*, *Klebsiella* and *Enterobacter*), Enterococci and *Pseudomonas* spp. Due to the fact that a prostatic involvement cannot be excluded based on clinical presentation alone, the use of antibiotics reaching therapeutic concentrations in the prostatic tissue is recommended by European Association of Urology (EAU) in 2017 .For this reason, the following regimens represent the most suitable therapeutic options for oral empiric treatment:

• trimethoprim/sulphamethoxazole 160/800 mg, twice daily

• levofloxacin 500 mg, once daily

• ciprofloxacin 500 mg, twice daily

• ciprofloxacin extended-release 1000 mg, once daily

• prulifloxacin 600 mg, once daily.

Few studies have evaluated the optimal treatment duration in male patients, but therapy is usually given for 14 days(European Association of Urology (EAU),,2017). A urologic visit is recommended to evaluate the presence of predisposing risk factors, except for healthy young patients at first UTI that responds quickly to antibiotic treatment (Hertting O., 2011). There are few data on the use of fosfomycin trometamol in the treatment of male UTIs, mainly because its penetration into the prostatic tissue is not well known. A prospective uncontrolled study, performed in 26 men undergoing a transurethral resection of the prostate and taking a single dose of 3 g of fosfomycin trometamol as prophylaxis, showed a tissue antibiotic concentration above the minimum inhibitory concentrations (MICs) of pathogens.

1.13.5Urinary tract infections in post-menopausal women

The urinary tract of elderly people is at high risk of infection, with a 3-fold higher risk in women than in men. The lack of oestrogenic stimulus causes a reduction in the concentrations of *Lactobacillus* in the bacterial vaginal flora, resulting in an increase in vaginal pH, which favors the adhesion of Gram-negative pathogens to epithelium (Ronald et al, 2009). Other risk factors for UTIs in post-menopausal women include urinary or faecal incontinence, neurologic bladder or a clinically significant post void residual, dementia, permanent catheter, vaginal atrophy and deficit of cell-mediated immunity. Treatment recommendations for management of acute UTIs in these patients are the same as those for adult female patients.

1.13.6 Urinary tract infections in diabetic patients

Diabetes mellitus is a predisposing risk factor for UTIs, even though the risk is increased only in female patients. In females with diabetes, UTIs are often associated with severe complications (i.e. pyelonephritis, urosepsis). Treatment recommendations for acute UTIs in this setting are the same as for those in the general population. However, the efficacy of short-term treatment is not supported by statistically significant clinical data.

1.13.7 Mechanism of pathogenicity for *E. coli* UTI

90% of the urinary tract infection (UTI) is caused by *E. coli* in anatomically-ordinary, unhindered urinary tracts. The microscopic organisms colonize from the excrement or perineal district and take the urinary tract to the bladder. Bladder diseases are 14-times more typical in females than guys by prudence of the abbreviated urethra. The ordinary patient with uncomplicated cystitis is an explicitly dynamic female who was first colonized in the digestive system with an uropathogenic *E. coli* strain. The creatures are impelled into the bladder from the periurethral locale during sex. With the guide of explicit adhesion they can colonize the bladder (Morrill et al., 2017).

The adhesion that has been most intently connected with uropathogenic *E. coli* is the P fimbria (or pyelonephritis-related pili [PAP]) (Lee, 2002). The letter assignment is gotten from the capacity of P fimbriae to tie explicitly to the P blood bunch antigen which contains a D-galactose-D-galactose. The fimbriae tie not exclusively to red cells yet to a particular galactose disaccharide that is found on the surfaces uroepithelial cells in roughly 99% of the populace.

The recurrence of the dispersion of this host cell receptor assumes a job in vulnerability and clarifies why certain people have rehashed UTI brought about by E. coli. Uncomplicated E. coli UTI for all intents and purposes never happens in people coming up short on the receptors.

Uropathogenic strains of *E. coli* have different determinants of harmfulness notwithstanding P fimbriae. E. coli with P fimbriae likewise have the quality for Type 1 fimbriae, and there is proof that P fimbriae are gotten from Type 1 fimbriae by inclusion of another fimbrial tip protein to supplant the mannose-restricting area of Type 1 fimbriae. Regardless, Type 1 fimbriae could give an advantageous component of adherence or assume a job in amassing the microscopic organisms to a particular manosyl-glycoprotein that happens in urine.

Uropathogenic strains of *E. coli* more often than not deliver siderophores that presumably assume a fundamental job in iron securing for the microorganisms during or after colonization (Kaper et al, 2004). They likewise produce hemolysins which are cytotoxic because of development of transmembranous pores in host cell layers. One system for getting iron and different supplements for bacterial development may include the lysis of host cells to discharge these substances. The action of hemolysis isn't constrained to red cells since the alpha-hemolysis of *E. coli* likewise lyse lymphocytes, and the beta-hemolysis repress phagocytosis and chemotaxis of neutrophils.

Another factor thought to be engaged with the pathogenicity of the uropathogenic strains of *E. coli* is their protection from the supplement subordinate bactericidal impact of serum. The nearness of K antigens is related with upper urinary tract diseases, and neutralizer to the K antigen has been appeared to bear the cost of some level of security in trial contaminations. The K antigens of *E. coli* are "capsular" antigens that might be made out of proteinaceous organelles related with colonization (e.g., CFA antigens), or made of polysaccharides. Notwithstanding their science, these containers might probably advance bacterial destructiveness by diminishing the capacity of antibodies as well as supplement to tie to the bacterial surface, and the capacity of phagocytes to perceive and inundate the bacterial cells. The best contemplated K antigen, K-1, is made out of a polymer of N-acetyl neuraminic corrosive (sialic corrosive), which other than being antiphagocytic, has the extra property of being an antigenic mask.

1.14 Symptoms of UTI

Not every person with an UTI has side effects, however the vast majority have in any event one. Symptoms may incorporate an incessant inclination to urine and an agonizing, consuming inclination in the zone of the bladder or urethra during urination. It isn't strange to feel dreadful and to feel torment when not urinating. Regularly, women feel an awkward weight over the pubic bone, and a few men experience a completion in the rectum. It is basic for an individual with a urinary disease to grumble that, regardless of the desire to urinate, just a modest quantity of urine is passed. The pee itself may look smooth or overcast, even ruddy if blood is available. A fever may imply that the contamination has come to the kidneys. Different side effects of a kidney disease incorporate agony in the back or side beneath the ribs, dizziness or vomiting.

1.15 Diagnosis of UTI

To see if you have an UTI, your PCP will test urine sample for pus and microorganisms. You will be approached to give a "spotless catch" pee test by washing the genital zone and gathering a "midstream" test of pee in a sterile compartment. This strategy for gathering pee keeps microbes around the genital zone from getting into the example and confounding the test outcomes.

1.16 Treatment of UTI

Urinary tract infection are basic bacterial diseases that influence the urethra, bladder, ureter or the kidney which require suitable anti-microbial mediations to counteract difficulties and further harms to these organs (Ohieku J.,2013)

UTIs are treated with antibacterial medications. The decision of medication and length of treatment relies upon the patient's history and the urine tests that recognize the culpable microorganisms. All things considered, numerous specialists approach their patients to take anti-toxins for up to 14 days to guarantee that the contamination has been relieved.

1.17 Aims and objectives

This aim of this study was to determine the prevalence and diversity of antibiotic resistance in *E. coli* from patients with suspected urinary tract infection. The pattern of antibiotic resistance is the main objective of this study.

Chapter 2

Materials & Methods

2 Materials & methods

2.1Study area

The study was carried out at Islami Bank Central Hospital in Dhaka, Bangladesh. The laboratory processing, analysis of data and the overall experiment was done in Microbiology Research Laboratory of Islami Bank Central Hospital.

2.2 Study duration

The study was conducted during the period May-August, 2019.

2.3 Sample size

A total of about 300 urine samples with positive *E.coli* growth were collected.

2.4 Materials

2.5 Culture media

Culture media utilized for bacterial separation and recognizable proof include:

2.5.1 MacConkey Agar

MacConkey agar is utilized for the separation and segregation of non-fussy (fastidious) gram-negative rods, especially individuals from the family Enterobacteriaceae. It additionally can separate between lactose maturing from non-aging microorganisms.

2.5.2 Mueller Hinton Agar

Mueller Hinton Agar is prescribed for assurance of defenselessness of microorganisms to antimicrobial operators disengaged from clinical examples. Kirby-Bauer et al suggested this vehicle for performing anti-microbial vulnerability tests utilizing a solitary circle of high fixation.. WHO Committee on Standardization of Susceptibility Testing has acknowledged Mueller Hinton Agar for deciding the weakness of microorganisms on account of its reproducibility.

2.6 Method

Sample Collection

2.6.1 Urine

All patients, male or female, associated with side effects with urinary tract disease (UTI with dysuria, hematuria, abrupt incontinence, or expanded recurrence of urination) were temporarily included(Ray J.,2015). The examples were for the most part gathered at early morning since it is increasingly focused and variations from the norm are simpler to recognize. As per NHS tests were generally mid-stream urine. This decreases the danger of the example being debased with microbes from hands, the skin around the urethra, the cylinder that does pee of the body.

The compartments were quickly sent to the microbiology research facility for plating. From the test of urine which is not centrifuged, a preheated and quickly cooled nichrome wire circle was utilized to move a proportion of the example (10 mL) on to the way of life media. The way of life media included MacConkey agar. They were then placed in a hatchery at 37 ºC for 24 h. Just examples demonstrating development of >10^5 CFU/mL (CFU: settlement framing unit), toward the finish of this period were considered as positive and further tests were completed on them (Ray J., 2015).

2.6.2 Colony-forming unit

In microbiology, a colony forming unit (CFU) is a unit used to appraise the quantity of reasonable microscopic organisms in an example. Suitable is characterized as the capacity to duplicate by means of parallel splitting under the controlled conditions. Province shaping units are utilized as a proportion of the quantity of microorganisms present in or on surface of an example. Settlement shaping units might be accounted for as CFU per unit weight, CFU per unit zone, or CFU per unit volume relying upon the sort of test tried. To decide the quantity of province shaping units, test was readied and spread on a surface of an agar and afterward brooded at some appropriate temperature for various days. The provinces that structure are checked. CFU isn't a measure for individual cells or spores as a province might be framed from a solitary or a mass of cells or spores.

Among 300 patients most of them showed 5×10^5 CFU/ml count. Some of the counts are given below:

Table 2.1: CFU count of UTI patients from Urine samples

Sample No	CFU/ml
1	5×10^5
2	5×10^5
3	2×10^5
10	4×10^5
29	10^5
35	3×10^5
65	5×10^4
76	10^3

2.6.3Antibiotic susceptibility testing (AST)

2.6.3.1 Disk Diffusion Method

Standard Kirby-Bauer disk diffusion technique was utilized to decide the antimicrobial specialist susceptibility profiles of the *E. coli* secludes for 16 anti-toxins. These antimicrobial specialists were picked based on their significance in treating human contaminations and based on their capacity to give assorted variety to portrayal of various antimicrobial operator classes (Parveen et al., 2018).Commercially accessible plates (Oxoid Limited, Hampshire, England) were utilized for the test.

2.6.3.2 Inoculation of the Muller Hinton Agar (MHA) plates

By the standard strategy for vaccination, an immunizing needle was contacted to a solitary well-disengaged state, and vaccinated into 2ml of clean saline arrangement. The turbidity of the effectively developing arrangement was then acclimated to a MacFarland 0.5 standard (3×108 CFU/ml). To vaccinate the agar medium, a sterile non-harmful cotton swab was plunged into the institutionalized suspension. Furthermore, overabundance stock was cleansed by squeezing and pivoting the swab immovably against within the cylinder over the liquid level. The swab was then streaked equally in three ways over the whole surface of the agar plates to get a uniform inoculum. A last breadth was made of the agar edge with the cotton swab. This plate was then permitted to dry for 3 to 5 minutes, before the circles were connected.

2.6.3.3 Placing the antibiotic discs on MHA plates

With a sterile forceps antibiotic soaked were applied to the surface of inoculated plates. To have proper contact with agar plate surface ,all the discs were delicately pushed down into the agar with sterile forceps. The plates were modified to upside down position and put in an incubator at 37°C.

2.6.3.4 Measuring zone

After incubation, the bacterial growth around each disc was observed. If the test organism is susceptible to a specific antibiotic an area of “no growth” will be observed .The plates were analyzed after 18 to 24 hours of incubation, and the distances across (in millimeters) of the reasonable zones of development restraint around the antimicrobial disks. Zone of inhibition were estimated by utilizing standard ruler (Clinical and Laboratory Standards Institute, 1997, 1999). By as per the understanding table (provided by Oxoid Limited, England) resistant, intermediate and susceptible condition was being measured.As per recommendation by the National Antimicrobial Resistance Monitoring System for *E. coli* resistant or susceptible condition was measured through break points.

The information was orchestrated in Microsoft Excel worksheet. Graphic factual capacities were utilized. Just the examples demonstrating development of E. coli in the way of life were incorporated into the examination for this investigation about anti-toxin affectability (Ray J., 2015).

Table 2.2: Concentrations and diffusion zone breakpoints for resistance for antibiotics tested in this study

Antibiotics	Abbreviation form	Disc drug concentration(μg)	Diffusion zone breakpoint (mm)		
			Resistant	Intermediate	Sensitive
Amikacin	AK	30	≤14	15-16	≥17
Azithromycin	AZM	15	≤13	14-17	≥18
Ceftazidime	CAZ	30	≤14	15-17	≥18
Ceftriaxone	CRO	30	≤13	14-20	≥21
Cefuroxime	CXM	30	≤14	15-22	≥23
Ciprofloxacin	CIP	5	≤15	16-20	≥21
Co-trimoxazole	SXT	10	≤10	11-15	≥16
Gentamicin	GEN	10	≤12	13-14	≥15
Levofloxacin	LVX	5	≤13	14-17	≥17
Meropenem	MEM	10	≤13	14-15	≥16
Netilmicin	NET	10	≤12	13-14	≥15
Nalidixic acid	NA	30	≤13	14-18	≥19
Nitrofurantoin	F	100	≤14	15-16	≥17
Pipercillin-tazobactam	TZP	110	≤20	21-22	≥23
Tobramycin	TOB	10	≤12	13-14	≥15

Chapter 3

Results

3 Results

3.1 Isolation of *E. coli* from UTI

For the detection of causal bacterial agent from urine, samples were inoculated on MacConkey agar plate. *E. coli* strains were isolated from McConkey agar plate.

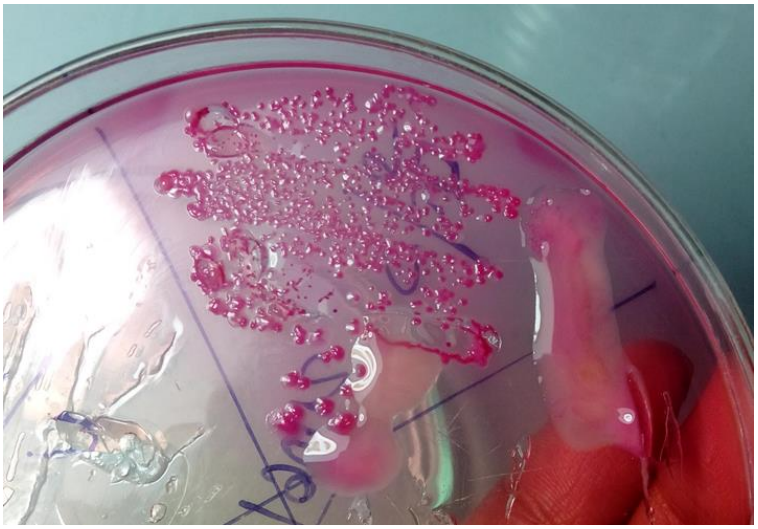

Figure 3.1: Close observation of *E.coli* growth on MacConkey agar plate

3.2 Distribution of UTI cases

3.2.1 Distribution of UTI cases according to sex

Among the 300 diagnosed positive UTI cases, only 90 cases were from male and the rest 210 cases were from female. The relative prevalence are presented in a pie diagram (Fig. 3.2)

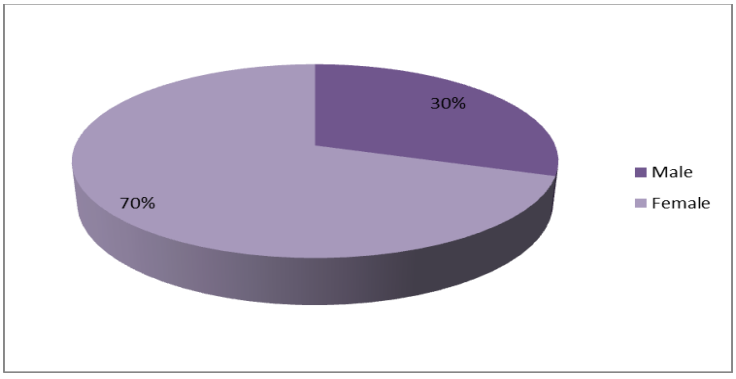

Figure 3.2: Distribution of UTI cases according to sex

3.2.2 Distribution of UTI cases in patients according to age

Age	Total	Male	Percentage	Female	Percentage
0-5	29	8	2.67	21	7
6-10	5	2	0.67	3	1
11-15	8	2	0.67	6	2
16-20	16	4	1.33	12	4
21-25	20	6	2	14	4.67
26-30	17	6	2	11	3.67
31-35	16	7	2.33	9	3
36-40	24	3	1	21	7
41-45	16	4	1.33	12	4
46-50	27	6	2	21	7
51-55	26	7	2.33	19	6.33
56-60	18	7	2.33	11	3.67
61-65	25	6	2	19	6.33
66-70	18	7	2.33	11	3.67
71-75	18	8	2.67	10	3.33
76-80	9	4	1.33	5	1.67
81-85	8	3	1	5	1.67

A variation of UTI distribution was observed among 90 positive UTI cases in male and 210 in female which means 30% are male and 70% of patients are female. Table 3.2 shows the number of UTI cases in different age group of male and female The relative prevalence are presented in a bar diagram (Figure 3.3).

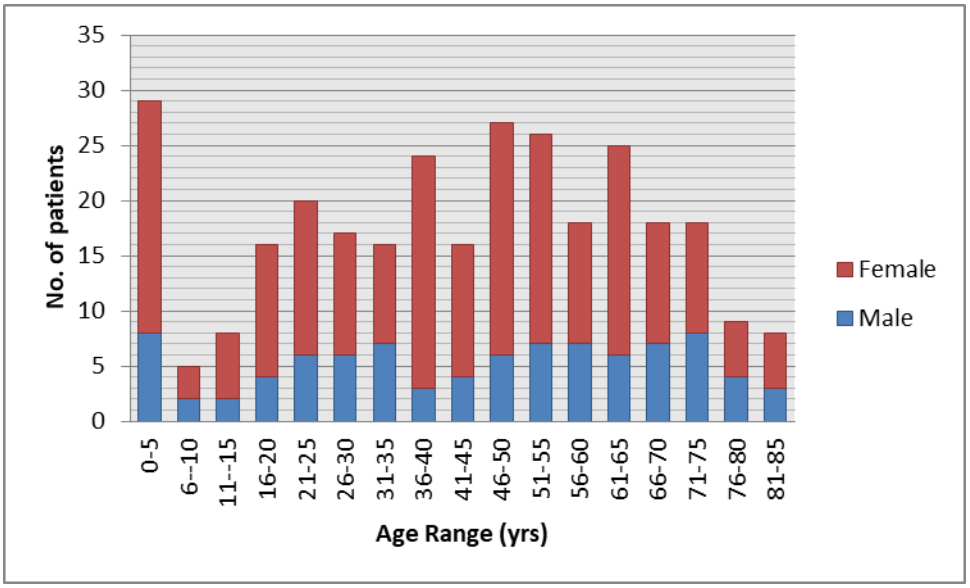

Figure 3.3: distribution of UTI among different age group of patients

According to bar diagram it can be said that female cases were much more significant than male cases. Patients with UTI cases mostly are female among different ages. In these age variations the age range of 0-5 , 36-40 ,46-50,51-55 and 61-65 are the higher range of female patients with UTI cases. At the very first with 0-5 age range and middle aged people are most affected by UTI.

3.3 Patients with different CFU/ml

Table 3.1: Patients with different CFU/ml count

CFU/ml	10^3	10^5	2×10^5	3×10^5	4×10^5	5×10^5
Number of patients	3	10	15	14	16	242

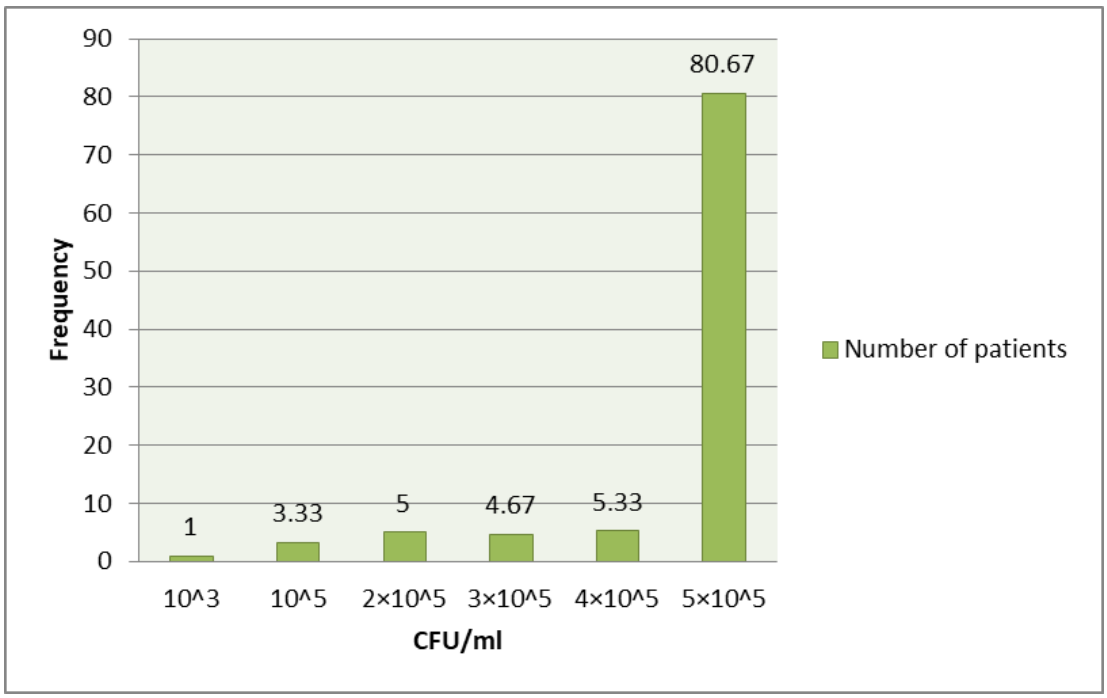

Figure 3.4: Patients with different CFU/ml count

According to the figure, 80.67 % of patients shows 5×10^5 count of CFU/ml. This count is most prominent among all the patients.

3.4 Antibiotic susceptibility test

3.4.1 Anti-biogram of *E. coli* strains from UTI

All of the 300 *E. coli* strains were tested for antibiotic susceptibility. The number and percentages of resistance and sensitivity are presented in Table 3.2 and Figure 3.6 respectively.

Table 3.2: Antimicrobial susceptibility results for 300 *E. coli* UTI isolates

Antibiotic	Sensitive	Percentage	Resistant	Percentage
Amikacin	283	94%	17	6%
Netilmicin	272	91%	28	9%
Tobramycin	254	85%	46	15%
Gentamicin	263	88%	37	12%

Azithromycin	67	22%	233	78%
Pipercillin-tazobactam	172	57%	128	43%
Meropenem	281	94%	19	6%
Ceftriaxone	122	41%	178	59%
Ceftazidime(3rd generation)	110	37%	190	63%
Cefuroxime(2nd generation)	124	41%	176	59%
Ciprofloxacin	135	45%	165	55%
Levofloxacin	145	48%	155	52%
Nalidixic Acid	94	31%	206	69%
Cotrimoxazole	100	33%	200	67%
Nitrofurantoin	260	83%	50	17%
Colistin	240	80%	60	20%

According to the table Azithromycin shows 233 or 78% resistant cases among 300 patients which is the highest among all the antibiotics. Nalidixic acid, Cotrimoxazole also shows most resistant among other antibiotics.

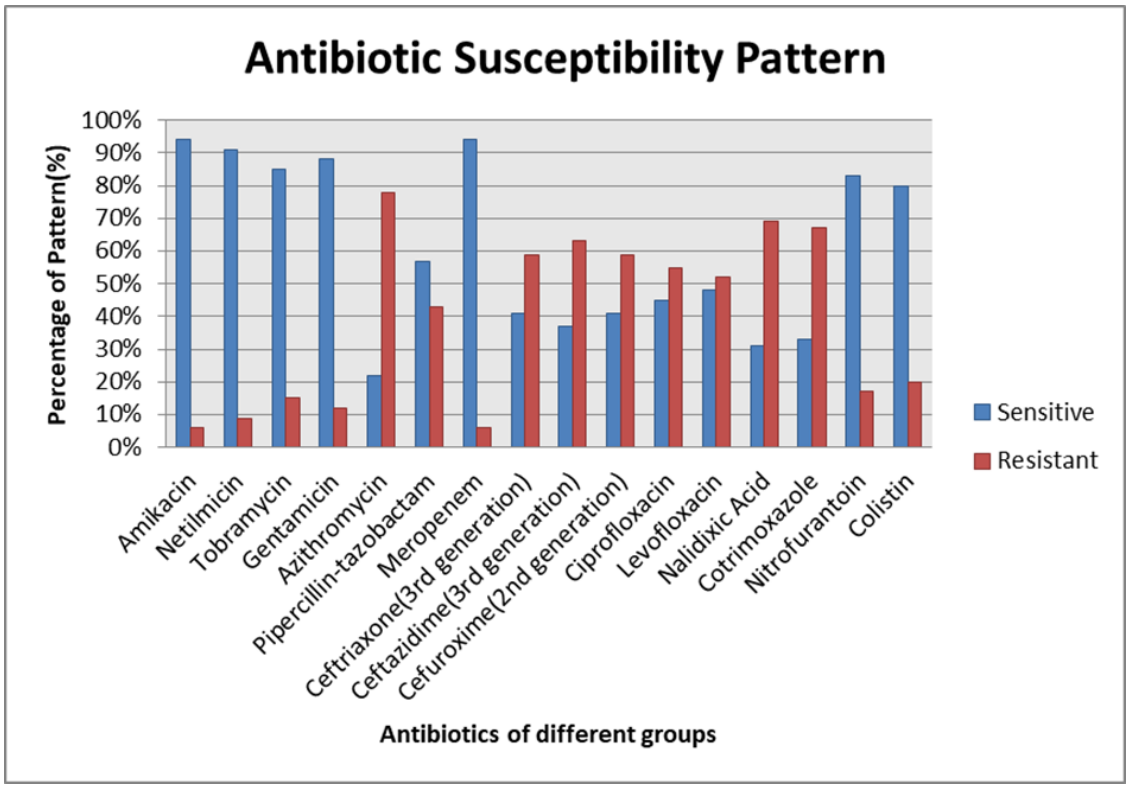

Figure 3.5: Antibiotic susceptibility pattern of 300 *E. coli* isolates from UTI

This table shows percentage of sensitivity and resistance among 300 patients with UTI. Meropenem and Amikacin are 94% sensitive whereas Azithromycin is 78% resistant, Nalidixic acid 69%, Cotrimoxazole 67%, Ceftazidime 63%, Ceftriaxone and Cefuroxime shows 59% resistance. Though some antibiotic shows sensitivity but most of them are resistant.

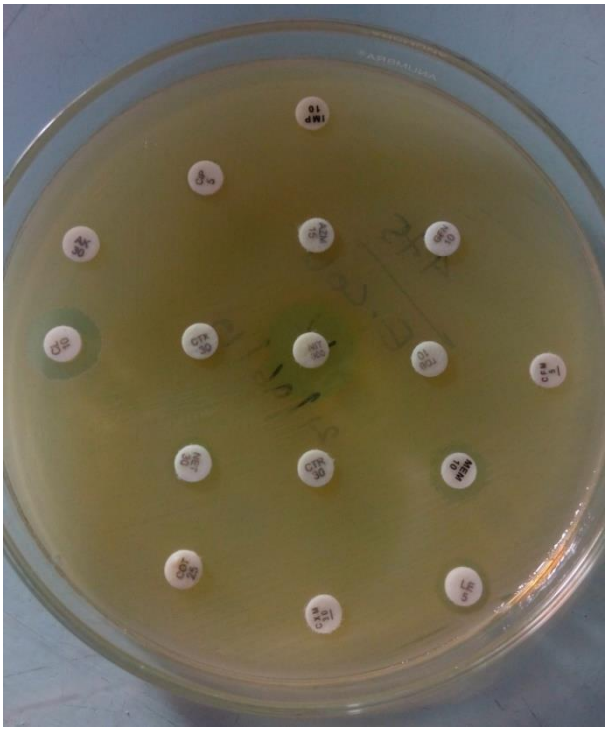

Figure 3.6: *E.coli* mostly resistance against antibiotics

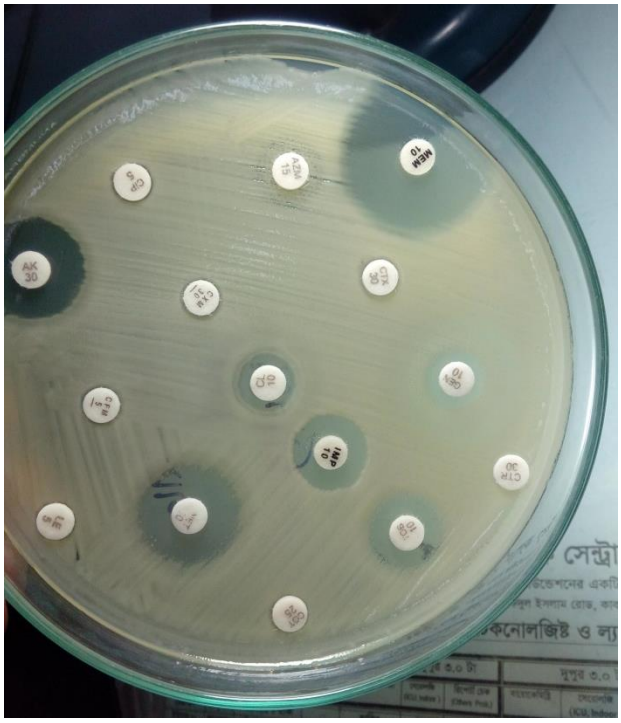

Figure 3.7: *E.coli* mostly sensitive against antibiotics

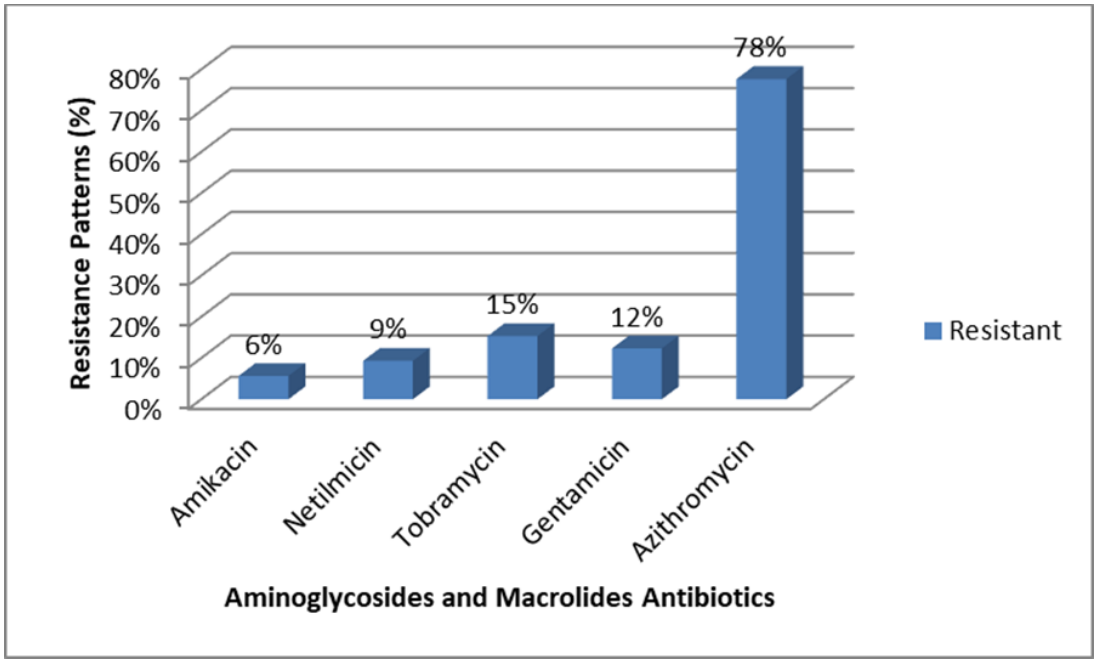

Figure 3.8: Resistance pattern of Aminoglycosides and Macrolides Antibiotic in percentage

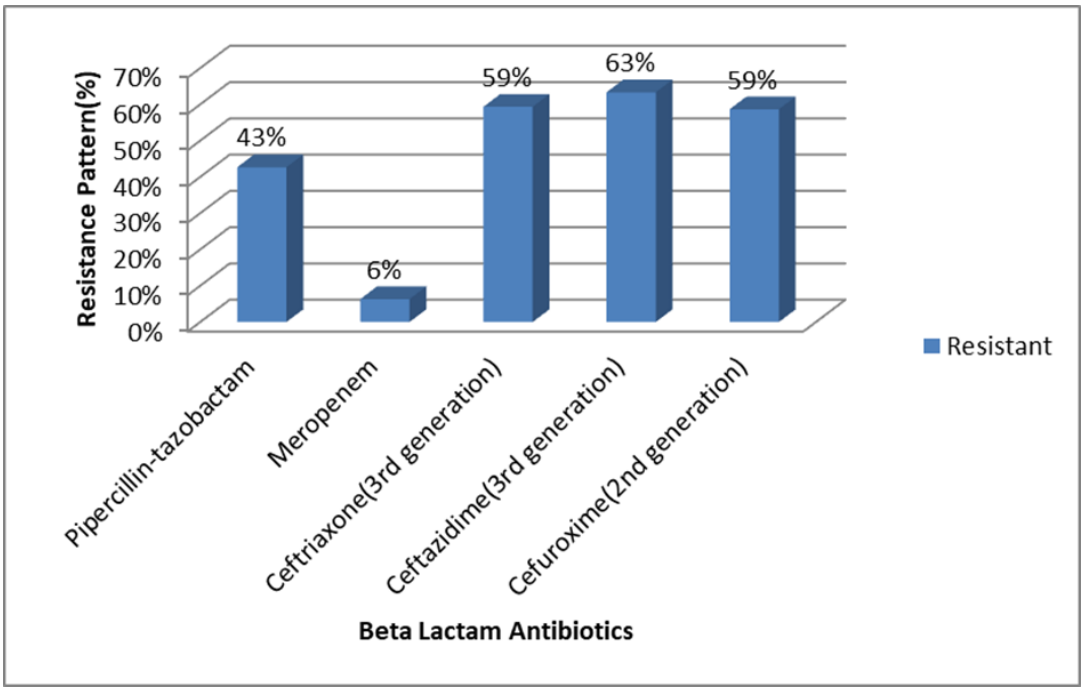

Figure 3.9: Resistance pattern of Beta Lactam Antibiotics in percentage

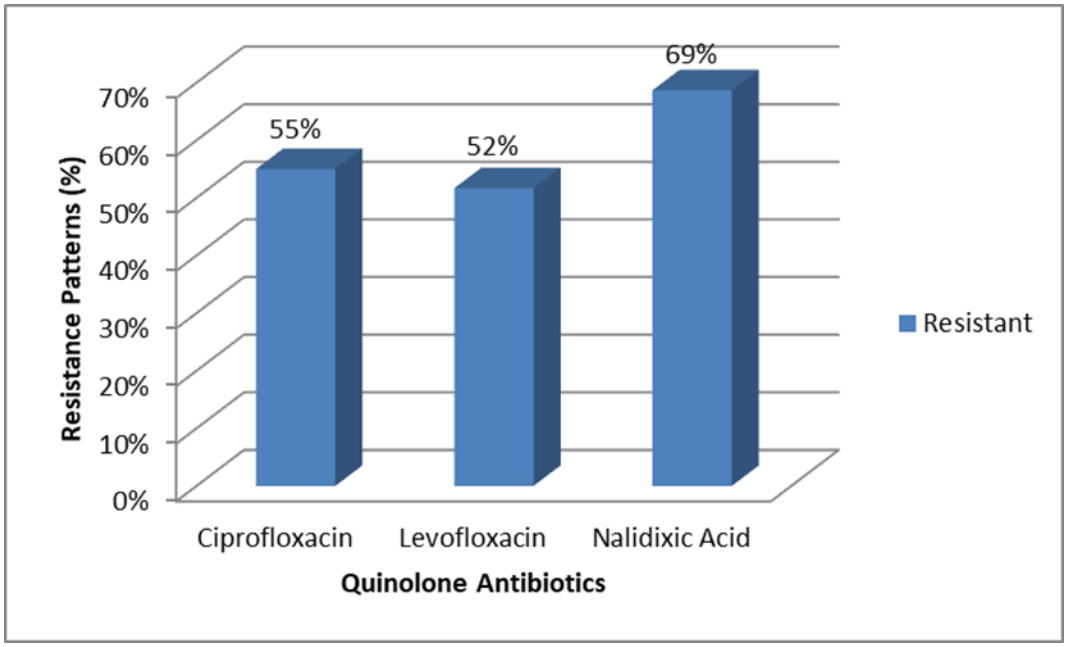

Figure 3.10: Resistance pattern of Quinolone Antibiotics in percentage

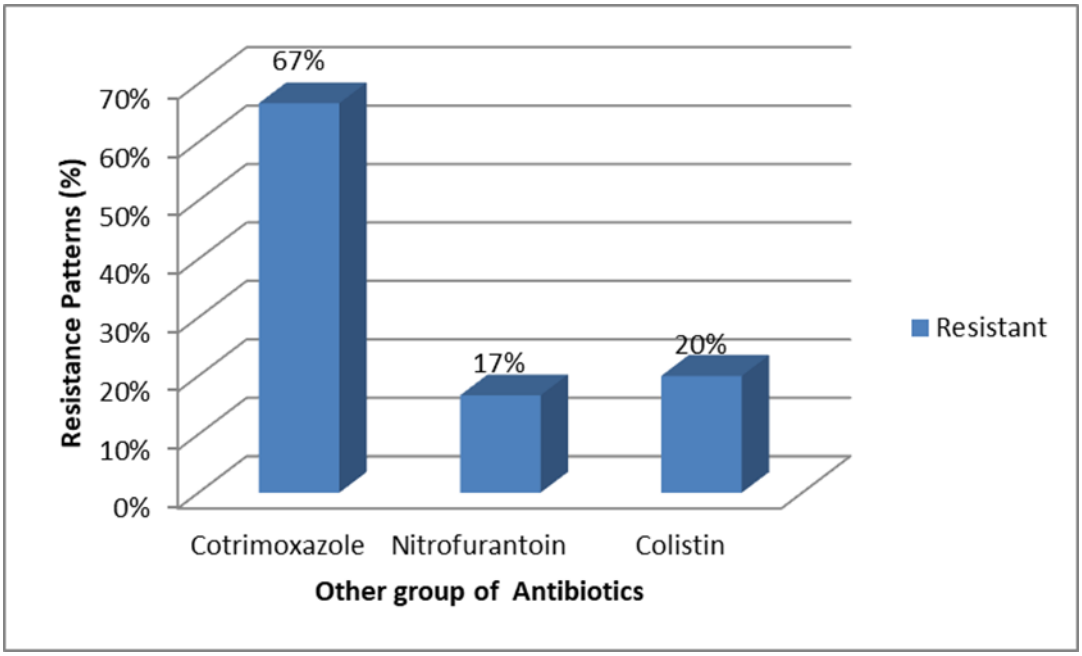

Figure 3.11: Resistance pattern of other groups of antibiotics in percentage

Table 3.3: Comparative data of resistance of *E. coli* isolated from UTI (All the data are given in percentage of resistance).

Antimicrobial agents	Bhowmik & Rashid , 2004	Lina *et al.*,2007	Majumder, et al. ,2011	B H N Yasmeen et al. ,2015	Majumder, et al. ,2016	Siddiqua M, Alam AN, Akter S, Ferdousi RS, 2017	Current Study , 2019
Amikacin (AMK)			2.3%	5.48%	1%	1%	6%
Gentamicin (GEN)	40%	39%	14%		10%	9%	12%
Azithromycin (AZM)		98%		66.08%		56%	78%
Meropenem (MEM)		0%	2%	22.69%	0%	3%	6%
Ceftriaxone (CRO)	5.5%	44%	24%	29.17%	51.5%	41%	59%
Ceftazidime (CAZ)		41%		7.4%	42%	50%	63%
Ciprofloxacin (CIP)	11.5%	77%	65.5%	40.31%	52%	38%	55%
Nalidixic Acid (NA)	40%	82%	75%		45%	74%	69%
Cotrimoxazole (SXT)	84%	78%	62%	45.45%	47%	43%	67%
Nitrofurantoin (F)	26.9%		9%	18.37%	12%	2%	17%

According to the table, most of the antibiotics grow in resistance over the years .Though some antibiotics for example Ciprofloxacin showed resistance of 77% in 2007 which lowered in 2019

to 55%.On the other hand, Ceftriaxone showed resistance of 44% in 2007 which rise to 59% in 2019.

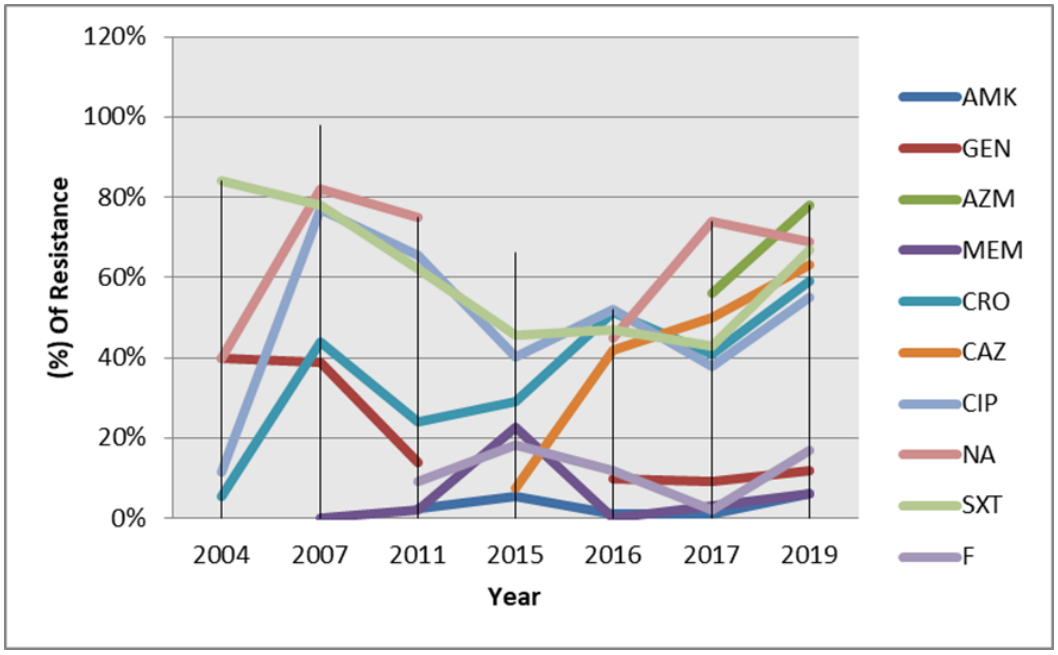

Figure 3.12: Antibiotic resistance over years in percentage

Table 3.4: Resistance pattern among patients

Number of Patients	Number and Percentage of patients sensitive to all antibiotics	Number and Percentage of patient resistant to one antibiotic	Number and Percentage of patients resistant to more than one antibiotic
300	13(4.3%)	19(6.3%)	289(89.33%)

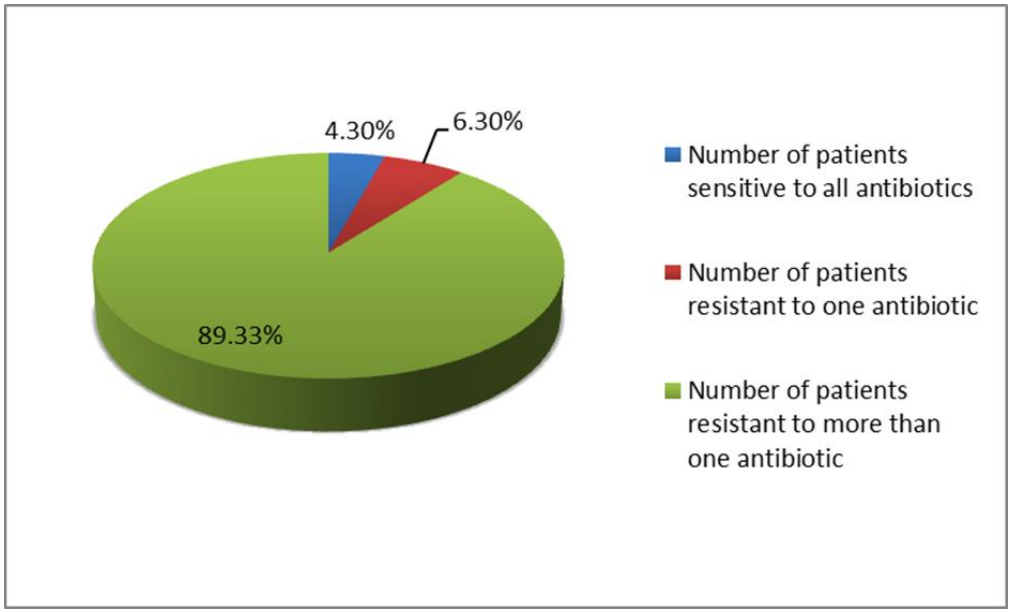

Figure 3.13: Resistance Pattern among patients

Among 300 patients 268 of them are resistant to more than one antibiotic.

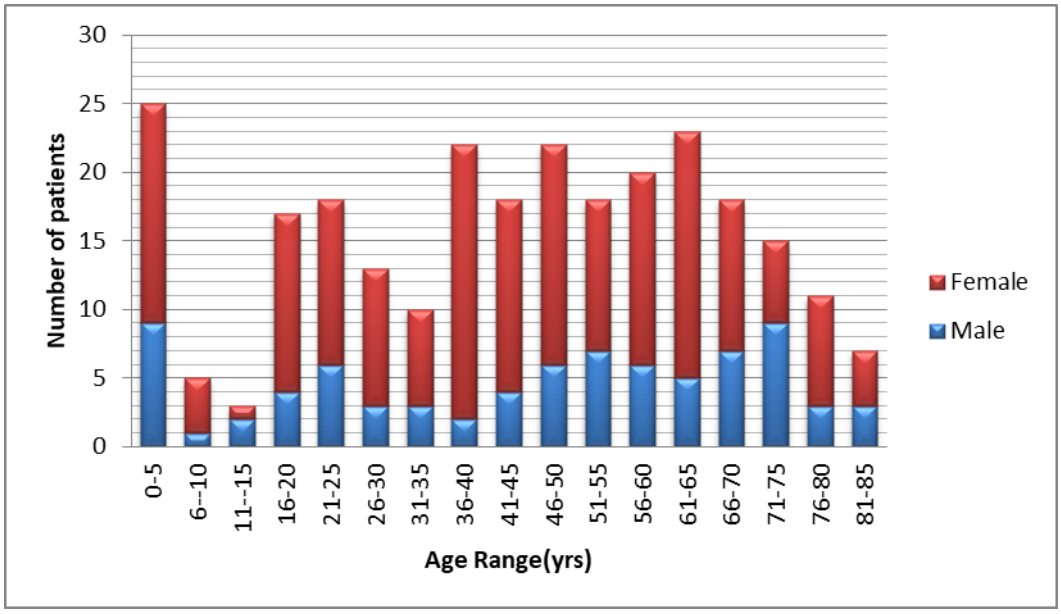

Figure 3.14: Multi-drug resistance pattern among different ages of patients

36-40 aged group shows mostly significant pattern of multi-drug resistance.

Chapter 4
Discussion

4 Discussion

Urinary tract infection (UTI) stays as one of the most widely recognized bacterial contaminations and second most normal irresistible ailment in network practice with roughly 150 million analyzed cases every year. Nearness of in excess of 105 life forms for every ml in a midstream test of urine alludes to huge bacteriuria and caused essentially by typical inside vegetation, *Escherichia coli*, which is in charge of more than 75 % of cases (Haque R., 2015) . The motivation behind this investigation was to segregating, recognizing the *E.coli* contaminants and distinguishing the obstruction example of *E.coli* disengaged from UTI suspected patients. Determination of sex of 300 patients revealed that 70% were female and 30% were male. So, it can be said that patients affected with UTI are mostly female. The age group of female of 0-5, 36-40, 46-50, 51-55 and 61-65 was highly susceptible to UTI. Age from 36-55 is vulnerable for female to have UTI.

Antibiotic resistance pattern showed that Azithromycin 78%, Nalidixic acid 69%, Cotrimoxazole 67%, Ceftazidime(3^{rd} generation cephalosphorin) 63% ,Ceftriaxone and Cefuroxime 59% ,Cirofloxacin 55% and Levofloxacin with 52% resistance were found from this study. On the other hand, Amikacin 6%, Netilmicin 9%, Tobramycin 15% and Gentamicin showed lower resistance. Among different groups of antibiotics, macrolides (Azithromycin) showed 78% resistance. Aminoglycosides (Amikacin, Netilmicin, Tobramycin and Gentamicin) showed much lower resistance. In addition, antibiotics of Beta lactam group showed higher resistance (Ceftriaxone, Cefuroxime, Ceftazidime). Quinolone (Ciprofloxacin, Levofloxacin,Nalidixic acid) group consisting of 3 antibiotics for this study showed much higher resistance. Other groups showed resistance for Cotrimoxazole only, rest are sensitive. So, the clear indication is towards Macrolides, Beta lactam and Quinolone group of antibiotics with higher resistance.

MDR of *E. coli* is also a concern, not only in Bangladesh but all over the world. In this study only 4.3% were sensitive against all antibiotics. 89.33% was resistant against more than one antibiotic and 6.3% were resistant to one antibiotic.

Bhowmick and Rashid (2004) , studied that Ceftriaxone showed 5.5% resistance , Lina *et al.,(*2007)studied an increase of 44% resistance which lowered according to the study of Majumder, et al. ,(2011) and B H N Yasmeen et al. ,(2015) to 24% and 29.17%.Then ,

Majumder, et al. ,(2016) there was an increase to 51.5%. Siddiqua M, Alam AN, Akter S, Ferdousi RS, (2017) showed 41% resistance which increase in the current study to 59%.

Siddiqua M, Alam AN, Akter S, Ferdousi RS, (2017) showed resistance of 1% for Amikacin, which increase to 6% in this study. Like this, Gentamicin showed resistance of 9% which increase to 12% in current study. Most of the antibiotics from the list showed an increase in resistance compare to 2017.

Various elements have been embroiled in expanding antibiotic resistance of microscopic organisms in Bangladesh. One of the most powerful factors is the simple accessibility of anti-microbials over the counter and the inclination of utilizing anti-toxins for generally indications. Along these lines, this is identified with access to drugs. This expanding dismal pattern requests quick activity. Indeed, even in the United States, it is assessed that up to half of anti-microbial remedies are unnecessary. Thus, appropriate control of anti-infection use, consequently diminishing the choice weight on life forms, isn't a simple methodology(Ray J.,2015).

Some shortcomings about the lacking of clinical data will be highly appreciated. This investigation depended on review research center information just so we neglected to give data on classification of UTI patients whether symptomatic or asymptomatic, entangled or uncomplicated. Further, dissemination of patients dependent on the wellsprings of contamination like catheter-related, network obtained or nosocomial likewise couldn't be referenced (Haque R.,2015).

Chapter 5
Conclusion

5 Conclusion

Urinary tract infection is a typical medical issue around the world; the study of disease transmission and antibiotic resistance of related microbes shifts from district to area and may vary contingent upon whether it happens in the community or in the hospital. Globally, the expanding rate of antibacterial obstruction including our nation is believed to be identified with mistaken systems of antibio-treatment. So it ought to be intently checked both at the provincial and national levels. The systems by which *E. coli* ends up impervious to anti-infection agents fluctuate incredibly with the anti-toxins .As a portion of the anti-infection opposition is expanding, contrasted and earlier years, there ought to be kept checking of this information to assess pattern of obstruction of antimicrobials for supported improvement of experimental treatment. *E.coli* was the most widely recognized etiological specialist and stays vulnerable to aminoglycosides and nitrofurantoin. The discoveries of this examination work demonstrate that Urinary tract disease is a typical between the two sexual orientations with higher pervasiveness among female because of their physiology. Likewise, age is a significant factor where moderately aged individuals are increasingly influenced alongside newborn children. Beta lactam anti-toxins, quinolones and macrolides are higher in obstruction, which can be a risk to the general population. In spite of the fact that anti-infection use has demonstrated to be gainful in checking the contamination however devouring more anti-toxins are destructive for our body. By devouring more anti-infection agents microscopic organisms transforms into safe then anti-microbials will be not any more powerful for treating ailment. Or maybe we should drink a lot of water and keep up cleanliness to remain sound. Patients experiencing long haul treatment are additionally helpless against the disease because of damp hospitalized conditions , plant source like cranberry juice is similarly powerful in battling the contamination and can be utilized as a choice to check the pathogen causing UTI.

5.1 Recommendations

Antibiotics cannot be exchanged for different kind of infections because of their specificity toward one infection. At the point when anti-infection agents are utilized effectively, they are normally sheltered with few reactions.

Antibiotics can prompt symptoms that may extend from being an irritation to life-threatening. Dose of antibiotics should be balanced dependent on the particular qualities of the patient, similar to kidney or liver capacity, weight, or age along with infants or aged one with kidney or liver ailment, in pregnant or breastfeeding women, and in numerous other patient gatherings. Medicinal services suppliers or health care workers can survey every patient separately to decide the right dose of antibiotic.

For each contamination the correct decision cannot be antibiotic. For instance, most sore throats, hack and colds, influenza or intense sinusitis are viral in inception (not bacterial) .So they needn't bother with an anti-microbial. These viral contaminations are "self-constraining", which means your own invulnerable framework will as a rule kick in and fend the infection off. Truth be told, utilizing anti-infection agents for viral contaminations can build the hazard for anti-toxin opposition, bring down the choices for future medications if an anti-toxin is required, and put a patient in danger for reactions and additional expense because of pointless medication treatment (Beyer M.,2018).

Directed coordination and counteractive action methodology to quit spreading anti-infection is recommended. Instruction and guidance expected to improve antibiotic use and to anticipate sepsis.

In Bangladesh unethical drug promotion and showcasing of unacceptable and pointless medications has been seen significantly. A therapeutic actioner can endorse any medication utilized for the basic cold to malignant growth. Also, polypharmacy is extremely regular among the rustic medicinal professionals with antibiotics and nutrients endorsed widely. This ought to be ceased right away

To decrease the chance of “collateral damage” development before utilizing antibiotics and utilizing appropriate antibiotics (if conceivable, thinking about utilizing nitrofurantoin,

fosfomycin, or pivmecillinam as first-line antibiotics) ought to be performed according to regional susceptibility data.

The utilization of suitable antibiotic dosages, not underdoses, to conceivably reduce mutatnt development; and the utilization of antibiotics for fitting terms to lessen repetition (proper de-heightening with rehashed culture). Likewise, to counteract abuse of anti-microbials, self-prescription, or over-the-counter (OTC) accessibility ought to be restricted by instruction or guideline. Besides, focusing on cleanliness, particularly people who travel to endemic regions or who are regularly in conditions with a high danger of presentation to anti-microbial safe microorganisms (e.g., social insurance units), could lessen the opportunity of colonization by safe miniaturized scale life forms. At last, the utilization of non-antimicrobial prophylaxis could viably decrease the aggregate sum of antibiotic utilization.

By following these instructions in Bangladesh the threat of antibiotic resistance to common people can be reduced to safe position. People should come forward to increase awareness for themselves and the future generation.

Chapter 6
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Appendices

APPENDIX I

Composition of the media used:

MacConkey agar

Bacto peptone	17.0 g
Protease peptone	3.0 g
Lactose	10.0 g
Bile salt	1.5 g
NaCl	5.0 g
Agar	15.0 g
Neutral red	0.03 g
Crystal violet	0.001 g
Distilled water	1000 ml

P^H 7.2

Sterilized at 121ºC under 15 lbs/in^2 pressure for 15 minutes

Mueller-Hinton Agar

Beef extract	2.0 g
Acid hydrolysate of Casein (technical)	17.5 g
Starch	1.5 g
Agar	17.5 g
Distilled water	1000 ml

pH 7.3

Sterilized at 121ºC under15 lbs/in^2 pressure for 15 minutes

APPENDIX II

Composition of chemicals and reagents

Normal saline

NaCl	0.85g
Distilled water	1000 ml