The Urinary Microbiome

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Contributor: Veronika Friedrich , Hae Woong Choi

The recent discovery of the urinary microbiome bolstered the notion that microbes might play a role in bladder cancer. Although microbial involvement in bladder neoplastic transformation and metastatic progression, except schistosomiasis, has not been established, accumulating research suggests that dysbiosis of the urinary microbiome can produce a chronically inflammatory urothelial microenvironment and lead to bladder cancer.

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1. Introduction

Bladder cancer (BC) is the sixth most common cancer in the United States; over 80,000 new cases were recorded in 2020 alone. Worldwide, more than 570,000 patients were diagnosed with BC, and over 200,000 patients died from the disease [1]. BC can be classified into non-muscle-invasive (NMIBC) and muscle-invasive (MIBC) bladder cancer. NMIBC comprises tumors isolated to the urothelium or lamina propria (stages 0 and 1), while MIBC refers to tumors that have invaded the muscle or deeper layers (stages 2–4) [2]. About 70–80% of newly diagnosed patients present with NMIBC, and only 10–20% show progression to MIBC [3]. As with other cancers, a higher tumor stage is associated with decreased survival rates; survival rates for NMIBC are approximately 80–90%, but drop to as low as 15% for MIBC [4]. Thus, depending on the stage, treatment options vary greatly, with intravesical Bacillus Calmette-Guerin (BCG) therapy following transurethral resection of bladder tumors (TURBT) being the standard treatment for intermediate- or high-risk NMIBC patients [5]. Despite these and other treatment options, bladder cancer shows a high recurrence rate. About 70% of patients who do not undergo cystectomy will experience recurrence of the disease [4]. This, combined with a mortality rate that has remained unchanged for over 30 years, has prompted investigations into other potential treatment methods.

The risk of developing BC increases with age, and men are affected nearly four times more frequently than women worldwide [6]. The most common risk factor for BC is smoking, followed by heavy alcohol use and occupational exposure to polycyclic aromatic hydrocarbons or aromatic amines. Chronic inflammation is now considered as an additional risk factor. Chronic inflammation, which is related with BC, can be attributed to host defense mechanisms for microbial infection or cellular injury in reaction to stressors. Schistosomiasis, in particular, is a well-known parasitic infection that can lead to BC [7]. Another player involved in the pathogenesis of BC is the urinary microbiome [8]. The bladder, which was previously thought to be sterile, is now known to harbor its own set of commensal microorganisms [9][10]. Not only does the composition of the urinary microbiome differ between sexes, it also varies between healthy individuals and BC patients [5][11]. Commensal microorganisms in the urinary bladder may be required for maintaining homeostasis and preventing an unwanted inflammatory response. However,

disruption in the urinary microbiome can also initiate proinflammatory responses by triggering immune cell infiltration [12], thereby establishing a chronic inflammatory milieu and eventually leading to the development of BC [12][13].

The fact that the urinary microbiome can play a unique carcinogenic role makes it a potential candidate for use as biomarkers in BC [14]. In addition, modifying the urinary microbiome could potentially be used as a therapeutic option in conjunction with conventional treatments [15][16][17].

2. Differences in Urinary Microbiome Sample Collection Methods

Collecting specimens from voided urine is noninvasive and convenient for both clinicians and patients when analyzing the urinary microbiome. However, since the urethra and genital tract are inhabited by a variety of microorganisms, analysis of voided urine samples misrepresents the actual composition of the urinary microbiome. Therefore, additional methods are routinely employed to gather urine samples for analysis. The aforementioned voided urine (sometimes referred to as “midstream urine”) approach is the only non-invasive method and the easiest to perform, making it a popular choice in many clinical studies; however, its disadvantages include contamination and consequent inaccuracy. Placing a catheter transurethrally to collect urine is a more invasive alternative, but it considerably reduces the risk of contamination [18]. Suprapubic aspiration, in which a syringe is injected into the bladder to acquire a urine sample, permits the most precise characterization of the urinary microbiome. This prevents the urine from coming in contact with other tissue areas, minimizing the risk of contamination [19][20]. The primary drawbacks of this method are its high degrees of invasiveness and pain [21]. For accurate characterization of the composition of the urinary microbiome, suprapubic aspiration continues to be the preferred method. Because multiple different factors such as pain and required level of sterility must be taken into consideration, individual patients benefit from a collection method that is appropriate for their unique medical situation. In addition to collecting urine, bladder mucosal tissue samples should also be analyzed to identify the bladder mucosa-associated microbiome [22].

As will be presented later, some bacterial genera are much more abundant in the microbiota isolated from the urine of patients with BC compared to that of healthy patients. Recently, “five suspect genera”, namely *Akkermansia*, *Bacteroides*, *Clostridium* sensu stricto, *Enterobacter*, and *Klebsiella* were found to be over-represented in BC tissue samples when compared to urine samples from the same group of BC patients [23]. This difference implies that these genera directly interact with the bladder mucosal tissue, which suggests that they are potentially associated with the oncogenic transformation process occurring at the bladder mucosa [23]. The bladder-mucosa-related microbiota is distinct from the urinary microbiota and provides the optimal test sample for characterizing the relationship between bacteria and cancer [23]. A more precise characterization of the changes in microbiota composition throughout the progression of BC could offer new potential for developing screening or monitoring tools for BC diagnosis.

3. Urinary Microbiome in Healthy Individuals

To comprehend the association between the urinary microbiome and the progression or treatment of BC, the characteristics of a non-diseased, healthy human bladder microbiome must be identified. Compared to other microbiomes found throughout the body, the urinary microbiome has not yet been adequately defined [24]. The few clinical trials that have been conducted typically suffer from constraints such as small sample size, imprecise method of urine collection, and high heterogeneity in characteristics such as race and age [25]. Defining a single general set of bacteria that constitutes a healthy bladder is thus challenging, but comparative studies have shed some light on overlapping and diverging urinary microbiome compositions. In terms of microbiomes of other organs, the gut shows different microbiome compositions depending on the sex of the individual [26][27]. Considering the substantial anatomical and physiological disparities between the lower urinary tract of men and women, the urinary microbiome of the bladder unsurprisingly shows differential stratification as well (**Table 1**) [9][10]. One study found that the most prevalent phyla shared among men and women were *Firmicutes,* followed by *Actinobacteria, Bacteroidetes,* and *Proteobacteria*. In terms of genera, however, the two diverge; healthy females show an abundance of *Streptococcus, Lactobacillus,* and *Prevotella*, while males have greater numbers of *Lactobacillus, Corynebacterium,* and *Gardnerella* [28]. Yet another study characterized healthy male bladders by an abundance of *Enterococcus*, *Proteus,* and *Klebsiella*, and healthy female bladders show dominance of *Lactobacillus* [10]. An independent study also confirmed the abundance of *Lactobacillus, Prevotella,* and *Gardnerella* in the female microbiome [29]. Even though *Lactobacillus* seems to be associated with a healthy urinary microbiome in women in particular, not all strains of the genus seem to be entirely favorable; Pearce et al., reported that the strain *L. gasseri* was more often found in women with urinary urge incontinence, while *L. crispatus* was more frequently cultured in healthy control subjects [30]. Acid-producing *Lactobacillus* species can contribute to keeping pathogenic bacteria unable to survive in a more acidic environment at bay, and thus assume a protective role in the bladder [10]. Indeed, a recent study found that *Lactobacillus* in the bladder can protect the host from uropathogenic *E. coli* infection by triggering type I IFN production [31]. Price et al. found that *Lactobacillus* in women showed no correlation with various factors such as age but discovered that other genera do differ among age groups; *Gardnerella* and *Escherichia* were more commonly found in younger and older women, respectively [32]. Both studies noted that genera composition is highly variable between individuals, once again substantiating the fact that a single, clear definition of what comprises a healthy urinary microbiome is difficult to provide [28][32].

Table 1. Urinary microbiome in a healthy human bladder.

Type of BC	Enriched Genera	Sex	Urine Collection	Reference
Healthy subjects only	*Staphylococcus, Streptococcus, Lactobacillus*	F	Midstream urine	Curtiss et al. [33]
Healthy subjects only	Genera of the phyla *Firmicutes*, *Actinobacteria*, *Bacteroidetes*	F, M	Midstream urine	Lewis et al. [9]
Healthy subjects only	*Lactobacillus, Streptococcus*, *Gardnerella, Escherichia*	F	Transurethral catheter	Price et al. [32]

Type of BC	Enriched Genera	Sex	Urine Collection	Reference
Healthy subjects only	M: *Enterococcus, Proteus*, *Klebsiella* F: *Lactobacillus*	F, M	Midstream urine, transurethral catheter	Fouts et al. [10]
Healthy subjects only	*Lactobacillus*, *Prevotella*, *Garnderella*	F	Midstream urine	Siddiqui et al. [29]
Healthy subjects only	*Lactobacillus*, *Staphylococcus*	F	Transurethral catheter	Pearce et al. [30]
Healthy subjects only	*Lactobacillus, Gardnerella, Gardnerella/Prevotella*	F	Transurethral catheter	Pearce et al. [34]
Healthy subjects only	*Lactobacillus*, *Gardnerella*, *Corynebacterium*	F	Midstream urine, transurethral catheter	Thomas-White et al. [35]
Study on microbiome in men with kidney stones	*Prevotella*, *Lactobacillus*	M	Transurethral catheter	Xie et al. [36]

Table 1 provides an overview of selected studies that have attempted to characterize the urinary microbiome of healthy males and females. Notice the inconsistent methods of urine collection, in addition to the variability in subject gender, race, and age. Overall, it can be seen a dominance of *Lactobacillus, Prevotella,* and *Streptococcus*. Further studies, ideally separately for different age groups, races, and genders, should be performed to more closely discern which members of the urinary microbiome are consistently found in the bladders of healthy individuals.

4. Urinary Microbiome in Bladder Cancer Patients

Urinary dysbiosis can be defined as the loss of beneficial bacteria from a healthy urinary bacterial community [37]. As was previously described, determining commensal microbes as “beneficial” is challenging; additionally, several studies have reported interpersonal variation even among healthy patients [8][24][37]. Recently, dysbiosis of the urinary microbiome has been linked to pathological conditions such as urinary urge incontinence, interstitial cystitis, and overactive bladder [10][29][30][38]. Although a similar speculation was made for BC patients, it is still unknown whether dysbiosis of the urinary microbiome at baseline precedes and is a risk factor for BC, or whether BC is responsible for the alteration of the microbiome. To date, few studies analyzing the urinary microbiome in bladder cancer have been performed, limiting our ability to draw definitive conclusions. A summary of the results from the available studies is given in **Table 2**.

Table 2. Urinary microbiome in bladder cancer patients.

Type of BC	Enriched Genera	Sex	Description	Urine Collection	Reference
Mixed	*Fusobacterium, Actinobaculum*,	M	No difference in alpha diversity	Midstream urine	Bucevic Popovic et al. [8]

Type of BC	Enriched Genera	Sex	Description	Urine Collection	Reference
	Facklamia, *Campylobacter*				
Mixed	*Acinetobacter*, *Anaerococcus*, *Rubrobacter*	M	Increased bacterial richness in cancer, significant beta diversity	Midstream urine	Wu et al. [14]
Recurrent NMIBC	*Staphylococcus*, *Streptococcus*, *Prevotella*	M	Increased bacterial richness in cancer, no differences in Shannon and Simpson indices, species diversity higher in recurrence group, more OTUs in cancer	Midstream urine	Zeng et al. [39]
MIBC, NMIBC	*Bacteroides*, *Akkermansia*, *Klebsiella*	M, F	No relationship found between microbiota in NMIBC and MIBC	Transurethral resection (tissue)	Mansour et al. [23]
MIBC, NMIBC	*Lactobacillus*, *Corynebacterium*, *Streptococcus*	M, F	No relationship found between microbiota in NMIBC and MIBC	Transurethral resectoscopy (urine)	Mansour et al. [23]
MIBC	*Bacteroides*, *Faecalibacterium*	M, F	Decreased bacterial richness in cancer	Midstream urine	Chipollini et al. [40]
Mixed	*Brucellaceae*, *Acinetobacter*, *E.Shigella*, *Proteobacteria*	M, F	Study on noncancerous vs. cancerous tissue;lower Shannon diversity in cancerous tissue, lower degrees of species richness and diversity in cancer	Tissue collected intraoperatively	Liu et al. [22]
Mixed	*Actinomyces*, *Achromobacter*, *Brevibacterium* phyla: *Actinobacteria*, *Proteobacteria*	M, F	No difference in alpha diversity but higher beta diversity	Midstream urine, Transurethral catheter	Hussein et al. [41]
Mixed	*Veillonella*, *Corynebacterium*	M	Significantly higher evenness parameter in BC group	Transurethral catheter	Oresta et al. [42]

ifferences were observed in alpha diversity compared to healthy individuals [8]. A subsequent study confirmed that only beta diversity is elevated in cancerous bladder samples [41]. Other independent studies reported that the genera *Brucella, Acinetobacter,* and *E.Shigella* were commonly present in BC patients; however, bacterial richness and diversity were significantly reduced in their urinary microbiome [22][40].

In contrast to the relationship between the urinary microbiome and BC, the bacterial diversity in BC increases with an increase in the genera *Acinetobacter*, *Anaerococcus*, *Rubrobacter*, *Staphylococcus*, *Streptococcus*, and *Prevotella* [14][39]. The genus *Acinetobacter*, the incidence of which was found to be elevated in BC specimens, has also been reported to contribute to the development of multidrug resistance and cause a variety of diseases, including pneumonia and bloodstream infections [43][44]. Multiple studies demonstrate an increase in the phylum

Proteobacteria in BC tissues. Other studies examining gut pathogenesis also found that dysbiosis of genera within this phylum are correlated with Crohn's disease and colitis-associated colorectal cancer [45][46]. Therefore, an increase of *Acinetobacter*, and more broadly *Proteobacteria*, could be employed as dysbiosis markers to diagnose BC. Oresta et al. noted that *Ruminococcus* and *Bifidobacterium*, which were reduced in BC patients, are genera known to be anti-inflammatory and important in mucosal homeostasis [42][47][48]. A decrease in beneficial bacteria such as these may provide "bad" bacteria with a permissive environment to stimulate inflammation and oxidative stress. They also suggest that tumor and microbiome may interact, where tumor formation breaks down the normal urothelium and favors attachment and proliferation of certain taxa [42]. As mentioned before, the development and progression of BC is thought to be influenced by many factors, which seems to result in a differential incidence and mortality rate between men and women. Even though the incidence is higher in men, poorer outcomes and higher death rates are associated with BC in women [49][50]. Studies have implicated hormones as playing an important role in BC, and more recently, sex-associated differences in the urinary microbiome have been emphasized [50][51]. Just as in healthy subjects, the urinary microbiome thus also shows variation between male and female bladder cancer patients: while the genera *Pelomonas, Corynebacterium,* and *Finegoldia* are more abundant in men with BC, women with BC show higher levels of *Lactobacillus, Actinotignum,* and *Prevotella* [41]. Oresta et al. found male BC patients to show an abundance of *Veillonella* and *Corynebacterium*, while Wu et al. reported an abundance of *Acinetobacter*, *Anaerococcus*, and *Rubrobacter* [14][42]. Although sex differences in the urinary microbiome were observed in recent studies, a more extensive mechanistic investigation is required to establish a link between these differences, the higher incidence of BC in men and worse disease progression in women.

Mechanically, urinary microbiota, which can attach to bladder mucosal surfaces, can undertake continuous translocations or transient tissue invasions [12]. Furthermore, bacteria present in the bladder can create biofilms that allow for continuous and extended direct contact with the urothelium, although it is unclear whether urinary microbiota can form biofilms in the bladder [12]. Due to the fact that a variety of bacteria create proteases that can act intracellularly and/or extracellularly, perturbing the natural process of extracellular matrix (ECM) renewal in the bladder can result in an altered and potentially cancer-promoting extracellular milieu [12][52]. It has been found that *Pseudomonas aeruginosa* secretes alkaline proteases predominately when cultivated in anaerobic conditions (probably within bladder tumor masses) [12]. These alkaline proteases degrade components of the ECM and inhibit lymphocyte proliferation by degrading and inactivating IFN-γ [53]. Additionally, proteases can cleave the IL-6 receptor, the FAS ligand, TNF, and its receptors [52][54][55]. Therefore, urinary microbiota and their released proteases may influence the integrity of the urothelial barrier, ECM development, and immunological repertoire in the bladder. This altered bladder tissue can thus potentially facilitate the development of the bladder tumor microenvironment.

The studies cited in this research show only a small number of overlapping genera and high variability even within a single study. Moreover, there is a substantial degree of interindividual variability in the microbial composition of both cancer patients and healthy individuals [8][40]. As was described in the previous section, the method of sample collection is a major factor determining the disparate results of urinary microbiome investigations. Some of the studies were conducted with people of a single sex, while others included participants of both sexes. Additional

characteristics, such as geographic location, age, and race, play a significant influence in the difficulty of identifying bacterial genera that are related to BC globally.

References

1. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, 2020. CA Cancer J. Clin. 2020, 70, 7–30.
2. Lenis, A.T.; Lec, P.M.; Chamie, K.; Mshs, M.D. Bladder Cancer: A Review. JAMA 2020, 324, 1980–1991.
3. Inamura, K. Bladder Cancer: New Insights into Its Molecular Pathology. Cancers 2018, 10, 100.
4. Berdik, C. Unlocking bladder cancer. Nature 2017, 551, S34–S35.
5. Andolfi, C.; Bloodworth, J.C.; Papachristos, A.; Sweis, R.F. The Urinary Microbiome and Bladder Cancer: Susceptibility and Immune Responsiveness. Bladder Cancer 2020, 6, 225–235.
6. National Cancer Institute. Cancer Stat Facts: Bladder Cancer. Available online: https://seer.cancer.gov/statfacts/html/urinb.html (accessed on 4 July 2022).
7. Mostafa, M.H.; Sheweita, S.A.; O'Connor, P.J. Relationship between schistosomiasis and bladder cancer. Clin. Microbiol. Rev. 1999, 12, 97–111.
8. Bucevic Popovic, V.; Situm, M.; Chow, C.T.; Chan, L.S.; Roje, B.; Terzic, J. The urinary microbiome associated with bladder cancer. Sci. Rep. 2018, 8, 12157.
9. Lewis, D.A.; Brown, R.; Williams, J.; White, P.; Jacobson, S.K.; Marchesi, J.R.; Drake, M.J. The human urinary microbiome; bacterial DNA in voided urine of asymptomatic adults. Front. Cell Infect. Microbiol. 2013, 3, 41.
10. Fouts, D.E.; Pieper, R.; Szpakowski, S.; Pohl, H.; Knoblach, S.; Suh, M.J.; Huang, S.T.; Ljungberg, I.; Sprague, B.M.; Lucas, S.K.; et al. Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder associated with spinal cord injury. J. Transl. Med. 2012, 10, 174.
11. Pederzoli, F.; Ferrarese, R.; Amato, V.; Locatelli, I.; Alchera, E.; Luciano, R.; Nebuloni, M.; Briganti, A.; Gallina, A.; Colombo, R.; et al. Sex-Specific Alterations in the Urinary and Tissue Microbiome in Therapy-Naive Urothelial Bladder Cancer Patients. Eur. Urol. Oncol. 2020, 3, 784–788.
12. Alfano, M.; Canducci, F.; Nebuloni, M.; Clementi, M.; Montorsi, F.; Salonia, A. The interplay of extracellular matrix and microbiome in urothelial bladder cancer. Nat. Rev. Urol. 2016, 13, 77–90.

13. Kiraly, O.; Gong, G.; Olipitz, W.; Muthupalani, S.; Engelward, B.P. Inflammation-induced cell proliferation potentiates DNA damage-induced mutations in vivo. PLoS Genet. 2015, 11, e1004901.
14. Wu, P.; Zhang, G.; Zhao, J.; Chen, J.; Chen, Y.; Huang, W.; Zhong, J.; Zeng, J. Profiling the Urinary Microbiota in Male Patients With Bladder Cancer in China. Front. Cell Infect. Microbiol. 2018, 8, 167.
15. Takahashi, T.; Kushiro, A.; Nomoto, K.; Uchida, K.; Morotomi, M.; Yokokura, T.; Akaza, H. Antitumor effects of the intravesical instillation of heat killed cells of the Lactobacillus casei strain Shirota on the murine orthotopic bladder tumor MBT-2. J. Urol. 2001, 166, 2506–2511.
16. Seow, S.W.; Cai, S.; Rahmat, J.N.; Bay, B.H.; Lee, Y.K.; Chan, Y.H.; Mahendran, R. Lactobacillus rhamnosus GG induces tumor regression in mice bearing orthotopic bladder tumors. Cancer Sci. 2010, 101, 751–758.
17. Naito, S.; Koga, H.; Yamaguchi, A.; Fujimoto, N.; Hasui, Y.; Kuramoto, H.; Iguchi, A.; Kinukawa, N.; Kyushu University Urological Oncology Group. Prevention of recurrence with epirubicin and Lactobacillus casei after transurethral resection of bladder cancer. J. Urol. 2008, 179, 485–490.
18. Perez-Carrasco, V.; Soriano-Lerma, A.; Soriano, M.; Gutierrez-Fernandez, J.; Garcia-Salcedo, J.A. Urinary Microbiome: Yin and Yang of the Urinary Tract. Front. Cell Infect. Microbiol. 2021, 11, 617002.
19. Eliacik, K.; Kanik, A.; Yavascan, O.; Alparslan, C.; Kocyigit, C.; Aksu, N.; Bakiler, A.R. A Comparison of Bladder Catheterization and Suprapubic Aspiration Methods for Urine Sample Collection From Infants With a Suspected Urinary Tract Infection. Clin. Pediatr. 2016, 55, 819–824.
20. Gajdács, M.; Ábrók, M.; Lázár, A.; Burián, K. Microbiology of urine samples obtained through suprapubic bladder aspiration: A 10-year epidemiological snapshot. Dev. Health Sci. DHS 2019, 2, 76–78.
21. Badiee, Z.; Sadeghnia, A.; Zarean, N. Suprapubic Bladder Aspiration or Urethral Catheterization: Which is More Painful in Uncircumcised Male Newborns? Int. J. Prev. Med. 2014, 5, 1125–1130.
22. Liu, F.; Liu, A.; Lu, X.; Zhang, Z.; Xue, Y.; Xu, J.; Zeng, S.; Xiong, Q.; Tan, H.; He, X.; et al. Dysbiosis signatures of the microbial profile in tissue from bladder cancer. Cancer Med. 2019, 8, 6904–6914.
23. Mansour, B.; Monyok, A.; Makra, N.; Gajdacs, M.; Vadnay, I.; Ligeti, B.; Juhasz, J.; Szabo, D.; Ostorhazi, E. Bladder cancer-related microbiota: Examining differences in urine and tissue samples. Sci. Rep. 2020, 10, 11042.
24. Pohl, H.G.; Groah, S.L.; Perez-Losada, M.; Ljungberg, I.; Sprague, B.M.; Chandal, N.; Caldovic, L.; Hsieh, M. The Urine Microbiome of Healthy Men and Women Differs by Urine Collection

Method. Int. Neurourol. J. 2020, 24, 41–51.

25. He, S.; Li, H.; Yu, Z.; Zhang, F.; Liang, S.; Liu, H.; Chen, H.; Lu, M. The Gut Microbiome and Sex Hormone-Related Diseases. Front. Microbiol. 2021, 12, 711137.
26. Kim, Y.S.; Unno, T.; Kim, B.Y.; Park, M.S. Sex Differences in Gut Microbiota. World J. Mens Health 2020, 38, 48–60.
27. Fransen, F.; van Beek, A.A.; Borghuis, T.; Meijer, B.; Hugenholtz, F.; van der Gaast-de Jongh, C.; Savelkoul, H.F.; de Jonge, M.I.; Faas, M.M.; Boekschoten, M.V.; et al. The Impact of Gut Microbiota on Gender-Specific Differences in Immunity. Front. Immunol. 2017, 8, 754.
28. Modena, B.D.; Milam, R.; Harrison, F.; Cheeseman, J.A.; Abecassis, M.M.; Friedewald, J.J.; Kirk, A.D.; Salomon, D.R. Changes in Urinary Microbiome Populations Correlate in Kidney Transplants With Interstitial Fibrosis and Tubular Atrophy Documented in Early Surveillance Biopsies. Am. J. Transplant. 2017, 17, 712–723.
29. Siddiqui, H.; Nederbragt, A.J.; Lagesen, K.; Jeansson, S.L.; Jakobsen, K.S. Assessing diversity of the female urine microbiota by high throughput sequencing of 16S rDNA amplicons. BMC Microbiol. 2011, 11, 244.
30. Pearce, M.M.; Hilt, E.E.; Rosenfeld, A.B.; Zilliox, M.J.; Thomas-White, K.; Fok, C.; Kliethermes, S.; Schreckenberger, P.C.; Brubaker, L.; Gai, X.; et al. The female urinary microbiome: A comparison of women with and without urgency urinary incontinence. mBio 2014, 5, e01283-4.
31. Song, C.H.; Kim, Y.H.; Naskar, M.; Hayes, B.W.; Abraham, M.A.; Noh, J.H.; Suk, G.; Kim, M.J.; Cho, K.S.; Shin, M.; et al. Lactobacillus crispatus Limits Bladder Uropathogenic E. coli Infection by Triggering a Host Type I Interferon Response. Proc. Natl. Acad. Sci. USA 2022, 119, e2117904119.
32. Price, T.K.; Hilt, E.E.; Thomas-White, K.; Mueller, E.R.; Wolfe, A.J.; Brubaker, L. The urobiome of continent adult women: A cross-sectional study. BJOG 2020, 127, 193–201.
33. Curtiss, N.; Balachandran, A.; Krska, L.; Peppiatt-Wildman, C.; Wildman, S.; Duckett, J. A case controlled study examining the bladder microbiome in women with Overactive Bladder (OAB) and healthy controls. Eur. J. Obstet. Gynecol. Reprod. Biol. 2017, 214, 31–35.
34. Pearce, M.M.; Zilliox, M.J.; Rosenfeld, A.B.; Thomas-White, K.J.; Richter, H.E.; Nager, C.W.; Visco, A.G.; Nygaard, I.E.; Barber, M.D.; Schaffer, J.; et al. The female urinary microbiome in urgency urinary incontinence. Am. J. Obstet. Gynecol. 2015, 213, 347.e1–55.e11.
35. Thomas-White, K.J.; Kliethermes, S.; Rickey, L.; Lukacz, E.S.; Richter, H.E.; Moalli, P.; Zimmern, P.; Norton, P.; Kusek, J.W.; Wolfe, A.J.; et al. Evaluation of the urinary microbiota of women with uncomplicated stress urinary incontinence. Am. J. Obstet. Gynecol. 2017, 216, 55.e1–55.e16.

36. Xie, J.; Huang, J.S.; Huang, X.J.; Peng, J.M.; Yu, Z.; Yuan, Y.Q.; Xiao, K.F.; Guo, J.N. Profiling the urinary microbiome in men with calcium-based kidney stones. BMC Microbiol. 2020, 20, 41.
37. Brubaker, L.; Wolfe, A.J. The female urinary microbiota, urinary health and common urinary disorders. Ann. Transl. Med. 2017, 5, 34.
38. Choi, H.-W.; Lee, K.-W.; Kim, Y.-H. The Microbiome's Function in Disorders of the Urinary Bladder. Appl. Microbiol. 2021, 1, 445–459.
39. Zeng, J.; Zhang, G.; Chen, C.; Li, K.; Wen, Y.; Zhao, J.; Wu, P. Alterations in Urobiome in Patients with Bladder Cancer and Implications for Clinical Outcome: A Single-Institution Study. Front. Cell Infect. Microbiol. 2020, 10, 555508.
40. Chipollini, J.; Wright, J.R.; Nwanosike, H.; Kepler, C.Y.; Batai, K.; Lee, B.R.; Spiess, P.E.; Stewart, D.B.; Lamendella, R. Characterization of urinary microbiome in patients with bladder cancer: Results from a single-institution, feasibility study. Urol. Oncol. 2020, 38, 615–621.
41. Hussein, A.A.; Elsayed, A.S.; Durrani, M.; Jing, Z.; Iqbal, U.; Gomez, E.C.; Singh, P.K.; Liu, S.; Smith, G.; Tang, L.; et al. Investigating the association between the urinary microbiome and bladder cancer: An exploratory study. Urol. Oncol. 2021, 39, 370.e9–370.e19.
42. Oresta, B.; Braga, D.; Lazzeri, M.; Frego, N.; Saita, A.; Faccani, C.; Fasulo, V.; Colombo, P.; Guazzoni, G.; Hurle, R.; et al. The Microbiome of Catheter Collected Urine in Males with Bladder Cancer According to Disease Stage. J. Urol. 2021, 205, 86–93.
43. Ketter, P.M.; Yu, J.J.; Guentzel, M.N.; May, H.C.; Gupta, R.; Eppinger, M.; Klose, K.E.; Seshu, J.; Chambers, J.P.; Cap, A.P.; et al. Acinetobacter baumannii Gastrointestinal Colonization Is Facilitated by Secretory IgA Which Is Reductively Dissociated by Bacterial Thioredoxin A. mBio 2018, 9, e01298-18.
44. Dijkshoorn, L.; Nemec, A.; Seifert, H. An increasing threat in hospitals: Multidrug-resistant Acinetobacter baumannii. Nat. Rev. Microbiol. 2007, 5, 939–951.
45. Gevers, D.; Kugathasan, S.; Denson, L.A.; Vazquez-Baeza, Y.; Van Treuren, W.; Ren, B.; Schwager, E.; Knights, D.; Song, S.J.; Yassour, M.; et al. The treatment-naive microbiome in new-onset Crohn's disease. Cell Host Microbe 2014, 15, 382–392.
46. Bonnet, M.; Buc, E.; Sauvanet, P.; Darcha, C.; Dubois, D.; Pereira, B.; Dechelotte, P.; Bonnet, R.; Pezet, D.; Darfeuille-Michaud, A. Colonization of the human gut by E. coli and colorectal cancer risk. Clin. Cancer Res. 2014, 20, 859–867.
47. Di Giacinto, C.; Marinaro, M.; Sanchez, M.; Strober, W.; Boirivant, M. Probiotics ameliorate recurrent Th1-mediated murine colitis by inducing IL-10 and IL-10-dependent TGF-beta-bearing regulatory cells. J. Immunol. 2005, 174, 3237–3246.

48. Burrello, C.; Garavaglia, F.; Cribiu, F.M.; Ercoli, G.; Lopez, G.; Troisi, J.; Colucci, A.; Guglietta, S.; Carloni, S.; Guglielmetti, S.; et al. Therapeutic faecal microbiota transplantation controls intestinal inflammation through IL10 secretion by immune cells. Nat. Commun. 2018, 9, 5184.
49. Noon, A.P.; Albertsen, P.C.; Thomas, F.; Rosario, D.J.; Catto, J.W. Competing mortality in patients diagnosed with bladder cancer: Evidence of undertreatment in the elderly and female patients. Br. J. Cancer 2013, 108, 1534–1540.
50. De Jong, J.J.; Boormans, J.L.; van Rhijn, B.W.G.; Seiler, R.; Boorjian, S.A.; Konety, B.; Bivalacqua, T.J.; Wheeler, T.; Svatek, R.S.; Douglas, J.; et al. Distribution of Molecular Subtypes in Muscle-invasive Bladder Cancer Is Driven by Sex-specific Differences. Eur. Urol. Oncol. 2020, 3, 420–423.
51. Goto, T.; Miyamoto, H. The Role of Estrogen Receptors in Urothelial Cancer. Front. Endocrinol. 2021, 12, 643870.
52. Vollmer, P.; Walev, I.; Rose-John, S.; Bhakdi, S. Novel pathogenic mechanism of microbial metalloproteinases: Liberation of membrane-anchored molecules in biologically active form exemplified by studies with the human interleukin-6 receptor. Infect. Immun. 1996, 64, 3646–3651.
53. Horvat, R.T.; Parmely, M.J. Pseudomonas aeruginosa alkaline protease degrades human gamma interferon and inhibits its bioactivity. Infect. Immun. 1988, 56, 2925–2932.
54. Mullberg, J.; Durie, F.H.; Otten-Evans, C.; Alderson, M.R.; Rose-John, S.; Cosman, D.; Black, R.A.; Mohler, K.M. A metalloprotease inhibitor blocks shedding of the IL-6 receptor and the p60 TNF receptor. J. Immunol. 1995, 155, 5198–5205.
55. Kayagaki, N.; Kawasaki, A.; Ebata, T.; Ohmoto, H.; Ikeda, S.; Inoue, S.; Yoshino, K.; Okumura, K.; Yagita, H. Metalloproteinase-mediated release of human Fas ligand. J. Exp. Med. 1995, 182, 1777–1783.