Urinary Tract Infections (UTIs): A Review on Currently Available Therapeutic Choices for UTIs

By

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing my 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

The thesis titled “Urinary Tract Infections (UTIs): A Review on Currently Available Therapeutic Choices for UTIs” submitted by Tamjid Rohman (ID: 18346006) of Spring, 2022 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics statement

I Tamjid Rohman, School of Pharmacy, Brac University, declare that this study does not involve any animal or biological samples.

Abstract

Urinary tract infections (UTIs) are commonly occurring infections that have become a major public health problem worldwide. They can be caused by both Gram-negative and Gram-positive bacteria, as well as by some species of fungi. However, Gram-negative bacteria, especially, *Escherichia coli* (UPEC), and *Klebsiella pneumonia* are primarily responsible for UTIs. Infections initiate in the urinary tract and may eventually culminate in severe complications such as kidney damage, bacteremia, or even death. Age, gender, anatomy, and ethnicity are the main risk factors for UTIs. Women and elderly people are most susceptible to UTIs due to their anatomical features and age. Currently, therapeutic options for managing UTIs include both antibiotic and non-antibiotic therapy. Though antibiotics represent first-line treatment choices for UTIs, the emergence of multidrug-resistant bacterial strains often results in treatment failure and recurrent infections. These factors further add to patients' sufferings and the economic burden of the disease. Research is currently underway to develop better diagnostic tools, drug therapies, and efficient delivery systems to tackle antimicrobial resistance. This review aims to provide an overview of UTIs and currently available therapeutic options, particularly focusing on challenges associated with the development of antimicrobial resistance (AMR) and multi-drug resistance (MDR) in UTI therapy.

Keywords: Urinary tract infections; Antibiotic therapy; Non-antibiotic therapy; Antimicrobial resistance (AMR); Multi-drug resistance (MDR); Metagenomic sequencing (MGS); Nanoparticles (NP).

Dedication

Dedicated to my parents for their constant support.

Acknowledgment

I would like to start by showing my heartiest gratitude to Almighty Allah (SWT), who has granted me unbounded blessings, opportunity, kindness, strength, and enormous patience, enabling me to finish this project paper.

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List of Acronyms

UTI	Urinary Tract Infection
UPEC	Uropathogenic *Escherichia coli*
AMR	Antimicrobial resistance
ESBLs	Extended spectrum β-lactamases
MDR	Multi-drug resistance
MDRO	Multi-drug resistant organisms
MGS	Metagenomic sequencing
NP	Nanoparticles
RUTI	Recurrent UTI
BBD	Bladder dysfunction

Aim and objectives

1. To summarize the impact of urinary tract infections (UTIs) in the healthcare community.
2. To focus on currently available therapeutic choices for treating UTIs.
3. To highlight the most potential emerging therapies for treating UTIs and managing AMR and MDR.
4. To outline how metagenomic sequencing (MGS) and nanoparticle (NP) strategy will play a promising role in the future for diagnosing and managing UTIs.
5. To emphasize how antimicrobial resistance (AMR) and multi-drug resistance (MDR) provoke treatment failure.

Chapter 1

Background

1.1 Introduction

Urinary tract infection (UTI) is a commonly occurring infection health concern worldwide. In 2019, over 404.6 million suffered from this bacterial infection, and around 1,98,433 – 2,59,034 people lost their lives due to UTIs (Zeng, 2022). The urinary tract consists of a collection of structures responsible for eliminating urine from the body. It has two parts- the upper urinary tract consists of two kidneys and ureters and the lower urinary tract consists of the bladder and urethra. Microbial infections occurring in any component of the urinary tract are known as urinary tract infections (UTIs) and can lead to serious complications (Medina & Castillo-Pino, 2019). Among the variety of complications that fall under the umbrella of this alarming infection are urosepsis, complicated and uncomplicated UTIs, acute pyelonephritis, chronic pyelonephritis, interstitial pyelonephritis, cystitis, renal and perirenal abscess, prostatitis, urethritis, recurrent UTIs, and severe kidney obstacle even kidney damage (Pulipati et al., 2017).

The major risk factors for UTIs include age, gender, anatomy, and ethnicity. According to the analysis of Millner & Becknell, 2019, it has been identified that other than bacterial infection, unhealthy lifestyle, kidney abnormalities, some foreign substances, use of a catheter, menopause, and defective urinary tract by birth is also the most common underlying risk factors of UTIs. People of all ages can be infected by UTIs, but elderly people are more susceptible to this infection. In addition, UTIs are more prevalent in women than in men (Millner & Becknell, 2019). Several microorganisms can be responsible for UTIs, but the majority of the infections are caused by bacteria especially gram-negative ones: *Escherichia coli* (UPEC), and *Klebsiella pneumonia*

(Medina & Castillo-Pino, 2019). UTIs are associated with complications such as severe lower back pain and difficulty during urination, feeling of frequent urination even after having an empty bladder, burning sensation and bleeding during urination, and urethral or vaginal discharge (Massa et al., 2009).

Diagnosis of UTIs is achieved by routine urine analysis. Physicians usually initiate antibiotic or nonantibiotic therapy for treating UTIs, but antibiotic therapy is the most preferable treatment option (Piñeiro Pérez et al., 2019). First-line antibiotic choices for UTIs include fosfomycin, trimethoprim or sulfamethoxazole, and nitrofurantoin (Holm et al., 2019). Additionally, inappropriate or unnecessary use of antibiotics can cause life-threatening conditions, sometimes the presence of certain bacteria such as *Escherichia coli* (UPEC), *Klebsiella. pneumonia, Enterococcus spp., Proteus mirabilis, Proteus aeruginosa, Staphylococcus aureus*, and *Candida spp*. and the activity of these bacterial enzymes obstacle the antibiotic therapy and causes antimicrobial resistance (AMR) and multidrug resistance (MDR) (Hossain et al., 2020). If left untreated, such infections can become life-threatening for the patient. Therefore, efflux pump inhibitors, beta-lactamase inhibitors, membrane permeabilizer, and other alternative nonantibiotic therapies are used for the management of antimicrobial resistance (AMR) and multidrug resistance (MDR)- associated UTIs (Annunziato, 2019). Sometimes inappropriate diagnosis and treatment, and overuse of antibiotics can cause life-threatening antimicrobial resistance (AMR) and multidrug resistance (MDR) which ultimately cause treatment failure and severe complications even death. In this case, better diagnostic methods and innovative approaches could be utilized such as metagenomic sequencing (MGS), a novel diagnostic method (Smelov et al., 2016), and nanoparticle (NP) therapies can be used as an alternative treatment strategy, which is still under investigation for proper practice (Sánchez et al., 2021).

This comprehensive review will provide an overview of UTIs and provide up-to-date information on currently available therapeutics to treat UTIs, focusing on challenges associated with the development of antimicrobial resistance (AMR) and multidrug resistance (MDR) in UTI therapy. The review will begin with the etiology of urinary tract infections (UTIs) in humans and it will particularly focus on the prevalence of UTIs, the pathogenesis of this infection, the classification of UTIs, then the major causes and risk factors of it, the most common signs and symptoms, diagnosis processes, combination therapies for UTIs, the practice of some emerging therapies for the treatment of UTIs, and overall treatment processes, also some limitations of antibiotic therapies, mechanism of AMR and finally some alternative treatment options.

1.2 Epidemiology

Urinary tract infections (UTIs) are most common in outpatients and the prevalence increases with age. In women, the rate of UTIs is higher than in men, as shown in Table 1. About 50−60% of adult women suffer from UTIs in a lifetime. Furthermore, women aged over 65 years are twice as susceptible to UTIs. Increased sexual activity is one of the major risk factors for UTIs in young women, which may reoccur within 6 months. Pyelonephritis, a less frequently occurring but more serious urinary tract infection can even cause hospitalization (Medina & Castillo-Pino, 2019). In children, urinary tract infections (UTIs) are the second most prevalent bacterial infection, 8% of girls and up to 2% of boys aged around 7 years suffer from this infection. Children younger than 1 year, neonates, young infants, toddler girls, and uncircumcised boys are more vulnerable to UTIs and end up with different kinds of structural and functional urinary tract abnormalities (Millner & Becknell, 2019). Geriatric patients over 65 years suffer from UTIs due to various bacterial infections, which cause hospitalizations in 15.5% of patients and 6.2% of patients end up with death. Increasing age is a serious risk factor for UTIs and is associated with anatomic abnormalities

of the urinary tract, prostatic hyperplasia, uncontrolled diabetes mellitus, and vaginal atrophy (Cortes-Penfield et al., 2017). Similarly, 1 to 4% of pregnant women suffer from acute cystitis, and 4 to 10% from asymptomatic bacteriuria which causes pyelonephritis with preterm labor and low birth weight (Curtiss et al., 2017) and also causes some other symptoms which are discussed in Table 4 (Massa et al., 2009).

1.3 Prevalence of UTIs

The prevalence of UTI mainly depends on age, gender, anatomy, and ethnicity. Infants are more susceptible to UTIs than elderly children. Besides, the ratio of the infected female is higher than the man (Pulipati et al., 2017) and the prevalence is higher in white children than in black children (Millner & Becknell, 2019). Table 1 summarizes the prevalence of UTIs in men and women at different age groups.

Table 1 *UTIs' prevalence for certain age groups with gender* (Pulipati et al., 2017)

Age (years)	Female prevalence (%)	Male prevalence (%)
<1	Around 1% of females suffer from UTIs due to anatomic or functional urinary tract abnormalities	Around 1% of males suffer from UTIs due to anatomic or functional urinary tract abnormalities
1-5	4.5% of females suffer from UTIs for having congenital abnormalities and vesicoureteral reflux	0.5% of males suffer from UTIs for having congenital abnormalities and uncircumcised penis
6-15	4.4% of females have UTIs due to vesicoureteral reflux	0.5% have UTIs for the reason of vesicoureteral reflux
16-35	20% of females suffer from UTIs for unhealthy sexual intercourse, use of the diaphragm, and having previous UTIs	0.5% of males suffer from UTIs due to anatomic abnormalities and rectal intercourse
35-65	35% of females suffer from this infection due to gynecological surgery, bladder prolapse, and previous UTIS	20% of males suffer from this infection due to prostate hypertrophy. obstruction, catheterization, and some
>65	Around 40% of females suffer from UTIs because of a deficiency of estrogen and loss of vaginal lactobacilli bacteria	Around 35% of males suffer from UTIs because of incontinence, long-term catheterization, and the use of condom catheters

1.4 Etiology and development of UTIs in human

Urinary tract infection occurs when bacteria gain entry into the urinary tract by the urethra and multiply. Bacteria are usually not present in urine. Urine contains waste products, salts, and different kinds of fluids and is considered sterile. Etiological agents for UTIs include bacteria: *Escherichia coli* (UPEC), *Klebsiella pneumonia, S. saprophyticus,* and *Proteus*, Fungi: Candida, Aspergillus, and viruses: adenovirus can cause urinary tract infections. (Medina & Castillo-Pino, 2019)

There are four routes by which microorganisms can enter the urinary tract; infection in ascending route, lymphatogenous spread, hematogenous spread, and direct extension from other organs (Pulipati et al., 2017). In this case, uropathogenic *Escherichia coli (UPEC), K. pneumonia, S. saprophyticus, Enterococcus spp., Group B Strep., P. mirabilis, P. aeruginosa*, *S. aureus,* and *Candida spp*. are responsible for UTIs but uropathogenic *Escherichia coli* (*E. coli)* (UPEC) and *Klebsiella pneumonia* are accounted for the most prevalent bacteria that causes both complicated and uncomplicated UTIs. The prevalence of complicated UTIs is 65% and uncomplicated UTIs is 75% (Nzalie et al., 2016).

Table 2 *Classification of urinary tract infections (UTIs)* (Kot, 2019) and (Pulipati et al., 2017)

Based on clinical aspects and rate of severity	**Based on risk factors**	**Based on infection source**	**Based on urinary tract anatomy**
1. **Urosepsis:** Untreated UTIs spreads into the kidney and cause sepsis and septic shock. **2. Pyelonephritis:** Due to the presence of gram-negative bacteria infection occurs in the urinary tract. **3. Cystitis:** Infection and inflammation in the bladder	**1. Complicated UTIs:** -Doesn't respond to traditional treatment. -Abnormalities in the urinary tract. -Renal functional impairment. **2. Uncomplicated UTIs:** -Renal functional impairment doesn't happen. - Functional or anatomical anomalies in the urinary tract don't occur.	1. From the hospital 2. Community-acquired 3. Due to the lack of healthcare facilities	**1. Upper urinary tract infections:** It includes pyelonephritis, renal abscess, and perirenal abscess (Pulipati et al., 2017) **2. Lower urinary tract infections:** It includes cystitis, prostatitis, and urethritis (Pulipati et al., 2017)

1.5 Recurrent UTI

According to an observational study, if a person suffers from uncomplicated UTIs more than 3 times within a year or 2 uncomplicated and symptomatic UTIs within 6 months due to the presence of gram-negative and gram-positive bacteria especially uropathogenic *Escherichia coli* (UPEC) and some non- *Escherichia coli* and accompanied by significant complications, then the condition can be defined as recurrent UTI (RUTI) (Epp et al., 2010). Previously occurring urinary tract infections, bowel and bladder dysfunction (BBD), age groups, altered estrogen levels, and gender are the major risk factors for recurrent UTIs. Around 10-15% of women aged over 60 commonly suffer from recurrent UTIs as their immunity declines with age. As a result, the patient faces some serious complications such as kidney infections, permanent kidney damage, and urethritis (de Nisco et al., 2019).

1.6 UTI in children and elderly people

The incidence of urinary tract infection is the second most common in children. Approximately 8% of girls and up to 2% of boys aged ≤7 years are commonly infected by UTIs (Millner & Becknell, 2019) and show malaise and fever along with abdominal pain-like symptoms (Lee & Neild, 2007a). During the age of one, around 0.7% of girls and 2.7% of boys suffer from UTIs for the presence of bacteria in their periurethral area and infection further spreads in the bladder and even in the kidney. For infants, symptoms are nonspecific they suffer from feeding disorders, diarrhea, vomiting, and gaining weight slowly, one study shows that the percentage of incidence of UTIs is 5% for girls and 20% for boys. In the first 6 months, the risk rate of developing UTI is 10 to 12-fold higher in boys but after one year this rate becomes higher in girls (Leung et al., 2019).

Escherichia coli is primarily responsible for UTIs in children. Urologic anomalies in children, like vesicoureteric reflux, help ascend bacteria from the bladder to the kidney and causes UTIs in children. The presence of foreign substances such as stones or the use of catheters is also a reason for UTIs in children. There are some other significant reasons such as parenchymal renal anomalies, dysfunctional bladder emptying, detrusor muscle instability, constipation in children, diabetes mellitus by birth, immunodeficiency, obesity, and vitamin D deficiency in children can cause UTIs (Leung et al., 2019) and shows the symptoms which are mentioned in Table 4 (Massa et al., 2009).

The immune system becomes weaker with age, about 15.5% of patients aged over 65 years become hospitalized, and 6.2% of patients died because of urinary tract infections (Cortes-Penfield et al., 2017). In this case, urinary tract abnormalities increase urinary incontinence and urinary retention, long-term hospitalizations, and the use of urinary catheters are the main challenges associated with UTIs. Also, uncontrolled diabetes mellitus in elderly patients and the use of sodium-glucose cotransporter 2 inhibitors such as canagliflozin can cause UTIs and eventually damage the kidney. One study represents, that more than 90% of case elderly patients suffer from uncomplicated cystitis because of gram-negative bacteria such as *E. coli*, 15% of case infections occur due to *Klebsiella oxytoca*, and 7% of case elderly patients face UTIs for the presence of Proteus mirabilis (Cortes-Penfield et al., 2017).

1.7 UTI in pregnant women

Physiological changes such as hormonal changes, increase in uterus size, and bladder reflux during the early stages of pregnancy often put women at risk of suffering from urinary tract infections. One study showed that 2–12% of women were affected by asymptomatic infection, bacteriuria

(ASB) (Ghouri et al., 2019) during the first trimester, which is diagnosed and treated by routine screening, and antibiotics whereas in nonpregnant women asymptomatic infection was not treated with antibiotics. In this case, the major risk factors are kidney infections, pyelonephritis, premature birth of the baby, low birth weight, sepsis, and even intra-uterine growth retardation (Kazemier et al., 2015). In pregnant women, unnecessary use of antibiotics can lead to the development of antimicrobial resistance (AMR) and this can be more harmful as resistant bacteria can easily be transmitted to the neonates during birth, and sometimes may cause teratogenic effects such as spontaneous abortion (Ghouri et al., 2019). In 63–85% of cases, *Enterobacteriaceae with Escherichia coli* is the most responsible pathogen that is found in the gastrointestinal tract and causes UTIs.

1.8 Causes and risk factors of urinary tract infections

The major causative factor behind UTIs is microbial infections, typically it occurs due to the presence of different kinds of bacteria, viruses, and fungus. In addition, an unhealthy lifestyle can also contribute to this risk. Less intake of water, fluids, and healthy food and a lack of physical activity can increase the risk of UTIs (Hossain et al., 2020). Also holding urine for hours makes the bladder suitable for the growth of bacteria. Kidney abnormalities such as dysplastic kidney, renal scarring, and vesicoureteric reflux can cause urinary tract infections (de Nisco et al., 2019). The presence of foreign substances like kidney stones, tumors in the urinary tract, and the use of indwelling catheters and nephrostomy tubes can cause urinary tract infections (Lee & Neild, 2007b). Besides, the anatomy of the female urethra is another reason for UTIs as the length of the female urethra is shorter than the male, making it more susceptible to bacterial infection as the bacteria can easily reach the female bladder. Furthermore, after menopause, the reduction of circulating estrogen levels in females causes a change in the urinary tract which makes it more susceptible to

infections. In the case of babies, those born with a defective urinary tract cannot eliminate urine from the body by the normal process, in this case, urine in the urethra increases the risk of UTIs (Millner & Becknell, 2019). Moreover, the hospitalized and neurological patients who can't urinate on their own, in this case, the use of a catheter can make them more vulnerable to bacterial infection (Figure 1B). Furthermore, sexually active women are more susceptible to UTIs, and patients with suppressed or impaired immune systems such as diabetes patient body can't defend against microorganisms which makes them vulnerable to urinary tract infection (Millner & Becknell, 2019) and finally shows different types of sign and symptoms which are included in Table 4.

1.9 Pathogenesis of UTIs

Uncomplicated UTIs are typically common in women, children, and elderly individuals who do not have any structural or neurological urinary tract abnormalities. Along with uropathogenic *Escherichia coli* (UPEC), some other causative organisms such as *Klebsiella pneumoniae, Staphylococcus saprophyticus,* group B *Streptococcus* (GBS), *Proteus mirabilis, Staphylococcus aureus,* and *Candida spp.* can be responsible for Uncomplicated UTIs (Klein & Hultgren, 2020). These uropathogens can directly bind with the bladder epithelium and uroplakins. These uroplakins are an important component for the apical membrane of umbrella cell because it helps to form a crystalline array in the mammalian bladder tissue to give protection against the damage of agent in urine (Flores-Mireles et al., 2015). Generally, these uropathogens enter the urinary tract through the urinary meatus then ascending up the urethra and reached into the bladder lumen, and rarely enter through the bloodstream, infecting the urinary tract, and pathogenesis starts. When the uropathogens from the gut get contaminated in periurethral area then the uncomplicated UTIs start and eventually colonization occurs in the urethra and the organism migrates to the bladder (Klein & Hultgren, 2020). However, the expression of pili and adhesins can cause this colonization along

with the invasion of superficial umbrella cells and then it causes inflammatory responses in the host, and neutrophil infiltration occurs that starts to initiate extracellular bacteria (Flores-Mireles et al., 2015). Bacterial multiplication takes place; some evade the host immune system, and host cell invasion or morphological changes cause resistance to neutrophils and undergo biofilm formation. These bacteria damage the host cell by producing toxins and proteases and the release of some nutrients helps the bacteria to survive and ascend to the kidneys. Colonization of the kidney causes the production of bacterial toxins and damage to the host tissue (Burke et al., n.d.). If this infection is left untreated, the pathogen crosses the tubular epithelial barrier of the kidney and causes bacteremia (Flores-Mireles et al., 2015; Klein & Hultgren, 2020), shown in figure 1 panel A.

Complicated UTIs can occur in individuals who have compromised the urinary tract or host defense system, along with urinary obstruction, urinary retention due to neurological disorder, immunosuppression, renal failure or renal transplantation, pregnancy, and the presence of foreign substances (calculi and indwelling catheters) and other abnormalities. Along with uropathogenic Escherichia coli (UPEC), some other causative organisms such as Enterococcus spp., K. pneumoniae, and Candida spp. can be responsible for complicated UTIs (Flores-Mireles et al., 2015). The progression of complicated UTIs can be initiated by following similar preliminary steps (step 1-step 2) as uncomplicated UTIs (Flores-Mireles et al., 2015). The pathogen causes infection in the bladder, for this bladder needs to be compromised and here catheterization is the most common reason for a compromised bladder. The fibrinogen-accumulated urinary catheter enters the bladder through the urethra and makes a suitable environment for the uropathogen so that these are easily expressed to fibrinogen-binding proteins, initiate infection, and induce neutrophil infiltration. Then, the bacteria multiply and form biofilms. Further, bacterial toxins and proteases

are produced and damage the epithelial tissue of the host. After that, colonization of the kidney occurs, and toxin production consistently damages the tissue. Similarly, if this condition is left untreated, it results in complicated UTIs and eventually causes bacteremia when it crosses the tubular epithelial cell barrier (Klein & Hultgren, 2020), shown in figure 1 panel B.

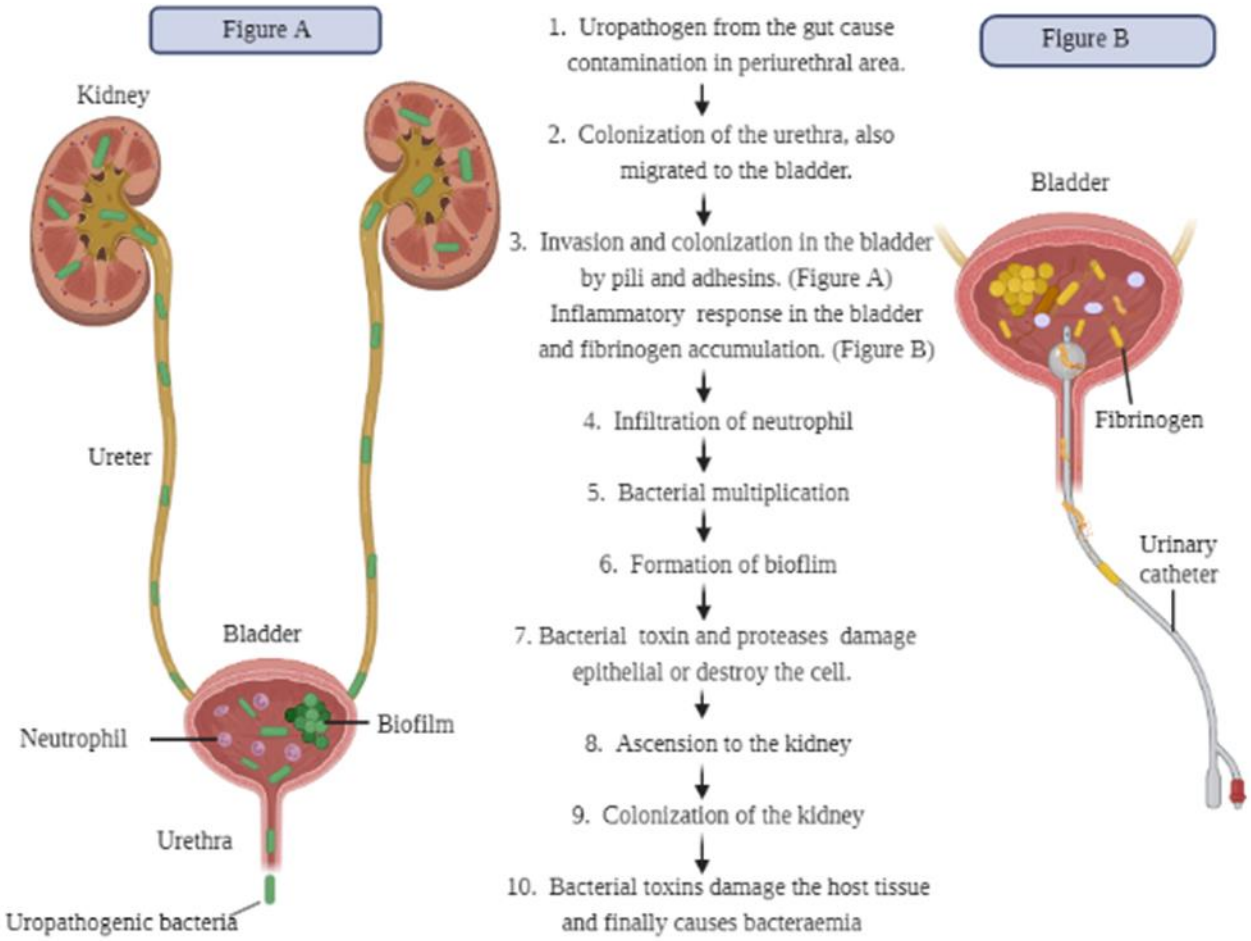

Figure 1: Pathogenesis of uncomplicated UTIs (Figure A) and complicated UTIs (Figure B) adapted from (Flores-Mireles et al., 2015). In the flow chart above step 1 shows, uropathogen from the gut causes contamination in the periurethral area. Then in step 2 colonization in the urethra and migration of the organism in the bladder. In step 3, invasion and colonization occur. Then after the infiltration of neutrophils (step 4), bacterial multiplication takes place (step 5) and eventually forms biofilm (step 6). Next, the cell gets destroyed due to the bacterial toxin and proteases (step 7) and ascends

to the kidney (step 8). Ultimately colonization of the kidney occurs (step 9) and this ended up causing host tissue damage and bacteraemia (step 10).

1.10 Catheter-associated- UTIs

Insertion of the catheter into the bladder causes inflammation which ultimately releases fibrinogen and this fibrinogen has deposited onto the catheter. Uropathogens absorb this deposition in many ways (Klein & Hultgren, 2020). For example, *E. faecalis* takes advantage of this condition and use fibrinogen as a food source with the help of proteases (GelE and SprE proteases). Also, this pathogen binds with fibrinogen with the help of endocarditis and biofilm-associated (Ebp) pilus, which promotes the formation of biofilm, and this biofilm is a large collection of uropathogens surrounded by a complex extracellular matrix that is composed of polysaccharides, proteinaceous fibres and extracellular DNA (Klein & Hultgren, 2020). However, biofilm protects the uropathogens from the host immune system and antibiotics (Flores-Mireles et al., 2015). Apart from this, Staphylococci use adhesins (clumping factor B: ClfB) as a virulence factor to adhere to the fibrinogen-covered catheter and ultimately cause bladder inflammation. Furthermore, Proteus species (*Proteus mirabilis)* have different virulence factors (mentioned in table 3), such as mannose-resistant Proteus-like (MR/P) fimbriae, pili, flagella, and urease which enhance the formation of bladder stones and crystalline biofilms and at the end causes bacterial persistence in the catheterized bladder. Production of urease causes hydrolysis of urea and from carbon dioxide and ammonia and influences the formation of calcium crystals and magnesium ammonium phosphate precipitate in the urine, resulting in increased pH level and forming crystalline biofilm to protect the pathogen. Mainly these crystalline structures hamper the proper urine drainage and enhance the development of pyelonephritis, shock, and septicaemia, also the formation of e

bacterial toxins haemolysin (HpmA) and Proteus toxic agglutinin (Pta) causes tissue destruction and bacterial spreading to the kidneys. (Flores-Mireles et al., 2015).

Table 3 *Virulence factors used by microorganisms* (Flores-Mireles et al., 2015)

Microorganism	**Virulence factors**	**Name of Virulence factors**	**Toxin**
UPEC	Pili and adhesins	**Pili:** F1C pili, P pili, S pili and Type 1 pili **Adhesin:** Dr adhesins	α-haemolysin (HlyA) and cytotoxic necrotizing factor 1 (CNF1)
Proteus mirabilis	Pili, fimbriae, and adhesins	**Pili:** mannose-resistant Proteus-like (MR/P) pili Fimbriae: non-agglutinating fimbria (NAFs) and P. mirabilis-like fimbria **Adhesins:** adhesion and invasion mediated by the Proteus autotransporter (AipA) adhesin and trimeric autoagglutinin autotransporter of Proteus (TaaP) adhesin	Haemolysins (HpmA and HlyA), Proteus toxic agglutinin (Pta)
Klebsiella pneumoniae	Pili	Type 1 pili and Type 3 pili	Not determined
Enterococcus faecalis	Pili and adhesins	endocarditis- and biofilm-associated (Ebp) pili, Ace adhesin and enterococcal surface protein (Esp adhesin	Not determined

Moreover, proteases and toxins, urease, and iron scavenging are some other virulence factors that are produced by the uropathogens to survive and grow within the bladder environment, which ultimately damages the host tissue (Klein & Hultgren, 2020). Proteases and toxins directly cause tissue damage and dissemination to the kidneys by secreting α-haemolysin (HlyA) and some toxins such as haemolysin (HpmA) and Proteus toxic agglutinin (Pta) produced by *P. mirabilis*. That forms pores in the umbrella cell, promoting lysis and spreading of the bacteria, ultimately causing

bladder and kidney damage. Also, uropathogens require iron for colonization and presistance and several siderophore systems are used by them for iron (Fe^{3+}) scavenging. However, these siderophore systems are potential targets for the development of vaccines and for designing small molecules, which get interfere due to the iron scavenging of uropathogens (Flores-Mireles et al., 2015)

Table 4 *Common signs and symptoms of UTIs* (Massa et al., 2009)

Area of Urinary Tract Infections	**Sign and Symptoms**
Cystitis in **bladder**	• Bleeding during urination • Burning sensation during urination • Lower back pain • Frequent urination
Acute **pyelonephritis** in the kidney	• Having a high fever • Severe pain and difficulty during urination • Frequent urination even after having an empty bladder • Serious pain in the upper back and side pain • Nausea and vomiting
Urethritis in **urethra**	• Urethral or vaginal discharge • Frequent urination • Burning sensation during urination • Itching and pain during urination • Presence of blood in semen or urine.

Chapter 2
Diagnosis of UTIs

Different kinds of UTIs (summarized in Table 2) trigger the clinical signs and symptoms of UTIs (mentioned in Table 4), and in this case, taking urine specimens is the first choice for diagnosing UTIs. Urinalysis, urine culture, and OTC dipstick testing are the most common methods to diagnose UTIs before starting antibiotic therapy (Wong, 2022). Cystoscopy, ultrasonography, CT scan or MRI, and scintigraphy are used for further testing. At first, it is essential to collect the urine sample, and store it at 4 ◦C temperature at least for 24 hours to prevent the proliferation of bacteria, and then sent it to the laboratory for the required test to identify the causative pathogens (Piñeiro Pérez et al., 2019). Urinalysis is a microscopic method that helps to analyze the appearance and the concentration of urine, the presence of foreign substances such as bacteria or crystals, and other markers, identify infections and kidney abnormalities, quantify total white blood cells, red blood cells, and also helps to screen for diabetes. Furthermore, urine culture or the bacteria culture test has been used for the regular follow-up to urinalysis by an automatic laboratory test which helps to identify and quantify specific urine substances and helps to select the most effective antibiotic for treating UTIs (Wong, 2022). But sometimes, it has become difficult to diagnose UTIs by analyzing urine due to the lack of patient seriousness (Brookes-Howell et al., 2019) in this case, ultrasonography has been used during the acute episode of UTIs for patients who are suspected to have recurrent or complications of UTIs (Piñeiro Pérez et al., 2019). Apart from this, in cystoscopy, an instrument with a camera is placed inside the urethra and bladder to analyze the inner condition urinary tract, and an eyepiece of this instrument helps to see the inner condition of the patient, and all this process is performed by a urologist (Flores-Mireles et al., 2015).

Chapter 3

Different therapeutic choices for treating UTIs

3.1 The management of UTIs

Some studies have shown that if UTIs are left untreated, they can spread and cause severe complications. Hence, lower urinary tract infection is easier to treat than the upper urinary tract. If untreated UTIs may spread to the upper urinary tract, and causing sepsis. The infection may also spread into the blood, resulting in serious illness (Piñeiro Pérez et al., 2019). Also, uncomplicated cystitis to septic shock occurs because of severe pyelonephritis, and for this kind of severe infection, broad-spectrum intravenous antibiotic therapy is required (Bader et al., 2017). However, drinking plenty of water, including garlic, cranberries, and blueberries in the diet, avoiding caffeine, alcohol consumption, and spicy food, reducing the number of carbohydrates from the diet, then taking pain relievers, D-mannose if needed, and using heating pads these are the general measure to manage the UTIs (Lee & Neild, 2007a). Furthermore, oral antibiotic therapy is the most common and effective treatment strategy to manage UTIs and the selection of antibiotics depends on the disease condition of the patient, local susceptibility patterns, types of microorganisms, and the types of UTIs (Piñeiro Pérez et al., 2019). Other than antibiotic therapy, nowadays there are also some alternative therapeutic options such as different types of vaccines, small compounds (silicide, mannosides, and phenyl), nutraceuticals (Cranberry and hyaluronic acid), immunomodulant agents (COX-2 inhibitor), and probiotics are used to manage UTIs effectively and these are known as non-antibiotic therapies, discussed in Table 6 (Loubet et al., 2020).

3.2 Antibiotic therapy

For treating UTIs, during the selection of antibiotics, physicians mainly focus on the effectiveness of antibiotics against the microorganism, infection severity, patient age factor, and the potentiality for antibiotic resistance. Antibiotics such as beta-lactams, penicillins, cephalosporins- Cephalexin, and Amoxicillin are used to treat UTIs, Macrolides- such as clarithromycin, azithromycin, and erythromycin are used when UTIs occur due to STD (sexually transmitted diseases), Aminoglycosides- gentamicin, amikacin, and tobramycin are used as combination therapy for the treatment of severe UTIs (Jacob, 2021), Tetracyclines- sumycin, and vibramycin, and Fluoroquinolones- Cipro, and Levaquin are also treat UTIs in adults and synthetic phosphonic acid derivative such as fosfomycinis used for the treatment of cystitis but applicable for more complicated UTIs (Jacob, 2021). However, currently, Fosfomycin, Trimethoprim or sulfamethoxazole, and Nitrofurantoin are well tolerated and used as first-line antibiotics for the treatment of acute and uncomplicated UTIs (Holm et al., 2019), as mentioned in Table 5(a).

3.3 Mechanisms of action of first-line antibiotics

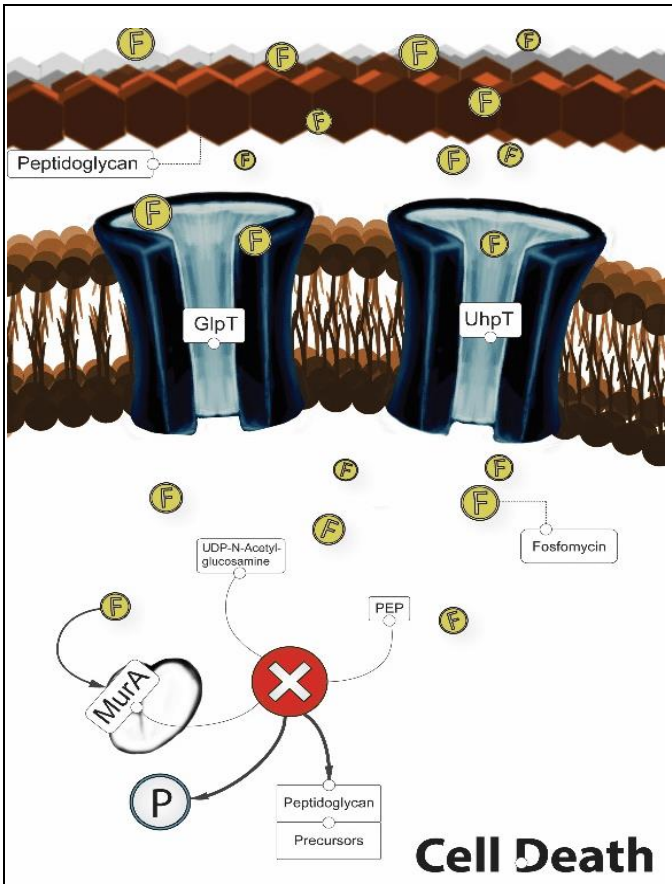

Figure 2(a): Mechanisms of action of fosfomycin. Fosfomycin irreversibly inhibits bacterial cell wall synthesis in the early stage of the peptidoglycan synthesis procedure which ultimately causes cell death (Raz, 2012).

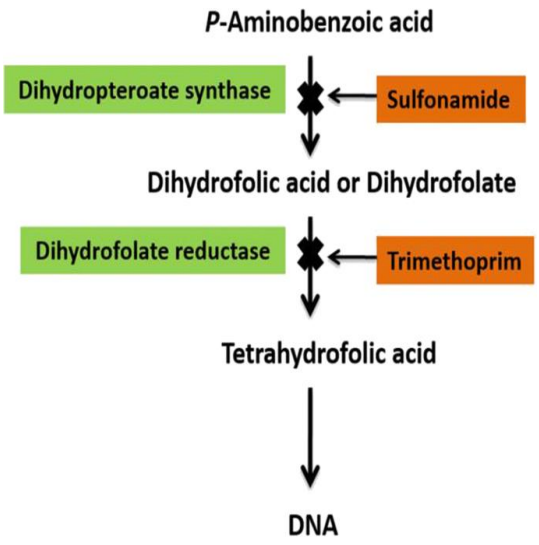

Figure 2(b): Mechanisms of action of trimethoprim or sulfamethoxazole: bactrim DS. Due to the structural similarity with para-aminobenzoic acid (PABA), sulfonamide works as an inhibitor of dihydrofolic acid synthesis and results in the lack of folic acid which ultimately causes bacteriostatic effect by preventing the tetrahydrofolate and, bacterial purines and DNA synthesis (Dowling et al., n.d.).

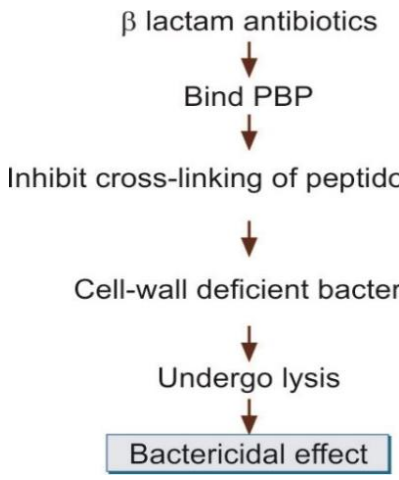

Figure 2(c): Mechanisms of action of pivmecillinam (Dowling et al., n.d.). Pivmecillinam is a prodrug of beta-lactam antibiotic mecillinam which is active against Gram-negative bacteria. It binds with enzymes, inactivates penicillin-binding protein 2 (PBP 2) that helps form peptidoglycans, and then inhibits bacterial cell wall biosynthesis which ultimately causes cell death.

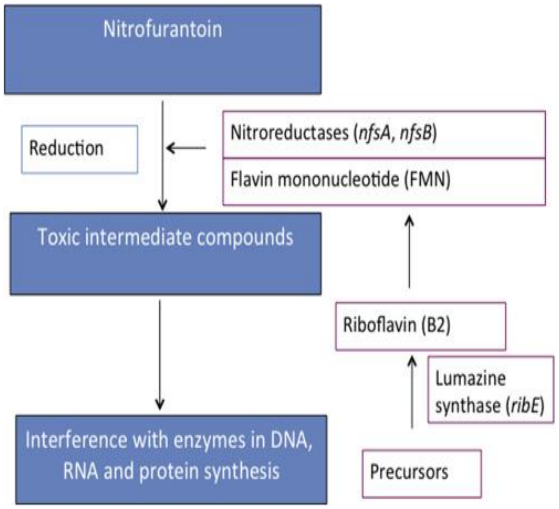

Figure 2(d): Mechanisms of action nitrofurantoin (Alam et al., 2019). Nitrofurantoin works as an oxygen acceptor, inhibits the bacterial cellular respiration process, and also interferes with the biosynthesis of bacterial nucleic acid which ultimately causes the bacteriostatic or bactericidal effect.

Table 5(a) *Selection of antibiotics for the treatment of UTIs* (Jacob, 2021)

Treatment option	Antibiotics	Doses	Class of antibiotic	MOA
First-line	Fosfomycin	A single dose (3 gm)	Phosphonic acid derivative	It inhibits the synthesis bacterial of the bacterial cell wall. (Raz, 2012)
	Trimethoprim or sulfamethoxazole: Bactrim DS	160/800 mg two times daily for three days	Sulfonamides	Inhibit dihydrofolic acid synthesis and prevent the tetrahydrofolate and synthesis, bacterial purines, and DNA synthesis. (Dowling et al., n.d.)
	Pivmecillinam	200 mg oral dose twice daily for 1 week or 3 times daily for 5 days	Beta-lactam (prodrug of beta lactam antibiotic: mecillinam)	Inhibitor of bacterial cell wall biosynthesis. (Dowling et al., n.d.)
	Nitrofurantoin macrocrystals: Macrodantin	100 mg two times daily for five days	Nitrofuran antibiotics	Interferes with the biosynthesis of bacterial nucleic acid (Alam et al., 2019)

Treatment option	Antibiotics	Doses
Second-line	• Ciprofloxacin: Cipro	250 mg two times daily for three days
	• Extended-release Ciprofloxacin: Cipro XR	500 mg daily for three days
	• Levofloxacin	250 mg daily for three days
	• Ofloxacin	A single dose of 400 mg or Daily 200 mg for three days
Third-line	• Amoxicillin	500 or 125 mg two times daily for a week
	• Cefdinir: Omnicef	300 mg two times daily for ten days
	• Cefpodoxime	100 mg two times daily for a week

Table 5(b) *Selection of antibiotics for the treatment of UTIs based on the disease condition of patients* (Frimodt-Møller, n.d.) and (Jacob, 2021)

Severe UTI with **high fever, chills, and hypotension**		
Treatment option	**Antibiotics**	**Considerations**
First-line	Ceftriaxone	For this antibiotic the rate of cross-reactivity is low. Patients with mild penicillin allergy can use this antibiotic safely.
Second-line	Gentamicin	It can cause kidney toxicity and ear toxicity, so it is important to take some caution.
Complicated or uncomplicated (Cystitis or lower UTI)		
Treatment option	**Antibiotics**	**Considerations**
First-line	Nitrofurantoin, Trimethoprim/sulfamethoxazole	• Nitrofurantoin is most effective against *E. coli* • In the case of Trimethoprim/sulfamethoxazole, it is important to consider the potassium level.
Second-line	Cephalexin	It is effective against *E. coli, Proteus,* and *Klebsiella pneumoniae*
Upper UTI and pyelonephritis		
Treatment option	**Antibiotics**	**Considerations**
First-line	Trimethoprim/sulfamethoxazole	A single dose of IV or IM injection can be administered before initiating the oral therapy
Second-line	Ciprofloxacin	It is the second choice if the patient cannot be able to tolerate Trimethoprim/sulfamethoxazole

Table 5(c) *Selection of antibiotics for the treatment of UTIs during pregnancy* (Bader et al., 2020a)

FDA-approved preferable antibiotics for pregnant women	Uses	Toxicity
Penicillins: Penicillin G, Amoxicillin, Ampicillin	The most commonly used antibiotic during pregnancy	The risk of toxicity is low
Cephalosporins: Cephalexin (1st generation), Cefuroxime (2nd generation), Cefepime (4th generation), Ceftriaxone and Ceftazidime (3rd generation)	Cephalexin, Cefuroxime, and Ceftriaxone are the commonly used antibiotics but Cefepime and Ceftazidime used with antipseudomonal coverage	Low risk
Lincosamides: Clindamycin	It is also a commonly used antibiotic and is mostly used in the patient with penicillin-allergic	Risk is low
Macrolides: Azithromycin, Erythromycin	Azithromycin is commonly used	Doesn't cause any serious toxic effects. Erythromycin can show greater GI side effects than azithromycin
Nitrofurans: Nitrofurantoin and Phosphonics	Commonly used only for the treatment of lower UTIs during pregnancy	Can cause hemolytic anemia and rarely teratogenesis
Sulfadiazine	Must be avoided during pregnancy	It has a high risk of antifolate teratogenesis and hyperbilirubinemia in 3rd trimester of pregnancy
Trimethoprim/sulfamethoxazole	Not recommended during pregnancy	It has a high risk of antifolate teratogenesis

3.4 Combination therapies

Some new antimicrobials which are resistant to inactivation by ESBLs can be used as a combination therapy with new classes of β-lactamase inhibitors, which target combinedly β-lactamases and K. pneumoniae carbapenemases (KPCs) and have been shown a promising effect against Enterobacteriaceae (Klein & Hultgren, 2020). Also, third-generation cephalosporin, ceftazidime has been shown effectivity against both Gram-positive and Gram-negative organisms, and when it has combined with β-lactamase inhibitor avibactam, then it has become able to effective against ESBL- and carbapenemase-producing Gram-negative bacteria. In some cases, ceftazidime–avibactam combination therapy against ESBL-, KPC- and AmpC-producing Gram-negative pathogens more future studies is required because this can be effective against a broad range of cephalosporin resistant Enterobacteriaceae family. However, effectivity of antibiotic–inhibitor therapies is reliant on the antimicrobial-resistance patterns which are encoded by each microorganism. The combinations of ESBLs and carbapenemases can show resistance to an antibiotic–inhibitor therapy. For example, combination of two β-lactam antibiotics along with a β-lactamase inhibitor can be effective against many carbapenem-resistant Enterobacteriaceae family but another type of ESBL (KPCs and SHVs), or AmpC enzymes are resistant(Flores-Mireles et al., 2015).

3.5 Side effects and contraindications of currently used antibiotic therapies

Antibiotic therapy has shown certain side effects, and among these, some are even serious and cause discomfort to the patient. Sometimes, inappropriate use of antibiotics can cause life-threatening reactions. Skin rashes, dizziness, yeast infections, nausea, and diarrhea are the most common side effects of antibiotic therapies. In some serious cases, *C. diff* infection can occur

because of some antibiotics, leading to serious diarrhea and severe colon damage, and even death. Additionally, the most common and serious negative outcomes of antibiotics are antibiotic resistance and multi-drug resistance (MDR) infections as a consequence of antibiotic-resistant Gram-negative bacteria which leads to treatment failure (Bischoff et al., 2018). Moreover, nitrofurans and sulfadiazine therapy can cause hemolytic anemia, hyperbilirubinemia, and teratogenesis, these are difficult to handle (Bader et al., 2020b). Although severe UTIs in pregnant are also difficult to treat due to some treatment limitations (Jacob, 2021), fluoroquinolones, sulfadiazine, and trimethoprim/sulfamethoxazole are not preferable for pregnant women or children as these can cause teratogenesis, nausea, vomiting, and even hyperbilirubinemia in pregnant women and it is also contraindicated in patients with severe renal failure (Table 5c) (Bader et al., 2020a). Similarly, amoxicillin can cause hepatitis along with diarrhea and rashes and it has been hypersensitive to penicillin therapy. Furthermore, dizziness, convulsions, the problem of hallucinations, hepatitis, various kinds of blood disorders, and photosensitivity are shown as side effects of ciprofloxacin and this antibiotic is also contraindicated in patients who have CNS disorders, G6PD deficiency, and also not preferable for pregnant women and children. Sometimes, Nitrofurantoin can show certain negative outcomes such as vomiting, peripheral neuropathy, and serious pulmonary reactions. This antibiotic does not apply to patients with G6PD deficiency, renal failure, porphyria, and neonates (Pulipati et al., 2017).

Chapter 4

Antimicrobial resistance and multi-drug resistance

4.1 Antimicrobial resistance (AMR) in the treatment of UTIs

The emergence and spread of antimicrobial-resistant microorganisms (AMRO), the antimicrobial resistance (AMR) arises when microorganisms such as parasites, viruses, fungi, and bacteria mutated over time and cannot respond to antibiotics, as a result, the infections become harder to treat, and even cause treatment failure, serious illness and increase the risk of spread, which even ended up with death (Hossain et al., 2020). Several studies emphasize, that bacteria are mostly responsible for urinary tract infections so unnecessary or inappropriate use of antibiotics and even the presence of some bacteria such as *E. coli, K. pneumonia, Enterobacter cloacae,* and *Proteus mirabilis* may rapidly develop antibiotic resistance which ended up causing failure of antibiotic therapy as a result infection becomes more serious which is a future threat to public health. It is believed that gram-negative bacteria are mostly responsible for antimicrobial resistance, as they synthesize extended spectrum β-lactamases (ESBLs) and also carbapenemases, which enable them to spread drug resistance rapidly. For example, *E. coli* and *K. pneumonia* can acquire resistance against third-generation cephalosporins (Matuszkiewicz-Rowińska et al., 2015).

There are four ways reported by which these bacteria resist the activity of antibiotics, and these are efflux pump, decreasing the uptake of antibiotics by changing the outer membrane's permeability or because of the presence of porins, inactivation of the enzyme, and modifying the target site (Annunziato, 2019). Primarily, antibiotics cannot be able to reach the target site for several reasons, as shown in the figure above. For example, the efflux pump is a common pumping process for the transportation of signaling molecules and some nutrients from bacterial cells. In some cases, antibiotics are pumped out of the cell by an efflux pump, resulting decrease in the

concentration of antibiotics inside the bacterial cell, further this causes the mutation of bacterial DNA and increases the pump of more antibiotics from the cell and also increases the risk of antibiotic resistance. Then, the permeability of the bacterial cell wall is decreased which changes the bacterial membrane and makes it more difficult for antibiotics to get inside the bacterial cell. Moreover, some bacterial enzymes destroy antibiotics such as β-lactamase enzymes of bacteria destroy the β-lactam ring of penicillins thus destroying the antibiotics, and also some enzymes are capable to modify the antibiotics by adding different types of chemical groups so that the binding of the antibiotics with the target is prohibited (Hasudungan, 2014). Furthermore, target alteration is another reason for antibiotic resistance inhibiting the binding of antibiotics and their potency. For example, in some cases, the target for antibiotics in bacteria can alter its composition and structure as a result antibiotics cannot be able to interact with the target which causes antibiotic resistance. Then again, some bacteria produce alternative proteins instead of desired ones which are inhibited by the antibiotic, as a result, antibiotics cannot be able to inhibit those proteins and resistance occurs. For example, Staphylococcus aureus is capable to interact with resistant gene mecA and make new penicillin-binding proteins, this protein has a low affinity to β-lactam antibiotics as a result resistance occurs and causes treatment failure (Piddock, 2014). Furthermore, structural alteration of the cell wall of bacteria is another reason for antibiotic resistance. The cell wall becomes different from the susceptible one for the antibiotics so antibiotics cannot interact with the cell wall, ultimately decreasing the uptake of antibiotics. (Martínez, J. L., 2014), also shown in Figure 3.

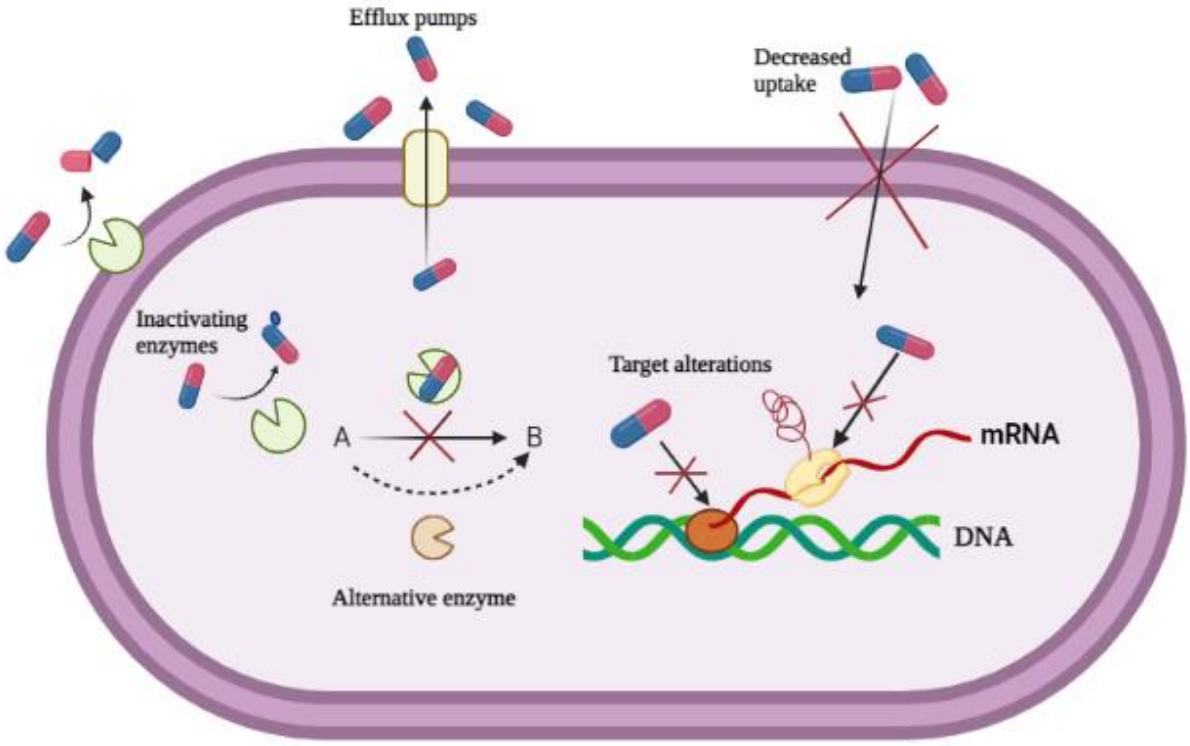

Figure 3: Mechanism of antibiotic resistance during UTIs (Annunziato, 2019). In figure 3, four ways of antibiotic resistance are shown, which are 1. efflux pump, 2. limiting uptake of antibiotics by changing the outer membrane's permeability, 3. modifying an antibiotic target, and 4. inactivating an antibiotic (Annunziato, 2019).

However, studies currently dictate that to prevent antibiotic resistance, existing antibiotics need to be replaced with new drugs. There are three types of antibiotic adjuvant strategies are used to control this antibiotic resistance and such classes of antibiotics are, a) efflux pump inhibitors: quinolines, thioridazine (TZ) derivatives, sulbactam, and clavulanic acid, b) beta-lactamase inhibitors: tazobactam, aminoglycosides, polimixyn B and c) outer membrane permeabilizer: polycationic/cationic antimicrobial peptide, caragenins and also some anti-virulence compounds are used such as- SAT-inhibitors, Cys-inhibitors (Annunziato, 2019).

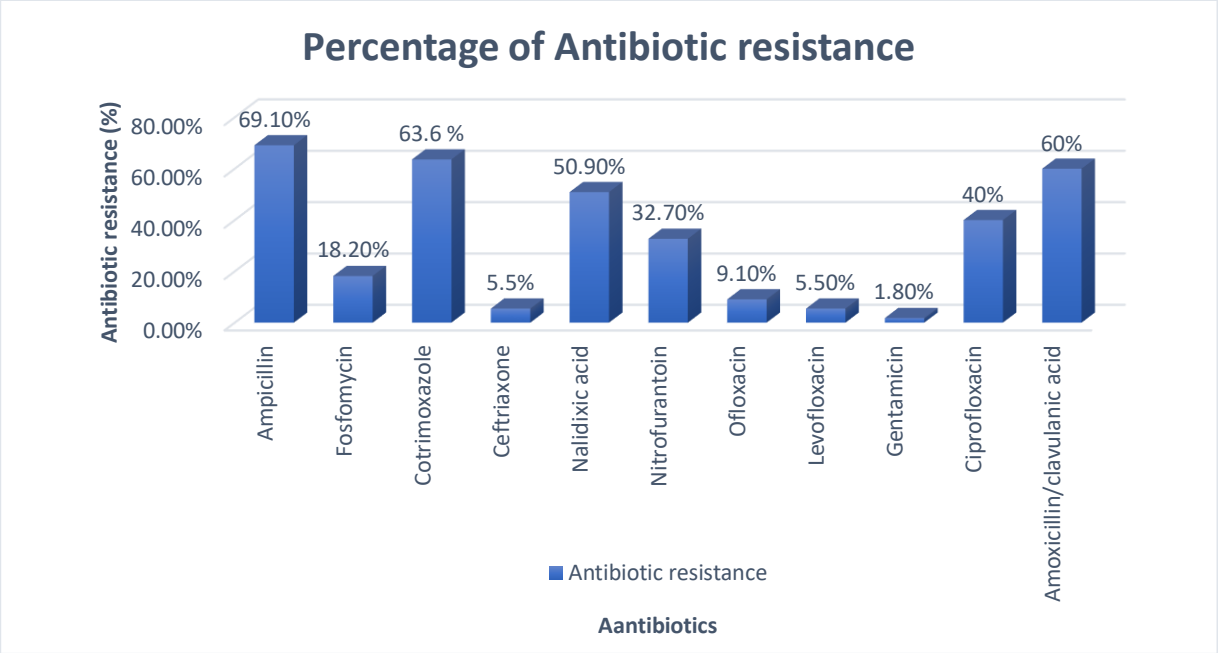

Figure 4:(A) Percentage of antibiotic resistance occurring with different antibiotics used in the treatment of UTIs. In Figure 4(A), the bar chart illustrates that most bacteria are resistant to ampicillin, cotrimoxazole, amoxicillin/clavulanic acid, and nalidixic acid. Whereas the percentage of antibiotic resistance is below 50% for ciprofloxacin and nitrofurantoin, apart from this the prevalence of antibiotic resistance is very low for ofloxacin, levofloxacin, ceftriaxone, and gentamycin and the percentage of it is 9.1%, 5.5% for both ceftriaxone and levofloxacin, and 1.8% respectively (Nzalie et al., 2016). (Lewis D A., 2013).

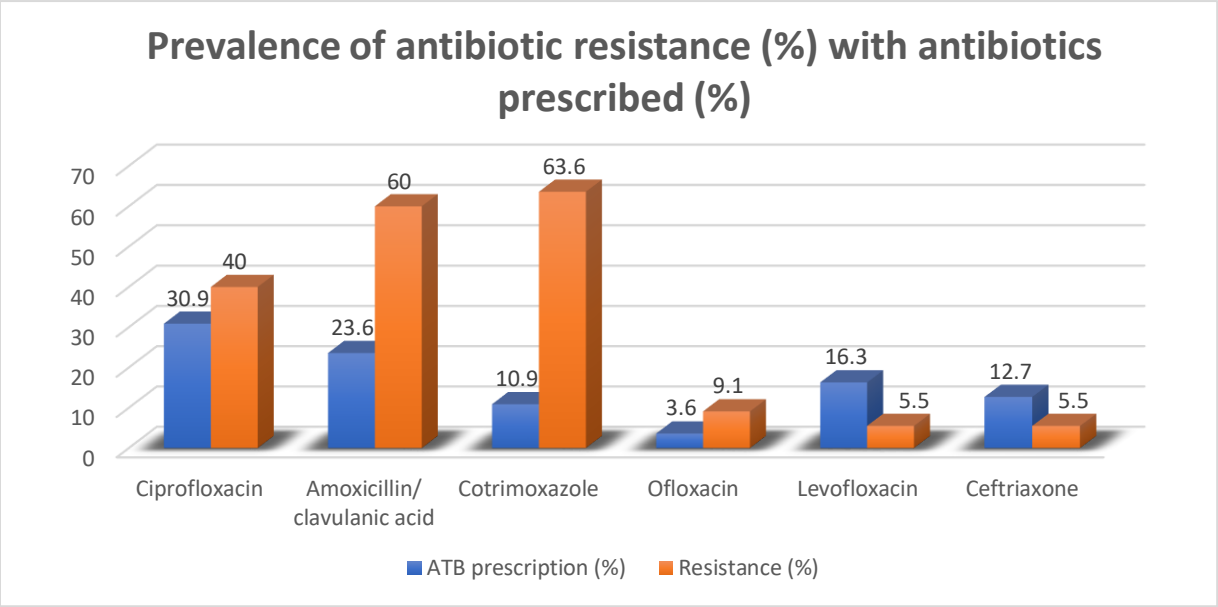

Figure 4(B): the prevalence of antibiotic resistance (%) with antibiotics prescribed (%), adapted from; (Nzalie et al., 2016). According to figure 4(b), it is also observed that microorganisms show the highest resistance to cotrimoxazole, amoxicillin, and ciprofloxacin. Also, amoxicillin/clavulanic acid is considered the second most prescribed antibiotic as 60% of most bacteria are resistant to this antibiotic Then, *Proteus mirabilis* is mainly resistant to cotrimoxazole, ampicillin, and fosfomycin. As a consequence of this antimicrobial susceptibility testing, it is observed that 40% of most bacteria are resistant to ciprofloxacin and for this reason, ciprofloxacin is the most prescribed antibiotic. Furthermore, bacteria are least resistant to levofloxacin and ofloxacin ((Nzalie et al., 2016).

4.2 Multi-drug resistance (MDR) in the treatment of UTIs

Some studies emphasize that multi-drug resistance occurs during UTI when multi-drug resistant organisms (MDRO) such as *E. coli, K. oxytoca, K. pneumoniae, Enterobacter spp, P. mirabilis*, etc., and some other Gram-negative uropathogens cause resistance to at least one or more kinds of antimicrobial substances and ultimately show serious complications even treatment failure (Mazzariol et al., 2017). Formation of biofilm helps to protect the uropathogens from the host immune system by providing a physical barrier to entering the antibiotics, resulting in multiple antibiotics failing to show the effectiveness. However, MDR can occur when Enterobacter produce various types of beta-lactamase enzymes which ultimately destroy the beta-lactam ring of several antibiotics. Hydrolysis of some antibiotics such as penicillins, first to third-generation cephalosporins and aztreonam occurs due to the presence of extended-spectrum beta-lactamases (ESBL) producers but this can be inhibited by clavulanic acid combinations. Also, some ESBL-producing bacteria often cause MDR as their plasmids carry some genes to the antimicrobial agents (aminoglycosides, fluoroquinolones, and sulphonamides). In the case of aminoglycosides, *E. coli, K. pneumoniae, P. aeruginosa,* and *Acinetobacter spp.* produce 16S rRNA methylases enzyme which eventually alters the target site and the drug is modified by some aminoglycoside-modifying enzymes such phosphotransferases (APH) and acetyltransferases (AAC) of those bacteria. Then fluoroquinolones resistance occurs when *E. coli, Klebsiella spp.,* and *P. aeruginosa* DNA gyrase and topoisomerase IV cause mutation of the target. Trimethoprim (sulphonamides) resistance occurs due to the overproduction of dihydropteroate synthase (DHPS), and dihydrofolate reductase (DHFS) enzymes in the presence of *E. coli* (Asmat et al., 2021). Moreover, the most frequently reported risk fact of MDR is previous antibiotic therapy, hospitalization, inappropriate antibiotic therapy, and urinary tract anomalies. Therefore, these observational phenomena indicate that

management of UTIs becoming more challenging for antimicrobial resistance and multi-drug resistance (MDR) pathogens. Hence, Fosfomycin is the most effective antibiotic against the most significant MDR pathogens CR-Kp, P. aeruginosa, ESBL producers, and VRE for the treatment of MDR UTIs. (Neuner et al., 2012). Apart from this some parenteral antibiotics such as aminoglycoside (Gentamicin, Amikacin), polymyxin (Tigecycline), Beta-lactam with a beta-lactamase inhibitor (Meropenem-vaborbactam) and some oral antibiotics therapy such as nitrofurantoin, amoxicillin-clavulanate (Penicillin with beta-lactam inhibitor), and pivmecillinam (synthetic penicillin) are also used for treating MDR UTI (Mahony et al., 2020). According to one recent analysis, some antimicrobial substances such as ceftolozane/tazobactam, meropenem/vaborbactam, plazomicin, and ceftazidime/avibactam are also used for managing MDR UTI (Bader et al., 2020b).

Extended-spectrum β-lactamases (ESBLs) such as cefotaximases (CTX-Ms) and oxacillinases (OXAs) are acquired by the Enterobacteriaceae family, frequently use of cephalosporin can establish an ideal environment for antibiotic resistance. ESBLs are plasmid-encoded or chromosomally encoded β-lactamases and have a broad spectrum of activity against penicillins and cephalosporins. However, it destroys the amide bond of the β-lactam ring, which ultimately inactivated the β-lactam antibiotics. ESBLs also carry some resistance genes that create resistance against aminoglycosides, sulfonamides, and quinolones, making the uropatogens that receive these plasmids and cause multidrug-resistant (Flores-Mireles et al., 2015). Furthermore, AmpC enzymes can hydrolyse penicillins, third-generation and extended-spectrum cephalosporins, and cephamycins, and also have the ability to form a resistance against β-lactamase inhibitors, such as clavulanate (Klein & Hultgren, 2020). Apart from this, carbapenemases are also a member of the ESBLs family, which have the ability to o inactive carbapenems and create resistance against

penicillins and extended-spectrum cephalosporins. The plasmids encoding the ESBLs CTX-Ms can create resistance against narrow-, broad- and extended-spectrum penicillins, cephalosporins, monobactams, and high-level resistance to cefotaxime. These mainly hydrolyse the β-lactam ring by a nucleophilic attack of a ring carbonyl carbon, ultimately opening the ring and inactive the substance (Flores-Mireles et al., 2015).

Chapter 5

Non-antibiotic therapy and new drug candidates

Although antibiotic therapies are the first-line treatment option for managing UTIs, because of limitations, contraindications, and treatment failure, sometimes it is needed to go for non-antibiotic therapies. Vaccines and low molecular weight compounds are currently used as standard non-antibiotic treatment options for UTIs. However, four types of vaccines are used as non-antibiotic treatment options for UTIs. Uro−Vaxom®, Urovac®, ExPEC4V, and Uromune® are the vaccines (Aziminia et al., 2019), which are mainly responsible to prevent recurrent UTIs by initiating an immune response to uropathogens and provide protection against the infection instead of killing the infectious pathogens (Loubet et al., 2020). Pillicide, mannoside, and hydroxamic acid are the common small compounds that help to prevent urine alkalization, reduce the formation of biofilm and also prevent the formation of pilli and ultimately help to manage UTIs, summarized in Table 6 (Loubet et al., 2020). Other than these, probiotics, bacteriophages, nutraceuticals, and acupuncture are also used for the treatment of UTIs. Sometimes decrease in the number of protective lactobacillus colonies in the vagina can increase the risk of UTIs in this case, oral or vaginal administration of probiotics helps to control the number of decreased lactobacillus and establish a protective barrier against the infection. Moreover, it has been established that acupuncture also helps manage UTIs by handling flow imbalances (Barea et al., 2020). Also, the proper practice of emerging therapies mentioned in table 7, will change the idea of treatment therapies (Flores-Mireles et al., 2015).

Table 6 *Non-antibiotic therapeutic options for the management of UTIs* (Loubet et al., 2020)

Non-antibiotic therapies	Advantages	Limitations
Vaccine: Vaccine targeting adhesion, bacterial toxins, capsule, and iron metabolism (Ellis and Kuehn, 2010)	• By targeting the adhesion vaccine blocks the attachment of liaison adhesin-host cell receptors which ultimately reduces bacterial colonization and protects the kidney and bladder. For example, the pili vaccine.	• In some cases, does not provide long-term protection.
	• Decrease the rate of renal injury and provide an effective immune response against pathogens by targeting toxins, and iron metabolism which ultimately reduces the virulence and the risk of recurrent UTI.	• Causes heterogeneity of the targets and bacterial membrane proteins
Small molecule compounds: Some low molecular weight compounds, typically bacterial substrates such as pillicide, mannoside, hydroxamic acid are used as a non-antibiotic therapy to inhibit UTIs.	• Pillicide inhibits the formation of pilli thus decreasing bacterial adhesion, virulence, and formation of biofilm (Åberg and Almqvist, 2007).	• Does not have enough in vivo test results. • No effective clinical study.
	• Mannoside reduces bladder colonization, inhibiting adhesion (Cusumano et al., 2011).	• Can cause side effects and show poor bioavailability
Nutraceutical: Cranberry**,** hyaluronic acid, and D-mannose are used as alternative treatment options.	• Cranberry blocks adhesion, motility, and biofilm formation, which affects UPEC strains, *P. aeruginosa,* and *P. mirabilis* (Ahuja et al.,1998).	• In some cases, the result is not promising enough.
	• Hyaluronic acid and D-mannose also reduce adhesion. In humans, hyaluronic acid gives a promising result and oral administration of D-mannose provides a fast effect (Constantinides et al., 2004).	

Immunomodulant agents: COX-2 inhibitors and extract of green tea work as immunomodulant agents for the management of UTIs (Moore et al., 2019)	• Inhibit inflammations and also the inflammation caused by cystitis.	• Does not provide significant clinical results.
	• Reduce the risk of recurrent UTIs.	• Mechanisms of action are not clear.
Probiotics Vaginal lactobacilli, E. coli 83972, and predatory bacteria are the common probiotics.	• Vaginal lactobacilli inhibit adhesion and virulence, produce natural antimicrobial compounds, and don't show serious side effects (Riaz et al., 2010). •	• Does not have enough in vivo test clinical test results
	• E. coli 83972 causes colonization of the bladder and also decreases the UPEC colonization (Sundén et al., 2010)	• No study has proven the effectiveness of predatory bacteria in the treatment of UTIs
	• Predatory bacteria inhibit the number of bacteria and also decrease the formation of biofilm. It is mainly effective against Gram-negative bacteria (Gupta et al., 2016).	
Bacteriophages	Bacteriophages directly kill the bacteria (Kuipers et al., 2019)	Required more human studies.
Acupuncture	Effectively reduce the risk of recurrence of UTI (Barea et al., 2020)	• Not applicable for all kinds of patients. • Heterogeneity of the treatment process.

Table 7 *Emerging therapies for treating UTIs* (Klein & Hultgren, 2020)

Treatment	Target agent	Target disease	Role
Anti-adhesive agents			
Mannosides	UPEC and type 1 pili	Acute cystitis	Reduce the bacterial burden and works as a prophylactic agent and reduce the bacterial burden in the bladder and kidney (Flores-Mireles et al., 2015).
Galactosides	UPEC and Fim-like pili	Chronic cystitis and pyelonephritis	
Vaccine			
FimCH	UPEC and type 1 pili	Cystitis	Reduce the UTI recurrence
$EbpA^{NTD}$	Enterococcus species	CAUTI- catheter-associated UTI	
4-component-2 capsular polysaccharides, mutated α-toxin, and clumping factor -A	Staphylococcus species	Staphylococcal UTI	Reduce the bacterial adhesion in the bladder and catheter
Others			
Probiotics	UPEC	rUTI	Reduce the frequency of infection
NSAIDs	UPEC	Chronic and recurrent cystitis	Inhibit the prostaglandin activity thus reduce the inflammation

According to the data shown in Table 5, maintenance of antimicrobial resistance (AMR), multidrug resistance (MDR), and the related toxicity due to the treatment of these are not that easy (Brookes-Howell et al., 2019). Even the alternative therapies also show some drawbacks, intravenous formulations such as vaccine doesn't provide lifetime protection. Small compounds which are used as an alternative treatment option, sometimes show poor bioavailability and don't have effective clinical studies. Then, the mechanism and effectiveness of immunomodulated agents are not well established. Also, acupuncture does not apply to all kinds of patients (Barea et al., 2020).

Chapter 6

Conclusion and future directions

6.1 Conclusion

UTI is increasingly becoming a serious and chronic health concern worldwide. So, selecting an appropriate therapeutic approach for treating this infection is important. In 63–85% of cases, gram-negative bacteria specifically *Escherichia coli* can be causes urinary tract infections (UTIs) (Ghouri et al., 2019) but there are some other reasons for UTIs. Microorganisms can enter the body through the urethra and undergo pathogenesis, further ending up by causing UTIs (Nzalie et al., 2016). When the symptoms are shown, the patient undergoes a diagnosis and, in this case, routine analysis of sterile urine has been the most used diagnostic method (Piñeiro Pérez et al., 2019). In some exceptional cases, cystoscopy, ultrasonography, and CT scan or MRI can be also used for diagnostic purposes. However, this study has aimed to address that antibiotic therapy can be the most preferable treatment approach, and currently, fosfomycin, trimethoprim or sulfamethoxazole, and nitrofurantoin have been used as first-line antibiotics therapy. If first-line therapy does not work properly due to some specifications, then second-line (ofloxacin, ciprofloxacin) or third-line (amoxicillin) antibiotic therapy will be required. This study has also highlighted that it is necessary to consider some factors during the selection of antibiotic therapy: the severity of infection, patient age, the potentiality for antibiotic resistance, and so on (Jacob, 2021). Antimicrobial resistance (AMR) and multi-drug resistance (MDR) have the most significantly highlighted drawbacks of treating UTIs. AMR and MDR ultimately turn into severe complications if left untreated. This study has focused on managing AMR, primarily, the replacement of antibiotics is required, if it fails then have to go for antibiotic adjuvant strategies to control this antibiotic resistance

(Annunziato, 2019). Additionally, it has been pointed out that Fosfomycin is most effective for managing MDR, along with this ceftolozane/tazobactam, meropenem/vaborbactam, plazomicin, and some parenteral antibiotics (Gentamicin, Amikacin, and Tigecycline) are also used (Mahony et al., 2020). The percentage of AMR can be different for various kinds of antibiotics, most of the bacteria have developed resistance to ampicillin, cotrimoxazole, and amoxicillin/clavulanic acid. On the other hand, this rate is low for gentamycin, ceftriaxone, and levofloxacin. It has been observed that according to the rate of antibacterial resistance, ciprofloxacin can be used as another most prescribed antibiotic (Nzalie et al., 2016). Currently, some effective combination therapies and non-antibiotic treatment approaches have been established, such as mannosides, antivirulence therapy: vaccines targeting bacterial adhesion, toxins and proteases, and siderophores, which will change the treatment strategy for UTIs in near future (Loubet et al., 2020). To conclude, this study will help to create awareness about UTIs, AMR, and MDR-associated UTIs by practicing the proper selection of antibiotics therapy.

6.2 Future directions

There are several scopes of improvement for the accurate identification of UTI-causing pathogens and effective treatment of UTIs to prevent their recurrence. First of all, routine analysis of sterile urine is commonly used to diagnose UTIs, but this analysis is applicable for culturable aerobic bacteria, and not for fastidious or anaerobic bacteria, in this case, a novel diagnostic approach is needed. Moreover, metagenomic sequencing (MGS) will work as a novel diagnostic approach that does not require any isolation of bacteria and provides more precise information and accurate results from a complex microbial environment, as a result, it becomes easier to select more specific antibiotics for treating UTIs (Smelov et al., 2016). MGS is a genome sequencing process where genetic material (DNA) has been extracted from urine specimens and filtered. After that, it has multiplied by cloning and then sequenced. All sequences are put together by the assembly method and investigate microbial genome sequencing results (Moustafa et al., 2018). For treating CAUTI and AMR and MDR-associated UTIs, several newly investigated nanoparticles (NP) have proved nontoxic to animal and human cells. Such nanoparticles are organic and inorganic (chemical/green synthesized) nanoparticles, nanodiamonds, and nanoparticles made of composite materials, which can be used as a promising treatment choice for UTIs. For example, carbon-based nanodiamonds have been working as a drug delivery system, which has antibacterial activity to inhibit bacterial proliferation by directly interacting with the chemical substances of the bacterial cell walls. Also, the trimeric thiomannoside cluster conjugated to nanodiamond particles has antiadhesive activity. However, the practice of nanoparticles to treat UTIs can be a potential treatment strategy shortly (Sánchez et al., 2021). Likewise, antiseptic agents including chlorhexidine nanoparticles work as a cover for foley catheters and can be effective against *E. coli*, *S. aureus,* and *C. albicans* during UTIs (Supranoto et al., 2015).

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