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Advances in experimental bladder models: bridging the gap between in vitro and in vivo approaches for investigating urinary tract infections

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Abstract

Urinary tract infections (UTIs) pose a substantial burden on global healthcare systems. When unraveling the complex pathophysiology of UTIs, bladder models are used to understand complex and multifaceted interactions between different components within the system. This review aimed to bridge the gap between in vitro and in vivo experimental bladder models towards UTI research. We reviewed clinical, animal, and analytical studies and patents from 1959 to the end of 2023. Both in vivo and in vitro models offer unique benefits and drawbacks in understanding UTIs. In vitro models provide controlled environments for studying specific aspects of UTI biology and testing potential treatments, while in vivo models offer insights into how UTIs manifest and progress within living organisms. Thus, both types of models are leading to the development of more effective diagnostic tools and therapeutic interventions against UTIs. Moreover, advanced methodologies involving three-dimensional bladder organoids have also been used to study bladder biology, model bladder-related disorders, and explore new treatments for bladder cancers, UTIs, and urinary incontinence. Narrowing the distance between fundamental scientific research and practical medical applications, these pioneering models hold the key to unlocking new avenues for the development of personalized diagnostics, precision medicine, and ultimately, the alleviation of UTI-related morbidity worldwide.

Keywords Urinary tract infections (UTIs), Bladder model, *In vitro*, *In vivo*, Organoids

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Background

Urinary tract infections (UTIs) are caused by microbial invasion of the genitourinary tract which extends from the renal cortex of the kidney to the urethral meatus [1]. UTI typically begins as a bladder infection (cystitis), however, it can progress to an acute kidney infection (pyelonephritis), which can lead to renal failure [2]. Complicated UTIs could result in serious complications such as urosepsis [3].

UTIs account for an estimated 25–40% of nosocomial infections in developing countries [4]. Approximately 25% of patients experience repeated infections within six months following the original episode, indicating a high recurrence [5]. Although seldom fatal [6, 7], UTIs are a debilitating disease for a significant percentage of female population and pose a significant health risk to persons with underlying medical conditions like diabetes mellitus [7]. Further, there are significant socio-economic consequences from the numerous UTI sequelae, including sepsis in the elderly, premature delivery in pregnant women, and renal scarring in youngsters [8].

Although human urinary tract was thought to be a sterile system until a decade ago, scientists have discovered and confirmed the existence of microorganisms (bacteria, viruses, fungi etc.) in the healthy human urinary tract by characterizing microbiome using metagenomics [118]. Despite these recent advancements, little is known about the bladder, urethra, and urinary microbiome (urobiome), which may contribute to urological diseases and UTIs [119]. The term "urobiome" collectively refer to both the microbiome and microbiota of the urinary tract [118]. It has been discovered that the urobiome, is composed not only bacteria but also a range of viruses, including bacteriophages and eukaryotic viruses [119].

It is known that around 5% of UTIs lead to complicated UTIs [120]. Urinary catheters are placed in approximately a quarter of all hospitalized patients during their hospital stay [9, 10]. More than 80% of UTIs are due to insertion of catheters [10]. Multiple medical problems, including catheter encrustation, bladder stones, septicemia, endotoxic shock and pyelonephritis, can result from catheter associated urinary tract infections (CAUTIs) [11]. The intricate interplay between bacterial pathogens and the host bladder environment is important in understanding the pathogenesis of UTIs [12]. To this end, bladder models serve as indispensable tools, offering researchers a window into the complex dynamics of UTIs at various levels of biological organization.

Urinary bladder models are representations or simulations designed to study the structure, function, and behavior of the bladder [13]. Various models have been developed to aid in the understanding of the bladder's complex physiology and to explore different aspects of urinary bladder-related research [14]. Applications of urinary bladder models include research in urology, physiology, urobiome, pharmacology, and the development of medical devices and treatments for conditions such as urinary incontinence, bladder cancer, and UTIs [15, 16].

Bladder models are designed as in vitro and in vivo tools to investigate UTIs [7, 17]. Scientists' inventiveness has produced a variety of in vitro models, each specifically designed to study the formation of biofilms by specific bacteria in specific settings [18, 19]. Many in vivo models were rapidly developed as a result of the success of in vitro models as well as their drawbacks, most notably their inability to accurately represent the host environment. Even though animal models have been designed to study UTI, the conditions that exist during human infection are different from those in animal models in terms of urine composition, residual urine in the human bladder, and constant flow of urine via the catheter [20]. It is one of the notable drawbacks associated with the utilization of in vivo bladder models. Various models inherently come with specific strengths and limitations, with some proving more apt for particular applications than others. The choice and establishment of an optimal model system is challenging and involve a thoughtful evaluation of researchers' preferences, practical considerations, and objective criteria [21].

This review comprehensively assessed diverse methodologies utilized in scientific research, emphasizing the distinctive features, practical utility, and inherent constraints associated with these models. A thorough exploration of different bladder model techniques provides valuable insights into the selection, application, and interpretation of these model systems, ultimately contributing to a more nuanced and informed approach to experimental design and data interpretation within the scientific community.

In vivo bladder models

Overview of animal models for studying UTIs

Small animal models (i.e.: rodents) are often employed to study the entire urinary tract, including the bladder [20]. Similarly to humans, animals also respond to infections and are used as models for research purposes [22]. These animals are easy to handle, accessible to multiple investigations, survive long enough to acquire the illness, suit the existing facilities for housing animals, are sufficiently massive to yield a large number of samples and be multiparous in order to yield many animals for each gestation [23]. Animals including cat, dog have been used as models to understand the composition, function, and role of the urobiome in health and disease (Table 1) [121, 122]. By introducing uropathogens into the bladder and monitoring the infection process, the host-pathogen interactions, bacterial colonization, and immune responses

Table 1 Comparison of different in vivo bladder models

Model	Animal	Organism/s used in the model	Study objectives	Results	Remarks	Ref
Murine model	Mouse	UPEC	To optimize a mouse model and validate microscopic methods for investigating host-pathogen relationships and disease development	• Bacterial loads in the bladder increased from 1 h after infection. • Nearly 50% of bacteria in the bladder were within an intracellular niche.	Flexible to study various bacterial strains including uropathogens, across a broad spectrum of mouse genetic backgrounds Feasible to identify host-pathogen interactions to determine the outcome of infections	[7]
	Mice	UPEC	To develop a method allowing direct comparison of female and male immune responses to UTI.	• After 24 h post-infection bacterial burden between male and female mice was equal. • A significant increase in the number of immune cells of infected female mice was observed. • No difference in the number of immune cells present in naïve animals of female and male mice.	Workable to study UTI and other bladder-associated diseases including prostatitis, bladder cancer, interstitial cystitis and under or over-active bladder syndrome	[63]
	Mice	UPEC	To study the effect of antibiotics in vivo, against pyelonephritis	• Among the UPEC strains studied, C175-94 strain was significantly high in the kidney tissues. • Cefuroxime concentration in urine was nearly 100-fold higher than that in serum. • Gentamicin was detectable in urine for a prolonged period, due to the accumulation in kidney tubular cells.	Feasible to study the effect of antibiotics on the urinary system.	[52]
	Mice	*Enterococcus faecalis*	To study the pathophysiology of *E. faecalis*-mediated CAUTIs	• The presence of silicone catheter tubing in the murine bladder elicited histopathological and immunological changes. • *E. faecalis* formed biofilms on silicone implants and persisted at high titers in the kidneys and bladders of the implanted mice.	A valuable tool to identify virulence determinants in order to target antimicrobials against enterococcal infections.	[64]
	Mice	*Klebsiella pneumonia*	To study the role of type 1 and type 3 fimbriae on the colonization of silicon tubes, inserted into the bladders of mice	• *K. pneumoniae* type 1 and type 3 fimbriae are required for colonization and persistence in the implanted silicon tubes.	Feasible for studying the role of virulence determinants on the formation of biofilms in CAUTIs.	[65]
	Mice	*Pseudomonas aeruginosa*	To understand the role of quorum sensing in the pathogenesis of UTIs.	• Quorum-sensing signals are critical for P. aeruginosa proliferation and pathogenicity during a UTI, and they may function as virulence factors.	Feasible for studying the role of virulence determinants in the pathogenesis of UTIs.	[66]
	Rat	*Proteus mirabilis*	To establish an improved urolithiasis model	• Following implanting a zinc disc in the rat bladder (after 5 days), infectious stones were formed, and progressively developed. • No change in Blood urea nitrogen values in all test animals. • $MgNH_4PO_4{\cdot}6H_2O$ was the primary struvite.	Possible to study the formation of infectious stones by introducing a foreign object into the bladder.	[27]
Rabbit model	Rabbit	*Escherichia coli*	To evaluate the efficacy of fleroxacin, trimethoprim-sulfamethoxazole, ampicillin and gentamicin in the treatments of CAUTI and Bacteriuria in the rabbit model	• *E. coli* was eliminated from the internal and external surfaces of the catheters by genatamicin, fleroxacin, and ampicillin. • Trimethoprim-sulfamethoxazole was not effective in eradicating bacteria from the surfaces of catheters.	Feasible to study the efficacy of antimicrobials against CAUTIs.	[48]

Table 1 (continued)

Model	Animal	Organism/s used in the model	Study objectives	Results	Remarks	Ref
Porcine model	Pig	UPEC	To establish a porcrine model (a novel large animal infection model) of cystitis to understand the pathogenesis of UPEC in the urinary tract.	• After inoculation with UPEC, significant bacteriuria persisted in the Porcrine bladder for prolonged periods. • Elevated levels of Granulocytes throughout the experiment were observed due to the progression of inflammatory response in infected pigs.	A useful tool (a large animal model) to study UTI pathogenesis.	[24]
Cat model	Cat	-	To compare differences in gut and urinary microbiota between cats with kidney stones and healthy cats. Further to evaluate the impact of an antibiotic on microbiota in both groups, and assess the role of specific intestinal bacteria in the formation of calcium oxalate stones	• Cats with kidney stones had less diverse gut microbiota, further reduced by antibiotic treatment in both groups. • The urinary microbiota in cats with kidney stones was more diverse and rich compared to healthy cats. • Lack of specific gut bacteria may contribute to kidney stone formation by hindering oxalate degradation.	Feasibe to study impact of urobiome on various disease states	[122]
Canine model	Dog	-	To compare the urine and fecal microbiota of dogs with urothelial carcinoma to healthy controls. Further to assess microbial diversity, composition, and explore similarities between canine and human bladder cancer microbiota	• The urine of dogs with urothelial carcinoma showed a significantly lower microbial diversity (α diversity), and altered microbial composition (β) diversity (with increased Fusobacterium) than healthy dogs, but not in fecal samples • The taxa found in canine urine and fecal samples were similar to those observed in humans with bladder cancer • Similar bacterial groups or species were found in the urine microbiota of both dogs and humans	Usefulness of dogs as a model for studies on bladder cancer and urine microbiota	[122]

can be closely observed. These models provide a useful platform to delve into the molecular and cellular mechanisms underlying UTI pathogenesis [24] including bacterial adherence, urothelial invasion, evading host defenses, and establishment of infections. Further, these bladder models serve as a testing ground for evaluating the efficacy of novel antimicrobial compounds [25] to assess the ability of these agents to combat bacterial colonization, reduce inflammation, and promote bacterial clearance within the urinary tract.

History of animals in UTI Research

As early as the late 19th century, animals were crucial for biological scientific research that advanced our knowledge of human physiology and disorders. The first record of bladder infection in animals dates back to 1873 when Fels and Ritter used urethral ligation to inoculate canine bladders and cause cystitis [26]. Satoh et al. (1984) described implanting a zinc disc in the bladder of rats and transvesical inoculation with *P. mirabilis*. This model was utilized to illustrate the significance of matrix production and biofilm formation in the emergence of UTIs [27].

Utilizing animals for biomedical research and education peaked, after the middle of the 20th century in USA and UK [28]. Animal models enabled researchers to study UTI pathogenesis including virulence factors of the pathogens, host immunological responses, and bacterial colonization [29]. Further, the effectiveness of different catheter coatings (i.e.: Gendine silicone coating [30], silver hydrogel-coating [30], silver coating [114]) in preventing UTIs has also been investigated using animal models [30]. However, it dropped sharply until the early 21st century [28]. Once again, annual increases have been observed (in the UK) due to the use of genetically modified animal technologies [28]. Furthermore, in the 20th and 21st centuries, the research studies gradually shifted from traditionally larger and more sensitive species (i.e.: dogs, cats, non-human primates, etc.) to smaller, less sensitive species (i.e.: fish, mice, and rats) [28]. It was found that innovative technologies can yield multifaceted results that decrease the annual use of animals in biomedical research [28]. Further, the identification and integration of non-animal alternatives replaced animal use for specific research and product development [28].

Available in vivo models

From a translational research standpoint, the selection of study species needs to be predicated on how closely the medical disorders under investigation resemble those

found in the human body. A model that gives anatomic, urodynamic, pathophysiological, histological, and biochemical values as close to those of humans as feasible would be ideal [31] (Table 1).

Non-human primates

"Non-human primates" refer to any primate species other than humans, including apes (i.e.: chimpanzees, bonobos, gorillas, and orangutans) as well as monkeys (i.e.: macaques, baboons, and marmosets) [32]. They represent the closest anatomical analogy, with two exceptions. Their left kidney is located lower in the abdomen than that of humans, and they have uni papillary kidneys [31]. However, a few experimental UTI studies involving non-human primates have been conducted thus far due to financial constraints and ethical issues [33]. Further, preclinical studies using primates evaluate the safety and effectiveness of potential medications and vaccines (i.e.: AIDSVAX [34], Flosint [34], Opren [115]).

Pigs

Recently, porcine species have been the most commonly utilized animal model for the assessment of UTIs [35]. Researchers have studied the range of host responses to pyelonephritis and the onset of renal scarring that can result from UTI using porcine infection models [29]. The anatomical structures of the urinary tract of humans are quite similar to those of pigs [29]. Compared to human ureters, porcine ureters are often longer and more twisted [36]. Human kidneys typically have four to eighteen papillae, whereas pigs have eight to twelve [37]. Furthermore, in terms of renal physiology, pigs' maximal urine concentration, glomerular filtration rate, and total renal blood flow are comparable to those of humans [38]. Interventions should ideally be performed on models weighing 35–40 kg because the urinary tract dimensions of such models are comparable to those of an adult human [39]. Accessibility, low cost and readily available tissue samples were further benefits of porcine models while preparation of physiological solutions, anesthesia, and extensive laboratory spaces are possible drawbacks [20].

Rats

Rats are often employed as in vivo models in bladder research due to their structural and physiological parallels to humans, which makes them useful for investigating a variety of bladder functions and diseases. The urinary bladders of rats and humans contain predominantly desmin intermediate filaments [40].

In 1995, Haraoka et al. implanted glass beads coated with bacterial biofilms into the bladder, which was followed by urethral clamping in order to simulate a renal infection in rats [41]. Recently, a non-surgical method was developed using rats and mice, in which the bladder is transurethrally opened to insert a polyethylene tube without any surgical manipulation [42]. According to Kadurugamuwa et al. (2015), the bladder can be inoculated with a particular quantity of bacteria once a sterile segment of the tube has been implanted, or the polyethylene tube can be colonized with pathogens before implantation [43]. Furthermore, the rat model is useful for analyzing the antibacterial and anti-encrustation capabilities of novel stents because it allows for the controlled experimental induction of urolithiasis and UTIs [44]. These in vivo models are induced by the intravesical instillation of bacterial suspensions, (i.e.:*S. aureus*, *E. faecalis*, *and P. aeruginosa)* [45]. The validation of ureteral and urethral stents is usually done using these animals. Cystotomies are performed in the ureter or bladder to implant ureteric stents [46].

Rabbits

Rabbits also have characteristics similar to humans that make them appropriate for some bladder research applications. Rabbits' urinary tract autonomic receptor distribution and their reaction to particular autonomic receptor stimulation are similar to those of humans [47]. However, rabbits are less frequently employed than rats.

In 1994, Bill Costerton created a catheterized rabbit model to investigate CAUTIs, which was then utilized to assess the impact of different antibiotics on *E. coli* biofilms that formed on these catheters and surrounding tissues [48]. Further, research has been done on rabbits to examine the biocompatibility of stent materials (31). However, due to the notable differences in urine composition between rabbits and humans, there is a dearth of scientific data discussing the validation of urinary stents in this animal model [49]. The use of the rabbit model has drawbacks such as the animals' comparatively bigger size, the increased care required during breeding, and the frequent occurrence of bladder stones as a result of the animals' inactivity in the cage or their inadequacy in having a proper toilet area [20]. However, the rabbit model can be used for a variety of in vitro and in vivo studies on Urinary bladder function [20].

Mice

In scientific research, the use of mice as bladder models is a frequent and useful method for studying UTIs. Mice share similarities in the anatomy and physiology of the urinary tract (including kidney, bladder and urethra) with humans [50]. Unlike other rodent models, mice do not naturally have vesicoureteral reflux which is similar to humans [51, 52]. Further similar to humans, in mice urothelial cells have more globoseries glycolipid receptors available for attachment [35]. Although there are differences, these similarities make mice suitable models

for studying certain aspects of UTIs. Mice are useful for large-scale research studies since they are cost-effective and of a manageable size for handling. Further, they can be housed in controlled environments, and their small size allows researchers to use fewer resources compared to larger animal models [53]. Mice can be genetically modified to replicate specific conditions or susceptibilities to infections (53) which allows researchers to study the impact of genetic factors on the development and progression of UTIs. UTIs can be induced in mice by introducing uropathogenic bacteria into their urinary tracts using various methods, including catheterization or direct injection, allowing researchers to control the timing and nature of the infection [54]. Researchers can easily monitor the progression of UTIs in mice and collect samples for analysis. This includes assessing bacterial load, studying the histopathological changes in the bladder tissue, and evaluating the effectiveness of potential treatments [54]. While mice provide valuable insights into UTIs, it's important to acknowledge the limitations of using mice for in vivo studies. Some aspects of mouse physiology (i.e. urodynamics), may differ from humans. Further immunological aspects such as balance of leukocyte subsets, Toll receptors, Ig subsets, the B cell (BLNK, Btk, and λ5) and T cell (ZAP70 and common γ-chain) signaling pathway components, Thy-1, γδ T cells, cytokines and cytokine receptors, Th1/Th2 differentiation in mice cannot be directly extrapolated to humans [54, 55]. Further, a difference was found in the bacterial load for mice and human in causing UTI. In humans, a significant microbial colony counts with $\geq 10^5$ microorganisms per ml of urine with the presence of at least one of the signs or symptoms (i.e.; fever (38^0C), urgency, frequency, dysuria, or suprapubic tenderness) was diagnosed as UTI [56]. However, as reported by previous studies, a bacterial load exceeding 10^4 CFU/bladder is generally sufficient to cause an infection in the murine bladder [2, 57]. The immune system of mice may respond differently to bacterial invasion compared to humans (i.e.: TLR11 [54–58], TLR9 [55], B cell CD5 and CD23 expression [55]), potentially requiring a different threshold of bacterial load to trigger a UTI [54, 55].

Some strains of mice including BALB/c mice [59], DDD [60], C3H/HeN [61], C3H/HeJ [61], DBA/2J [61], CBA/J [61] AKR/J [61], H5Rll [62] and CP9 [62] were identified to have vesicourethral reflux, where urine flows backward from the bladder into the ureters or kidneys [62]. Johnson and Brown (1996) experimented to eliminate the vesicourethral reflux of H5Rll and CP9 mice by reducing the infusion rate, inoculum volume (25 µl), by using less traumatic methods for euthanasia and organ harvest [62].

Therefore, while our murine model provides valuable insights into the mechanisms of UTI pathogenesis, translating these findings to human infections requires careful consideration of these interspecies differences.

Hence, researchers often integrate data from mice studies, other animal models, and clinical studies, to gain a comprehensive understanding of UTIs [29, 35].

Comparative analysis of different in vivo bladder models

Ethical considerations and challenges in working with in vivo models

Utilizing in vivo models in scientific study is essential to comprehending intricate biological processes, validating theories, and expanding our understanding of medicine [67]. However, there are important ethical issues and constraints that researchers must address when incorporating in vivo models into experimental studies [68]. The ethical implications of using live materials for research must be carefully considered as we dive deeper into the complex field of in vivo research. Considerations of ethics have been a major topic of discussion in the controversy surrounding animal experiments.

In response to ethical concerns, regulations and oversight mechanisms were implemented to ensure the humane treatment of animals in research. The first animal welfare laws were enacted by the British parliament in the 19th century, with subsequent legislation and guidelines providing further protections for animals used in research [69].

One of the earliest animal welfare laws, the Cruelty to Animals Act 1835, was enacted in the United Kingdom, primarily aiming to address cruelty to domestic animals but laid the groundwork for future legislation by recognizing the moral obligation to prevent unnecessary suffering in animals [69].

In the 20th century, the principles of the 3Rs in animal research: Replacement, Reduction, and Refinement emerged from ethical considerations, aiming to minimize the use of animals and reduce their suffering in scientific experimentation. The principle of the 3Rs also originated from the United Kingdom and was introduced by Russel and Burch in 1959 [70]. Each **R** represents a moral precept about the use of animals in research: **R**eduction is the use of techniques that permit the use of fewer animals in a protocol. **R**efinement is the use of techniques that prevent animal suffering, such as using analgesic regimens for pain relief during recovery and anesthesia during procedures, using non-invasive methods, creating safe and comfortable housing environments, and teaching animals to cooperate with procedures. **R**eplacement involves using alternative models in place of animals, such as cell cultures, microbes, or other invertebrates [68–70].

Although in vivo testing is mandatory to answer certain research questions specialized technical skills are

required when conducting research with these living creatures. This includes tasks such as proper administration of substances, surgical procedures, and monitoring physiological parameters [71]. In order to investigate certain research problems, scientists frequently use particular in vivo models, such as distinct animal strains or genetically modified species [72]. Nevertheless, obtaining and maintaining these models might be challenging. A substantial amount of effort and money must be dedicated to in vivo research projects. Ongoing financial assistance is necessary to maintain animal communities, provide appropriate housing, and guarantee ethical treatments [73]. The expense is further increased by the requirement for specialized facilities, equipment, and staff with the necessary training [20]. In vivo studies, especially those that involve long-term monitoring or the study of developmental processes can extend over prolonged periods [74]. A constraint may be the amount of time needed to track physiological changes, treatment responses, or the emergence of particular circumstances, particularly when prompt outcomes are desired. Animals used as in vivo models frequently exhibit genetic and physiological variations from humans. These differences may affect how the organisms react to interventions or treatments during experiments. When attempting to directly apply findings from in vivo models to humans, these discrepancies present a barrier.

In vitro bladder models

In vitro bladder models are innovative experimental platforms designed to replicate the intricate structure and function of the bladder in a controlled laboratory environment [22, 75]. These models utilize a combination of cell cultures [76], biomaterials [76] and advanced imaging techniques to mimic the complex cellular interactions and physiological processes that occur in the bladder. By providing a realistic representation of bladder biology, in vitro models offer researchers a powerful tool to study bladder development, disease mechanisms, drug testing, and personalized medicine approaches [17, 77]. This cutting-edge technology has enormous potential to improve clinical outcomes for patients with bladder-related illnesses and to further our understanding of bladder health.

Numerous advanced in vitro model systems have been employed to comprehend the key concepts that govern the formation of microbial biofilms, to study parameters crucial for CAUTI etiology, catheter encrustation and to assess potential treatments or intervention tactics [78]. Accurately representing the physical and chemical parameters existing in a catheterized human bladder [79], the bladder model systems have shown that microbial biofilm formation is significantly impacted by flow dynamics, nutrient accessibility, nutritional composition, and other physicochemical features [79]. Thus, in vitro models allow for the initial screening of novel concepts and potentially high throughput testing while bypassing the ethical concerns, high complexity and greater expenses related to in vivo testing (Table 2).

Components of the in-vitro bladder model

The bladder model is a complete closed-drainage system that resembles the urinary bladder [80]. The key components of the model can be categorized into four groups based on the distinct features of the human urinary bladder and urine flow, which consist of constant urine excretion, one-way flow from the kidney to the ureter, cyclical bladder urine retention and irregular urination. These comprised the waste liquid collection and storage devices, the bladder models (in conjunction with the infection carrier), the input and output pathways of the culture medium, and the power sources for transmitting urine flow (gravity, pump, combined agitation, or rotation to the latter). All the components collectively mimic the human urinary bladder system [21].

Different materials used to mimic the bladder

Only a few studies utilized plastic bags [81] to simulate the bladder while most studies utilized glass bottles or cylinders with a capacity of 200 to 700 mL as the bladder models [80]. Some researchers have utilized double-walled glass vessels to symbolize the "bladder" [82, 83]. Its external chamber surrounds the internal vessel that has its own inlet and outlet. Water at 37 ^{0}C circulates in the outer chamber of the double-walled glass vessel, to retain the inner vessel temperature at the body temperature. The inner vessel consists of artificial urine, microbial culture, balloon and the catheter tip during model operation [80].

Infection carrier

P. mirabilis was the main typical strain used as the infection carrier in most of the studies [80]. *P. mirabilis* has a unique ability to produce crystalline biofilms, owing to ureolytic biomineralization, ultimately leading to encrustation and blockage of urinary catheters [84]. Other than *P. mirabilis*, some studies have use d *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Providencia stuartii*, *Morganella morganii* and *Proteus vulgaris* as the infection carriers [82] (Table 2).

Growth media

According to the existing literature, researchers have used tryptone liquid culture [85], Muller Hinton Broth (MHB) [17], human urine [15] and artificial urine [80] as the growth media (Table 3).

Urine is a natural choice for in vitro research of UTIs, as its components undoubtedly have the potential to

Table 2 Comparison of different in vitro bladder models

Model	Characteristics				Aim of the study	Research findings	Remarks (Success/Failures)	Figure
	Type of material used for bladder	Growth medium	Pump	Micro organisms (Infection carrier)				
Dynamic in-vitro bladder model by Abbott et al. [17]	Glass vessels	MHB supplemented with glucose-6-phosphate	Peristaltic pumps	*Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter cloacae*	To determine the relationship between urinary fosfomycin exposures, microbiological effect and fosfomycin resistance	*E. coli* and *E. cloacae* with MIC > 16 mg/L and all *K. pneumoniae* isolates tested were not reliably destroyed when exposed to normal urinary fosfomycin concentration	This model describes the processes of gastrointestinal absorption, distribution into systemic circulation and excretion into the bladder as it contains intestinal, circulatory, and bladder compartments separately which is an added advantage compared to other in vitro bladder models. Although this is more accurate than static models, it still does not fully stimulate the complexity of the human bladder environment including immune responses, uroepithelium.	Figure 1 (A)
A bladder model modified by Rad et al. based on Stickler et al. [19]	Glass chamber (200mL)	Synthetic urine	Pump (Not specified)	*P. mirabilis*	To study the ability of allicin to inhibit *P. mirabilis*-induced struvite crystallization and blockage in urinary catheters	Allicin prevented the *Proteus*-induced urine crystal formation and catheter blockage by inhibiting pH rise and lowering Mg^{2+} and Ca^{2+} deposition	Compared to static models, these models accurately represents the human bladder environment. However, this may not fully simulate the dynamic conditions of a functioning bladder (periodic filling and emptying cycles, the absence of immune responses, uroepithelium, and the mechanical properties of the bladder). Thus, these models may limit the applicability of the findings to real-life scenarios.	Figure 1 (B)
A model developed by King, Winters and Stickler [87]	Fermentation flask (100mL)	Sterile pooled urine	Peristaltic pump	*Escherichia coli*, *Klebsiella pneumoniae*, *Providencia stuartii*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Enterococcus feacalis*	To assess the activity of mandelic acid against bacterial colonization on catheters	Mandelic acid eliminated established *E. coli* infection on the urinary catheter and exhibited some bactericidal activity against the other bacterial species tested.		Figure 1 (B)
Bladder model modified by K.A. Getliffe [88]	Glass vessels	Synthetic urine	Peristaltic cassette pump head	*Proteus mirabilis*	To compare the effectiveness of bladder wash-outs (1% mandelic acid, Suby G or 0.9% saline) to reduce catheter encrustation	Encrustation was significantly reduced in catheters washed out with Suby G and mandelic acid.	Minimize the risk of airborne microbial contamination, since all air entering the model system (collecting vessel and reservoir), passes through 0.2 µm pore size filter units. However, it does not fully simulate the complexities of the human bladder environment.	Figure 1 (C)
Bladder model modified by Xu et al. based on Morris, stickler and Winters [18]	Fermentation flask	Artificial urine	Pumps (not specified)	*E. coli*, *S. aureus*, *P. Mirabilis*	To assess the ability of ciprofloxacin-incorporated waterborne polyurethane (WBPU) polymers to prevent in vitro bacterial biofilm formation	Ciprofloxacin-incorporated WBPU polymers effectively inhibited in vitro biofilm formation	It simulates the human bladder environment compared to static models. However, as factors like immune response, tissue interactions, and the dynamic nature of urine flow in the body are not accounted. Further, this model does not fully represent the complexities of a living human bladder.	Figure 1 (B)

Table 2 (continued)

Model	Characteristics				Aim of the study	Research findings	Remarks (Success/Failures)	Figure
	Type of material used for bladder	Growth medium	Pump	Micro organisms (Infection carrier)				
Bladder model modified by Gaonkar et al. [89]	Tube	Sterile urine	Gravity	*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Candida albicans*, Clinical isolates --methicillin-resistant *Staphylococcus aureus* (MRSA), van comycin-resistant *Enterococcus faecium*, *Proteus mirabilis*, *Enterobacter aerogenes*, and *Klebsiella pneumoniae*.	To assess the long-term effectiveness of latex and silicone catheters impregnated with (1) chlorhexidine and silver sulfadiazine; (2) chlorhexidine, silver sulfadiazine, and triclosan in inhibiting bacterial adherence	Synergistic combinations of triclosan, silver sulfadiazine, and chlorhexidine-impregnated catheters demonstrated broad-spectrum, long-term resistance against microbial colonization on the catheter outer surface	This model precisely controls the exposure of catheters to antiseptic agents, providing consistent and reproducible conditions for testing antimicrobial efficacy. Further, there is an ability to directly compare different antiseptic-impregnated catheters under standardized conditions. However, as urine flows through the gravity, flow rate cannot be controlled.	Figure 1 (D)
Bladder model modified by Cox et al. [16]	Polythene box ($15\times21\times8$ cm^3) reaction vessel	Artificial urine	peristaltic pumps	*Proteus*	To test the susceptibility of urinary catheter materials for encrustation	Ca/Mg deposits adhered firmly to the catheter surface for a period of one week. The chemical components of deposits were the same as the encrusting deposits that occur *in vivo.*	The pH electrode allows to monitor the pH of the content throughout the experiment. Further, a large number of catheter samples can be experimented simultaneously. However, it does not fully represent the complexities of a living human bladder.	Figure 1 (E)
Bladder model modified by Chua et al. [15]	Polypropylene vessel (250 mL)	Pasteurized human urine	Peristaltic pumps	*Escherichia coli*	To establish an in vitro bladder model to prevent CAUTI	Heavy *E. coli* biofilm colonization was seen on the surfaces of control and silver-hydrogel catheters, but not on the catheter coated with anti-microbial peptide (CP11-6 A)	This model used pasteurized human urine and showed no drastic change in pH compared to synthetic urine. The thermocouple-controlled heat pad is one step forward from the other in vitro models which used a double-walled glass vessel with circulating water at $37\ ^0$ C to maintain body temperature. However, this model cannot fully simulate the biological complexity of the human urinary tract, limiting the model's ability to predict clinical efficacy accurately.	Figure 1 (F)

Table 2 (continued)

Model	Characteristics				Aim of the study	Research findings	Remarks (Success/Failures)	Figure
	Type of material used for bladder	Growth medium	Pump	Micro organisms (Infection carrier)				
Bladder model modified by Azevedo et al. [90]	Reaction vessel	Artificial urine	Peristaltic pumps	*Delftia tusurhatensis*, *E. coli*	To evaluate the role of *E. coli* and *Delftia tusurhatensis* on urinary catheters	*E. coli* and *D. tsuruhatensis* cooperated when sharing the same environment under dynamic conditions, facilitating their sustained presence within biofilm communities.	Unlike static models, this allows the simulation of intermittent urine flow rates mimicking physiological conditions of the human bladder and provides more accurate data on biofilm formation and catheter colonization under varying flow rate conditions. This model further allows a correct simulation of intraluminal colonization, as flow is restricted to the catheter lumen. However, this does not fully simulate the complexities of the human bladder environment.	Figure 1 (G)
Bladder model modified by Lehman & Donlan [91]	Flask	Artificial urine	Pumps (not specified)	*P. mirabilis* and *P. aeruginosa*	To evaluate the effectiveness of a phage cocktail treated hydrogel- silicone catheters to inhibit mixed-species (*P. mirabilis* and *P. aeruginosa*) biofilm formation	Pretreated hydrogel urinary catheter with a phage cocktail significantly reduced mixed-species: *P. mirabilis* and *P. aeruginosa* biofilm formation.	This model mimics the conditions of the human urinary tract to study CAUTIs, providing a controlled environment to test the efficacy of bacteriophage therapy. However, it does not fully represent the complexities of a living human bladder including immune responses, urine flow dynamics, and interactions with host tissues.	

Table 3 Comparison of different growth media utilized in in vitro models

Growth medium	Method	Advantages	Disadvantages
Human Urine [15]	Urine pooled from 1–3 donors, adjusted dilution, concentration, and pH according to specific gravity and osmolality	• Promotes diverse uropathogens growth • Human urine includes elements beyond synthetic recipes • Supports for denser biofilm formation compared to synthetic urine	• Expensive due to the associated costs for collection, storage, processing, quality control, disposal, and transportation • Variability makes it challenging to maintain consistency in experimental conditions • Obtaining a large amount of human urine for large-scale studies or long-term experiments is difficult • Collecting and using human urine for research purposes needs ethical concerns
Artificial urine media [80]	Similar to physiological ranges of osmolality, pH, and composition of human urine	• Able to precisely formulate reproducible results • Allow to manipulate for target investigations • Free of pathogens, minimizing contamination risks • Long-term stability	• Requires expertise in formulation • Production expenses may be higher • Limited mimicry as it may not fully replicate natural urine
MHB culture medium (supplemented with glucose-6-phosphate) [17]	Well-defined, commercially available bacterial growth medium	• Relatively easy to prepare, often requiring minimal additional components • Offers consistency and reproducibility ensuring the reliability of the results • Cost-effective compared to more specialized culture media (i.e.: Artificial urine)	• It does not accurately mimic the composition of human urine
Tryptone liquid culture [85]	Well-defined, commercially available bacterial growth medium	• Provides a rich source of nutrients (i.e.: peptides, amino acids, vitamins, minerals) that support the growth and metabolic activity of bacteria • Offers consistency and reproducibility across experiments • Relatively inexpensive compared to specialized culture media • Easy to prepare and handle, requiring minimal additional components and simple protocols	• Lacks the complexity and specificity of specialized culture media designed to mimic human urine

significantly impact bacterial proliferation. However, there are certain opposing drawbacks to utilizing this method. The most significant of these is that urine varies greatly in terms of its composition, capacity to promote growth, and key physical characteristics including pH, osmolality, and colour [75, 86]. While pooled urine can temporarily serve as a standard product, enormous volumes would be needed to conduct a comprehensive set of comparative trials, which would present issues with stability, sterility, and storage [75]. One potential solution could be to utilize "artificial standard urine". However, it's unclear what the precise components should be in such a medium. It appears that until 5% brain-heart infusion broth or 1% tryptone soy broth is added, synthetic urine that has been developed does not promote bacterial development to a satisfactory degree [75].

Bacterial growth media like tryptone liquid culture [85], MHB [17] are rich in proteins and peptides derived from casein and soy, and provide a broad range of nutrients to support diverse microbial growth that may not perfectly stimulate the urinary environment. Further these growth media contain only basic salts like sodium chloride, while artificial urine media includes a broader range of inorganic salts (i.e.: sodium sulfate, ammonium chloride, calcium chloride, magnesium chloride) which mimic the ionic strength and electrolyte balance of urine.

The power devices to facilitate urinary flow

Based on the prevailing literature researchers had used fluid gravity [81], pump [17] and rotating devices into the models to provide urine transmission and urination power.

Comparison - types of in vitro models used to simulate bladder environments

Advantages and limitations of in vitro models to study UTIs

Currently, there is no perfect experimental model to study UTIs, due to the shortcomings of both in vitro and in vivo models. Nonetheless, a more thorough understanding of UTIs, biofilm formation, pathophysiology, treatment options, and coating techniques is possible to be gained with the information gathered from these in vitro models.

One of the main benefits of in vitro model applications is that they provide a controlled and precise environment, allowing researchers to manipulate specific variables and conditions with high accuracy [91]. This

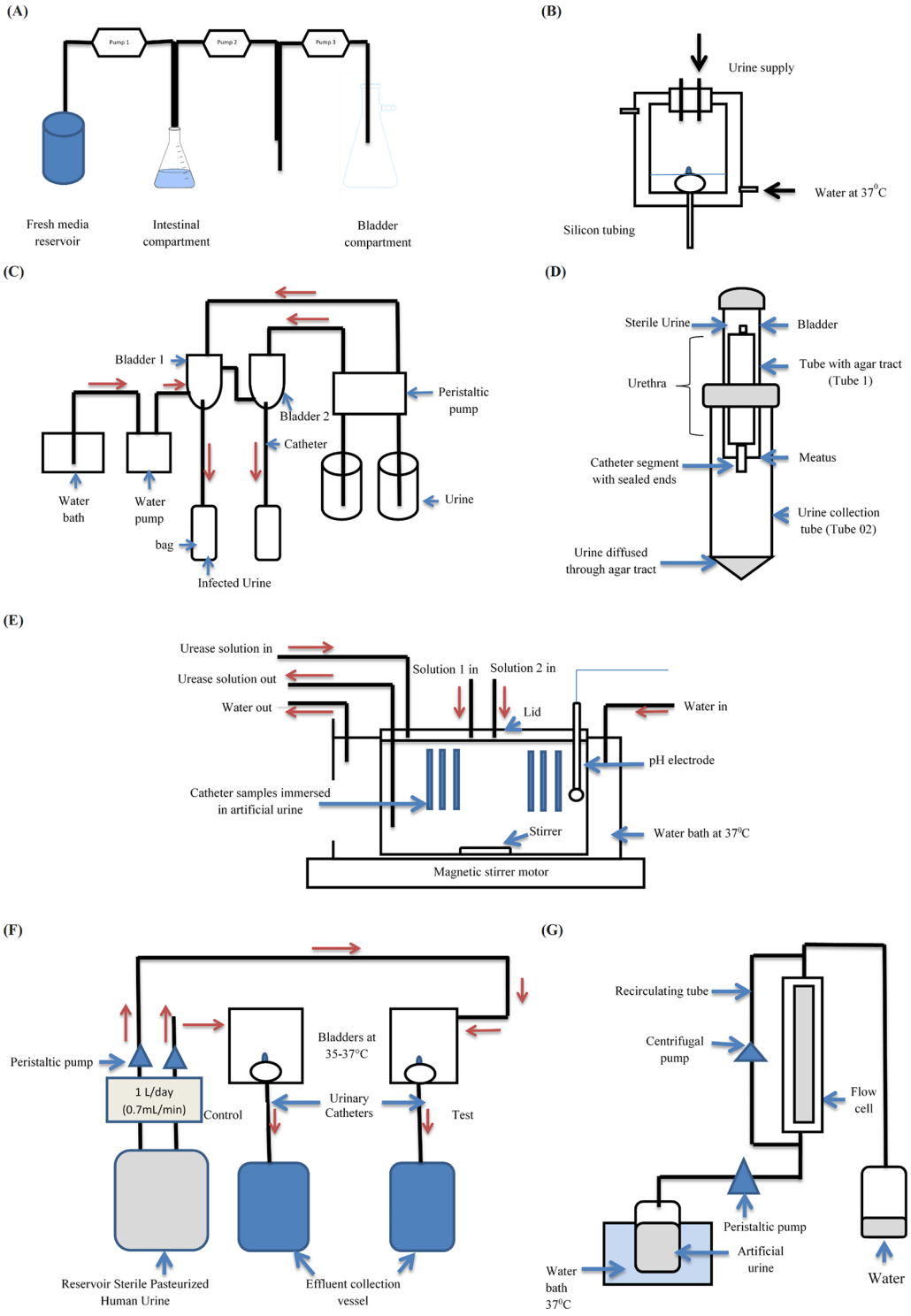

Fig. 1 Bladder models established in different studies **(A)**: Abbott et al., 2018; **(B)**: Rad et al., 2019; **(C)**: Getliffe, 1994; **(D)**: Gaonkar et al., 2003; **(E)**: Cox et al., 1987; **(F)**: Chua et al., 2017; **(G)**: Azevedo et al., 2017

involves adjusting variables including particular bacterial strains that are introduced to the model and the environmental conditions in which the tests are being carried out [82]. This control enables the study of strain-specific virulence factors. Further, isolating particular variables of interest is made possible in vitro by precise control. A methodical examination of the effects of distinct factors on UTI-related processes can be facilitated by researchers' ability to change one variable at a time while maintaining the stability of others [91]. This method aids in

recognizing important factors and comprehending how they affect infection.

Since there are no costs involved with maintaining and caring for animals, conducting research in vitro can be more affordable than using in vivo models [25]. Further, in vitro studies enable the simultaneous testing of multiple conditions, facilitating the rapid screening of potential therapeutics or interventions. In vitro models provide a compassionate substitute for animal testing in some UTI research scenarios, addressing ethical issues related to it.

In contrast, the major limitation of in vitro models is that they may lack the complex biological and physiological characteristics of a living organism (i.e.: composition of three cell layers, apical membrane, immune responses, potentially overlooking critical aspects of UTI progression. A crucial component of UTI pathogenesis is the complex interaction between bacteria and the human immune system. It is frequently left out of these models while in vivo models allow researchers to study the intricate interactions between bacteria and the host, offering an understanding of the immune response and microbial behavior [91].

Further, a significant difference exists in the expression of the asymmetric unit membrane by urothelial cells in vitro and in vivo, which is required to understand the correlation between in vitro and in vivo findings. The asymmetric unit membrane is a specialized apical membrane feature of urothelial cells, characterized by crystalline lattice consisting four key uroplakin proteins (UPK1A, UPK1B, UPKII, UPKIII) [92]. These uroplakins create plaques that are essential to the bladder epithelium's mechanical stability and permeability barrier [93]. The expression of asymmetric unit membrane in vivo is influenced by the complex three-dimensional architecture of the bladder, exposure to urine, and interaction with other cell types and extracellular matrix components [94, 95]. Conversely, the lack of these in vivo stimuli such as three-dimensional architecture, low diffusive water and urea permeabilities, differentiation markers of the umbrella cells, high transepithelial resistances (resistance of 20,000 $\Omega \cdot cm^2$ or greater in native tissues) and amiloride-sensitive transport system, causes urothelial cells grown in vitro to frequently change expression of uroplakin. It results decreased production of asymmetric unit membrane plaques [92]. Cell differentiation and membrane protein expression can vary significantly depending on the two-dimensional nature of cell culture, the absence of mechanical strain, and the nutritional environment in the in vitro models [96]. In vivo models are invaluable in understanding the cellular mechanisms in the bladder epithelium [97]. Jafari and Rohn had developed an improved barrier-forming, terminally differentiated, 3D urine-tolerant human urothelial model (3D-UHU) [96]. This urothelial model exhibited morphological similarities with human bladder urothelium, consisting of terminally differentiated umbrella-like cells at the apical surface, multiple intermediate cell layers and a single basal cell layer at the basal membrane. The model also showed similar CD phenotypes to human bladder tissue including basal cells, $CD271^{+,}$ and $CD227^{+}$ umbrella like cells [96]. Further Sharma et al. developed a human bladder-chip model using bladder microvascular endothelial cells and umbrella cells. Most epithelial cells had expressed cytokeratin markers including CK7 and CK8, indicating their uroepithelial nature, while some endothelial cells also expressed CK7 [98].

The findings' practical significance may be limited by the fact that the controlled environment of in vitro models might not accurately represent the dynamic and complex settings found in a living organism.

Validation of bladder models

Validation is an important aspect of an in vitro bladder model. Validation should be done before utilizing the bladder model for any experiment to generate reproducible results throughout experiments.

Validation of a bladder model involves ensuring the model accurately, representing the biological and physiological characteristics of the human bladder [111, 112] in scientific research. Validation is crucial especially when these models are used for drug testing [111, 112]. Criteria validity and predictive validity are two essential components of validation [113]. Predictive validity measures the model's accuracy in predicting in real-world scenarios, while criterion validity tests, how well a measure predicts an outcome based on a gold standard [113].

Further, in vitro and in vivo correlation is also essential for validating. It is important to see that the in-vitro models can accurately predict the biological behavior observed in in vivo systems. A study done by Conte et al. in 2023, demonstrated the efficacy of bovine lactoferrin (bLf) in preventing recurrent UTIs through both in vitro and in vivo experiments. In vitro studies were performed using T24 human bladder cancer cell line (HTB-4) demonstrated that bovine lactoferrin inhibited the growth of bacteria under controlled laboratory conditions. Further, in vivo study also showed similar results when administrating the bovine lactoferrin to experimental subjects. This further highlight the potential of bovine lactoferrin as a promising therapeutic intervention for managing UTIs [102].

Model	Validation process	Reference/s
In vitro Dynamic flow model	• A model's reproducibility was validated to ensure that each piece of biomaterial in the model showed similar levels of encrustation with no differences in the amount of encrustation on each piece. Three pieces of a material (i.e.: Percuflex), each 3 cm long, on each mandrel was used. Four vessels were set up with urine flow for two weeks. After this period, encrustation on each piece of Percuflex was measured, focusing on the amounts of calcium and magnesium, which represent hydroxyapatite and struvite encrustations, respectively. • The performance of a dynamic encrustation model was tested in two ways: 1. Samples from the material were tested in a dynamic model with urine flow for two weeks to measure calcium and magnesium buildup. 2. The results from this dynamic model were compared with a static model by Tunney et al., (1996) where materials were placed in stationary artificial urine with minimal movement.	[112]
In Vitro Encrustation model	Validation of the in vitro encrustation model comprised 5 parts; i. Determination of amount of calcium leaching from a glass model / container was done by placing deionized water in it for 5 days. Calcium levels were measured before and after, and the experiment was triplicated, and average results was taken. ii. Quantification of calcium leaching from catheter materials was done subtracting inherent calcium in polymers and urological devices from the total after urinary exposure. Using acidified lanthanum chloride, calcium was removed and measured daily over 5 days, followed by assessing remaining calcium in the polymers. iii. Comparison of the encrustation index of single-source urine and pooled urine from three men without urolithiasis was done to verify reproducibility of the experiments. iv. Quantification of the encrustation in vitro was done using different antimicrobials (i.e.; vancomycin and gentamicin) treated fresh human urine and different catheter materials (i.e.; Hyaluronic acid-coated polyurethane and medical-grade silicone membranes). Urine was delivered at constant flow rate (i.e.; 0.5 mL/min) to materials to be tested. Experiments were repeated five times to measure encrustation. v. Assessing encrustation in vivo was done implanting the same biomaterial into the bladders of 20 male Wistar rats. HA-coated and silicone disks (3 mm diameter, 1 mm thick) were used and left for 9 weeks, with prophylactic amoxicillin administered subcutaneously. Urine samples were collected for culture and pH measurement. After retrieval, encrustation was quantified using atomic absorption spectroscopy.	[113]

Significance of experimental in-vivo and in-vitro bladder models for clinical/ other applications

Other than studying UTIs, experimental bladder models play a vital role in expanding our understanding of bladder-related disorders, and their usefulness extends to different clinical and scientific applications. These experimental models, which can range from complex in vivo systems to in vitro cell cultures, offer crucial information that can be applied to the diagnosis, management, and prevention of bladder-related diseases.

These bladder models play a major role in the investigation of interstitial cystitis, a painful chronic illness characterized by pain and discomfort in the bladder [99]. These models allow researchers to identify possible therapy targets and create focused therapies to reduce symptoms and enhance the quality of life for affected persons by simulating the inflammatory processes and cellular alterations linked to interstitial cystitis. Bladder cancer, another prevalent and challenging health issue, benefits immensely from experimental models that facilitate the study of tumor initiation, progression, and response to treatment [100]. These models facilitate the testing of new therapeutic drugs, investigation of the genetic and molecular elements driving the development of cancer, and enhance our knowledge of the tumor microenvironment [25]. To date, the most widely utilized in vitro bladder cell model is comprised of cultivated urinary bladder cells. These models are often made up of isolated bladder cancer cell lines and are reliable in vitro models for researching the processes leading up to the formation of bladder cancer as well as for assessing the effectiveness of anti-neoplastic medications [101]. The first human urinary bladder cancer cell line designated as RT4, was established by Rigby and Franks in 1970 [102].

In preclinical research, bladder models are a useful resource that lets scientists evaluate the effect of potential drugs on bladder pathology and function in a systematic way. This is especially crucial when considering overactive bladder, a common disorder marked by the involuntary contraction of the bladder muscles, which can result in symptoms including incontinence, urgency, and frequency [103]. Further, Trospium chloride (TrCl) was found to be efficacious when loaded onto degradable poly(lactide-co-glycolide)-based polymer-carriers in in vitro experiments using a porcine bladder model [104].

Bladder organoids

Organoids are 3D structures grown from stem cells in the laboratory that mimic the structure and function of organs [105]. Similarly, a bladder organoid is also created from stem cells or tissue samples of the bladder into a 3D structure, on the outside of a living organism that imitates the anatomy and physiology of the bladder [106]. These bladder organoids can be employed in controlled

Table 4 Comparison of different organoids used as bladder models

Cell line	Organism/s	Aim/s	Findings	Reference
5637 (HTB-9) cells	UPEC with and without a hemolysin gene mutation	• To establish a 3D organoid model of human urothelium and to assess the interaction of 5637 (HTB-9) cells with UPEC strains	• 5637 human bladder cells cultured for ten days in the rotating wall vessel (RWV) bioreactor, formed 3D structures that exhibited structural organization and cellular markers typically found in human urothelium that has undergone differentiation. • 5637 organoids showed increased and more selective expression of some markers (E-cadherin, cingulin, cytokeratin 20, uroplakin) that are normally present in differentiated human urothelium compared to the same cell line cultured in monolayers.	[110]
Human bladder epithelial progenitor cells (HBEP, HBLAK)	*E. faecalis*	To create an organotypic urine-tolerant human urothelium that may be utilized to investigate host-uropathogen interactions, therapies, and resolution.	• Both HBEP and HBLAK organoids had the correct spatial expression of some vital biomarkers including Cytokeratin and Uroplakin-II. • *E. faecalis* established significant intracellular colonies inside the intermediate and basal cells of the organoids.	[107]
Mouse bladder organoids from C57BL/6 wild-type or mT/mG mice	UPEC	To investigate how early invasion of the bladder wall by solitary bacteria can protect UPEC from the effects of antibiotics and the response of neutrophil swarms, using an organoid model	In bladder organoid model, solitary bacteria invaded deeper layers of the bladder wall early in infection, independent of the formation and rupture of intracellular bacterial communities (IBCs). These solitary bacteria, which resemble quiescent intracellular reservoirs (QIRs), evade destruction by antibiotics and neutrophils. They are distinct in morphology from bacteria within IBCs, suggesting that QIR-like bacteria can form early during infection.	[111]
• 5637 human bladder epithelial carcinoma cell line (procured from ATCC HTB-9TM) • Human primary bladder epithelial cells • Human Bladder Micro-vascular Endothelial cells (HMVEC-Bd)	UPEC strain CFT073	To develop and characterize a bladder-chip model that mimics bladder structure by co-culturing human bladder epithelial cells with bladder microvascular endothelial cells, using a device that exposes epithelial cells to urine and endothelial cells to culture media	Neutrophils rapidly recruited from the vascular channel to infection sites formed swarms and extracellular traps, but this did not prevent the formation of intracellular bacterial communities (IBCs). Antibiotics showed delayed effectiveness in eliminating bacteria within IBCs, and in some cases, bacteria were not eradicated at all. During recovery periods, bacteria rapidly proliferated in IBCs, leading to new infection sites through bacterial shedding and host cell exfoliation.	[99]
HBLAK human bladder progenitor cells	UPEC strains UTI89, CFT073, *E. coli* 83,972 *Enterococcus faecalis* EF36, EF77 and *Streptococcus agalactiae*	To develop and utilize an immune-responsive three-dimensional urine-tolerant human urothelial model to study UTIs	3D urine-tolerant human urothelial model (3D-UHU) was stratified into 7–8 layers with distinct cell types, after 18–20 days. The apical surface differentiated into CD227 + umbrella-like cells expressing key urothelial markers (uroplakin-1 A, II, III, and cytokeratin 20) and a glycosaminoglycan layer, while intermediate and basal cells (CD271+) were present underneath. The model showed effective barrier function and expressed proteins like E-cadherin, claudin-1 and – 3, and ZO-1. Infection with both Gram-negative and Gram-positive bacteria led to increased pro-inflammatory cytokines and chemokines, mimicking human UTI. This model offered potential for exploring host-pathogen interactions and host urothelial immune responses.	[97]

laboratory settings for scientific studies on bladder development, disorders, and evaluating therapeutic and diagnostic effects [106, 107]. This approach is an efficient component for researching the cellular and molecular aspects of epithelial differentiation.

In comparison to bladder organoids, other in vivo and in vitro models have some disparities. In 2D cell culture models, they are inadequate to accurately represent the complex nature of in vivo structures including the extracellular matrix and the cell-to-cell interactions that take place in 3D spaces [108]. In animal models, there are limitations to accurately mimic human bladder-related pathologies and responses to treatments, highlighting specific anatomical, physiological, and immunological differences between animals and humans [107]. According to the review of Mestas et al., the immune response in animals like mice differs from humans in terms of cytokine profiles and immune cell functions, impacting the body's response to bladder infections [116]. Mice have a lower proportion of neutrophils (10–25%) and a higher proportion of lymphocytes (75–90%) in peripheral blood compared to humans, who have 50–70% neutrophils and 30–50% lymphocytes [116]. This difference affects the immune response to bladder infections and

the effectiveness of treatments targeting these cells. TLR9 is expressed on all myeloid cells, plasmacytoid dendritic cells (DCs), and B cells in mice, while it is only expressed on B cells and plasmacytoid DCs in humans [96, 116]. These differences can also impact the immune response to bacteria. In addition, CD33 marker is expressed on granulocytes in mice but on monocytes in humans, impacting how these cells respond to pathogens and the effectiveness of treatments based on these cell types [117]. Further, in mice, serum IgA is mostly polymeric, while it is monomeric in humans. This affects mucosal immunity and the response to pathogens in the bladder. Regarding the anatomical disparities in bladder size and capacity, the adult human bladder typically has a capacity of about 400–600 ml whereas it is about 0.15–0.30 ml in mice. This can also impact on bladder-related pathologies [54, 96].

Consequently, there is a critical need for alternative approaches that can better capture the complexity of bladder-related conditions and improve the conversion of research findings to clinical practice. The major advantages of 3D culture methods are the ability to capture in vivo microarchitecture and the ability to mimic cell-to-cell interactions more accurately and precisely [107]. Further, with the use of organoids for drug screening and toxicity assessment, drug discovery may be simplified and cost-effective in the future [107]. Although bladder organoids represent a significant advancement in in vitro bladder modeling, they are not exempt from constraints. Keeping bladder organoids viable in urine over extended periods is difficult, which limits their use in long-term studies. Further, some key biomarkers like cytokeratin 20 are not correctly expressed making incomplete disease modeling [106] (Table 4).

Prospects for advancing UTI and biofilm research using experimental models

The prospects for advancing UTIs and biofilm research using experimental models are promising with several key avenues for exploration and innovation. These prospects have the potential to advance our knowledge of UTIs, enhance therapeutic approaches, and aid in the creation of preventative measures. The detection and development of new antibiofilm agents will be a key focus of future research. Discovering compounds that particularly target the formation of biofilms or disrupt existing biofilms will be crucial to the development of successful treatment approaches. Further investigations into the host immune response in relation to UTIs and biofilm formation ought to be the main emphasis of future research. Gaining knowledge of the complex interactions between biofilm-associated pathogens and the immune system might help discover possible targets for immunomodulation and provide important information about how diseases progress. Translating laboratory results into clinical applications requires corporate collaboration as well as support from researchers and physicians. The development of new treatments, diagnostic tools, and prophylactic measures for UTIs and biofilm-related problems will be sped up by overcoming the disparity between bench research and clinical practice.

Conclusion

Both in vivo and in vitro models offer unique benefits and drawbacks in understanding UTIs. In vitro models provide controlled environments for studying specific aspects of UTI biology and testing potential treatments, while in vivo models offer insights into how UTIs manifest and progress within living organisms. Thus, both types of models are leading to the development of more effective diagnostic tools and therapeutic interventions against UTIs. Moreover, advanced methodologies involving three-dimensional bladder organoids have also been used to study bladder biology, model bladder-related disorders, and explore new treatments for bladder cancers, UTIs, and urinary incontinence. Narrowing the distance between fundamental scientific research and practical medical applications, these pioneering models hold the key to unlocking new avenues for the development of personalized diagnostics, precision medicine, and ultimately, the alleviation of UTI-related morbidity worldwide.

Abbreviations

UTI	Urinary Tract Infection
CAUTI	Catheter Associated Urinary Tract Infection
CFD	Computational Fluid Dynamics
UPEC	Uropathogenic Escherichia coli
TrCl	Trospium Chloride
MHB	Muller Hinton Broth
TLR	Troll Like Receptor

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

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Consent for publication

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