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REVIEW

Rewriting the urinary tract paradigm: the urobiome as a gatekeeper of host defense

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Abstract

The urobiome, or urinary tract microbiome, has emerged as a crucial component in maintaining urinary health and defending against infections. Recent advances in next-generation sequencing (NGS) have debunked the long-held belief that the urinary tract is sterile, revealing a unique ecosystem of microorganisms. The urobiome interacts with the urothelium and mucosa-associated lymphoid tissue (MALT) to support local immunity, playing an integral role in defending the urinary tract against pathogens. Through complex communication processes like quorum sensing, the urobiome regulates microbial behavior and controls interactions with host tissues, helping to prevent pathogen colonization and infection. However, dysbiosis in the urobiome can disrupt this balance, making the urinary tract more susceptible to infections, including urinary tract infections (UTIs). Studies have highlighted specific microbial compositions associated with both healthy and disease states, suggesting that shifts in the urobiome may correlate with various urological diseases. Furthermore, microbial diversity within the urinary tract differs by factors such as age and gender, reflecting the dynamic nature of the urobiome. Future research focusing on the interplay between the urobiome, host immune defenses, and pathogenic mechanisms may lead to innovative diagnostic and therapeutic approaches. Understanding how microbial composition changes during disease states could enable targeted treatments, potentially reducing reliance on antibiotics and minimizing resistance issues. The urobiome thus represents a promising frontier in urology, with implications for enhancing urinary health and treating infections more effectively.

Keywords Urinary tract · Microbiome · Urobiome · Bacteria

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Introduction

The human body contains an enormous number of bacteria, archaea, viruses and fungi [1] that interact with the host. Over the past 25 years, our understanding of human-associated microorganisms has changed significantly, thanks in part to research conducted as part of the Human Microbiome Project (HMP) [2]. It is worth noting that microorganisms that colonise the human body not only outcompete microbial pathogens, but also promote the development of immunity and synthesise compounds that affect the host state [3, 4]. At present, it is well known that microbiota-host symbiosis is essential for maintaining health due to its impact on immunological, hormonal, metabolic and neurological functions.

It has been shown that the microbiome of each human ecological niche (skin, respiratory tract, gastrointestinal tract, vagina, urinary tract) differs significantly, mainly due to different functions, but also as a result of oxygen availability and secretion of the corresponding tissues [2, 5, 6]. The diversity of the microbiome also varies significantly between healthy individuals [2]. Literature data show that the maternal microbiota, as well as the mode of birth, strongly influence the child's skin, gut and oropharyngeal microbiome [7, 8]. Next-generation sequencing (NGS) identifies the composition of microbes in each niche [9], including microorganisms not previously cultured [10].

Work on the microbiome has focused mainly on the gastrointestinal tract because of its apparent richness and diversity, especially at the lowest levels. The gastrointestinal tract is a high-mass niche containing approximately 300–500 different species [11], predominantly anaerobes and gram-negatives [12]. Homeostasis within the microbiota of the GI tract is critical for maintaining its function, including metabolising dietary components, stimulating immunity via synthesised peptides, and maintaining the integrity of the intestinal barrier [13–15]. Alterations in the gut microbiota appear to be at the root of many diseases affecting different organs [11, 16–19]. Furthermore, research on the gut microbiota has already demonstrated that the established balance between immune tolerance and immune response may influence the development and progression of immune-related diseases such as allergy, asthma or inflammatory bowel disease [20, 21]. Relatively little is known about the microbiome of other sites of the human organism.

The last decade has seen a change in the paradigm of sterile urine [22]. Notably, in the 1970s and 1980s, British microbiologist Rosalind Maskell successfully cultured bacteria from urine samples of patients with urinary symptoms, challenging the prevailing belief in urinary tract sterility [23, 24]. Despite the rigor of her work, her findings were largely dismissed by the scientific community at the time, reflecting the strength of the sterile urine dogma that dominated urological thinking for decades [25]. Indeed, the urine of a healthy person, with its acidic pH and high concentration of urea and substances with antimicrobial properties, has been treated as an extreme habitat for microorganisms that cannot survive in such conditions. However, with the use of new techniques of microbiome analysis scientists clearly demonstrated that the urinary tract is not sterile.

The discovery of the urobiome shed more light on the number of links that control the biological phenomena that occur in the bladder. The urobiome appears to play a crucial role in the communication between the urothelium, mucosa-associated lymphoid tissue (MALT) and macrophages, which ensures the proper immune function of this organ.

The observation that the healthy urinary tract is not sterile comes with the advancement of the Human Microbiome Project (HMP) (2007), the first large-scale human microbiome mapping project using culture-independent techniques [2, 26]. The modern discovery of microbiota in the urinary tract began with the study by Nelson et al. (2010), who conducted experiments in the urine of asymptomatic men with sexually transmitted diseases [27] and then in healthy women [28, 29]. NGS approach led the researchers to identify symbiotic bacteria in the bladder [27–29]. There is a microenvironment in the urinary system that includes groups of microorganisms and therefore their genomes and metabolites. The general term urobiome refers to both the microbiota and the urinary tract microbiome [30].

Research into the urobiome is primarily revealing its changes in relation to different diseases. While our knowledge of the life of bacteria and their interaction with a host in symbiotic coexistence (mainly from the well-studied gut microbiome), it seems quite clear that the urobiome cooperates with the tissues of the urinary tract at the molecular level. For example, the mechanism of local immunity in the bladder is complex and involves the urobiome, urothelium and MALT. Urinary tract homeostasis depends not only on host-commensal communication, but also on the well-known communication between bacteria themselves (quorum sensing - QS) [31].

The aim of this narrative review is to outline the interactions between the urobiome and the host in order to understand the importance of these interactions in the pathophysiology of various urinary tract diseases.

In order to comprehensively assess the topic, the following steps were taken in the literature search: (1) Identification of the research question: "What is known about the urobiome and the interactions between the urobiome and the human organism?" (2) Identification of relevant studies: PubMed and Embase databases were searched using the following keywords: urobiome, urinary tract microorganisms, urinary tract immunity, urinary tract infection, quorum

sensing. We also performed a manual reference search for relevant reviews describing the above aspects. We searched for English-language studies with no restrictions on publication date. Data were collected and presented in a narrative form.

Urobiome research and analytical methods

For years, urobiome researchers focused on cultivable microorganisms, mainly pathogens, and the methods used to isolate bacteria from urine were applied to infections and focused on uropathogens. Media and growth conditions were established for aerobic and fast-growing bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* or *Enterococcus faecalis* [32, 33].

New laboratory techniques such as: polymerase chain reaction (PCR), expanded quantitative urine culture (EQUC), whole genome sequencing (WGS) and NGS have confirmed the urobiome existence. Amplification-based methods targeting hypervariable regions (16 S rRNA hypervariable regions) and shotgun sequencing have gained popularity in recent years due to their ability to provide both species-level taxonomic resolution and functional genomic information. These approaches – along with the new ones orienting only on functional level like metatranscriptomics, metaproteomics and metabolomics - allow researchers to rapidly compare and contrast microbial communities in different environments of the human body [34].

In recent years, one of the preferred techniques for urobiome assessment has been 16 S rRNA sequencing. This NGS amplicon-based approach has made it possible to study microbial communities, their genomes and their functions with greater sensitivity than ever before. However, this sensitivity is a double-edged sword because these tools also efficiently detect [35] contaminant DNA and cross-contamination, which can confound the interpretation of microbiome data. In contrast to other human niches, urobiome analysis is challenging due to the small sample biomass and potential sample contamination during sample collection and analysis [36].

The urobiome biomass of healthy individuals varies from 10^2 to 10^4 CFU/mL of urine (colony forming units per millilitre) [37]. Current clinical microbiology protocols, such as those outlined by the Infectious Diseases Society of America (IDSA) [38], the Clinical and Laboratory Standards Institute (CLSI) [39], or the European Association of Urology (EAU) [40, 41], typically define urinary tract infection (UTI) based on the presence of $\geq 10^5$ CFU/mL in midstream urine samples. Noteworthy, suprapubic urine aspiration and transurethral catheterisation are two methods that protect against contamination of the urobiome sample intended for analysis, particularly the bladder microbiome [42, 43]. However, it should be emphasized that during catheterisation of the urinary bladder, contamination may also occur, e.g. with bacteria living in the urethra. Urine samples collected into a container very often contain contamination of the urethra, vulva and vagina microbiome. However according to Wolfe et al. [29] suprapubic urine collection is similar to catheterised urine samples in terms of microbiome composition, whereas voided samples are significantly different, suggesting that the urobiome of the urethra and bladder are different. Supporting this, a comparative analysis revealed that bladder microbiota are significantly different from those found in the urethra, periurethral area, and voided urine. While the urethral and periurethral microbiota are similar, the urethral microbiota differ from voided urine, whereas periurethral microbiota do not [44]. These findings reinforce the anatomical and microbial distinction between niches of the lower urinary tract. Nevertheless, in contexts where direct urethral swabbing is not feasible—such as in large-scale population studies or protocols requiring repeated sampling—voided urine may serve as a practical, though imperfect, surrogate for characterizing the urethral microbiome.

The diagnostic threshold of bacterial load $\geq 10^5$ CFU/mL in midstream urine samples was primarily developed in the context of symptomatic women and may not be universally applicable across patient populations or urine collection methods. These guidelines often fail to adequately differentiate between symptomatic infection and asymptomatic bacteriuria (ASB), the latter of which can present with similarly high bacterial counts. In fact, ASB is common in certain populations, such as elderly individuals, pregnant women, and patients with long-term catheterization, and does not necessarily indicate pathology. This diagnostic ambiguity highlights the limitations of relying solely on microbial load to assess urinary tract health. Consequently, clinical evaluation—particularly the presence or absence of patient-reported symptoms—remains essential for accurately claiming “bladder health”. Recognizing these limitations is important when interpreting urobiome data and reinforces the need for more refined, symptom-inclusive diagnostic frameworks However, this assumption—linking high microbial biomass solely with infection—oversimplifies the diagnostic picture. Evidence suggests that asymptomatic individuals, especially females, may present with high bacterial loads, including counts $\geq 10^6$ CFU/mL of *Escherichia coli*, without clinical signs of infection [45–47]. This challenges the traditional notion that high CFU levels always signify disease. The current absence of a universal CFU threshold or standardized microbial composition to differentiate between health and disease underscores the need for symptom-based assessment.

Urothelium - anatomical unit in the bladder and its functions

The urothelium is a structurally and functionally complex organ. It acts not only as a barrier, but also as an active communicator between the bladder environment and the human organism. It regulates local and systemic homeostasis by receiving mechanical, chemical, thermal and biological stimuli and transmitting signals from the bladder to the nervous system [48–52]. Therefore, this cross-talk creates a brain-bladder axis that determines the proper functioning of the muscle layer of the bladder and the regeneration of urothelial cells [51].

The urothelium is the inner layer of the bladder wall and has complex sensory and transduction capabilities [52]. The innervation of the bladder consists of afferent fibres (Aδ and C) and the submucosal plexus, whose axons penetrate the entire thickness of the urothelium [53]. Within the urothelium, there is an interaction between its cells and autonomic nerves, and the gap junctions between urothelial cells allow the transmission of signals from the innermost layer to the muscle layer [52].

Urothelial cells express receptors and ion channels on their surface and release some transmitters and mediators. By receiving information about changes in intravesical pressure and bladder position or movement of surrounding organs, they transmit information throughout the bladder wall and modulate the activity of sensory neurons by secreting relay molecules (e.g. adenosine triphosphate, nitric oxide, acetylcholine, nerve growth factor) [48, 49, 52, 54, 55].

Urothelial cells have an immense regenerative potential in response to damage through increased production of growth factors (e.g. epidermal growth factor and keratinocyte growth factor) [51, 56, 57]. This regenerative potential is dependent on contact with acidic urine and the function of the local immune system [58].

The biological activity of the urothelium is continuously dependent on its interaction with the nervous system. This dependency also affects the distribution and efficiency of immune system elements in the bladder wall as one of the important factors in antibacterial defence, including the degradation of lymphocytes and cells that synthesise and secrete IgA [48].

Disorders of bladder wall innervation, such as in neurogenic bladder, result in disruption of transitional epithelial continuity, loosening of intercellular junctions, increased urothelial permeability and increased susceptibility to infection [53, 59, 60]. In bladder innervation disorders, urinary stasis predisposes to bacterial growth and more frequent infections, and the increase in intravesical pressure leads to hypoxia of the urothelial cells [48]. In addition, the lack of influence of the nervous system on the urothelium causes disturbances in the secretion of growth factors, epithelial regeneration, the function of receptors and adhesive molecules, but also in the functioning of defence mechanisms, predisposing to infections [61, 62].

All of these changes can affect the functioning of the normal urobiome within the bladder. On the other hand, it should be considered whether bladder dysbiosis in the course of its primary dysfunction and recurrent urinary tract infections may further worsen the functioning of an already damaged bladder.

The local immune system of the bladder - MALT

Mucous membranes are a functional, active barrier against the penetration of pathogens and are commonly colonised by commensal organisms with which they co-operate. This is due to the extensive system of lymphatic tissue associated with the mucous membranes - MALT.

MALT can be divided into inductive sites and effector sites depending on its function [63]. In the inductive sites (secondary lymphoid tissues), IgA class switching and B cell clonal expansion occur in response to antigen-specific T cell activation, thereby initiating the immune response. After activation, T and B cells migrate from inductive sites to effector sites [64]. In effector sites (diffuse lymphoid present in all mucosal tissues), secretion and transport across the mucosal epithelium of special secretory IgA (S-IgA −2 linked IgA molecules) occurs [65]. The cellular composition of MALT effector sites includes B cells, CD4 + and CD8 + T cells, antigen-presenting dendritic cells, macrophages, and occasionally mast cells and eosinophils in the interfollicular region. They thus contain all the cell types necessary to initiate an immune response [63, 65, 66]. In MALT there are specialised epithelial cells called M cells. They are responsible for transporting microorganisms as well as macromolecules and soluble molecules to the subepithelial lymphoid tissues [63, 67]. Mucosal lymphoid organs develop during embryogenesis in the absence of microbial stimulation, and lymphoid tissues develop in adults after exposure to microbes or as a result of inflammation [68].

The microbiota influence the development of the host immune system by stimulating it to respond more effectively to pathogen attack and by inhibiting the colonisation of the mucosal surface by pathogenic microorganisms through bacterial antagonism [69]. Commensal bacteria - microbiota - can cause local or systemic infections under certain conditions [70]. Therefore, the immune system must effectively control the inflammatory response to simultaneously

prevent commensals from entering systemic sites and allow them to remain and function on mucosal surfaces.

MALT, which is constantly exposed to the presence of antigens, has an important function in controlling the immune response and avoiding unnecessary or excessive inflammatory responses to 'innocent antigens'. The mucosal immune system must be able to recognise pathogens, distinguish them from commensals and mount an appropriate response against the former [68]. This immune response is facilitated by numerous mucosal lymphoid tissues that collect antigens from mucosal tissues and effectively present them to lymphocytes in a manner that results in a rapid and robust immune response to pathogens [71]. The structure of the lymphoid organs maximises encounters between naive lymphocytes, antigens and antigen-presenting cells, and provides a microenvironment that supports the proliferation, differentiation and survival of activated cells [68, 72]. The peripheral lymphoid organs support immune responses appropriate to the tissue they drain and the type of antigen with which they come into contact. Although MALT sites are anatomically separate, antigen presentation and B cell activation at one site may result in IgA secretion elsewhere, making mucosal-associated lymphoid tissues a functional unit [63].

Macrophages – emerging role among immune cells

Macrophages play a remarkable role in limiting infection as an important link in the anti-infective response. These cells rest and hide beneath the epithelium and remain in communication with endothelial cells that modulate the immune response [73]. Macrophage migration into the urothelium is dependent on the CX3CL1 receptor [73, 74]. The vital defence mechanism in the urothelium depends on the recruitment of macrophages to the urothelium. They are involved in maintaining homeostasis, during infection they participate in antibacterial defence, while the absence of macrophages hinders the immune response. They also correlate with the number of neutrophils at the site of infection (a decrease in the number of macrophages causes an increase in the number of neutrophils) and are responsible for reducing the number of inflammatory molecules derived from mature neutrophils [73]. For example, the immune response to E. coli infection is critically regulated by macrophages through their effective phagocytosis of E. coli in the uroepithelium, and their absence is associated with worsening of the infection [73, 75]. These cells recognise pathogens and then participate in the recruitment of neutrophils and monocytes to the site of infection by producing cytokines [76].

Bacteria talk – the QS phenomenon

Because of the vast number of microorganisms in the environment they share, they must develop different mechanisms and strategies to compete with other species for ecological and nutritional niches. Bacteria also develop many molecular mechanisms that enable them to survive in different environments and under stress. Overall, responses to environmental factors include oxygen free radical tolerance, energy metabolism, toxin-antitoxin systems, malnutrition signalling and quorum sensing [77].

Bacterial cells can communicate within species or between species by molecular mechanisms defined as QS. In addition, the transfer of information can even be used to interact with higher organisms [78]. QS is a phenomenon of bacterial communication consisting of the synthesis and secretion of signalling molecules into the environment that are involved in the regulation of various physiological processes. This phenomenon is widespread in the microbial world and occurs in many species of symbiotic and pathogenic bacteria [79]. The QS mechanism is involved in many cellular processes such as bacterial DNA replication, conjugative transfer of plasmids, bioluminescence, synthesis of toxins, enzymes, polysaccharides and antibiotics. It affects the mobility of microorganisms, their ability to colonise and form a biofilm [79, 80]. Different bacterial species have their own specific QS system [80].

Signalling molecules called autoinducers (AIs) play a key role in communication between bacteria. These particles move from the cytoplasm to the extracellular environment either by diffusion or by active transport. They accumulate as the number of bacteria in the environment increases. When the concentration of AI exceeds the threshold, gene expression changes and all cells in the bacterial population acquire new characteristics. The QS controls about 10% of the bacterial genome, including functional genes: genes necessary for bacterial growth and life, genes for adaptation to a foreign environment, controlling the placement of bacteria in a group (biofilm formation), synthesis of virulence factors and other components involved in interaction with another organism [78–81].

The QS is the basis for the functioning of different bacterial strains in natural niches. Many strains of commensal bacteria have the ability to synthesise AI-3 signalling molecules that enable interspecies communication: bacteria - bacteria, and bacterial cell - host factors. The number of AI-3 signalling molecules produced depends on: bacterial density and results in the activation of virulence genes. Autoinducers (AI-3) are also involved in the expression of genes that control bacterial motility and biofilm formation. Gram-negative bacteria: *E. coli*, *Pseudomonas*, *Proteus*,

Serratia are examples of bacteria that use QS to control the expression of virulence factor genes [79, 80].

Thus, in short, the mechanism of the QS phenomenon can be described in three steps: first - members of the group secrete AI, second - AI are received by receptors located in cell membranes or cytoplasm of cells of organisms living in the same environment (both bacteria of the same species and bacteria of other species), third - activation of the expression of the relevant genes [79].

The QS mechanism is used by both saprophytic and pathogenic microbiota. Interfering with this mode of communication may in the future be used to change the way bacterial infections are treated, either through the use of inhibitors of AI receptors or of the AI itself. This is a tempting alternative in the era of excessive antibiotic therapy, which leads to chronic sterilisation of the body [78].

The urobiome: insights from recent research

An increasing number of studies point to a unique microbiota inhabiting the urinary tract, with a composition specific to both men and women. Most of the urobiome research is related to the adult population and its characteristics, including the composition of individual groups of microorganisms and their role in the urinary tract, are still not fully known [82, 83]. Determining the origin of the child's urobiome is important because the microbiome niches are structured after birth and we now know that changes in the urobiome can trigger the development of disease [81–85].

Storm et al. found in their pilot study that the urobiome is detectable as early as 2 weeks of age. They found that the urobiome differed between males and females, and in females between 3 groups (under 3 years, between 3 and 12 years and over 12 years), which was associated with toileting ability in younger children and puberty in older children. They note that in the male group, the most abundant genera were *Prevotella*, *Staphylococcus*, *Corynebacterium*, *Streptococcus* and *Winkia*, with a more stable composition during growth than in females. In the female group, the most abundant genera were *Lactobacillus*, *Bifidobacterium*, *Veillonella*, *Prevotella*, *Winkia* and *Schaalia*, with greater diversity in the younger cohort and higher prevalence of *Lactobacillus* or *Bifidobacterium* in the post-pubertal group [86]. Urobiome analysis of children younger than 2 years was performed by Kinneman et al. using 16 S rRNA sequencing technique and demonstrated the dominant presence of *Prevotella*, *Peptoniphilus*, *Escherichia*, *Veillonella* and *Finegoldia* in the niche [87]. To add, the urobiome of children of different ages and sexes has been compared with the microbiomes of other urogenital niches: bladder, urethra, perineum, vagina and foreskin. The composition of the microbiome of the bladder and urethra, vagina and perineum of girls varies significantly with age [86].

In adult females, the bladder microbiota has been found to be dominated by *Lactobacillus* and to a lesser extent by *Gardnerella*, *Streptococcus* or *Corynebacteria* [82, 88]. Importantly, some *Lactobacillus* species, with their ability to maintain low pH by producing lactic acid and hydrogen peroxide, can inhibit the growth of uropathogens such as *Escherichia coli* or *Klebsiella pneumoniae* [82, 89, 90]. Lewis et al. reported that the urobiome of adult males was less heterogeneous than that of females, with the absence of Actinobacteria and Bacteroidetes and the presence of the Firmicutes phyla. However, these analyses were performed on voided urine samples [83]. On the other hand, Wojciuk et al. in their elegant review stated that the male urobiota is dominated by Gram-positive bacteria, among these *Lactobacillus*, *Sneathia*, *Veillonella*, *Corynebacterium*, *Prevotella*, *Streptococcus* and *Ureaplasma*, as present in a healthy urobiome [3].

Overall, the most common phyla in the urinary tract are Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes. However, the majority of urinary tract bacteria (up to 60%) detected with the advent of new diagnostic tools have not yet been described [90]. Among the genera, *Lactobacillus* and *Streptococcus* have been reported as the most common. These lactic acid bacteria also colonise other human systems where they play a protective role against pathogens [91]. Studies also show that different groups of bacteria may play the same role in maintaining homeostasis [33, 92].

It should be emphasised that bacteria interact with many environmental toxins such as heavy metals, polycyclic aromatic hydrocarbons, pesticides, ochratoxins, plastic monomers and organic compounds. After some toxins are removed from the blood by renal filtration, their storage in the bladder provides sufficient time for the urinary microbiota to interact with and metabolise these compounds. These changes may increase or decrease the risk of diseases such as renal pathology and urinary tract cancer, as well as cognitive impairment, which can be caused by toxins [3]. Similar to the gut microbiota, the urinary tract ecosystem, and consequently its metabolic profile, has been shown to be influenced by external environmental factors, including disease, diet, stimulants, physical activity, as well as age and gender [3, 93]. All of this makes the urinary metabolic profile very complex and variable, making it difficult to determine the exact relationships between the microbiome and humans [3]. However, studies have proposed the existence of a 'core' bladder microbiota, a subset of bacteria present in varying amounts in the urinary tract.

To the best of our knowledge, changes in the urinary microbiota have been associated with the occurrence of various diseases. Bacterial changes observed in the urinary

tract are associated with an increased risk of incontinence or overactive bladder, urinary tract infection (UTI), neoplasia and nephrolithiasis [10, 91], as discussed below.

Urge urinary incontinence (UUI)/Overactive bladder (OAB) Lower urinary tract symptoms (most commonly manifested as incontinence and overactive bladder) are one of the best-studied urobiome-related conditions, along with urinary tract infections. Pearce et al. showed an overall decrease in Lactobacillus in the UUI group and an increase in *Gardnerella* in the no UUI group [88]. The decrease in urinary microbiota diversity was correlated with higher urinary incontinence severity and poorer response to anticholinergic treatment [94, 95]. Elsewhere, the presence of *Lactobacillus gasseri* was associated with the presence of UUI [82]. Kun et al. studied women with OAB and compared the results with the severity of symptoms. Surprisingly, they concluded that more severe symptoms correlated with increased urobiome diversity and richness. They also identified eight genera that correlated with the severity of specific OAB symptoms. These included *Campylobacter*, *Porphyromonas* and *Bosea*, which were associated with worse nighttime frequency [96].

Urinary tract infections (UTIs) With the discovery of the physiological microbiota in the urinary tract, the global definition of UTI seems to be going out of date. Across the board, the most common uropathogens are gram-negative bacteria. The urobiome in relation to UTI is best studied in adult women. Using an improved quantitative urine culture technique, Price et al. found a higher prevalence of *E. coli* in the UTI group and *Streptococcus /Gardnerella* in the non-UTI group, as well as lower species-level diversity and richness in the UTI group (88). Even a genus that is generally considered to be pathogenic - *E. coli* - has strains that can be protective. They have been shown to be beneficial by secreting escherichelin, which can counteract opportunistic infections by *Pseudomonas aeruginosa* [97]. *Lactobacillus crispatus* has been observed to be beneficial in recurrent UTI, with preventive properties of such bacterial intervention when given intravaginally [98]. This genus has been associated with the secretion of large amounts of the D-isomer of lactic acid, which inhibits pathogens [99]. Thomas-White et al. investigated the urobiome in women undergoing urogynaecological surgery and assessed the risk of postoperative UTI. They reported that the preoperative abundance of played a protective role against the development of UTI after surgery [100].

In children, the most common bacteria causing UTI are *E. coli*, *K. pneumoniae*, *P. mirabilis*, *M. morganii*, whereas Gram-positive bacteria (e.g. *E. faecalis*, *S. aureus*) are a rather rare cause [84]. Furthermore, as pointed out by Lei et al. and Shrestha et al., the bacterial composition of the urobiome changes with age: in younger children, whose urine pH is more acidic, infections with *E. coli*, *K. pneumoniae* or *E. faecalis* are more common. In contrast, in older children, P. mirabilis and P. aeruginosa are more often responsible for UTIs [101, 102]. The presence of *Achromobacter* has been demonstrated in children after exposure to antibiotics [80].

Neoplasm: In patients with bladder cancer, *Fusobacterium* was found to be a bacterial marker of the disease, and within this genus, *Fusobacterium nucleatum* was also responsible for some risk of GI cancer. Similar observations have been made in relation to *Campylobacter hominis* [103]. Wu et al., in their study of male patients with bladder cancer, found that there was diversity in the urobiome between cancer patients and controls. They found some different genera more abundant in the bladder cancer group, including *Acinetobacter*, *Anaerococcus*, *Rubrobacter*, *Sphingobacterium*, *Atopostipes*, *Geobacillus* (95). Xu et al. found an enrichment of *Streptococcus* in patients with urothelial carcinoma (96).

Urolithiasis Urolithiasis is a common urological disease with a prevalence of 1–13% worldwide [104]. A growing body of research suggests a link between the gut microbiome and the development of urinary calculi. Most kidney stones are composed of calcium oxalate. The GI tract contains several bacteria involved in oxalate degradation, the most important of which is *Oxalobacter formigenes* [18]. Colonisation with these anaerobic gram-negative bacteria has been associated with a 70% reduction in recurrent calcium oxalate stone formation [105]. Urease-producing bacteria (*Proteus*, *Haemophilus*, *Corynebacterium*, *Ureaplasma*) also favour struvite stone formation, and one of the main pathways is by increasing urinary pH and decreasing citrate levels [106], There is evidence that urinary tract enrichment of *E. coli* may be associated with the presence of calcium-based stones [107]. The co-occurrence of urinary tract infection and urolithiasis has been reported, as has the presence of bacteria directly in the kidney stone [85, 108].

Neurogenic bladder In the paediatric population, the main cause of neurogenic bladder is myelomeningocele. Changes in bladder innervation can lead to high bladder pressures with a high risk of UTI, vesicoureteral reflux, hydronephrosis and renal damage. Filler et al. showed that 50% of children with spina bifida will have a UTI by 15 months of age and 44% will have a UTI by 15 years of age [109]. Based on the National Spina Bifida Patient Registry, more than 70% of paediatric patients with spina bifida use clean intermittent catheterisation, antibiotics and anticholinergic medications. This population presents a diagnostic challenge

in distinguishing asymptomatic colonisation from symptomatic infection. Forster et al. investigated the urobiome in patients with neurogenic bladder and their study showed that the most common genera were unspecified *Enterobacteriaceae*, *Klebsiella*, *Staphylococcus* and *Streptococcus*. They found a slight difference between patients catheterised *via* the urethra (with a higher presence of Staphylococcus) and *via* the Mitrofanoff stoma (higher presence of *Enterobacteriaceae*). However, they did not find any changes in the composition of the urobiome in the presence of comorbidities (UTI or asymptomatic bacteriuria) [110]. In adults with neurogenic bladder (NB) due to spinal cord injury, it was concluded that patients with catheterisation had a higher abundance of *Enterobacteriaceae* and that this bacterial family increased with the duration of the primary disorder [111].

Role of the urobiome as an integral link in the immune defence process

The environment consisting of all the bacterial microorganisms present in the bladder, the proteins and metabolites produced by the microbiota, and host proteins and metabolites has been shown to influence the well-being of the human body, including urinary tract homeostasis [112, 113]. Studies in mice have shown that the absence of microbes in the urine correlates with a weakened immune system, leaky gut, and behavioural and neurological disorders. These disorders are most likely caused by the lack of appropriate signalling compounds produced by the urinary microbiota [28].

The urinary system, and in particular the mucosal lining of the bladder, has a complex, integral antimicrobial defence system. This unique barrier includes physical, biological and immunological factors. Comprehensive action of innate and acquired immune response pathways - humoral and cellular response cells constitute and create an integral, systemic immune response system focused on defence against pathogenic microorganisms. Protective physicochemical factors include: flushing of bacteria with an intense flow of urine, clumping and immobilisation of bacteria by mucus, production of organic acids by the urinary tract mucosa, which maintains an acidic pH (low pH inhibits bacterial growth) [58].

Adhesion of bacterial cells to the uroepithelium is the initiating critical moment of infection. Uropathogenic strains of bacteria are equipped with a large number of adhesive receptors that allow them to aggregate and adhere to mucosal cells. The first line of defence against infection is to prevent pathogens from adhering to the bladder mucosa [58]. Commensal bacteria provide a barrier of sorts by blocking access of uropathogenic bacteria to the uroepithelium and by producing antibacterial components. These include: the presence of glycosaminoglycans and sulphur residues on the mucosal surface, sloughing and apoptosis of infected squamous cells, Tamm-Horsfall protein, blocking the interaction of bacterial fimbriae with uroplakin receptors [58].

In addition, systems that block the use of ferric ions by pathogenic strains are involved in antibacterial immunity. The urinary tract is a niche with limited availability of ferric ions, which are crucial for bacterial metabolism and thus their life. Uropathogenic bacteria have mechanisms to obtain ferric ions, i.e. siderophores - small, highly active molecules secreted by pathogens in iron-deficient states. They are therefore one of the virulence factors. Inhibiting the production or function of siderophores helps to attenuate bacterial pathogenicity. For example, receptor-induced lipocalin 2 inhibits Fe binding by siderophores [58].

The innate immune system comprises several components, such as: (i) numerous toll-like receptors within the uroepithelium (the basis for activation of immune response pathways), (ii) plasma proteins, chemokines and cytokines, (iii) cellular components, (iv) antimicrobial peptides (B-defensin, cathelicidin), (v) local microbiota in the genitourinary and gastrointestinal tracts, which help to control bacterial urinary tract infections. The most important cellular component is the neutrophil, which is responsible for reducing the mass of pathogenic bacteria. Neutrophil migration is enabled by the expression of intracellular adhesion molecule 1 (ICAM-1) by epithelial cells and β2 integrin (CD11b/CD18) by neutrophils [58, 114].

The innate immune system, which plays a role in protecting the urinary tract, functions acutely and generates a very rapid response to microbial contamination to eliminate invading uropathogens [114]. When innate immune cells encounter potential pathogens, they activate intracellular signalling cascades that lead to the production of antimicrobial mediators, cytokines and chemokines that organise the local immune response [114]. Epithelial cells contribute significantly to innate immunity by communicating with haematopoietic cells, producing cytokines and chemokines, secreting proteins and antimicrobial peptides that kill invasive pathogens [77, 115].

The interaction between the aforementioned urobiome, uroepithelium and lymphoid tissues allows the maintenance of homeostasis and appears to play a key preventive role against the development of infection.

Therefore, UTI should be considered as the result of disturbances between them and have several causative factors. These factors are: dysbiosis - disturbance in the composition of the urobiome, impairment and dysfunction of the uroepithelial barrier, neurogenic disturbance of the bladder wall, invasion and development of uropathogenic bacterial strains in the bladder niche [116]. In each case of UTI, one of these

causative components predominates, but they remain interdependent. For example, the use of antibiotics inhibits uropathogens but also causes microbial changes in the bladder niche. While antibiotic therapy suppresses inflammation, it also promotes the development of resistance and disturbs the balance in the system [116]. It has been suggested that bacteria residing in the urinary tract may become uropathogenic depending on changes in the urobiome [58].

UTI should therefore be considered as the result of a multi-level complex response to bacterial virulence factors and host defence capabilities. The uropathogenicity of the bacterial strain is determined by genes encoding virulence factors, so genetic properties underlie this interaction. Gene expression and metabolic studies have helped to understand the dynamics and regulation of bacterial virulence. Understanding the complex, multi-level interaction between host and pathogen is key to understanding the process of pathogen colonisation of the urothelium and the development of infection [116].

Conclusions

Considering that research on the urinary tract microbiome has been going on for about 10 years and different methods of analysis have been implemented, further studies and standardisation of protocols will certainly allow to deepen the knowledge of the urobiome.

The composition of microorganisms in the urobiome, as in other microbiomes, is associated with specific health states, which may vary according to gender, age and a variety of environmental factors [30]. Little is known about the metabolites of urobiome microorganisms. Bacteriophages and phages have also been identified in the urobiome, but their role in UTIs remains unknown [117].

The urinary tract microbiome plays an important role in keeping the bladder healthy by maintaining its homeostasis, i.e. maintaining its urothelial integrity and neurotransmission. The urobiome has also been shown to prevent urinary tract infections [118]. A deeper understanding of the healthy urobiome in healthy individuals and identifying the changes in microbial composition that occur during urinary tract disease may change the way we treat urinary tract disease in the future.

As mentioned above, in response to a UTI, the human body engages a mechanical defence barrier, innate and acquired immune response pathways, but as emphasised in this paper - the urobiome appears to play a particularly biological role. The question that remains is where the urobiome fits into the anti-inflammatory defence. For some researchers, the urobiome may play a greater role in defending against the development of infection caused by pathogens than the body's immune response [116, 119].

Further work is needed to identify definitive and mechanistic links between the urinary microbiome and host health and urinary tract disease. Analysis of metabolic changes may be potentially useful as a future early diagnostic indicator of disease, but at this stage further studies in large populations are required to identify disease-specific profiles that could form the basis of future diagnostic tests [93]. Understanding the interaction of these mechanisms will allow new directions in the treatment of one of the most common clinical problems, UTI.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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