# A Review on

# In vitro and In vivo model advancements for the study of Escherichia coli-induced Urinary tract infections

## 1. Abstract

Urinary tract infections (UTIs) are bacterial infections that affect public health and are caused more frequently due to Uropathogenic Escherichia (UPEC). UPEC uses a wide range of virulence factors like adhesins, biofilm formation, fimbriae, and immune evasion techniques for persistent, recurrent infection and antimicrobial resistance. The need for improved diagnostic methods, treatments, and protective actions is raised due to the emergence of Multidrug-resistant UPEC strain which has complicated the present treatment plans. Traditional methods like Invitro & Invivo models helped in studying the UTI host-pathogen interaction & pathogenesis but have constraints in studying long-term infections and also in recreating human urinary tract conditions. The latest innovations in experimental models such as bladder organoids, dynamic microfluidic systems, and murine & zebrafish models improved physiological relevance and understanding of UPEC behaviour and newer treatment methods. These models helped us deepen our knowledge of antibiotic resistance, biofilm dynamics, and host immune responses which enabled us to develop novel therapeutic approaches. UTI research has fastened after technological innovations in genome & transcriptomic analyses, imaging techniques, and high-throughput screening. To deal with MDR UPEC, newer treatment methods like vaccines, phage therapy and anti-virulence agents are being delved into along with antibiotics. In addition, improved invitro and invivo models are used to develop vaccines specific to UPEC. This review focuses on the developments in experimental models and methods to study E.coli-induced UTIs, mainly focusing on its purpose in studying pathogenesis, improving preventive measures, and overcoming treatment challenges. These integrated innovations are critical to tackling the rising MDR UPEC and the need for personalized treatment to decrease the worldwide burden of UTIs.

## 2. Introduction

### Overview of urinary tract infections (UTIs)

Urinary tract infections are a type of bacterial infection that affects the bladder, urethra, and kidney which are the parts of the urinary system. Urinary tract infections are classified into complicated UTIs, mostly observed in healthy women, and uncomplicated UTIs, mostly caused by structural or functional abnormalities. [1]. For 75% of acquired UTIs, E.coli is responsible, and other microbes like Klebsiella pneumoniae & Staphylococcus saprophyticus are responsible for UTIs in some people. The recurrent infections caused by uropathogenic E.coli (UPEC) are due to the fimbriae which are important for attachment and biofilm formation which is important for persistence [2].

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Age, diabetes, and catheterization increase the susceptibility and severity of infection. The symptoms of pyelonephritis which is a type of upper UTI are mostly fever, flank pain, and nausea while symptoms of lower UTIs are dysuria & suprapubic pain. The prognosis for detection of causative pathogen is mostly urinalysis and urine culture while the medication used mostly are antibiotics such as nitrofurantoin or trimethoprim-sulfamethoxazole [3]. Nevertheless, in the case of UPEC due to its increased anti-microbial resistance, the treatment methodologies have become difficult. As UTI is the major reason for Gram-negative sepsis, it significantly impacts public health and is one reason for the high healthcare costs [4].

**Importance of Escherichia coli as a primary pathogen in UTIs**

80-90% of community-acquired UTIs are caused by Escherichia coli (E. coli). These are also known as Uropathogenic E.coli. These strains have diverse pathogenicity factors such as fimbriae and adhesins, enabling E.coli to attach and colonize the urinary tract [5]. Its ability to form biofilm is the major reason for its persistence, recurrence (majorly in women having recurrent UTIs), and antibiotic resistance. Some studies like Phylogenic studies show the relation between UPEC strains (for example those in group B2) and increased pathogenicity and antimicrobial susceptibility [6].

Besides, the adaptive mechanisms of E.coli help it to grow in nutrient-dense environments such as urine. Factors like age, diabetes, and catheterization also affect the rate of susceptibility and severity of infection. Due to these traits of UPEC, there is a need for newer treatment and prevention methods and these traits make UPEC one of the major challenges for public health [7], [8], [9].

**Challenges in understanding pathogenesis and treatment strategies**

Due to the complex host-pathogen relationship and increasing antibiotic resistance, studying and developing treatment plans for UPEC-induced UTIs is becoming difficult [10]. UPEC utilizes pathogenicity factors such as Intracellular bacterial communities (IBCs) and quiescent reservoirs to escape immune responses and initiate infections. The identification of universal therapeutic agents is difficult due to the genetic diversity among the UPEC strains which is due to the mobile genetic elements [11].

Alternative therapies such as anti-virulence agents, vaccines, and immunomodulators are needed due to the emerging multi-drug-resistant strains mainly those with extended-spectrum beta-lactamases (ESBLs) which are limiting the treatment options [12]. Some non-pathogenic strains protect virulent bacteria, and because of this asymptomatic bacteriuria showcases diagnostic and therapeutic predicament. To combat these predicaments, a deeper study is required to understand the UPEC's adaptive methods, and innovative diagnostic & personalized therapeutic approaches are needed [13], [14], [15].

**Role of experimental models in advancing UTI research**

In studying the host responses, pathogenesis, and potential treatment methods of E.coli as a part of UTI research, the experimental models have been a significant help. One of the major used models in UTI

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research are Murine model which simplifies various mechanisms of E.coli such as biofilm formation, intracellular colonization, and immune evasion [16]. Some of the models help in understanding the relationship between genetic factors and susceptibility which helps us to find the innate immunity and specific cytokine's role in disease effects [17].

The advanced germ-free and humanized mice model systems helped us to understand the role of microbiota in influencing UTI susceptibility. The innovative Invitro models such as 3D bladder organoids present a human-relevant platform for understanding UPEC-host relations and also contribute to innovating novel therapeutics [18]. The experimental models are essential in identifying biomarkers, studying recurrent mechanisms, and also innovating targeted treatments regardless of their inability to replicate the human UTI complexity [19], [20], [21].

## 3. Historical Perspective

### Early approaches to study E. coli-induced UTIs

Historically the major focus of UPEC research was on its pathogenicity, mechanism, and genetic diversity. MALDI-TOF bio typing which is used to analyse protein signatures, profiles the bacterial isolates due to which bacteria is identified in clinical samples. Some proteomic techniques such as 2D gel electrophoresis were also used for diagnosing UTIs by their biomarker identification [22]. Molecular cloning techniques isolated and characterized various essential factors such as fimbriae adhesins, iron acquisition systems, and toxins while genomics studies looked over the virulence genes. Methods based on PCR gave quick UPEC strain identification surpassing other usual methods in terms of efficiency [23]. Epidemiological studies showed the impact of UPEC strains on various populations also showcasing their relationship with recurrent UTIs. In addition to this, many in-vitro models also tested UPEC's ability in case of adherence, host cell invasion, and immune response evasion which helped us to study the infection mechanism. All these formed a strong foundation for the innovation of diagnostic methods and treatments [24], [25], [26].

### Limitations of traditional models

In traditional models, many limitations caused various problems in studying the infection mechanisms and treatment methods. The use of animal models like porcine and murine systems was also restricted due to their limited ability to be manipulated genetically, greater cost, and ethical restrictions. One of the major limitations of animal models is their inability to replicate human urinary tract physiology inclusive of urine composition, host immune responses, and interaction of microbial flora [27]. These models were further restricted due to their inability to study biofilm formation which is the major factor for persistent UPEC resistance. Also, the focused study on specific UPEC strains often failed to observe the diversity of pathogens causing UTIs. Many models worked on simplistic experimental conditions and they poorly stimulated factors such as urine flow, susceptibility, and nutrient availability [28]. In addition to this, most

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studies are conducted for very little time and this averts the observation of recurrent and long-time infections. All these limitations demanded newer innovations that could study complex human UTIs and come up with accurate diagnoses and effective treatment methods [29], [30], [31].

### Transition to advanced in vitro and in vivo techniques

Understanding urinary tract infections has advanced remarkably with the highly developed Invitro and in vivo models. Invitro techniques, including human bladder epithelial cell models & flow chamber systems, permitted a comprehensive study of UPEC attachment, invasion, and the uropathogenic cascade. Introducing the hydrodynamic conditions in these models has shown important processes such as secondary colonization and changes in bacterial shape which significantly improved the infection dynamic studies [32]. In addition to this, the incorporation of T24 epithelial cell lines into these models has significantly shown cranberry proanthocyanidin dose-dependent effects which reduces UPEC attachment. This showed evidence that dietary interference can prevent UTIs. Harmonizing with these discoveries, Caenorhabditis elegans utilization authenticated that cranberry consumers have seen decreased virulence of E.coli strains cultured in their urine. This showed that the synergism of in vitro and in vivo has significantly helped in understanding the e.coli's bacterial mechanism, pathogenesis, and potential prophylactic treatments. [33].

In Invivo developments, the breach between real-world infections and laboratory findings has been bridged by models such as murine models and the use of multi-drug-resistant E.Coli clinical isolates. Detailed estimation of therapeutic efficacy, infection progression, and also treatment evaluation such as intravenous colistimethate sodium (CMS) is permitted using these models [34]. The studies have shown that CMS has the potential to reduce bacterial load, and inflammation, and also reach good concentrations in urine to support their ability to treat multidrug-resistant e.coli-induced UTIs. Altogether, these techniques improve the understanding, prevention, and treatment of E.coli-induced UTIs highlighting their advancements [35], [36], [37].

## 4. In Vitro Models

### Static and Dynamic Culture Models

The study of urinary tract infections caused by E.coli is significantly enhanced by the recent advancements in static and dynamic in-vitro models. These in-vitro models resemble urinary tract shear stress and mimic human bladder conditions which provides a much more realistic model for studying bacterial behaviour and biofilm formation[38]. These biofilms play a very significant role in the persistence of infection and provide resistance to treatments. These dynamic models are advantageous for studying phenomena like “rolling-shedding-refilling” colonization which is important for understanding E.coli behavior and developing UTI treatments [39]. Dynamic models, like microfluidic-based systems, allow real-time monitoring of the progression of an infection, including bacterial filamentation and dispersal. Dynamic systems highlight the

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significance of urine-induced morphological variability that is essential for studying E.coli behavior under physiological conditions. Unlike nonstandardized models, these dynamic models evolve that enhance our understanding of bacterial infections, and contribute to much more effective treatments [40].

E.coli colonization on catheters is found to be accurately similar in a dynamic catheterized bladder model that mimics human infection conditions. This research highlights the role of type 1 fimbriae in catheter colonization and found that E.coli cells lacking these fimbriae were outcompeted by wild-type strains. This emphasizes the importance of type 1 fimbriae in the persistence of infection [41]. To gain a comprehensive understanding of CAUTI pathogenesis this study emphasizes combining in vitro findings along with in vivo expression analysis. To develop prevention and treatment strategies, identifying specific virulence factors were necessary [42]. In addition to that understanding the overlap of virulence factors in these dynamic models in UTIs is very crucial for vaccine development. This is feasible by identifying unique factors for catheter colonization, which could accelerate the effectiveness of developing prevention and treatment strategies [43].

**Organoid Models**

The development of the human urothelial organoid model represents a significant advancement in studying UTIs induced by E.coli. These organoid models mimic the bladder epithelium, allowing the study of pathogenic interaction in the physiological environment [44]. Organoid models support long-term culture enabling us to study chronic infections and bacterial colonization dynamics. This model provides insights into host-pathogen interaction that includes the study of E.coli adherence and invasion, which are necessary for the study of infection mechanisms [45]. Organoid models offer a controlled environment for dissecting molecular responses without any complexities of whole animal systems when compared to traditional animal models. Organoids derived from individual patients pave the way for understanding variations in susceptibility and responses to treatment and designing much more personalized medicine approaches in UTI management [46]. Advanced organoid systems incorporate elements in the urinary tract microenvironment, that offer a more accurate representation of infection conditions [47]. The development of a bladder organoid that resembles the stratified structure of the epithelium in the human bladder, provides high-resolution live cell imaging of UPEC (Uro-Pathogenic Escherichia coli) infection dynamics. UPEC rapidly invades the superficial umbrella-like cells in the organoid lumen and proliferates to form intracellular bacterial communities (IBCs) that cause infection. Individual bacteria penetrate deeper into bladder wall layers and exhibit distinct morphology that protects against neutrophil and antibiotic attacks that cause the persistence of bacterial infection and potential recurrence of the infection. This study highlights the utility of organoid models for studying the UPEC infection mechanism and emphasizes the need for novel therapeutic strategies targeting superficial and deep-seated bacterial colonies to prevent recurrent infections in the urinary tract [48].

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## Microfluidic and Organ-on-a-Chip Systems

Microfluidic chips serve as a crucial tool for studying E.coli-induced UTIs. These devices resemble the urinary tract environment, including fluid flow and cellular interactions. This provides controlled settings for examining E.coli behavior [49]. They enable high-output screening of multiple antibiotics and tailor personalized models according to individual patient profiles for testing antibiotic efficiency. Integrating sensors into these microfluidic chips allows real-time monitoring of infection progression and treatment efficiency, which enhances our understanding pathogenesis of E.coli [50]. Organ–on–chips models provide a platform to study the host-pathogen interaction which enhances our understating of infection dynamics. It can recreate human UTI conditions that allow details studies of E.coli colonization and antibiotic testing under controlled conditions [51]. Microfluidic organ-on chips replicate the urinary tract microenvironment to study the colonization of E.coli, biofilm formation, and antibiotic resistance mechanisms. They facilitate the detailed exploration of immune responses, bacterial evasion strategies, and real-time assessment of antibiotic susceptibility efficacy. Tailoring of patient-specific personalized therapies can be aided by the integration of patient-derived cells into individual infection models [52]. The Brimor chip model enables continuous observation of E.coli biofilm development and antibiotic-resistant dynamics using confocal microscopy. This is a user-friendly model that supports the study of the emergence and proliferation of antibiotic-resistant bacteria within the biofilm, which provides insights into the mechanism that drives resistance and supports basic research in this critical area[53].

## 3D printed Models

The current advancement in 3D printing technology has led to the development of novel tools for studying E. coli-induced urinary tract infections. To measure impedance based on the antibiotic susceptibility of bacteria, a fully 3D-printed impedance-based biosensor has been designed. This is one of the rapid, non-invasive, and quick methods used to detect bacterial infections. This method is adopted for monitoring E. coli-induced UTIs by assessing the real-time activity and antibiotic resistance mechanisms adopted by the bacteria in the bladder environment [54]. To provide an efficient study on UTIs, A modular 3D Printed peg Biofilm device provides a flexible platform. This device features customizable pegs that mimic natural environments like bladder walls in medical devices. This facilitates antibiotic susceptibility testing and E.coli biofilm resistance. It allows precise handling of individual biofilms and simulates bladder conditions, which paves ways to better study in biofilm behavior and improves treatment efficiency[55] .3D Printing of antimicrobial materials provides better solutions for combating antimicrobial resistance (AMR) in UTIs. The bladder conditions are replicated in the form of antimicrobial polymers and biodegradable scaffolds, which provides a better platform for studying E.coli formation and resistance mechanisms when compared to traditional methods. These materials are designed to provide improved efficacy of treatments, localized drug delivery, and reduce systemic side effects. Personalized 3D printed models tailor advanced research in, novel approaches for UTI management and addressing AMR. [56].

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## 5. In Vivo Models

### Murine Models

E.coli exhibits much higher rates of active cell division in kidneys and urine when compared to bladder during urinary tract infection. Bacteria that can survive and trespass the effects of antibiotics are majorly non-dividing cells across the infection sites. This indicates that the non-dividing cells are resistant to antibiotics. The infection of E.coli and the response of bacteria to antibiotics is significantly affected by the strain of bacteria and the local microenvironment [57]. Human cystitis and pyelonephritis caused by E.coli can be effectively studied using a Murine model. It provides an excellent system for studying the pathogenesis of bacteria causing UTIs that ultimately leads to the development of better treatment strategies. The critical aspects of E.coli infections studied using murine models include the formation of intracellular bacterial communities within the epithelial cells in the bladder lining which contributes to infection and resistance of bacteria against treatments. Murine models also allow the assessment of host-pathogen interaction like studying immune response and facilitate the testing of therapeutic inventions aimed to treat UTIs.[58].

### Non-Murine Mammalian Models

Non-murine models have an important role in filling the gap between the in-vivo and in-vitro investigations which gives an insightful awareness about E.coli-induced urinary tract infections (UTIs). These models are crucial for the accurate investigation of the mechanism of infection and the development of effective treatments [57]. The progress from the generalized in-vitro models to more advanced systems, plays an important role in the study of E.coli infection, by replicating the human bladder environment. The combination of in-vitro and in-vivo approaches offers to create the models that address the gap in UTI research [59]. In terms of both complicated and uncomplicated UTIs, the initial cause of infection is uropathogenic E.coli. Considering the pathogenic mechanisms of E.coli, includes observation and understanding of bladder epithelium, seizing and forming bacterial colonies which is crucial for developing therapeutic strategies [60].

### Zebrafish Models

For the Study of E.coli-induced UTIs, zebrafish is an important tool. These models illustrate the bacterial virulence, and host-pathogen interactions and study the mechanisms of infection. The embryos of zebrafish were used for the calculation of different strains of extraintestinal pathogenic E.coli-like (ExPEC) Strains, which successfully replicate the bacterial distribution and host immune responses. The research aimed towards the specific difference between strains in ExPEC virulence and contributes towards the study of host-pathogen interaction in real-time [61].

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During the study of zebrafish embryos that were infected by uropathogenic E.coli (UPEC), the results tell about the immune responses activated during infection. The dynamic investigation of UPEC infections on a cellular level, comprising inflammatory and defense responses, informs about the specific genes and pathways included in host-pathogen interactions [61], [62].

When zebrafish larvae are infected by E.coli exhibit a state where bacteria lose their cells to seize the immune responses and antibiotics also known as L-form switching of bacteria. The L-form bacteria continues within the host tissues, continuing the repetitive occurrence of UTIs. This centered on the usefulness of zebrafish in the study of bacterial adaptations and determining the mechanisms during UTIs [63].

## 6. Technological and Methodological Advances

### Genomic and transcriptomic analyses in experimental models

For the genomic and transcriptomic study of Escherichia coli-induced UTI, where primarily antibiotics are used as treatment. Particularly for another way of solution, the receptors of host cells and pathways are focused on insisting on overcoming the new challenges of antibiotic resistance. Some bioinformatical approaches came into light as the methodology of these issues, such as gene ontology (GO)analysis, Kyoto encyclopaedia of gene and genome (KEGG) analysis, and protein-protein interaction (PPI) network analysis are used to identify the biomarkers and pathways which are involved in the UTI pathogenesis. [64]. The methodologies resulting in certain specifications as the key involvement of the TNF-α pathway in the identification of UTI as it shows crucial roles in immune response and inflammation, and it is shown by KEGG analysis. By this, the novel treatment of UTI can be targeted by the identification of signalling pathways and certain genes. For the identification of pathways biomarkers can be used by the identification of hub genes and therapeutics can be targeted. The development of new therapeutic sources and diagnostic tools in the focus of UTI, the GEO database can be used. [65].

### Imaging techniques for real-time tracking of infection

For the bacterial infection, the rapid and accurate diagnostic methods are focused. Rapid diagnostics help to initiate a proper antimicrobial therapy, especially in the case of UTI quick and efficient diagnostics and therapy are needed. Methods like large volume solution scattering imaging (LVSi) system are used for quick analysis rather than the traditional methodology which was time-consuming. [66]. The system records the phenotypic features like shape, size, and movement of bacteria present in the solution, helping fast analysis of the sample. After the identification approach, the data processing and analysis takes place comprising quick analysis of similarly shaped bacteria and distinguishing between other shapes of bacteria and the presence of urine particles in them, allowing quick and accurate clinical diagnostics. [67]. The process gives a broad spectrum of identification by short video capturing through LVSi. The accuracy rate in the detection

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of UTI was 92.3% by this method. This technology plays a crucial role in the enhancement of clinical diagnostics and therapy by reducing testing period and analysis accuracy. [68].

**Advances in co-culture systems (host-pathogen interactions)**

Urinary tract infections are common and for women, it is a big concern, as half of them experience it once in their life. UTI gets complicated by certain species like proteus mirabilis in patients with certain conditions. The current scenario of research centralizes the idea of identification of additional virulence factors and improving UTI prevention and treatment by developing vaccines against E.coli. And p. mirabilis. In this, the increasing antibiotic resistance implies a negative impact on the microbiota, which limits the strategies of treatment. [69]. The host-pathogen interaction comprises the structural, genetic, immunological, and microbiological aspects of interaction during UTI infection. A shift in the treatment approach by targeting more pathogen-specific therapies of bacterial replications rather than depending on broad-spectrum antibiotics. Determination of bacterial virulence at the host-pathogen interaction as the focus in the process offers the potential for more effective and sustainable UTI treatment [70], [71].

**High-throughput screening for therapeutic interventions**

The study of UTI developed the strategies of promoting a high-throughput assay to interpret the effect of compounds while biofilm formation by uropathogenic Escherichia coli. (UPEC)UMN026, which is known for causing primary infections. In the assay, the resazurin and crystal violate staining in a 384-well microplate format with optimum conditions like specific time and incubation period. [72]. Certain approaches like the Z'- factor, signal-to-noise effect, and edge well effects are used for the validation of quality parameters. The antibiofilm at sub-inhibitory concentrations of known bacterial compounds was successfully detected in the assay, this provides a tool for the potential screening of antibiofilm therapies aimed at UPEC. [73].

The recent studies imply enhancement of strategies by making a cost-effective and rapid process of antibiotic resistance testing by using sugar-induced bacterial release i.e., 13-Dococenamide for filling fluorescein. This method is standardized for CLSI specialized for 12-well microdilution strip, which captures fluorescence signals in the optoelectric device, and allows the accurate identification of antibiotics within 8 hours of sample collection. [74]. Clinical tests show 94.3% of UTI-infected patients matched with the standard disk diffusion results, as the new approach results in quicker and more accurate results rather than the traditional one, and being affordable makes it one of the best options for alternatives. [75].

## 7. Applications in Drug Discovery and Therapeutics

**Testing novel antibiotics and alternative therapies**

In the exploration of new therapeutics, such as new antibiotics and vaccines for Escherichia coli. Infected UTI, and many other types includes many complex processes including different steps of drug inventions

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like research and development in the laboratory, pre-clinical trials in different stages, and clinical trials on animals, volunteers, and patients. [76]. The process starts with the bacterial processes specifically targeted by designed novel antibiotics i.e. synthesis of the bacterial cell wall and protein, DNA replication, and efflux pump used by bacteria to conquer resistance mechanisms. The tests of the laboratory check out the efficiency and resistance against MDR strains or multi-drug resistance strains, by practicing MIC or minimum inhibitory concentration testing and synergy studies. [77]. Later animal tests exert the efficiency of antibiotics by clarifying the infections in the models that are stimulating UTIs or system of infections, parallelly pharmacokinetics and safety will be considered. Once the results of preclinical tests are favorable, the processes of clinical trials will proceed by getting measures of safety, efficiency, and long-term performance. The trials of phase 1 ensure tolerance in healthy individuals, while phases 2 and 3 show the effect of E. coli. Infection in patents and filter the treatment method or pattern. [78]. The development of resistance and making sure the sustained efficacy is tracked down by post-market surveillance. Even after these efforts, some challenges also become evident as evolving resistance, high development costs, and safety concerns keep on, bringing out the complexity of introducing effective antibiotics for E. coli. Infections. Supervisory approval by bodies like the FDA and EMA marks the final landmark, enabling the introduction of the antibiotics market and real-world applications. [79], [80], [81].

**Role of in vitro and in vivo models in vaccine development**

In the way of developing vaccines for e.coli. Induced UTI research and testing plays an important role by introducing in-vitro and in-vivo models. By these models, scientists understand the biology of pathogens against the vaccines, the efficiency of host and pathogen immune systems and responses towards potential vaccine candidates, and their efficacy in human trials [63], [82].

The in-vitro model needs a controlled experimental laboratory and techniques like bacterial culture, cell line, etc. can be used. These experiments play a crucial role in the early stage of vaccine development by analysis of the combination of immune responses and bacterial processes. Researchers isolate and characterize the culture of uropathogenic Escherichia coli. (UPEC), which is known as the primary cause of UTIs, for the recognition of virulence factors and biofilm formation [83]. In the process of vaccine development, these process helps to analyze bacterial factors like proteins, toxins, and other factors as potential antigens. Besides that, the analysis of cell-based immune responses helps researchers explore how immune cells like macrophages or dendritic cells show response against these antigens, which will provide direction on how cytokines are released and antibodies are produced. Mostly in-vitro models are used for testing the antibodies that are released by the anticipation of vaccine candidates to stop bacterial growth and imitate urinary tract conditions. Additionally, these models confirm the vaccine safety by going through a cytotoxicity test which acknowledges the potential negative or undesirable changes in the human cell line [84]

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On the contrary, The in-vivo model includes animals like mice for catalyzing real-world conditions, and for the evaluation of the safety, efficacy, and immunogenicity of vaccines. These models duplicate the human UTIs to estimate the efficiency of the vaccine in reducing the bacterial load of the urinary tract and preventing colonies in it [85]. The research frequently focuses on different types of infection such as cystitis( bladder infection) or pyelonephritis (kidney infection) which ensures the wide potential for protection and prevention. In-vivo models compress into the vaccine-induced immune responses, which include activation of T-cells, production of antibodies, and generation of memory immune cells for long-term protection. Besides this, it helps in the optimization of different vaccine delivery methods such as oral and intramuscular routes, which calculates the immunity of durability [86].

Both the models (in-vitro and in-vivo) are crucial in the development of vaccines for ecoli. Induced UTIs by providing an understandable insight into the biology of pathogens, immune responses, and vaccine efficacy. Together they fill the gaps between initial research and clinical trials, which ensures the efficacy and safety of vaccine candidates before they reach human trials [87].

**Insights into resistance mechanisms of Uropathogenic E. coli (UPEC)**

The leading cause of urinary tract infection is uropathogenic ecoli. (UPEC) and unfolded the advanced mechanism to avoid the host immune responses and resist the antibiotic treatment. These mechanisms make the UPEC infection risky and challenging to treat, mainly with the increase in commonness of multi-drug-resistant strains. UPEC secretes certain enzymes such as β-lactamases, together with extended-spectrum β-lacatmases (ESBLs) and carbapenemases, which degrades β-lactam antibiotics, showing them ineffective [88]. Besides, UPEC can modify their targeted sites, such as penicillin-binding protein and DNA gyrase by decreasing the efficacy and binding potential of antibiotics. The efflux pump actively throws out antibiotics from the bacterial cell, reducing the concentration of intracellular drugs, while creating certain changes in the porin and outer membrane i.e. lipid bilayer, and then decreasing the permeability of the drug [89].

Expect antibiotic resistance, UPEC infection uses virulence factors to set up the infection and escape host defenses. Facilitation of union of uroepithelial cells and fimbriae (including type 1 and P fimbriae), permitting colonization and biofilm formation, which guards UPEC from antibiotics and immune responses. Biofilms and cytoplasmic pools permit bacteria to continue in the urinary tract, leading to repetitive infections [88], [90]. UPEC also secures immune detection by altering the lipopolysaccharides creating a protective capsule and overpowering the immune cell activation, such as neutrophil responses. Additionally, it involves host cells for nutrients like iron, by constructing siderophores which collect iron from host protein garnering bacterial growth and survival [91].

The contributing combination of these virulence and resistance mechanisms makes it progressively more difficult to treat UPEC infection, especially in the involvement of multi-drug resistant strains. Commonly,

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the antibiotics are not effective against these strains, where carbapenem-resistant UPEC, poses a remarkable threat in the healthcare background [90], [92], [93].

## 8. Conclusion

The advancements in experimental models have significantly revolutionized the study of Escherichia coli-induced urinary tract infections (UTIs), providing a deeper understanding of the pathogenesis, host-pathogen interactions, and potential therapeutic interventions. From the development of dynamic in vitro systems like microfluidics and bladder organoids to in vivo models like murine and zebrafish systems, each model has contributed unique insights into the molecular mechanisms of UTI progression and persistence. These innovations have bridged the gap between traditional methodologies and the complexities of human UTI conditions, enabling the identification of critical virulence factors, biomarkers, and resistance mechanisms.

Furthermore, technological advances in imaging, genomic analyses, and co-culture systems have facilitated real-time tracking of infections and high-throughput screening for therapeutic interventions. Such progress has not only enhanced our understanding of antibiotic resistance in uropathogenic E. coli (UPEC) but also opened avenues for developing personalized medicine, alternative therapies, and vaccines. Despite these achievements, challenges like replicating human urinary tract physiology and addressing the genetic diversity of UPEC strains persist, demanding continued innovation and interdisciplinary collaboration.

In conclusion, the integration of advanced in vitro and in vivo models with cutting-edge technological tools represents a pivotal step toward addressing the global burden of E. coli-induced UTIs. This multifaceted approach holds promise for improving diagnostics, innovating treatment strategies, and ultimately reducing the prevalence and recurrence of these infections. Continued efforts in refining these models and expanding their applications will be instrumental in advancing UTI research and achieving meaningful clinical outcomes.

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