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Uropathogenic *Escherichia coli* Virulence and Antibiotic Resistance

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Abstract

Urinary tract infections (UTIs) are among the most common infectious diseases worldwide, causing chronic and recurrent issues that significantly impact work efficiency, personal responsibilities, quality of life, and sexual health. UTIs represent an exceedingly common condition affecting individuals of all ages and genders. Women exhibit a higher prevalence of UTIs compared to men, influenced by various clinical factors such as anatomical disparities, and hormonal effects. *Escherichia coli* (*E. coli*), typically intestinal flora, can thrive in the urinary system, leveraging virulence factors such as type 1-fimbriae, P fimbriae, S fimbriae, and alpha-hemolysin (HlyA) for invasion, colonization, and evasion of host defenses. A major healthcare concern is the increasing antibiotic resistance, particularly due to extended-spectrum beta-lactamases (ESBLs) like TEM, SHV, and CTX-M, which degrade beta-lactam antibiotics but can be inhibited by clavulanic acid, tazobactam, or sulbactam. OXA β-lactamases add to this resistance, especially against oxacillin and penicillin. The emergence of carbapenem-resistant Enterobacteriaceae, resistant to nearly all β-lactam antibiotics, further complicates treatment.

Keywords: *E. coli*, Antibiotic resistance, Virulence factor, urinary tract infection

Introduction:

Urinary tract infections (UTI), a common type of bacterial infection, occur when bacteria inhabiting the bladder and surrounding the urethra are expelled during micturition. The bladder is also susceptible to bacterial colonization. Notably, women, compared to men, have a shorter distance to the bladder, facilitating easier access for bacterial colonizers through the female reproductive system (Geerlings, 2016). Additionally, the proximity of the female urethral opening to the rectum increases the likelihood of bacterial transfer to the urethra. Everyday urogenital manipulations or medical interventions further contribute to this risk. The diagnosis of a UTI involves evaluating symptoms and confirming the presence of infection through a positive urine culture. Typically, a bacterial count of 10^5 colony-forming units (cfu)/ml is considered the threshold for bacteriuria across various patient groups. Women may exhibit typical urinary symptoms, but a significant portion (up to 20%) might still test negative in bacterial cultures. This variability in results depends on the specific threshold used in the testing process (Foxman, 2010). UTIs represent a widespread occurrence of bacterial infections, impacting approximately 150 million individuals globally each year. Presently, the economic implications of these infections, encompassing healthcare expenses and productivity losses, amount to an estimated US $3.5 billion

annually in the United States alone (Flores-Mireles et al., 2015). UTIs pose a significant health challenge, contributing to morbidity in various demographic groups, including infant boys, elderly men, and females across all age ranges. The associated complications are substantial and encompass recurrent infections, pyelonephritis leading to sepsis, renal damage, particularly in young children, pre-term birth, and adverse effects stemming from frequent antimicrobial usage. These effects include the development of high-level antibiotic resistance **(**Flores-Mireles et al., 2015)**.** The year 2019 saw a substantial global health burden from UTIs, with around 404.61 million recorded cases, leading to 236,790 deaths and causing 520,200 Disability-Adjusted Life Years (DALYs). Significantly, there was a remarkable rise in mortality rates, indicating a 2.4-fold growth from 1990 to 2019. Simultaneously, there was a worrisome increase in the age-standardized mortality rate (ASMR), which went up from 2.77 per 100,000 to 3.13 per 100,000 over the same time frame. The age-standardized incidence rate (ASIR) continuously showed prominence in places with a higher socio-demographic index (SDI), which corresponds to noticeably increased trends in ASMR. Conversely, countries with a lower SDI or those that were initially burdened with a greater prevalence have had a significant decrease in burden rates over the past three decades (Yang x et al., 2022.). Gram-negative bacteria, primarily *Escherichia coli* (*E. coli*), stand as the main pathogens responsible for UTIs, causing approximately 80% of UTIs, followed by other Enterobacteriaceae species like *Proteus mirabilis* and predominantly *Klebsiella pneumoniae* (*K. pneumoniae*) (Mazzariol et al., 2017). Beta-lactams, commonly prescribed for UTI treatment, are facing a challenge due to the emergence of extended-spectrum beta-lactamases (ESBL) enzymes synthesized by specific bacteria, including certain strains of *E. coli.* These enzymes can break down penicillins, various generations of cephalosporins, and monobactams, such as aztreonam. Notably, there are three primary groups of ESBL enzymes, namely TEM, SHV, and CTX-M, and their activity can be hindered by clavulanic acid, tazobactam, or sulbactam (Naziri et al., 2020). OXA β-lactamases are prominent in conferring resistance against β-lactam antibiotics, demonstrating substantial resistance, particularly against oxacillin and penicillin (Sadeghi et al., 2023). Compounding the healthcare challenge is the swift emergence of carbapenem-resistant Enterobacteriaceae, showcasing resistance to nearly all β-lactam antibiotics and a substantial portion of other antibiotic classes (Gurung et al., 2020).

2. Classification of urinary tract infections

A complicated UTI is characterized by its correlation with structural or functional abnormalities that increase the likelihood of therapy ineffectiveness or the emergence of serious consequences. The anomalies include a wide variety of causes, such as the existence of foreign objects, obstructive diseases, neurogenic bladder, renal failure, renal transplantation, immunosuppression, the utilization of drainage devices, and incidents during pregnancy. Patients with complex UTIs are at significant risk of treatment failure and the possible development of severe consequences due to the wide range of illnesses falling under this group. The difficulty in handling complex UTIs stems from the diverse range of microorganisms involved, which includes multidrug-resistant bacteria, hence further complicating the treatment strategy (Lichtenberger & Hooton, 2008).

According to the most recent standards, lower or upper infections in women who are not pregnant and do not have any functional, anatomical, or comorbidities are considered uncomplicated UTIs. Normal kidney function, the absence of any additional conditions that can raise the risk of a UTI, and the absence of any anomalies in the urinary system are the characteristics of an uncomplicated UTI (Negri et al., 2024). The classification differentiates between lower UTI, characterized by symptoms limited to the lower urinary tract such as dysuria, urgency, frequent urination, or pain above the symphysis, and upper UTI, which encompasses symptoms such as flank pain, pain upon tapping the kidneys, and fever (>38 °C). Furthermore, it is important to distinguish between clinically symptomatic UTI and asymptomatic bacteriuria (Wagenlehner et al., 2011).

3. *E. coli* general characters

E. coli is a type of bacteria that is a gram-negative, non-sporulating, rod-shaped, and facultative anaerobe. It is commonly found in the intestines of humans. The gastrointestinal tract is the main habitat of *E. coli*, although typically found in our daily nourishment and water, easily establishes itself as a secondary habitat in our surroundings. The abundance of *E. coli* strains varies in the prevailing temperature and nutrient levels, whereas in humans, their occurrence is influenced by a range of host attributes including body size, diet, intestinal structure, and resident microorganisms (Tenaillon et al., 2010).

4. *E. coli* pathogenesis and its virulence

E. coli, which is a major cause of UTIs, demonstrates variation in its disease-causing mechanisms, classified into four primary phylogroups (A, B1, B2, and D)(Bien et al., 2012). These phylogroups are differentiated based on the existence of genetic Pathogenicity Islands (PAI) and the manifestation of several virulence markers, such as adhesins, toxins, surface polysaccharides, flagella, and iron-acquisition systems (Bien et al., 2012). During the development of UTIs, *E. coli* follows a complex pathogenic process reported by (Terlizzi et al., 2017). This process involves.

(a) Initial colonization of the periurethral and vaginal areas, which then extends to the urethra.

(b) Ascending into the bladder and thriving as individual cells in the urine.

(c) Attaching to the surface of the bladder and interacting with the protective defenses of the bladder's epithelium.

(d) Forming biofilms.

(e) Invading and reproducing, leading to the formation of Intracellular Bacterial Communities (IBCs) in the bladder, with quiescent intracellular reservoirs (QIRs) residing in the underlying urothelium.

(f) Colonizing the kidneys, causing damage to the host tissue, and increasing the risk of bacteremia/septicemia. Surprisingly, bacterial reproduction inside the IBC can reach significant amounts, reaching up to $10^{\wedge 5}$ bacteria per cell, and involves changes in shape as bacteria leave the afflicted cell to infect nearby cells. The complex pathogenic process highlights the ability and endurance of UPEC during UTIs (Spaulding & Hultgren, 2016).

4.1. Adhesion

Adhesins are a diverse group of sticky proteins that are put together by bacteria and can facilitate the binding of receptors to surfaces and surface colonization. The whole-genome sequencing of multiple standard library UPEC strains during the previous 20 years has shown each strain has a variety of potential and recognized adhesins, some of which have been shown to improve the strain's capacity to colonize the urinary tract (Zhou et al., 2023). Attachment to host cells can occur using both fimbriae and afimbrial adhesins. Fimbriae, or pili, are complex structures regulated by gene clusters that encode fimbrial subunits. The genome of *E. coli* has many operons that encode different forms of fimbriae, highlighting the diversity and complexity of these structures (Lüthje & Brauner, 2014). Type 1 fimbriae plays a crucial role as a virulence factor in UPEC, even though these pili are seen in strains that are not always pathogenic. The interaction between FimH, a constituent of Type 1 fimbriae, and its receptors is crucial in facilitating adhesion, invasion, and the development of IBCs (Eto et al., 2007). P-fimbriae are conventionally associated with pyelonephritis. Unlike Type 1 fimbriae, which identify receptors containing mannose residues, the PapG adhesin linked to P fimbriae exclusively attaches to Galα1-4Gal structures found in glycolipids of the host cell membrane. Crucially, the attachment of P fimbriae is not hindered by mannose. The ability of various classes of PapG to recognize Galα1-4Gal is dependent on the presence of adjacent carbohydrates. The diverse binding affinities of PapG variants are crucial in defining their preferences for distinct tissues. This complex system explains how P fimbriae, using the adhesion facilitated by the PapG adhesin, can demonstrate a subtle tissue-specificity that is impacted by the distinct identification of Galα1-4Gal and the varied carbohydrate surroundings in host tissues (Strömberg et al., 1990; Stromberg et al., 1991).

S-fimbrial adhesins have a crucial function in facilitating adhesion by attaching to receptors that contain sialic acid. The Sfa determinant, which was obtained from urinary tract isolation, has undergone thorough examination and analysis. The intricate composition of the S fimbrial adhesin consists of four proteins, wherein SfaA functions as the primary fimbrillin protein, while SfaG, SfaS, and SfaH serve as secondary components. Gene study and sequencing revealed that SfaS serves as a sialic-binding S-specific adhesin. Moreover, it has been shown that the production of S fimbriae is strongly dependent on the surrounding environment, highlighting the significant impact of external factors on the presence of S fimbrial adhesins (Schmoll,' et al., 1990).

4.2. Toxins

Alph-hemolysin (HlyA) is a toxin that is released and acts as a pore-forming substance, known for its ability to destroy red blood cells. Nevertheless, it is crucial to acknowledge that the cytotoxic properties of HlyA extend beyond its influence on red blood cells. This toxin exhibits cytotoxicity against various types of nucleated cells, including immunological cells, endothelial cells, and epithelial cells found in the urinary tract. The harmful consequences of HlyA on different cell types emphasize its wide-ranging and influential ability to cause cell death, thus underlining its importance in the field of urinary tract pathophysiology (Island et al., 1998; Mobley,' et al., 1990). Further, (Gur et al., 2013) have reported a newly discovered relationship between HlyA and natural killer (NK) cells in the urine bladder, as revealed in subsequent investigations. This process occurs when bacteria attach to NK cells using type 1 fimbriae, resulting in the eventual destruction of NK cells by HlyA. Significantly, NK cells have a vital function in immune responses to

infections by stimulating the release of tumor necrosis factor-alpha (TNF-α). Nevertheless, HlyA hinders the pro-inflammatory reaction by inhibiting NK cells, so impeding the usual release of TNF-α in response to UPEC infection. This illuminates an extra level of HlyA's influence, uncovering its regulatory function in modifying immune responses to UTIs.

4.3. Bacterial self-defense

The ability to resist the bactericidal effects of serum provides a notable advantage to extraintestinal pathogenic *E. coli* when entering the bloodstream. To bypass the natural defenses, present in serum, such as complement and antimicrobial peptides, requires an intricate interaction of several components. *E. coli* utilizes multiple strategies to resist the effects of serum, such as the synthesis of defensive extracellular polysaccharide capsules and the activation of proteins that hinder or disrupt the complement cascade (Miajlovic & Smith, 2014).

5. Antimicrobial resistance

The prevalence of antibiotic resistance in uropathogens has been rising, which has led to a rise in treatment failure rates, patient morbidity, medical expenses, and the need for broadspectrum medicines, which has exacerbated antibiotic resistance (Ku et al., 2024). This is particularly evident as members of the Enterobacteriaceae family are increasingly acquiring ESBLs, including cefotaximases (CTX-Ms) and oxacillinases (OXAs), as well as AmpC-type β-lactamases and carbapenemases. The emergence of MDR in these uropathogens amplifies the threat, so a scientific approach is essential for responding to this challenge associated with combating bacterial infections and their resistance (Flores-Mireles et al., 2015).

5.1. Extended-spectrum β-lactamases (ESBLs)

ESBLs belong to the class A β-lactamases, constituting a rapidly evolving category of enzymes. These β-lactamases possess the capacity to hydrolyze and induce resistance to oxy-imino cephalosporins, including cefotaxime, ceftazidime, ceftriaxone, cefuroxime, and cefepime, along with monobactams such as aztreonam. ESBL-producing strains of *E. coli* and *K. pneumoniae* stand out as the primary pathogens in UTIs, presenting considerable challenges (Abdallah et al., 2023; Kayastha et al., 2020; Peirano & Pitout, 2010).

The plasmids that carry the ESBLs known as CTX-Ms constitute a distinct plasmid phylum in terms of phylogeny, setting them apart from other plasmid-encoded β-lactamases. CTX-Ms exhibit activity against a wide spectrum of antibiotics, including narrow-, broad-, and extended-spectrum penicillins, as well as classical and extended-spectrum cephalosporins, and monobactams. Notably, they impart significant resistance to cefotaxime. Within community-associated isolates, CTX-Ms stand out as the most prevalent β-lactamases, typically being encoded on plasmids alongside other resistance genes (Bradford, 2001; Gupta & Bhadelia, 2014; Paterson, 2006).

Over the last decade, there has been widespread documentation of plasmid-mediated CTX-M-type ESBLs. These enzymes are predominantly found in pathogens acquired in the community, particularly in *E. coli*. These β-lactamases pose a significant challenge to the effectiveness of all β-lactam antibiotics, except for carbapenems and cephamycins. Furthermore, their location on plasmids is associated with various non-β-lactam resistance markers. As a result, they present a substantial threat to the successful treatment of UTIs caused by community-acquired *E. coli* (Coque, 2008.).

Antibiotic resistance is becoming a significant issue all over the world because of the proliferation and widespread presence of TEM ESBL types in *E. coli* bacteria. Scientists have discovered a variety of TEM ESBLs in *E. coli* (Cantón & Coque, 2006; Paterson & Bonomo, 2005; Pitout & Laupland, 2008). Our understanding of the evolution of ESBLs primarily stems from investigations into TEM and SHV enzymes. These enzymes are predominantly structural variants of TEM-1, TEM-2, and SHV-1 penicillinases. Notably, nosocomial Enterobacteriaceae populations exhibit a significant prevalence of these β-lactamases, contributing to a high ampicillin resistance rate, particularly around 50% in *E. coli*. This prevalence plays a vital role in the global dissemination of TEM and SHV ESBLs**.** Detailed analyses of the nucleotide sequences of bla_{TEM} and bla_{SHV} ESBL genes, in comparison to their parental penicillinases, have uncovered numerous point mutations. These mutations lead to amino acid substitutions within the β-lactamase polypeptide. Many of these substitutions have been identified to impact both the structure and activity of the enzyme in various ways (Gniadkowski, 2001)**.**

5.2. OXA (Oxacillin-hydrolyzing) beta-lactamases

OXA refers to beta-lactamases that can hydrolyze oxacillin. The word "bla_{OXA}" denotes the gene responsible for encoding these enzymes. OXA beta-lactamases have been detected in many bacterial species, such as *Acinetobacter baumannii*, which is a well-known opportunistic pathogen recognized for its resistance to multiple drugs. The bla_{OXA} genes frequently correlate with resistance to carbapenems, which are regarded as antibiotics of last resort. (Poirel & Nordmann, 2006; Queenan & Bush, 2007; Tacconelli

et al., 2018). The OXA β-lactamases, initially identified as among the earliest β-lactamases, were initially infrequent and consistently plasmid-mediated, falling within the molecular class D β-lactamases. Initially, their substrate range was confined to penicillins, but some later acquired the ability to induce resistance to cephalosporins. In the 1980s, there was a notable emergence of *Acinetobacter baumannii* isolates resistant to carbapenems, characterized by plasmid-encoded β-lactamases (OXA-23, OXA-40, and OXA-58) classified as OXA enzymes due to their sequence resemblance to earlier OXA β-lactamases**.** Carbapenem-resistant OXA β-lactamases (OXA-48) have migrated to Enterobacteriaceae and are increasingly implicated in causing carbapenem resistance. The rise of OXA enzymes capable of conferring resistance to carbapenems, particularly in *Acinetobacter baumannii*, has transformed these β-lactamases from a minor concern into a substantial challenge, posing a significant threat to the clinical efficacy of carbapenems (Evans & Amyes, 2014).

5.3. New Delhi. Metallo-beta-lactamase (NDM)

NDM is a highly influential factor in the field of antibiotic resistance, posing a substantial worldwide health issue. NDM, initially discovered in *K. pneumoniae* in India, is classified as a metallo-beta-lactamase. This enzyme group is recognized for its capacity to provide resistance to a wide spectrum of beta-lactam antibiotics, including important drugs such as carbapenems (Yong et al., 2009). The high prevalence of NDM-producing bacteria highlights the need to urgently address this resistance mechanism, as these organisms frequently demonstrate resistance to numerous antibiotics. The bla_{NDM} genes possess a high degree of mobility, as they are frequently found on mobile genetic components. This characteristic enables them to be transferred across different strains and species of bacteria, hence promoting the spread of resistance (Nordmann et al., 2012). The ability of NDM-mediated resistance to spread is highlighted by the fact that it not only makes treatment options for different infections more complicated but also increases the possibility of its transmission. Developing new antibiotics, exploring alternative therapy, and imposing strict infection control methods are crucial in addressing the increasing problem of antibiotic resistance associated with NDM (Fomda et al., 2014; Lutgring & Limbago, 2016).

5.4. *Klebsiella pneumoniae* carbapenemase (KPC)

The presence of the KPC is a major worry when it comes to antibiotic resistance, especially because it is linked to the carbapenem-resistant Enterobacteriaceae group. KPC enzymes are a type of beta-lactamases that can break down carbapenem antibiotics. As a result, these powerful medications become useless in treating bacterial infections (Yigit et al., 2001). Initially discovered in the early 2000s, KPC has quickly spread worldwide, adding to the growing problem of bacterial infections that are resistant to several drugs (Munoz-Price et al., 2013). The bla_{KPC} gene is frequently present on plasmids, which enables its horizontal transfer among many bacterial strains and species (Mathers et al., 2013). The transferability of germs increases their adaptability, which poses a significant risk to treatment alternatives. The transmission of KPC-mediated resistance is linked to healthcare facilities, highlighting the urgent requirement for infection control measures and surveillance to restrict its spread (Munoz-Price et al., 2013). Furthermore, it is imperative to develop novel antibiotics and alternative treatments to mitigate the influence of the bla_{KPC} gene on the effectiveness of current antimicrobial drugs (Queenan & Bush, 2007).

5.5. Quinolone resistance

The extensive utilization of quinolone antimicrobials in clinical settings has resulted in the development of resistance due to genetic changes in DNA gyrase and DNA topoisomerase IV. These mutations decrease the ability of the drug to bind to the enzyme-DNA complex. Mutations in regulatory genes also affect the expression of efflux pumps, expanding the range of substances they may transport to include quinolones. The gradual build-up of these genetic changes in response to selective pressure leads to the emergence of highly resistant strains. Resistance encoded in plasmids is attributed to Qnr proteins, which shield target enzymes from the effects of quinolones, a mutated aminoglycoside-modifying enzyme that also alters specific quinolones and mobile efflux pumps. Plasmids carrying these mechanisms frequently include extra antimicrobial resistances and can transfer multidrug resistance, encompassing quinolones (Hooper & Jacoby, 2015).

5.6. Prevalence of resistance over time

The proof is clear and worrying antimicrobial resistance (AMR) among UPEC has been a problem in both private community labs and public hospital labs for over 12 years. What's surprising is that the AMR rates are consistently higher in public hospitals. Almost all antibiotics showed a clear increase in resistance rates over time in both private community labs and public hospitals. So, it's a fact that resistance to antibiotics has gone up in both lab settings, with public hospitals having consistently higher rates. This shows we urgently need to do something, especially in the community where private labs handle most outpatient cases. Understanding this, we need to focus on community labs to tackle the growing problem of antibiotic resistance in the wider community (Keighley et al., 2022). AMR, as reported by the World Health

Organization (WHO) on 21 November 2023, not only leads to death and disability but also carries significant economic consequences. According to the World Bank, AMR could lead to an extra $1 trillion in healthcare expenses by 2050, and annual economic losses of $1 trillion to $3.4 trillion in gross domestic product (GDP) by 2030. The global increase in antibiotic resistance is a major concern, reducing the effectiveness of common antibiotics against widespread bacterial infections. The 2022 Global Antimicrobial Resistance and Use Surveillance System (GLASS) report highlights worrisome resistance rates among prevalent bacterial pathogens, such as 42% for third-generation cephalosporin-resistant *E. coli* and 35% for methicillin-resistant *Staphylococcus aureus* in 76 countries. Moreover, in 2020, 1 in 5 cases of UTI caused by *E. coli* showed decreased susceptibility to common antibiotics such as ampicillin, co-trimoxazole, and fluoroquinolones. This makes treating common infections successfully more difficult.

Conclusion

Urinary tract infections (UTIs) are a serious global health concern that is more common in women because of anatomical and hormonal reasons. The frequent and chronic nature of UTIs places significant financial strain on top of their adverse effects on quality of life. The primary cause of infection is *E. coli*, which utilizes virulence factors like fimbriae for adhesion, toxins like Alpha hemolysin for cellular damage, and genes like *traT* for serum resistance to survive and proliferate in the urinary tract. Effective treatment of UTIs is made more difficult by the growing issue of antibiotic resistance, which is caused by the overuse and abuse of antibiotics. The emergence of carbapenem-resistant Enterobacteriaceae and Extended-Spectrum Beta-Lactamases (ESBLs) underlines the critical need for new antimicrobial approaches. A variety of strategies is needed to address UTIs, including encouraging appropriate use of antibiotics, improving preventive measures, and going research into novel treatments. Addressing the virulence and resistance mechanisms of UTIs can enhance patient outcomes and reduce the worldwide effects of this widespread infectious illness.

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