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Urinary tract infection caused by antibiotic-resistant uropathogenic *Escherichia coli*: a major public health concern

Infecção do trato urinário causada por Escherichia *coli* uropatogênica resistente a antibióticos: um importante problema de saúde pública

Infección del tracto urinario causada por *Escherichia coli* uropatógena resistente a los antibióticos: un importante problema de salud pública

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Resumo
A infecção do trato urinário (ITU) causada por *Escherichia coli* uropatogênica (UPEC) é uma das infecções bacterianas mais prevalentes e uma das principais causas de morbidade relacionada à saúde e custos hospitalares em todo o mundo. A crescente resistência bacteriana a antibióticos administrados de forma rotineira para indivíduos com infecções bacterianas está se tornando uma fonte significativa de preocupação. Pesquisas mostram que a UPEC está se tornando mais resistente a várias classes de antibióticos, incluindo fluoroquinolonas, beta-lactâmicos e aminoglicosídeos, como resultado de determinantes genéticos de resistência e clones multirresistentes. O conhecimento da etiologia local e do perfil de sensibilidade dos uropatógenos mais comuns aos antibióticos deve orientar as decisões na terapia empírica de ITU não complicada. Dada a alta prevalência de ITU e clones multirresistentes, medidas preventivas, como o desenvolvimento de uma vacinação eficiente, são essenciais. O presente trabalho é uma revisão integrada da literatura que sintetiza informações sobre ITUs causadas por *E. coli* uropatogênica resistente a antibióticos e considera as implicações práticas dos principais resultados da pesquisa.
Palavras-chave: Infecções do trato urinário; *Escherichia coli* uropatogênica; Resistência a antibióticos; Vacinas; Saúde pública; Prejuízos financeiros.

Abstract
Urinary tract infection (UTI) caused by uropathogenic *Escherichia coli* (UPEC) is one of the most prevalent bacterial infections and is a major cause of health-related morbidity and hospital costs worldwide. The rising bacterial resistance to routinely given antibiotics for infected individuals is becoming a significant source of concern. Current research shows that UPEC is becoming more resistant to multiple antibiotic classes, including fluoroquinolones, beta-lactams, and aminoglycosides**,** as a result of genetic determinants of resistance and multidrug-resistant clones. Knowledge of the local etiology and the sensitivity profile of the most common uropathogens to antibiotics should guide decisions in the empirical therapy of uncomplicated UTI. Given the high prevalence of UTI and multidrug-resistant bacteria, preventative measures such as the development of an efficient vaccination are essential. The current work is an integrated literature review that synthesizes information on UTIs caused by antibiotic-resistant uropathogenic *E. coli* and considers the practical implications of key research results.
Keywords: Urinary tract infections; Uropathogenic *Escherichia coli*; Antibiotic resistance; Vaccines; Public health; Economic burden.

Resumen
La infección del tracto urinario (ITU) causada por *Escherichia coli* uropatógena (UPEC) es una de las infecciones bacterianas más prevalentes y es una de las principales causas de morbilidad relacionada con la salud y costos hospitalarios en todo el mundo. La creciente resistencia bacteriana a los antibióticos que se administran de forma rutinaria a las personas infectadas se está convirtiendo en una fuente importante de preocupación. La investigación actual muestra que la UPEC se está volviendo más resistente a múltiples clases de antibióticos, incluidas las fluoroquinolonas, los betalactámicos y los aminoglucósidos, como resultado de los determinantes genéticos de la resistencia y los clones resistentes a múltiples fármacos. El conocimiento de la etiología local y el perfil de sensibilidad de los uropatógenos más comunes a los antibióticos debe orientar las decisiones en la terapia empírica de la ITU no complicada. Dada la alta prevalencia de infecciones urinarias y de bacterias multirresistentes, las medidas preventivas como el desarrollo de una vacunación eficaz son fundamentales. El trabajo actual es una revisión

integrada de la literatura que sintetiza información sobre las infecciones urinarias causadas por E. coli uropatógena resistente a los antibióticos y considera las implicaciones prácticas de los resultados clave de la investigación.
Palabras clave: Infecciones del tracto urinario; Escherichia coli uropatógena; Resistencia a antibióticos; Vacunas; Salud pública; Carga económica.

1. Introduction

Urinary tract infections (UTIs) are the leading cause of sepsis in hospitalized patients, coming at a significant cost and driving a heavy blow to both the patient and society (Sood *et al*., 2015; Strehlow *et al*., 2006). Approximately 150 million people around the world develop UTIs annually (O'Brien *et al*., 2016). However, since this is seldom reported a disease, it is challenging to precisely estimate its prevalence. This scenario is compounded by the fact that an accurate diagnosis requires both specific symptoms and a positive urine culture. It is taxing to reach a definitive diagnosis of the infection since each group of infected individuals (e.g., newborns, children, pregnant women, and the elderly) exhibits distinct symptoms. The most frequent bacteria that cause UTIs are *Escherichia coli*, *Proteus mirabilis*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Staphylococcus saprophyticus* (Flores-Mireles *et a*l., 2015). Considering the high prevalence, UTI significantly impacts the patient's quality of life and the health system. Financial implications include medical appointments, diagnostic exams, medications, and losses caused by absenteeism from work. UTI affects both sexes and all ages, with a higher prevalence among women. Although short-term antibiotic regimens for uncomplicated UTI provide a rapid resolution of symptoms in the majority of patients, the risk of recurrence is considerable.

The majority of UTIs begin with a bladder infection (e.g., cystitis) caused by pathogenic bacteria colonizing the perineum, passing through the urethra, and infecting the bladder. If the cystitis is not treated, bacteria can spread into the ureters and reach the kidneys, leading to pyelonephritis—an infection that causes irreversible organ damage. In severe instances of pyelonephritis, invasive bacteria may occasionally breach the epithelial and endothelial barriers in the kidney, gain access to the host bloodstream, and induce bacteremia and systemic infections. The occurrence and severity of UTIs are related to aspects such as the pathogen's virulence, anatomical characteristics of the host, efficiency of the host immune system in controlling the infection, and availability of appropriate therapeutic interventions. First-line antibiotics for treating acute, uncomplicated UTIs include nitrofurantoin, trimethoprim-sulfamethoxazole (TMP-SMX), and fosfomycin (Gupta *et al*., 2011). The drug of choice is influenced by many factors, including cost, pharmacokinetics, resistance patterns, and the patient's clinical condition.

Multidrug-resistant (MDR) bacteria are responsible for about 25,000 deaths and accumulate €1.5 billion in expenditures annually in Europe (Bryce *et al*., 2016). Moreover, treatment with antibiotics can cause long-term changes in the vaginal and gastrointestinal natural microbiota (Stapleton, 2017). Consequently, niches that the changed microbiota has vacated might enhance the likelihood of colonization with MDR bacteria, especially uropathogenic *Escherichia coli* (UPEC), the most common bacterial cause of UTI. UPEC is becoming increasingly resistant to drugs used as the first choice in the empirical treatment of UTI. Current studies demonstrate the increasing resistance of UPEC to various classes of antibiotics related to the presence of genetic determinants of resistance and multidrug-resistant (MDR) clones. Given the high frequency of UTI and MDR bacteria, prevention such as developing an effective vaccine is crucial. This review intends to characterize the financial burden arising from the treatment of UTI, the importance of UPEC as a UTI causative agent, the pathophysiology of UTI, the current knowledge on antibiotic resistance in UPEC, and the effectiveness of prophylactic vaccines.

2. Methodology

The current study is an integrated literature review that synthesizes information on UTIs caused by antibiotic-resistant uropathogenic *E. coli* and incorporates the relevance of important research' findings in practice. Literature review from

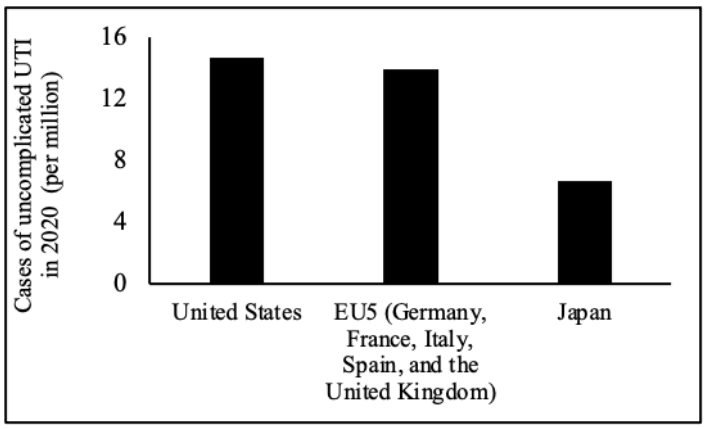
bibliographic research in reliable scientific databases such as Science Direct, Scopus, Wiley Online Library, and Scielo were used. To search for scientific articles, we used terms such as "urinary tract infection," "uropathogenic *Escherichia coli*," "cystitis," "pyelonephritis," "fluoroquinolones," "fosfomycin," nitrofurantoin," "trimethoprim–sulfamethoxazole," "beta-lactam," "Uro-Vaxom," "Solco-Urovac," and "Uromune." Criteria such as articles mostly in English and preferably published between the years 2010 to 2021 were adopted for the selection of the most relevant publications on urinary tract infection caused by antibiotic-resistant uropathogenic *E. coli*. However, it is important to note that although a few articles used in this review were published before 2011, they were still included in the present study due to their scientific importance, and most of them have more than one hundred citations.

3. Results and Discussion

3.1 UTI is a prevalent and costly public health concern

UTI is the second-most prevalent illness in the senior population and in long-term care facilities (Genao & Buhr, 2012). It is also the leading cause of bacterial infection-related hospitalization (Genao & Buhr, 2012). UTI is responsible for about 7 million office visits and 1 million emergency visits in the United States each year, resulting in 100,000 hospitalizations (Foxman, 2003). Symptomatic UTIs were the most common form of infection in surveys of antibiotic usage for health care-associated infections (HAIs) in European long-term care facilities and accounted for 31.2% of all HAIs in 2010 and 22.3% in 2013. Figure 1 shows the total cases of uncomplicated UTI in 2020 in the United States, EU5 (Germany, France, Italy, Spain, and the United Kingdom) and Japan (*Uncomplicated Urinary Tract Infection Epidemiology Forecast Report 2021-2030*, 2021).

Figure 1. Total occurrence-specific cases of uncomplicated UTI in the year 2020.

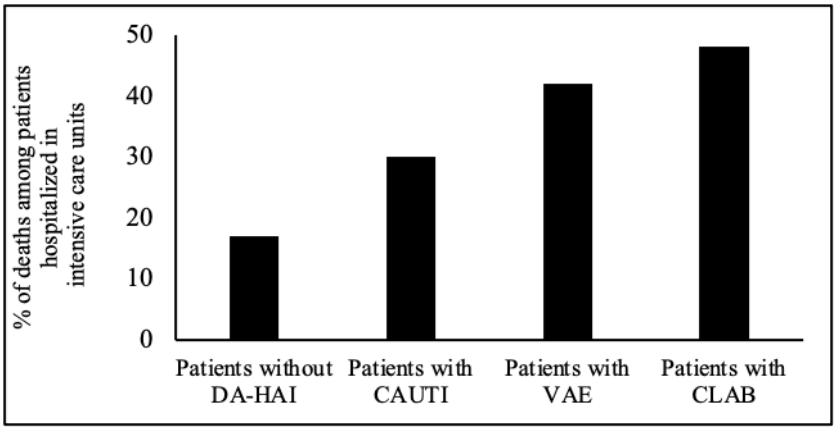


Source: Adapted from *Uncomplicated Urinary Tract Infection Epidemiology Forecast Report 2021-2030* (2021).

The disease affects individuals of all ages, particularly young and sexually active women. By the age of 24, approximately one-third of all women in the United States will have experienced at least one episode of UTI (Foxman, 2003). In the United Kingdom, it is predicted that half of all women will experience an episode of UTI at least once in their lives, as opposed to only one in every 2,000 healthy males (*NHS choices: urinary tract infections in adults*, n.d.). Women are more susceptible than men to acquiring UTIs due to their shorter urethral length, frequent vaginal colonization, and difficulty with urine flow and complete bladder emptying caused by urinary retention (Chu & Lowder, 2018). The recurrence of UTI is a typical phenomenon. Approximately 40% to 50% of women who have had one episode will have one or more additional episodes throughout their lives (Foxman, 2014). Patients who require monitoring of urinary output and need to be catheterized, particularly children, as well as elderly individuals with diabetes, are also at a higher risk of developing UTI *(Harwalkar et al*.,

2015). The use of an indwelling urethral catheter is responsible for approximately 80% of catheter-acquired UTI. Figure 2 shows part of the International Nosocomial Infection Control Consortium (INICC) data from device-associated infection (Nicolle, 2014). This report highlights the importance of catheter-associated UTI, with 30.15% of fatalities among 1,844 patients hospitalized in 45 countries (Rosenthal *et al*., 2021). UTIs have a bimodal onset age, with one peak occurring in the first year of life and another peak occurring between 2 and 4, which correlates to toilet training (Korbel *et al*., 2017). The infection affects about 0.7% of girls and 2.7% of uncircumcised boys within their first year of life (Chang & Shortliffe, 2006). By the time teens reach 16 years, UTIs affect approximately 11.3% of girls and 3.6% of boys (Stephens *et al*., 2015). In individuals over the age of 70, both sexes suffer UTI, with a female to male ratio of 2:1 (Cove-Smith & Almond, 2007).

Figure 2. Percentages of deaths among patients hospitalized in 664 intensive care units in 133 cities, of 45 countries, from Latin America, Europe, Africa, Eastern-Mediterranean, Southeast-Asia, and Western-Pacific, from 2013 to 2018. DA-HAI, device-associated healthcare-associated infection; CAUTI, catheter-associated urinary tract infection; VAE, ventilator-associated event; CLAB, central line-associated bloodstream infection.



Source: Adapted from Rosenthal *et al*. (2021).

In the United States, the social impact of UTI, including healthcare expenditures and lost time at work, is estimated to be over US$3.5 billion per year (Flores-Mireles *et al*., 2015). A retrospective analysis of children hospitalized with UTI in the United States found that projected hospital expenditures for treatments in 2000 were US$2,858 per hospitalization, rising to US$3,838 by 2006 (Spencer *et al*., 2010). According to a cross-sectional survey conducted in France in 2012 and 2013, the average cost of care for a suspected UTI episode in women over 18 was €70, with a societal cost of €58 million (François *et al*., 2016). A retrospective study of 309 women with a history of UTI admitted to the Hesperia Hospital in Modena Italy between 2007 and 2010 indicated a mean annual direct cost per patient of €229 (Ciani *et al*., 2013). The average cost of a UTI treatment was €5,700, according to a multinational study conducted in eight countries (i.e., Hungary, Romania, Turkey, Italy, Greece, Spain, Israel, and Bulgaria), with significant variation between countries (Vallejo-Torres *et al*., 2018). Serious sequelae resulting from UTI (i.e., recurrences, pyelonephritis with sepsis, kidney injury, preterm delivery, and antimicrobial resistance due to the overuse of antibiotics) contribute considerably to the increased economic burden of this costly disease (Flores-Mireles *et al*., 2015).

3.2 Classification of UTIs and related symptoms

Symptomatic UTIs can be categorized as either urosepsis syndrome (the most severe form of UTI), pyelonephritis (or upper UTI, which involves a kidney infection), cystitis (or lower UTI, which involves bacteria in the bladder), and bacteriuria (bacteria in the urine). Asymptomatic bacteriuria (ABU) is defined as bacterial colonization of the urine in the absence of

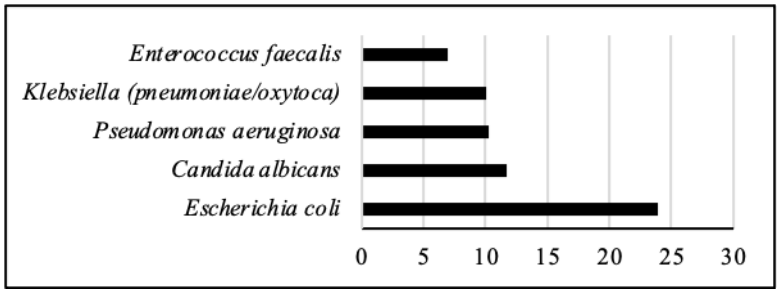
symptoms. The majority of ABU patients do not require treatment, and ABU colonization may help avoid infection by more aggressive bacteria (Darouiche *et al*., 2001). While cystitis episodes outnumber pyelonephritis occurrences, pyelonephritis accounts for most hospital admissions (Czaja *et al*., 2007; Ikäheimo *et al*., 1996; Stamm *et al*., 1991).

In clinical terminology, UTIs can be classified as either uncomplicated or complicated. This categorization is intended to aid in the identification of individuals who may require further diagnostic testing, broad-spectrum antibiotics, or treatment for extended periods. Uncomplicated UTIs generally afflict healthy patients with no anatomical or neurological disorders in their urinary tract system (Hooton, 2012). In contrast, complicated UTIs are associated with variables that impair the host's urinary tract or the immune system. Causes of complicated UTIs include urinary blockage or retention due to neurological conditions, immunosuppression (e.g., cancer, asplenia, HIV), pregnancy, presence of foreign bodies (e.g., calculi), renal failure, renal transplantation, and drainage devices (e.g., indwelling catheters) (Levison & Kaye, 2013). The most frequent symptoms of uncomplicated UTIs (e.g., cystitis) include frequent and urgent urination, dysuria (painful urination), suprapubic pain, nocturia (excessive urination at night), hematuria (presence of blood in urine), and malaise (feeling of debility). Complicated UTIs (e.g., pyelonephritis) are characterized by more severe symptoms, such as back and flank pain, fever, chills, malaise, nausea, vomiting, and anorexia (McLellan & Hunstad, 2016).

3.3 Causative agents of UTI

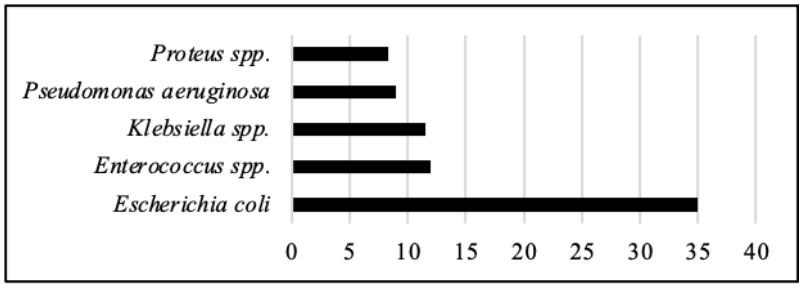
Gram-negative bacteria belonging to the *Enterobacteriaceae* family (e.g., *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Candida albicans*) are the most prevalent cause of UTIs (Nicolle, 2001). Indeed, gram-positive bacteria, especially *Staphylococcus saprophyticus*, play a significant role as an etiologic agent in the bacterial landscape of these infections (Nicolle, 2003). UPEC is the most common cause of both complicated and uncomplicated UTIs, accounting for more than 80% of uncomplicated UTIs (Brumbaugh & Mobley, 2012). A study conducted among women with cystitis in nine countries in Europe and Brazil between 2003 and 2006 revealed that UPEC is the most prevalent pathogen (on average 76.7%) in the human body, ranging from 74% in Austria to 85% in France (Naber *et al*., 2008). Epidemiological surveillance conducted in the United States in 2006 and 2007 showed that UPEC is the most frequent cause of UTI related to urinary catheters in intensive care units and the fifth most common pathogen in infections acquired in this sector (Hidron *et al*., 2009). Figure 3 depicts the distribution of the most prevalent bacteria for catheter-associated UTIs in the United States in 2016, whereas Figure 4 shows the main pathogens related to UTIs in Europe in 2011 and 2012 (WHO, 2019).

Figure 3. Distribution of the main pathogens for catheter-associated UTIs reported in the United States in 2016.



Source: Adapted from WHO (2019).

Figure 4. Distribution of the main pathogens related to UTIs in Europe, 2011-2012.

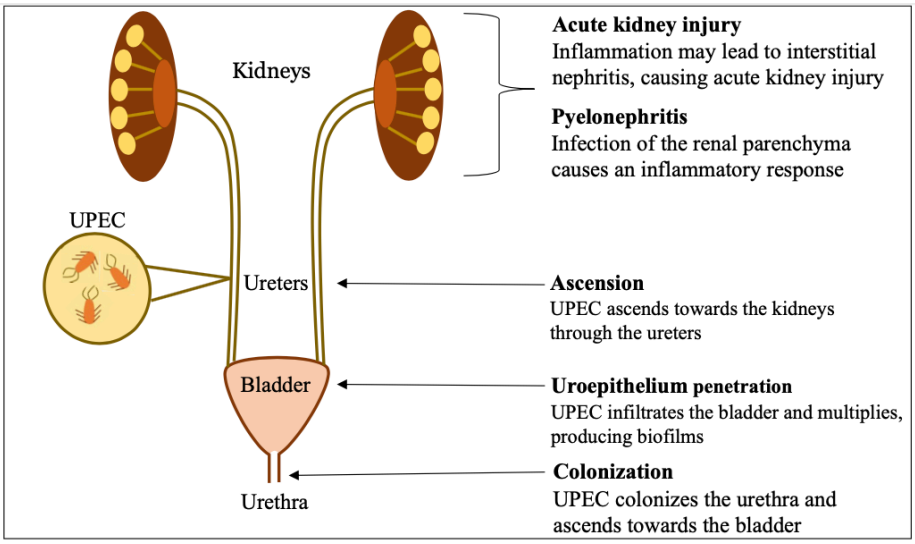


Source: Adapted from WHO (2019).

3.4 Pathophysiology of UTI caused by UPEC

The individual vulnerability to UTI is determined by various factors, including the interaction between UPEC virulence factors and the host immune response as well as renal anatomical abnormalities (e.g., hydronephrosis, hydroureter, vesicoureteric reflux, and urethral obstructions) (Ditchfield *et al*., 2004). Under normal circumstances, UPEC is usually eliminated by urinary flow, antimicrobial proteins in urine (e.g., Tamm–Horsfall and ribonuclease 7), and to a lesser extent immunoglobulin A (IgA) and polymorphonuclear cells present on the vesical surface (Spencer *et al*., 2011). If UPEC is not eliminated, the bacteria can colonize the urothelium, causing damage to the bladder epithelium and eventually leading to an infection (e.g., cystitis). UPEC can also spread into the ureters and reach the kidneys, leading to pyelonephritis. Figure 5 depicts the pathogenesis of UTI caused by UPEC.

Figure 5. Pathogenesis of UTI caused by UPEC.



Source: Goulart, D. B. (current manuscript).

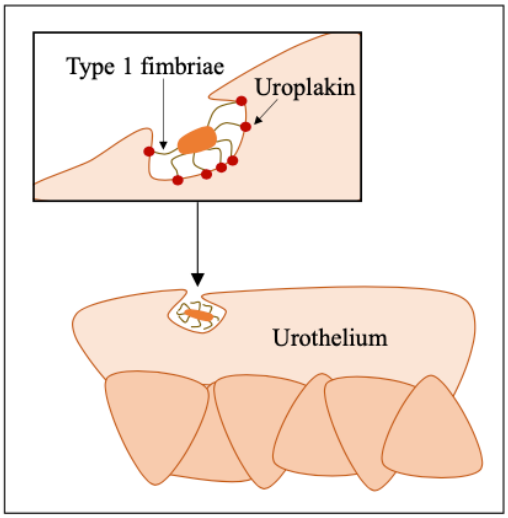
3.4.1 Infection with UPEC begins in the lower urinary tract

A UTI usually begins with periurethral contamination by UPEC residing in the gut, followed by urethral colonization and pathogen migration to the bladder. UPEC virulence factors include toxins (cytotoxic necrotizing factor type 1), hemolysins, surface features that aid in the evasion of host defenses (capsule and O antigen), fimbrial adhesins, and iron acquisition systems (Ruiz *et al*., 2002). Fimbrial adhesins are crucial for bacterial colonization because they recognize specific receptors present in the stratified squamous epithelium of the urethra, bladder, and ureters (Nielubowicz & Mobley, 2010). The combination of the different virulence factors raises UPEC's pathogenicity and increases the likelihood of further UTI

episodes. The attachment of UPEC to urogenital epithelial cells, which is mediated by adhesins, is the first step in bacterial colonization (Figure 6). Among the several fimbriae identified are Type 1, Type P, and common pilus (ECP) (Nielubowicz & Mobley, 2010). Type 1 fimbriae are heteropolymers formed by a larger subunit (FimA) and three smaller subunits (FimF, FimG, and FimH). During the early stages of UPEC pathogenesis, FimH adhesin, found at the tip of type 1 fimbriae, binds to the uroplakin expressed at the surface of bladder urothelial cells (Flores-Mireles *et al*., 2015). This adhesion induces actin rearrangement and subsequent internalization of UPEC via unknown mechanisms (Wu *et al*., 1996).

UPEC can undermine host defenses and resist antibiotic therapy inside the host cell. The host responds to the UPEC invasion by launching a strong immune reaction. Toll-like receptor 4 (TLR-4), expressed on the membrane of bladder epithelial cells, combines with a cluster of differentiation 14 (CD14) and detects lipopolysaccharide (LPS) from UPEC (Frendéus *et al*., 2001). Consequently, adenylyl cyclase 3 (AC3) is activated, and cyclic AMP (cAMP) is produced, leading to the exocytosis of vesicular UPEC through the apical plasma membrane (Bishop *et al*., 2007). Eventually, UPEC subverts the immune system, invades the urothelial cell's cytoplasm, and quickly proliferates, creating intracellular bacterial communities (IBCs) (Wright *et al*., 2007). IBCs keep UPEC wrapped in a polysaccharide-rich matrix and protected by a coating of uroplakin (Anderson *et al*., 2010). IBCs constitute quiescent intracellular reservoirs of microorganisms causing recurrent urinary infections and protect the bacteria from succumbing to the host's immune system (Jhang & Kuo, 2017). A recurrent UTI is defined as three or more UTIs in less than a year, as well as two or more recurrences in less than six months (Terlizzi *et al*., 2017). IBCs pass through a series of phases before forming biofilm-like communities inside the surface cells. UPEC eventually separates from the biofilm and enters the bladder lumen. These escaping UPEC subsequently rebind to the epithelium, resulting in the formation of another IBC (Anderson *et al*., 2004).

Figure 6. The attachment of UPEC to urogenital epithelial cells, which is mediated by adhesins, is the first step in UPEC colonization.



Source: Goulart, D. B. (current manuscript).

3.4.2 Ascension to the kidneys and possible complications

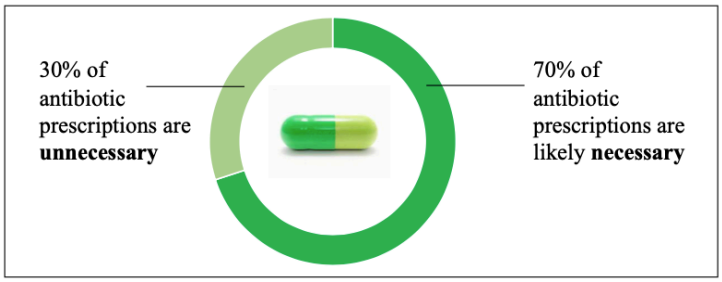
The extracellular survival of UPEC is accomplished by the secretion of several factors, including cytotoxic necrotizing factor 1 (CNF1), toxin alpha-hemolysin (HlyA), and siderophores (Emody *et al*., 2003). Toxins and proteases generated by UPEC destroy host urothelial cells, releasing vital nutrients that aid bacteria to survive and ascend via ureters to the kidneys (Flores-Mireles *et al*., 2015). In the kidney, pyelonephritis-associated type P pili bind globoside-containing glycoproteins that line the renal tissue, allowing UPEC colonization (Lane & Mobley, 2007). If the host's immune system is

unable to combat the local infection, UPEC releases tissue-damaging toxins, allowing bacteria to pass through the tubular epithelial barrier and into the bloodstream, resulting in bacteremia (Flores-Mireles *et al*., 2015).

3.5 Antibiotic treatment of UTI

The primary goal of UTI treatment is to eliminate the current infection to achieve a sterile urinary tract. The second goal is to investigate all risk factors for UTI, and third, prevent the recurrence of infection (Osamwonyi & Foley, 2017). Currently, most UTI can be treated with antibiotics without resorting to the hospitalization of patients. Antibiotic treatment of asymptomatic bacteriuria is indicated only for individuals undergoing urological procedures, including vasectomy, cystoscopy, ureteroscopy, and male circumcision (Walsh & Collyns, 2017). However, a more severe UTI with a high risk of complications may require hospitalization and treatment with intravenous antibiotics. In general practice, UTIs are the second most prevalent reason for antibiotic prescriptions (Petersen & Hayward, 2007). According to the 2018 Update on Antibiotic Use in the United States Progress and Opportunities Report, 30% of antibiotic prescriptions are unnecessary in doctor's offices and emergency departments (Figure 7).

Figure 7. Antibiotic use in doctor's offices and emergency departments in the United States.



Source: Adapted from CDC (2018).

When choosing the most appropriate antibiotic for UTI treatment, several factors should be considered, including individual patient risk (e.g., allergies and contraindications) and antibiotic pretreatment; the bacterial spectrum and susceptibility profile; antimicrobial agent effectiveness demonstrated in clinical studies; epidemiological implications and adverse effects (Wagenlehner *et al*., 2011). Additionally, choosing the appropriate antibiotic involves considering the results of urine culture, which is the gold standard for diagnosing UTI. This method may also be used to estimate bacteriuria levels. Nonetheless, microbiological laboratories have not standardized the minimum amount of bacteriuria that indicates a UTI. The threshold used in many laboratories is 10^5 colony-forming units (CFU)/mL urine (Schmiemann *et al*., 2010). Others establish a lower threshold of 10^3 CFU/mL to identify a broader spectrum of infections (Hilt *et al*., 2014).

An antibiotic used to treat UTI must meet specific criteria, including being active against the most common uropathogens, being excreted in its active form in the urine by glomerular filtration, having an adequate and inhibitory concentration in urine and tissues (in the case of pyelonephritis), and being active at urinary pH (Moura *et al*., 2009). Other variables, such as the treatment cost, availability, pharmacokinetics, and pharmacodynamics of the chosen antibiotic, are critical to successfully treating UTI and preventing the occurrence of MDR bacteria. Treatment usually lasts 7 to 14 days and varies depending on the location of the UTI and the patient's reaction to the medication (Makvana & Krilov, 2015). Acute uncomplicated cystitis is treated with nitrofurantoin monohydrate (100 mg twice daily for 5 days), trimethoprim-sulfamethoxazole ([TMP-SMX], 160/800 mg twice daily for 3 days), pivmecillinam (400 mg bid for 3 to 7 days), fosfomycin trometamol (3 g in a single dose), fluoroquinolones (FQs) such as ofloxacin, ciprofloxacin, and levofloxacin (3-day regimens), and beta-lactam antibiotics such as amoxicillin-clavulanate, cefaclor, cefdinir, and cefpodoxime-proxetil (3- to 7-day

regimens) (Gupta *et al*., 2011). Except for nitrofurantoin and fosfomycin, which are solely used to treat cystitis, the Clinical and Laboratory Standard Institute uses serum concentrations of these antimicrobial drugs rather than urine concentrations (Moura *et al*., 2009). A urine culture followed by a susceptibility test should be conducted in individuals with suspected pyelonephritis, and empirical therapy should be modified based on the uropathogen. Recommended treatment includes oral ciprofloxacin (500 mg twice daily for 7 days), oral FQs such as ciprofloxacin (1,000 mg extended-release for 7 days), or levofloxacin (750 mg for 5 days), and oral TMP-SMX (160/800 mg twice daily for 14 days) (Gupta *et al*., 2011). Oral beta-lactam antibiotics are less efficient than the available options in pyelonephritis treatment. Table 1 summarizes the most frequently used oral therapeutic regimens for the treatment of UTI.

Table 1. Most frequently used oral therapeutic regimens for the treatment of UTI.

Antibiotic	**Oral dosage**	**Clinical indication**
Amoxicillin	500 mg, 3 times per day	Cystitis
Amoxicillin-clavulanic acid	500 mg, 3 times per day or 875 mg, 2 times per day	Cystitis
Cefixime	400 mg, once per day	Cystitis caused by resistent uropathogens, pyelonephritis
Cefpodoxime	100 ou 200 mg, 2 times per day	Cystitis, pyelonephritis
Ciprofloxacin	500 mg, 2 times per day	Cystitis, pyelonephritis
Fosfomycin	3 g per day (one or two days)	Cystitis
Levofloxacin	500 or 750 mg per day	Cystitis, pyelonephritis
Nitrofurantoin	100 mg, 2 times per day	Cystitis
Trimetoprim- sulfametoxazol	160 mg + 800 mg, 2 times per day	Cystitis

Source: Goulart, D. B. (current manuscript).

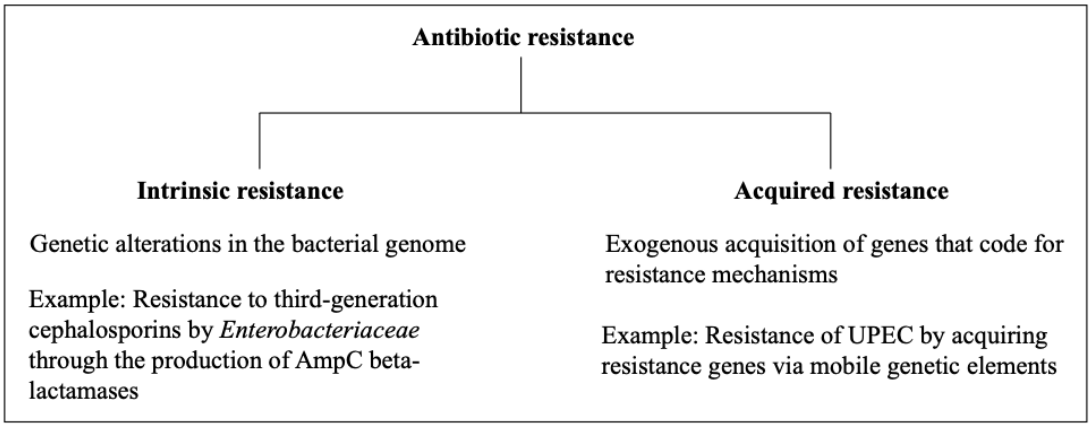
3.6 Resistance to antibiotics in UPEC strains

Antibiotic usage has become more indiscriminate over the years, resulting in MDR bacteria, a significant public health issue worldwide. Infection by MDR pathogens has substantial implications, including increased mortality, prolonged hospital stays, a lack of prophylactic options for patients undergoing surgery, and higher treatment expenses. MDR bacteria are responsible for about 25,000 deaths and 1.5 billion euros in expenditures per year in European Union countries (*Draft global action plan on antimicrobial resistance. Report by the WHO Secretariat*, 2015). Research reveals that more than 10% of UPEC causing cystitis are MDR, presenting resistance to at least three antimicrobial classes (Kahlmeter, 2003). According to the Drug Resistance Index, UTIs grew increasingly challenging to treat between 1999 and 2010 due to rising antibiotic resistance (*Center for Disease Dynamics, Economics & Policy*, 2018). The situation becomes more dramatic if we consider that few new antibacterial compounds have been developed in recent years, potentially causing common and easily treated diseases to become deadly because of a lack of treatment alternatives.

Antimicrobial resistance can be divided into two major groups, intrinsic resistance and extrinsic (or acquired) resistance. Intrinsic resistance is characterized by an inherent insensitivity of the bacteria to antibiotic activity. An example is the resistance to third-generation cephalosporins by bacteria belonging to the *Enterobacteriaceae* family through the production of AmpC beta-lactamases (Shah *et al*., 2004). Acquired resistance is a significant challenge to public health because of its high prevalence in important pathogens causing human illness. Bacteria can develop resistance through two primary mechanisms, i) genetic alterations in the bacterial genome, which can alter the therapeutic targets of antibiotics or ii) the exogenous acquisition of genes that code for resistance mechanisms. Figure 8 depicts a schematic representation of bacterial intrinsic and acquired antibiotic resistance. Resistance can be developed by UPEC by acquiring resistance genes via

mobile genetic elements such as transposons, plasmids, gene cassettes in integrons, or alterations in the regulatory locus on the bacteria's chromosome (Paniagua-Contreras *et al*., 2017; Tong *et al*., 2017). These plasmids can be transmitted horizontally between bacteria of the same species or between different species. There are different risk factors for developing MDR bacteria, including antibiotic usage before an episode of UTI, prolonged hospitalization, medical devices such as urinary catheters, other underlying illnesses, and advanced age (Walker *et al*., 2016). In addition to antibiotic resistance per se, a study has shown a positive correlation between the number of virulence factors, resistance spectrum, and recurrence rate (Liu *et al*., 2013). The more virulence factors a UPEC strain has, the greater the number of antibiotics it is resistant to, which leads to an increase in recurrences of UTIs.

Figure 8. Intrinsic and acquired antibiotic-resistance in bacteria.



Source: Goulart, D. B. (current manuscript).

According to European research intended to gather information on UPEC resistance in females affected with acute uncomplicated UTI, resistance to ciprofloxacin and trimethoprim is becoming more severe. Alternatively, resistance to mecillinam [amdinocillin (United States adopted name)] and nitrofurantoin remains low. Since 2000, resistance to TMP-SMX has increased significantly in Spain (25% to 37%), Sweden (9% to 17%), Germany (23% to 37%), and the UK (13% to 46%), to ciprofloxacin in Spain (15% to 31%), Sweden (0% to 7%), Germany (2% to 21%), and the UK (1% to 15%), to mecillinam in Spain (1% to 6.5%), and to nitrofurantoin in the United Kingdom (0% to 6%). Since 2008, resistance to ciprofloxacin has increased significantly in the United Kingdom (1% to 15%) and Sweden (3% to 15%), as well as resistance to nitrofurantoin (0% to 6%) and TMP-SMX (13% to 46%) in the United Kingdom (Gunnar Kahlmeter *et al*., 2015).

The Infectious Diseases Society of America and the European Society of Clinical Microbiology and Infectious Diseases have proposed that the cutoff point for empirical treatment of uncomplicated UTI be up to 20% antimicrobial resistance (Gupta *et al*., 2011; Wagenlehner *et al*., 2011). This fact has led to changes in the choice of empirical therapy for UTI. An example is that TMP-SMX is no longer recommended as the first-line antibiotic for empirically treating UTIs due to the increased resistance rate. Considering the current resistance scenario, nitrofurantoin and fosfomycin seem to be the best options for the empirical treatment of acute cystitis (Gunnar Kahlmeter *et al*., 2015).

3.6.1 Resistance to fluoroquinolone

This class of antibiotics has a wide spectrum of activity and is effective against gram-negative and gram-positive bacteria. Clinical cure rates of UTIs have been reported to be in the range of 93% to 97% of treated patients (Walker *et al*., 2016). Microbiological cure rates show similar success in patients infected with nonresistant UPEC. According to a study of male veterans, ciprofloxacin was the most commonly recommended antibiotic for outpatient UTIs, administered in 62.7% of

cases (Drekonja *et al*., 2013). However, because of the risk of cartilage injury, FQs are not recommended for pregnant women, children, teenagers, or high-performance athletes because they might cause tendonitis even after short-term use (Moura *et al*., 2009). FQ antibiotics inhibit DNA synthesis by inhibiting DNA gyrase and bacterial topoisomerase IV, enzymes involved in the DNA replication (Hooper, 2001). The tetrameric enzyme DNA gyrase comprises two GyrA and two GyrB subunits, essential for maintaining the DNA's topology. Topoisomerase IV is also a tetrameric enzyme of two ParC and two ParE subunits involved in chromosome segregation during DNA replication. Resistance to FQs is primarily caused by chromosomal mutations in the genes that code for DNA gyrase and topoisomerase IV. A correlation was observed between the minimum inhibitory concentration values for ciprofloxacin and differences in *gyrA* gene sequencing, indicating that the primary mechanism of resistance to ciprofloxacin in *E. coli* is the accumulation of mutations in *gyrA* (van Hees *et al*., 2011). Mutations in *gyrB* are considered rare, while mutations in *parC* and *parE* are generally combined with mutations in *gyrA*, suggesting that DNA gyrase is the primary target for FQs (Moon *et al*., 2010). Another mechanism of resistance to FQs is reduced concentration of drugs in *E. coli* by the over-expression of the multidrug efflux pump AcrAB-TolC through mutations or insertions in the regulatory genes (Swick *et al*., 2011). Quinolone resistance may also be transmitted by a plasmid, which allows quinolone and multidrug resistance to proliferate quickly among *Enterobacteriaceae* (Paterson, 2006).

The development of resistance to FQs has been observed worldwide, and it is most likely related to the overuse of these antibiotics (Zurfluh *et al*., 2014). The use of FQ antibiotics in the month leading up to UTI episodes and healthcare-related infections are considered risk factors for ciprofloxacin-resistant UPEC in male patients with UTI (Colodner *et al*., 2008; Smithson *et al.*, 2012). FQ resistance is seen in approximately 25% of UPEC isolated from catheter-associated UTI in intensive care units (Hidron *et al*., 2009). UPEC has shown substantial resistance to FQs, according to reports from several countries. More than 20% of UPEC isolated from individuals with community-acquired uncomplicated UTI and more than 50% of UPEC isolated from complicated UTI exhibited resistance to FQs in various regions of the world. Researchers have shown a significant prevalence of FQ resistance in UPEC in India (more than 60%) (Prasada *et al.*, 2019). From 1994 to 2005, the bacterial spectrum in urine samples collected from urological patients admitted to a hospital in Germany remained constant for 12 years, with UPEC representing approximately one-third of the isolated bacteria. UPEC resistance to ciprofloxacin increased from 4% in 1994 to 15% in 2005, according to the same research (Wagenlehner *et al*., 2008). FQ resistance was found in around 30% of UPEC from patients with UTIs in Poland (Michno *et al*., 2018). Resistance to ciprofloxacin grew dramatically in Switzerland from 1.8% to 15.9% during ten years (1997–2007), coinciding with increased ciprofloxacin usage (Blaettler *et al*., 2009). In São Paulo, Brazil, from 2000 to 2003, UPEC resistance to FQs (e.g., ciprofloxacin and norfloxacin) increased from 9% to 14%, and in patients aged over 60 years, resistance to ciprofloxacin reached 24.3% (Kiffer *et al*., 2007). Similar results were described by other studies carried out in other countries (Araújo *et al*., 2011; Omigie *et al*., 2009). There was a gradual increase to 41% and 54% of UPEC resistant to ciprofloxacin isolated from the urine of community and nosocomial-acquired infection in a Mexican cancer hospital, respectively. In the United States, 12.1% of UPEC isolates from individuals with acute uncomplicated and complicated pyelonephritis were resistant to ciprofloxacin between 2013 and 2014 (Talan *et al*., 2017). Fluoroquinolone resistance is a major public health issue, and indiscriminate use of these drugs must be avoided if their efficacy against essential diseases is to be preserved.

3.6.2 Resistance to fosfomycin

Fosfomycin is produced from phosphonic acid that has been used in clinics for over 40 years, mostly for single-dose (3 g) oral treatment of uncomplicated UTI. Peak urine concentrations occur within 4 hours and stay high (>128 mg/L) for 24 to 48 hours, long enough to suppress most UTIs (Patel *et al*., 1997). Fosfomycin has a broad antibacterial spectrum, acting against a range of anaerobic pathogens, gram-positive and gram-negative bacteria. The antibiotic works as a bacterial cell wall

inhibitor by interfering with peptidoglycan biosynthesis's initial step. It binds to UDP-N-acetylglucosamine-3-O-enol-pyruvyl transferase and acts as a phosphoenolpyruvate analog. The binding prevents UDP-N-acetylglucosamine-3-O-enol-pyruvate from being formed from UDP-N-acetylglucosamine and phosphoenolpyruvate. Fosfomycin is moderately effective against *E. coli* strains and various other uropathogenic bacteria that cause uncomplicated UTI (Moura *et al*., 2009). With fosfomycin therapy, microbiological cure rates ranged from 80% to 83%, whereas clinical cure rates of UTI ranged from 87% to 93% in research conducted on nonresistant UPEC in Germany, Belgium, and Spain (Kresken *et al*., 2016). However, in a retrospective study of 41 hospitalized patients treated with fosfomycin, the overall microbiological cure rate for UTI caused by multidrug-resistant UPEC was significantly lower than the previous study, reaching 59% (Patel *et al.*, 1997). A single dose of fosfomycin tromethamine was successful in clinical studies in individuals with acute uncomplicated UTI, with bacteriological eradication rates of 75% to 90% obtained 5 to 11 days following therapy (Patel *et al.*, 1997).

In *E. coli*, fosfomycin enters the bacterial cell through two distinct transport systems: the glycerol-3-phosphate transporter (GlpT) and the hexose-6-phosphate transporter (UhpT). Fosfomycin resistance in *E. coli* can be caused by reduced drug uptake, enzymatic drug inactivation, or target alteration (Castañeda-García *et al*., 2013; Lucas *et al*., 2018). Reduced drug uptake results from chromosomal mutations (e.g., point mutations, insertions, deletions) in the *uhpT* and *glpT* structural genes or regulators, which cause defects in one or both transport systems (Kadner & Winkler, 1973; Kahan *et al*., 1974). It is the primary mechanism through which fosfomycin resistance develops. Fosfomycin-modifying enzymes have recently emerged as a way to inactivate the antibiotic. These enzymes can give resistance to fosfomycin by disrupting its epoxide ring and rendering it inactive (Thompson *et al*., 2015). FosA is the most often reported of the three primary classes of fosfomycin resistance enzymes (FosA, FosB, and FosX) among *E. coli* (Ho *et al*., 2013; Li *et al*., 2015). Fosfomycin resistance can also be caused by qualitative and quantitative changes of UDP-N-acetylglucosamine enolpyruvyl transferase (MurA), an enzyme crucial for peptidoglycan biosynthetic pathway (target alterations) (Karageorgopoulos *et al*., 2012). Fosfomycin resistance is uncommon in *E. coli* clinical isolates (typically less than 2%), owing to the substantial fitness cost of chromosome-encoded fosfomycin resistance (Falagas *et al*., 2019; Vardakas *et al*., 2016). In addition to the low level of resistance, the administration of fosfomycin in a single dose allows adequate levels of antibiotic for 72 hours, improving treatment adherence and, therefore, avoiding the appearance of relapses and the selection of resistant strains (Silva *et al*., 2008).

3.6.3 Resistance to nitrofurantoin

One of the most often recommended pharmacological prophylactic treatments, especially for women with recurrent UTIs, is taking a small dose of antibiotics daily or three times per week, reducing the risk of UTI by up to 85% (Albert *et al*., 2004). Nitrofurantoin is the first-line antibiotic of choice under these circumstances. It is important to note that this strategy has certain drawbacks, the most severe of which are oral and vaginal candidiasis and gastrointestinal disturbances. The typical length of this prophylaxis is six months, and in some situations, after discontinuing the medication, the frequency of UTIs returns to pre-therapy levels; in these circumstances, it may be prudent to extend the preventive measure (Ejrnæs *et al*., 2011). Nitrofurantoin is rapidly absorbed and eliminated into the urine by the kidneys. Because it does not reach an effective concentration in the blood or tissues, it is solely used to treat uncomplicated cystitis (Bryce *et al*., 2016). Nitrofurantoin is an antibiotic with low systemic absorption proven to be as effective as TMP-SMX in preventing UTI, with the added benefit of a reduced risk of resistance than TMP-SMX and FQs (McKinnell *et al*., 2011).

Nitrofurantoin works by inhibiting acetyl coenzyme A, which causes bacterial cell wall formation to be disrupted (Bailey *et al*., 1971). Bacterial flavoproteins convert nitrofurantoin to reactive intermediates that inactivate bacterial ribosomal proteins. The inactivation of ribosomal proteins prevents aerobic metabolism and synthesizes cell wall, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and eventually, protein (McOsker & Fitzpatrick, 1994). UPEC resistance to nitrofurantoin is

currently low, allowing it to be used as a first-line antibacterial drug. In 2008, researchers in Portugal conducted a study to determine the antibiotic susceptibility of the most common uropathogens isolated from uncomplicated acute cystitis (Silva *et al*., 2008). The data showed that 37.9% of UPEC were resistant to amoxicillin, 23.5% to TMP-SMX, 8.5% to FQs, and approximately 5% to the association of amoxicillin-clavulanic acid and cefuroxime. The lowest resistance levels were recorded for nitrofurantoin and fosfomycin, 2.1% and 0.6%, respectively. From 2013 to 2014, the prevalence of UPEC resistance from patients with community-acquired UTIs in Spain, Belgium, and Germany was below 1.5% (Kresken *et al*., 2016). The resistance of UPEC isolated from adults rose just slightly from 0.7% to 0.9% in a retrospective investigation conducted in the United States from 2003 to 2012 (Sanchez *et al*., 2016). Nitrofurantoin was shown to be effective against 95% of UPEC strains in urology clinics in the Netherlands (van der Donk *et al*., 2012). In Bosnia and Herzegovina, 8.23% of UPEC isolates from inpatients and outpatients were nitrofurantoin resistant (Abduzaimovic *et al*., 2016). Even though nitrofurantoin has been selected to treat uncomplicated UTI in several countries, there are concerns about its safety. For example, nitrofurantoin has been associated with hemolytic anemia in newborns when used by pregnant women in the last gestational trimester (Nordeng *et al*., 2013). Antibiotic therapy of UTI during pregnancy requires special considerations, with non-teratogenic antibiotics such as aminopenicillins, fosfomycin, and cephalosporins being preferred (Guglietta, 2017; Kalinderi *et al*., 2018).

3.6.4 Resistance to trimethoprim–sulfamethoxazole (TMP–SMX)

TMP-SMX has an antibacterial range that includes both gram-positive and gram-negative bacteria. It is a widely used first-line antibiotic for treating uncomplicated UTI. In prospective investigations, clinical cure rates from 87% to 88% and microbiological cure rates from 82% to 83% have been reported (Raz *et al*., 2002). TMP-SMX affects bacterial folic acid production by inhibiting dihydrofolate reductase (DHFR), responsible for catalyzing the reduction of dihydrofolate to tetrahydrofolate. In gram-negative bacteria, sulfonamide resistance is caused by the acquisition of dihydropteroate synthase (DHPS) genes in integrons that are not inhibited by the antibiotic. Only three TMP-SMX resistance genes have been discovered: *sul*1, *sul*2, and *sul*3 (Ben *et al*., 2017).

According to comparative research conducted in the United States, TMP-SMX resistance in UPEC collected from patients with symptoms of UTI in 1999–2000 and 2016–2017 did not differ substantially (Yamaji *et al.*, 2018). However, surveillance statistics show that resistance to TMP-SMX currently ranges from 16% to 36% worldwide (Khawcharoenporn *et al.*, 2013; McIsaac *et al*., 2013). TMP-SMZ should not be utilized in empiric therapy since most trials demonstrate resistance levels at or above the acceptable threshold of 20% (Bartoletti *et al*., 2016; van der Donk *et al*., 2012). From 2012 to 2015, UPEC isolated from urine samples obtained from a tertiary care hospital in Switzerland had a 24.5% resistance rate (Erb *et al*., 2018). In some European countries, including Romania, Belgium, Bosnia and Herzegovina, France, Germany, Spain, Poland, and Switzerland, UPEC resistance to TMP-SMX widely varied from 14.6% to 60% (Ciontea *et al*., 2018; Hitzenbichler *et al*., 2018; Kot *et al.*, 2016). In Ankara, Turkey, *E. coli* from urine samples from outpatients with acute cystitis showed a 44% resistance rate to TMP-SMX. Urinary tract isolates of *E. coli* in the United States in 2017 revealed a TMP-SMZ resistance rate higher than 24% (Critchley *et al*., 2019). In the Philippines, the frequency of TMP-SMZ-resistant *E. coli* was high, reaching 41.3% among women with acute uncomplicated UTI (Gangcuangco *et al*., 2015). *E. coli* from outpatients collected between 2012 and 2014 in Potohar, Pakistan, revealed the highest resistance to TMP-SMX (82%) among all tested antibiotics (Ali *et al*., 2016).

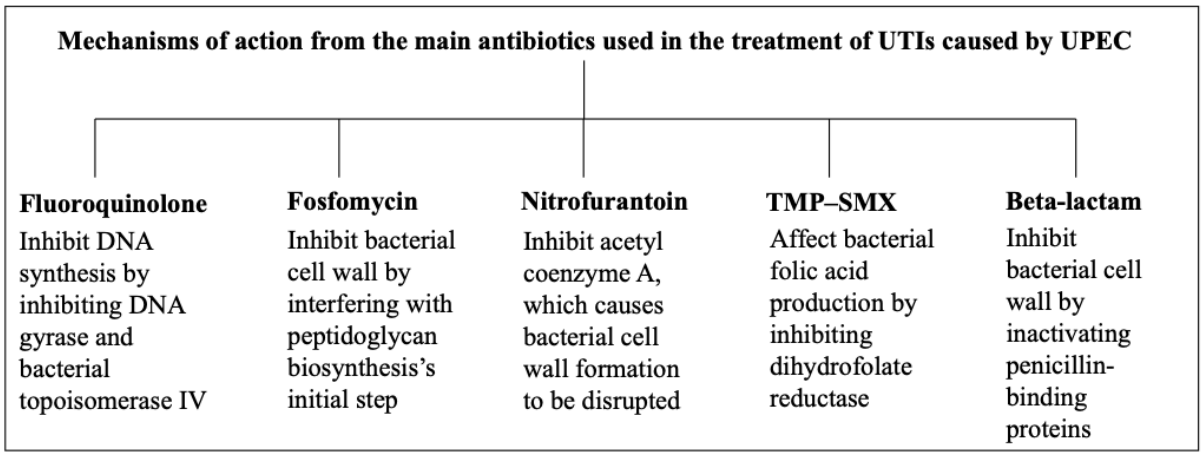
3.6.5 Resistance to beta-lactam antibiotics

Cephalosporins, ampicillin, amoxicillin, and amoxicillin-clavulanate are examples of oral beta-lactam antibiotics used to treat UTIs in outpatients. When first-line antibiotics are unavailable, beta-lactams may be utilized as alternative options.

However, since resistance rates for beta-lactam antibiotics are more significant than for other antimicrobials, treatment failure and reinfections are considered a significant concern (Gupta *et al*., 2011). The bactericidal effects of beta-lactams are achieved by inhibiting the production of bacterial cell walls (Park & Strominger, 1957). The polymer peptidoglycan that makes up the cell wall comprises glycan chains with attached peptides that crosslink neighboring glycans, forming a matrix structure. Beta-lactam antibiotics act by inactivating enzymes named penicillin-binding proteins, which cause polymer peptidoglycan synthesis to be disrupted (Tipper & Strominger, 1965). Resistance to beta-lactam antibiotics (e.g., ampicillin, amoxicillin, cephalosporins) is generally acquired by developing bacterial beta-lactamase enzymes (Dashti *et al.*, 2006). By catalyzing the hydrolysis of the amide group of the beta-lactam ring, beta-lactamase inactivates beta-lactam antibiotics. Extended-spectrum beta-lactamases (ESBLs) result from mutations in the ancestral enzymes *blaTEM-1*, *blaTEM-2*, and *blaSHV-1* and are frequently transmitted via plasmids (Dashti *et al*., 2006). Except for carbapenems, cephamycins, and beta-lactamase inhibitors, ESBL enzymes encode resistance to all beta-lactam antibiotics (Baudry *et al*., 2009). This happens because the plasmid encoding ESBLs normally encode other bacterial resistance genes, thus conferring resistance to other antibiotics such as aminoglycosides, sulfonamides, and quinolones (Asadi Karam *et al*., 2019). A strategy often used to combat antibiotic resistance associated with the presence of beta-lactamase is to administer, concomitantly with the antibiotic, clavulanic acid that binds to and inhibits beta-lactamase. However, the effectiveness of this strategy depends on the amount of beta-lactamases produced by the bacteria (Moura *et al*., 2009). Compared to non-producing ESBL bacteria, UPEC isolates with the ability to produce ESBL enzymes demonstrated higher rates of resistance to beta-lactams, aminoglycosides, and quinolone antibiotics (Shahbazi *et al*., 2018). Interestingly, isolates resistant to ampicillin are more prone to be resistant to amoxicillin-clavulanate, with researchers showing over 75% of *E. coli* isolates from UTI resistant to amoxicillin-clavulanate and about 72% to cephalosporins (Niranjan & Malini, 2014).

The prevalence of amoxicillin-clavulanic acid resistance rates varies regionally. Resistance to amoxicillin was found in about 40% of *E. coli* urine isolates collected from inpatients and outpatients in the Veterans Affairs Care System in the United States between 2009 and 2013 (Morrill *et al*., 2017). In Mexico, 23.6% of UPEC isolated from community and hospital-acquired UTIs were resistant to amoxicillin-clavulanic acid (Ramírez-Castillo *et al*., 2018). From 2015 to 2017, UPEC isolated from urine obtained from patients at tertiary care hospitals in Germany had a resistance rate of 5.3% to amoxicillin-clavulanic acid *(Hitzenbichler et al*., 2018). Between 2015 and 2017, 29.0% and 19.6% of UPEC isolates collected from outpatients in Romania and Bosnia and Herzegovina were resistant to amoxicillin-clavulanic acid. In Poland, between 2003 and 2006, 3.3% of UPEC in women with uncomplicated UTIs were resistant to amoxicillin-clavulanic acid (Naber *et al*., 2008). In a French prospective multicentre study from 2009 to 2010, UPEC resistance to amoxicillin-clavulanic acid was 37.6% (Lavigne *et al*., 2016). In England, amoxicillin-clavulanic acid resistance was found in 30% of *E. coli* isolates from hospitalized patients' urine (Abernethy *et al*., 2017). High levels of resistance to amoxicillin-clavulanic acid were observed in UPEC isolates from children suspected of UTI hospitalized in Nepal (48%) and from outpatients in Pakistan (71%) (Parajuli *et al.*, 2017). These findings show that UPEC resistance to amoxicillin-clavulanic acid differs among geographical locations and patient groups. As a result, the local susceptibility of *E. coli* should be used to guide empiric regimens for both uncomplicated and complicated UTIs. Figure 9 depicts the mechanisms of action regarding the described antibiotics used to treat UTIs caused by UPEC.

Figure 9. The primary antibiotics used to treat UTIs caused by UPEC and their mechanisms of action.



Source: Goulart, D. B. (current manuscript).

3.7 UPEC lineages and resistance profiles

Extraintestinal pathogenic *E. coli* (ExPEC) lineages are commonly studied using the multilocus sequence typing (MLST) method. MLST is a molecular typing technique that identifies strains of bacterial species (or other microbial species) from sequences at seven housekeeping loci. With MLST, a sequence type (ST) with a number identifier is allocated to *E. coli* strains, based on two standardized schemes (http://mlst.warwick.ac.uk [Achtman] and http://www.pasteur.fr/recherche/genopole/PF8/mlst/EColi. html [Pasteur]). ExPEC pandemic clones STs 69, 73, 95, and 131 were isolated from human infections such as UTIs and bloodstream infections *(Adams-Sapper et al*., 2013; Riley, 2014). These genotypes have become pandemic, accounting for more than half of all ExPEC infections worldwide (Adams-Sapper *et al*., 2013; Kallonen *et al*., 2017). The causes for this preponderance are unknown; nevertheless, the pathogenic potential is an important factor. While *E. coli* strains of STs 69, 73, and 95 generally remain susceptible to antibiotics and seldom demonstrate resistance to extended-spectrum cephalosporins, in particular, ST-131 strains show growing resistance to various antibiotic classes and account for 80 to 90% of MDR ExPEC (Griffin *et al*., 2012; Rogers *et al*., 2011).

ST-131 is largely responsible for spreading MDR *E. coli* strains across the world (Banerjee & Johnson, 2014; Sarkar *et al*., 2018). Clones of ST-131 have competitive advantages over other clones due to their specific genetic features, allowing them to spread quickly. For example, ST-131 has various drug resistance genes, including CTX-M-15 (confers resistance to penicillins, cephalosporins, and monobactams), TEM-1 and OXA-1 beta-lactamases, and *aa(6')-1b-cr* (Rogers *et al*., 2011). Furthermore, ST-131 is a member of the phylogenetic group B2, the most virulent group. ST-131 is a well-known UPEC strain that produces ESBL and is resistant to FQs (Kudinha *et al*., 2013). Among ESBL-producing or ciprofloxacin-resistant *E. coli* isolates from UTI, ST-131 has emerged as the most prevalent clone in Korea. UPEC strains belonging to ST-131 from a Saudi Arabian tertiary care facility were similarly linked to high levels of antibiotic resistance (Alghoribi *et al*., 2015). ST-131 (85.7%) and ST-69 (41.7%) lineages were found to have the highest rates of multidrug resistance among ExPEC isolates from a hospital in Iran (Hojabri *et al*., 2019).

ST-73 was the most prevalent clone isolated from community-acquired UTI in males (50%) and females (12%) in recent research conducted in Rio de Janeiro, Brazil, with the highest virulence score among other clones, including ST-69, ST-405, ST-131, ST-706, ST-14, ST-699, and ST-90 (de Souza da-Silva *et al*., 2017). ST-73 was the second most prevalent ST in a study of UPEC isolated from urine and blood samples in England, with ST-131 being the most prevalent (Siu *et al*., 2008). ST-73 strains with high similar PFGE types have been recovered from humans, cats, and dogs, suggesting that this genotype is

spread across species (Johnson *et al.*, 2008). The ST-73 lineage was the most prevalent among *E. coli* associated with bacteremia in England, and the bacteria were resistant to most antibiotics. ST-69 strains have been identified from community-acquired and healthcare-associated UTIs in numerous countries, including Denmark, Canada, Brazil, and the United States (Dias *et al*., 2009; Johnson *et al*., 2009; Manges *et al*., 2006; Skjøt-Rasmussen *et al*., 2013).

3.7.1 Other clonal ExPEC

ST-38 and ST-405 are also common among MDR *E. coli* strains. In a tertiary hospital in Korea, ST-38 and ST-405 accounted for 27.5% and 10.0% of CTX-M-producing UPEC, respectively (Kim *et al*., 2016). In a study of UPEC isolated from the urine of patients with community-acute pyelonephritis, 60% of ST-405 clones produced ESBL, and 40% of ESBL-producing clones were also TMP-SMX resistant (Bongyoung *et al*., 2020). Subsequent studies from different institutions in South Korea, China, Australia, and the United States have shown the presence of FQ-resistant *E. coli* isolates of the clonal group ST-1193 since 2012 (Kim *et al*., 2017; Li *et al.*, 2017; Platell *et al*., 2012; Tchesnokova *et al*., 2019). In a study conducted across multiple cities in the United States, MDR *E. coli* clone ST-1193 isolates were resistant to FQs (100%), TMP-SMX (55%), and tetracycline (53%) (Tchesnokova *et al*., 2019). According to the same research, ST-1193 is associated with individuals under age 40 and currently comprises about one-fourth of FQ-resistant clinical.

3.8 Immunostimulants and vaccines

Successful immunization against UPEC and other uropathogens can make a significant difference in patients' lives, especially those at risk for complicated UTIs or recurring UTIs. However, there is presently no vaccination available that targets uropathogenic bacteria specifically (Magistro & Stief, 2019). Two critical factors must be considered for the development of an effective vaccine for UTIs. First, because several *E. coli* are a part of the natural intestinal flora, vaccination may alter this bacterial population (McLellan & Hunstad, 2016). Second, the level of antibodies (IgG) that can reach the bladder lumen is unknown. Therefore, the increase in antibodies against UPEC in the serum may have a more marked effect against pyelonephritis, as these antibodies can more easily reach the kidney (McLellan & Hunstad, 2016). Several research groups have been working to identify specific UPEC components that might be used as vaccine antigens. An attractive candidate for the development of a vaccine would be an antigen specific to the pathogenic strains, which is expressed on the surface of the bacteria and is highly expressed during the infection. The ideal vaccine should elicit a robust humoral and cellular immune response whenever the UPEC strain reaches the urinary tract mucosa.

Uro-Vaxom is an immunostimulant that is currently on the market for the prevention of UTIs. This drug is administered orally and is composed of lyophilized membrane proteins from 18 different strains of UPEC (Mobley & Alteri, 2015). The dosing regimen consists of taking one capsule per day for three months, followed by a three-month break, and finally, another three months, when ten tablets per month is the recommended dosage (Magistro & Stief, 2019). Uro-Vaxom boosts cellular and humoral immunity by stimulating macrophages and lymphocytes and increasing endogenous IgA and IgG antibody levels (Osamwonyi & Foley, 2017). The safety and efficacy of this drug are well described, with headache and gastrointestinal problems being the most common adverse effects (Magistro & Stief, 2019; Osamwonyi & Foley, 2017).

Other immunostimulants, such as Solco-Urovac, which is given vaginally, and Uromune, administered sublingually, have recently been developed with promising outcomes. Both include bacterial lysates from various uropathogenic bacteria, not simply different UPEC strains (Magistro & Stief, 2019). Solco-Urovac is a polymicrobial combination of heat-inactivated uropathogens that is accessible in several European countries. *E. coli* comprises six of the ten strains present in this immunostimulant and a strain of *Proteus mirabilis*, *Klebsiella pneumoniae*, *Morganella morganii*, and *Enterococcus faecalis* (Asadi Karam *et al*., 2019; Genao & Buhr, 2012). Vaccination prevented urinary tract reinfection in 50% of women during 24

weeks and significantly delayed the reinfection interval compared to the placebo-treated group (Totsika *et al*., 2012). However, some studies revealed adverse effects such as pain, fever, burns, bleeding, vaginal itching, and nausea, which limited the vaccine's usage in the general population (Asadi Karam *et al.*, 2019). Furthermore, it was impossible to demonstrate its efficacy against heterologous strains or its ability to induce a lasting immune response (Totsika et al., 2012). Uromune is composed of bacterial lysates from *E. coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, and *Enterococcus faecalis*. The first study in the United Kingdom using the bacterial vaccine Uromune to treat women with recurrent UTIs revealed that after three months of treatment, 78% of the women with recurrent UTIs had not experienced any recurrence for 12 months (Yang & Foley, 2018). A retrospective cohort study comparing the efficacy of administering Uromune for three months to the efficacy of antibiotic therapy (i.e., SMX-TMP and nitrofurantoin) for six months revealed that 81% of women in the Uromune group did not experience any recurrence, while only 3% of patients in the antibiotic-treated group had no recurrence (Lorenzo-Gómez *et al.*, 2015). However, further research is needed to prove these positive findings and demonstrate Uromune's safety and usefulness. Several UTI vaccine options have been studied in animal models in recent years, but most have never been tested in humans.

Although the conjugated ExPEC4V vaccine candidate may carry up to 12 O antigens to provide wide coverage against most pathogenic *E. coli* infections, only four serotypes were included in this first-generation vaccine-based prevalence and antibiotic resistance. The four most frequent serotypes (i.e., O1A, O2, O6A, and O25B) were chosen for the ExPEC4V vaccine, which was toxicity tested in animals and first-in-human (phase 1). Although the findings of this clinical study indicated the vaccine's excellent safety and a substantial reduction in *E.-coli*-related UTI, they were insufficient to show a significant reduction in vaccine-specific *E. coli* UTI (Huttner & Gambillara, 2018). Recently, another experimental vaccine (FimCH) targeting the FimH antigen has completed phase I clinical trials with promising results. Vaccination of two cohorts of individuals with a 24-month history of recurrent UTI decreased infection by 70% to 74% after establishing FimH immunity. The number of UTIs caused by *E. coli* was reduced by 70% (Tamadonfar *et al.*, 2020). Based on these positive findings, the Food and Drug Administration has authorized the vaccine for compassionate use in patients infected with multidrug-resistant UPEC.

4. Conclusion

UTI is a critical public health concern because it raises morbidity and healthcare costs. UTIs, particularly those caused by UPEC strains, are among the most prevalent diseases in today's society, and complex and recurring UTI forms can make treatment more difficult. UPEC is the gram-negative bacterium most commonly linked to UTIs. UPEC colonizes the urinary system and can cause cystitis by ascending the urinary tract and into the bladder. UPEC can ascend the ureters to the kidney and cause pyelonephritis, a secondary infection that can lead to permanent kidney damage, renal failure, and death if left untreated. Currently, the treatment of UTIs is mostly empirical, with TMP-SMX, fluoroquinolones, beta-lactams, and aminoglycosides among the antibiotic classes utilized as therapeutic options. However, given the significant increase in UPEC resistance to these antibiotics, fosfomycin and nitrofurantoin appear to be the best alternatives for the empirical therapy of uncomplicated cystitis. Decisions in the empirical treatment of uncomplicated UTI should be guided by knowledge of the local etiology and the susceptibility profile of the most frequent uropathogens to antibiotics. Available treatments for UTIs have not changed much in recent years, do not prevent recurrences, and are being challenged by antibiotic resistance. The reduction and control of the transmission of bacterial resistance in UTIs require novel antimicrobial drugs, the prudent use of available antibiotics, new vaccines, and public health initiatives.

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