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Review

The Microbiome's Function in Disorders of the Urinary Bladder

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Abstract: The introduction of next generation sequencing techniques has enabled the characterization of the urinary tract microbiome, which resulted in the rejection of the long-held notion of urinary bladder sterility. Since the discovery and confirmation of the human bladder microbiome, an increasing number of studies have defined this microbial community and understand better its relationship to urinary pathologies. The composition of microbial communities in the urinary tract is linked to a variety of urinary diseases. The purpose of this review is to provide an overview of current information about the urinary microbiome and diseases as well as the development of novel treatment methods.

Keywords: urinary bladder; microbiome; disease

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1. Introduction

The Human Microbiome Project has examined the makeup of bacterial communities in a variety of human body niches, including the mouth cavity, skin, gastrointestinal system, and vagina, as well as their function in health and illness [1,2]. The urinary bladder was initially excluded from the research since urine was historically deemed sterile due to the notion that all microorganisms were harmful [2,3]. Microbial communities in the urinary tract were first discovered less than a decade ago. Numerous scientists have now verified the existence of a urinary microbiota [4,5]. Technological advancements will offer physicians better knowledge of the status of the urinary microbiome.

Recent research has shifted its emphasis to defining the microbiome and its relationship to human health. The beneficial role of the microbiota in preserving the human body's homeostasis is expected to provide a protective role against infections by forming a physical barrier, and adds to the immune system's development [2]. However, the detailed physiological impact of the urinary microbiome remains unknown. The changes in the urinary microbiota have been linked to the development of a variety of urinary diseases. These transitions will guide the management of a variety of common urinary diseases associated with changes in the urobiome [5].

Thus, understanding the human urinary microbial makeup and how it changes under pathological situations may facilitate the creation of novel preventive, diagnostic, and therapeutic methods. The current level of knowledge about the urine microbiome is given in this review, with an emphasis on its relevance for health maintenance and its involvement in disease development.

2. Main Body

2.1. Urine Collection for Urinary Microbiome Analysis

Voided urine specimens and midstream urine samples may contain bacteria from the uroepithelium, periurethra, or genital tract, therefore mischaracterizing the urinary bladder microbiome in favor of the urogenital microbiota [2,6,7]. Therefore, urine samples from females should be classified as genitourinary specimens based on unequivocal evidence

of vulvovaginal microbial involvement [5]. Contamination DNA is a significant problem in microbiome research, particularly in communities with low biomass. According to the study, the urine microbiota exhibits low biomass ($<10^5$ colony-forming units per milliliter, approximately) [8]. Anatomically, the bladder urethra is close to the vagina or the gut, a bacterial habitat with a larger microbial biomass, so considerable care must be taken to prevent contamination during sample collection, processing, and analysis [2,9].

Although standardizing specimen collection, preservation methods, and analytical methodologies require consensus, urine specimens obtained through suprapubic aspiration or transurethral catheterization are currently accepted as bladder specimens [10]. A comparative study of microbial communities in urine obtained via suprapubic aspiration or transurethral catheter demonstrated that the outcome is very similar regardless of the collection method, indicating that transurethral catheterized samples are widely accepted for studying the urinary bladder microbiome [2,10]. It is conceivable, however, that certain bacteria associated with the bladder mucosa are not identified in these types of urine samples [11]. Biopsies or tissue samples would be required for detection.

2.2. Identification of Urinary Microbiome

Metagenomic study with Next Generation Sequencing (NGS) enables the quantitative characterization of microbiomes, providing information on microbial populations and assisting in the discovery of hitherto uncultured microorganisms [12,13]. This method discovers anaerobic microbes with sluggish growth rates or bacteria with complicated nutritional requirements. The use of NGS has enabled the identification of commensal and pathogenic species, as well as the discovery of novel uropathogens [7,14,15]. Amplicon sequencing is a PCR-based study focusing on the 16S rRNA subunit (V1–V9) that is used to identify various bacterial species [12,16]. The urinary microbiome was discovered primarily through the use of 16S rRNA gene sequencing. Shotgun sequencing enables microbiome sequencing in a range of different samples, including fugus or virus [17,18].

However, since DNA sequencing-based techniques cannot demonstrate the bacteria's vitality, it is suggested to utilize the enhanced quantitative urine culture (EQUC) methodology for examining the viability of bacteria in urine [19]. This procedure involves plating a urine sample on a variety of media, including blood agar, chocolate, and colistin-nalidixic acid agars, then incubating them at 35 °C for 48 h under aerobic or anaerobic circumstances [2,20]. Urine culture methods have been enhanced in order to increase the variety of bacteria that may be detected. Thus, a combination of 16S rRNA gene sequencing with EQUC would give an accurate characterization of urinary tract microbial populations.

2.3. Urinary Microbiome

The human urinary microbiome studies have concentrated on defining microbial populations in either sex. Other confounding variables such as gender, race, or geographic distribution have not been evaluated for analyzing the urinary microbiome composition in the general population [2,4]. The urine microbiome of men and women is comparable at the phylum level when characterized by 16S rRNA sequencing. The majority of bacteria in both genders are members of the phylum Firmicutes. Actinobacteria, Bacteroidetes, and Proteobacteria make up the rest of the phyla [2,6]. Next, *Prevotella*, *Escherichia*, *Enterococcus*, *Streptococcus*, and *Citrobacter* are all common genera found in male and female urine microbiomes. The main variation in the makeup of the urinary microbiome between men and women is evident in that the number of *Lactobacillus* is more prevalent in females, whereas *Corynebacterium* and *Streptococcus* are more prevalent in males [2,6].

2.4. The Functional Role of the Urinary Microbiome

Although the unique roles of the urine microbiome have not been fully identified, it is believed that, similar to other mucosal areas such as the gastrointestinal and female reproductive tract, the microbiota inside this location are essential for urinary tract homeostasis [21]. Few studies are examining the unique function of the urine microbiota in home-

ostasis maintenance and the underlying processes [22]. The urine microbiota, like other human microbial communities, may play a role in regulating the immune response [22]. Indeed, it has been shown that some bacteria metabolites may have a role in regulating the immune response and inflammation associated with bladder disorders [23]. The abundance of certain resident macrophages in the urinary tract may indicate the critical role of host cell–microbial interaction in preparing the immune response to infection [24,25]. However, additional investigation of microbial-mediated processes of urinary tract homeostasis is required.

The microbiota has a significant impact on the creatures it inhabits. A recent study revealed some interesting findings by comparing germ-free (GF) mice to mice with a normal microbiota (i.e., specific pathogen-free, SPF) animals [26]. This study can examine the effect of microbiota on the bladder transcriptome. In general, GF mice have lower body fat and slower metabolic rates, smaller livers, a lower total surface area of the small intestine, and a bigger caecum [26,27] in comparison to other mice. This study reported that GF urinary bladders were 25% lighter than bladders from mice maintained under conventional SPF settings [26]. Additionally, there were differences in the expression of the uncharacterized immunoglobulin genes (Igkv1-122, Igkv4-68), but interestingly, the lack of microbiota had no effect on the expression of genes involved in microbe recognition and their products [26]. Some of the alterations in gene expression, such as circadian rhythm, extracellular matrix, and neuromuscular synaptic transmission support the notion that the microbiota has an effect on gene expression in the urinary bladder. The functional study of the urinary microbiome in the health and illness of the bladder can be enhanced by using GF mice. Numerous studies demonstrate a link between the urinary microbiome and bladder health. Assessing the impact of individual microbial strains requires the establishment of gnotobiotic mice, which can be generated by microbial reconstitution of GF mice with single organisms and defined mouse- or human-derived microbial consortia [28].

2.5. Urinary Microbiome of Healthy Women

Recently, research groups have started characterizing the female bladder microbiome. The bladder microbiome is dominated by organisms belonging to a few genera, most often *Lactobacillus*, *Gardnerella*, and *Streptococcus* [8,20,29]. The profile size is very small in comparison to microbiomes in other body sites [5]. *Lactobacillus crispatus*, *Gardnerella vaginalis*, and *Atopobieum vaginae* are bacteria that are usually found in healthy females [30]. *Lactobacillus* is the most common species identified in the urine microbiome of healthy females [6–8,20]. When women experience the decline or absence of estrogen, the urogenital tract undergoes significant changes such as modification of bladder epithelium and increased incidence of UTIs [31,32]. The presence of bacterial populations in the bladders of nonpregnant perimenopausal women was demonstrated by compelling enhanced culture and DNA sequencing data [32]. The relationship between microbial composition and menopause was further investigated on maternal bladder microbiota [33]. The enhanced quantitative urine culture and 16S rRNA gene sequencing of urine taken from young pregnant women (average age 30 years) via transurethral catheterization revealed that the major urotype is *Lactobacillus* (60%), followed by *Gardnerella* (25.7%). *Staphylococcus*, *Enterococcus*, or the family *Enterobacteriaceae* comprised a tiny percentage of individuals [33]. The *Lactobacillus* genus profile included *L. gasseri*, *L. jensenii*, *L. iners*, *L. johnsonii*, and *L. crispatus* [33]. Not all *Lactobacillus* species, on the other hand, are linked with a healthy microbiome. Reduction in *Lactobacillus* abundance has been associated with clinical conditions since its deficiency promotes the colonization of disease-causing uropathogens [7,9]. *Gardnerella* comes in second place in terms of abundance, with *Gardnerella vaginalis* being the most common species containing certain dangerous strains causing urinary tract infections (UTIs) in women [8]. The existence of these species in the bladder suggests that the vagina may be the primary source of the microbial community in the bladder [2,29,34]. The anatomical closeness of the vagina and the urinary system may provide a single urogenital microbiota in both niches. The study discovered a close similarity between the vaginal and bladder microbiota,

which is distinct from gastrointestinal microbial communities, after analyzing cultured bacteria from the female bladder and a detailed comparison of the bladder microbiome with the gastrointestinal and vaginal microbiomes [29]. A whole metagenome study of bacterial strains obtained from vaginal and bladder samples from the same donor found striking similarities between commensal members *Lactobacillus iners* and *Lactobacillus crispatus*, both of which are associated with good health [29]. A more recent study employing culturomics, particularly those targeting anaerobes, identified that 64.1% of the bacterial species recovered at least once from urine microbiota had previously been isolated from gut microbiota, whereas only 31.7% had previously been isolated from vaginal microbiota. These findings imply that many aerobic members of the microbiota in the urinary system come from the gut [35]. Therefore, advances in urine analysis technology would allow for the accurate characterization of urinary tract bacteria communities. However, the imbalance of the bladder microbiome can lead to various urinary diseases. Earlier research has shown correlations between the female bladder microbiome and post-instrumentation UTIs, responsiveness to hyperactive bladder therapy, and urgency urine incontinence (UUI) [4,5].

2.6. UTI and Urinary Microbiome

The study of the urine microbiome is becoming increasingly important due to the fact that changes in its composition have been linked to the development of many illnesses, mainly UTIs. UTIs are the body's second most frequent bacterial infection, accounting for around 8.1 million medical visits each year [36]. UTIs are prevalent in women and commonly reoccur. According to the National Health and Nutrition Examination Survey III data, UTIs occur in 53,067 cases per 100,000 women throughout their lifetime, compared to 13,689 cases per 100,000 males [37]. The greatest gender disparity comes between the ages of 16 and 35, when women are about 35 times more likely to be impacted [37]. Women have a greater incidence of urinary colonization than men. Anatomically, the vaginal cavity and rectal opening are located in close proximity to the urethral opening. Additionally, women have more wet periurethral regions, which are suitable growing grounds for bacteria [38]. Due to the shorter urethral length, the bacteria entering the urethra are more likely to ascend to the female bladder than the male bladder [38]. Uropathogenic *E. coli* (UPEC) is responsible for about 80% of infections. Although virulence characteristics like sticky fimbriae play a role in UPEC pathogenesis, predisposing host variables also play a role in UTIs, especially in people who have repeated episodes [39]. UTI is defined as an infection of the urethral cavity followed by an infection of the lower urinary tract up to the bladder, resulting in urethritis and cystitis, respectively [40]. When infections cause pyelonephritis in the kidneys they can even spread via the bloodstream, resulting in systemic infection (urosepsis) [41].

Patients with culture-confirmed UTI should receive oral antibiotics, depending on their clinical status [42]. Sulphonamides or first-generation cephalosporins are the most often prescribed oral antibiotics. However, there is rising worry about urinary pathogen resistance to these antibiotics, as seen by the increasing frequency of therapeutic failures following empiric therapy [43]. Recent examination of the impact of antibiotic prophylaxis on urinary microbiota [6] showed that when the urinary microbiota of preventive trimethoprim-sulfamethoxazole therapy and a healthy control group were compared, the antibiotic group substantially increased the number of pathogenic species while decreasing microbial diversity relative to the healthy control group. These results emphasize the need to show sensitivity when choosing optimum preventive regimens and indicate that probiotic prophylaxis may be more successfully explored. [6,44]. The comparative genomic analyses were performed on *E. coli* isolates from adult female bladders without signs of lower UTI, with a clinical diagnosis of UTI, or with lower urinary tract symptoms (LUTS) [45]. The genetic compositions of the *E. coli* isolates or the makeup of the complete urobiome was unable to differentiate between the urinary microbiomes of persons with UTI and those without LUTS [45]. This study suggests that UTI symptoms linked with

E. coli detection are more likely the result of microbiome composition. Recently, the study comparing urine next-generation sequencing (NGS) of patients with acute uncomplicated cystitis (AUC) and recurrent cystitis (RC) revealed differences in microbiome patterns [46]. Transurethrally obtained urine specimens from the RC group had substantially more microbiome diversity than the AUC group. *Pseudomonas*, *Acinetobacter*, and *Enterobacteriaceae* were identified in the urine NGS findings for the AUC group, while *Sphingomonas*, *Staphylococcus*, *Streptococcus*, and *Rothia* spp. were detected in the RC group [46]. Significant variations in bacterial diversity and patterning were seen between AUC and RC patients. This study suggests that AUC can be considered a transient infection produced by a single pathogenic organism, while dysbiosis seems to play a more significant role in the pathophysiology of RC [46,47]. RC may be linked with urinary tract dysbiosis, but more study is necessary [46,48].

Numerous UTIs go unreported and untreated, particularly in older individuals who frequently have polymicrobial UTI samples. The presence of significant uropathogenic species in mixed culture urine samples from older individuals, as well as resistance to first-line antibiotics with potentially enhanced resistance to ciprofloxacin and trimethoprim, was described [49,50]. Most notably, the study demonstrates that *E. coli* isolated from polymicrobial UTI samples is statistically more invasive than *E. coli* recovered from monomicrobial culture samples in in vitro epithelial cell infection tests [51]. *E. coli* contamination in polymicrobial UTI samples may offer an elevated danger to human health [51]. Furthermore, the function of *enterococci* in the pathogenesis of polymicrobial infections provides insight into the bacterial cooperation process. When virulent *enterococci* were evaluated in the presence or absence of *E. coli* strains in the in vivo *Caenorhabditis elegans* model, a synergistic impact on virulence was seen when *enterococci* and *E. coli* were compared to *enterococci* alone or *E. coli* alone [52]. *Enterococcus faecalis* has the ability to modify its immediate environment via signaling, therefore promoting the growth of other coinfecting organisms [52]. Increasing reports support that a single external pathogenic bacterial invasion is insufficient to account for UTI-associated disease in humans. Both an imbalance in the urine microbiota repertoire and polymicrobial pathogenic causes should be correlated.

Lactobacilli species such as *L. crispatus*, *L. iners*, and so on are commensal bacteria that reside in healthy females [30]. These *Lactobacilli* deficits have been linked to the colonization of UTI-causing uropathogens [2,7]. Predisposition to get UTI is linked with a decline of *Lactobacillus iners* in the patients who develop postoperative UTI [29,53]. The change in the urine microbiota, in conjunction with other risk factors (age and estrogen levels) contributes to the development of postoperative UTI with uropathogens such as *E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [29,53]. The vaginal microbiome can influence the host's susceptibility to UTI. According to the clinical study on young women with a history of recurrent UTIs, women with recurrent UTIs become more resistant when their vaginal microbiome is modified with probiotics such as *Lactobacillus crispatus* [54]. Intravaginal probiotic treatment with *L. crispatus* showed significant reduction in recurrent UTI associated with high-level vaginal colonization with *L. crispatus* [54]. The regulatory effect of the vaginal microbiome on UTI was further supported by the report that women who have bacterial vaginosis as a result of anaerobic *Gardnerella vaginalis* overgrowth experience more UTI than women who have healthy microbial communities consisting primarily of *Lactobacillus* [55]. Clinical studies indicate that the makeup of a woman's vaginal microbiome has an effect on her susceptibility to recurrent UTI [56]. Bladder exposure to *G. vaginalis* induces *E. coli* egress from latent intracellular *E. coli* reservoirs in the bladder and increases the risk of life-threatening *E. coli* [56]. *G. vaginalis* exposures were sufficient to induce bladder epithelial apoptosis and exfoliation, as well as interleukin-1 receptor-mediated kidney damage that persisted after *G. vaginalis* clearance from the urinary system [56]. This study provides the etiology of recurrent UTI, in which illness may be triggered by brief but potent urinary tract exposures to vaginal bacteria. Altogether, increasing evidence support the notion that single invasion by an external pathogenic bacterium fails to explain UTI-associated pathology in humans. The complete understand-

ing of UTI needs to consider both an imbalance in the urine microbiota repertoire and polymicrobial pathogenic sources [2,57].

2.7. Urinary Incontinence and Urinary Microbiome

Urinary incontinence (UI) is characterized by uncontrolled urine loss that is more prevalent in women. Urgency urinary incontinence (UUI) is a poorly understood urinary disease characterized by symptoms that overlap with urinary infection, such as urinary urgency and increased frequency of urination in conjunction with urine incontinence. Utilization of 16S rRNA gene sequencing to categorize bacterial DNA and EQUC methods to extract live bacteria from urine collected via transurethral catheter from women with UUI and a control cohort indicated an increase in *Gardnerella* and a reduction in *Lactobacillus* in the UUI microbiome [8]. *Lactobacillus gasseri* were identified more frequently in the UUI cohort and *Lactobacillus crispatus* were detected more frequently in controls [8]. Using both high-throughput sequencing and extended culture methods revealed statistically significant variations in the quantity and frequency of urinary microbiome, despite the fact that several risk factors for UI have been identified, including age, body mass index, parity, and hormones [8,58]. This result was further supported by a more large-scale study recruiting 123 women with mixed urinary incontinence (MUI) and 86 healthy controls using catheterized urine samples [34]. Patients with MUI exhibited an increased relative abundance of *Gardenerella* and *Prevotella*, whereas a decreased relative abundance of *Lactobacillus* [34]. This result is consistent with earlier findings in UI patients. However, a different study compared the bladder microbiome features of well-characterized asymptomatic women to those of women with mixed urinary incontinence (MUI) using catheterized urine samples and 16S rRNA sequencing [59]. This study showed there was no difference in the proportion of women with *Lactobacillus* predominance between adult women with MUI and age-matched asymptomatic women [5,59]. Therefore, additional research is required to optimize the tools for microbial identification and to develop a core microbial population in UI patients.

While *Gardnerella* has been characterized as a commensal component of the urinary microbiota, certain *Gardnerella* strains may be associated with specific diseases such as bacterial vaginosis, allowing for the separation of pathogenic and commensal *Gardnerella vaginalis* strains [60]. The contribution of *Gardnerella vaginalis* on UUI and overactive bladder (OAB) was mechanically examined and proven to impact the contraction of the detrusor muscle during micturition [61]. The urothelial bladder cell's exposure to *Gardnerella vaginalis* along with *E. coli* stimulates purinergic signaling pathways, which revealed a correlation between the Ca^{2+} influx and contraction of cells and the quantity of extracellular ATP generated by *E. coli* [61]. In comparison, *Lactobacilli* appears to alleviate this reaction by using extracellular ATP or generating inhibitory chemicals that function as receptor agonists or Ca^{2+} channel blockers [61]. Therefore, changes in the urine microbiota significantly contribute to UUI [8,62]. Additionally, a rise in the intensity of UUI symptoms is related to a decline in microbial diversity in women with UUI [62].

2.8. Interstitial Cystitis and Urinary Microbiome

Interstitial cystitis/bladder pain syndrome (IC/BPS) is a puzzling urological disease characterized by bladder pain, frequency, and urgency of urination in the absence of a normal UTI [63]. Management is challenging, and a solution remains elusive due to an unclear etiology and pathogenesis [64]. Although active UTI precludes diagnosis and empiric antibiotic therapy is typically ineffective, a bacterial etiology has never been ruled out in IC/BPS, and an association with bacteria has been suggested in a small but possibly significant number of IC/BPS patients [65–67]. Historically, microbiologic diagnosis of bladder infection relied on cultivation methods in which bacteria were grown from voided urine distributed on culture plates, which lacked the necessary nutrients and environmental conditions for the development of many bacteria. These established methods for studying bacteria, not only in patients with IC/BPS but also in those with suspected bacterial cystitis,

are insufficient for surveying the microorganisms present in patient samples [68]. The study compared the urinary microbiome from catheterized urine samples of women diagnosed with IC to age-matched women in a control group [69]. The urine of IC patients contained fewer distinct operational taxonomic units and was less likely to contain *Lactobacillus acidophilus* [69]. *L. acidophilus* was associated with less severe IC symptoms index scores. Although this study found no correlation between the presence of *Lactobacillus* species and cytokine levels, interleukin-4 had a positive association with higher IC symptoms' index scores [69]. The *Lactobacillus acidophilus* strain is a well-characterized probiotic bacteria that exhibited potential anti-inflammatory action in response to lipopolysaccharide (LPS) [70,71]. Therefore, the absence of the *Lactobacillus acidophilus* strain and increased levels of cytokines in individuals with IC indicate that a proinflammatory response mediated by bladder dysbiosis is very likely linked with the development of IC [70,71]. However, when the existence, variety, and abundance of species and genera of IC patients' midstream urine specimens were compared to control individuals in another study, there was no significant difference in species composition between IC/BPS and control participants, but the urine of IC/BPS individuals showed a tendency toward an excess of *Lactobacillus gasseri* but also a decreased prevalence of *Corynebacterium* [68]. There is another report that a midstream voided specimen from IC/PBS patients and asymptomatic controls showed no significant difference in urotypes across groups as determined by EQUC or 16S rRNA gene sequencing. As a result, although *Lactobacillus* strains are suggested to be associated with IC/BPS, more research is necessary to achieve agreement on both views.

2.9. Overactive Bladder Syndrome and Urinary Microbiome

Overactive bladder (OAB) syndrome is a chronic medical disease that has a significant influence on both men and women's quality of life [72]. OAB is characterized by "urinary urgency, frequently accompanied by frequency and nocturia, with or without urgent incontinence, in the absence of apparent pathology" [73]. Urgency is the primary symptom of OAB and is strongly related with frequent urges to pee during the day, nocturia, and incontinence. Nocturia has been identified as the most distressing symptom [74,75]. Numerous variables may contribute to OAB, and the primary reason may differ from person to person. The etiology of OAB is yet unknown. One study compared the microbiome of patients with OAB to the microbiota of healthy females without overactive bladder symptoms [76]. Female human bladder microbiomes are varied, with statistically significant differences between microorganisms identified in patients with OAB and controls [76]. Proteus was more commonly isolated from women with OAB than other bacterial genera and *Lactobacillus* was detected less frequently in OAB patients' urine than in controls' urine [76]. Another 16S rRNA sequencing finding from adult women who had catheterized urine samples immediately before urogynecological surgery were recently reported that *Lactobacillus* was the most abundant genus in bladder (30%) urine [77]. However, this report found that a link between OAB symptoms and the presence of Gram-positive anaerobic bacteria (*Atopobium vaginae* and *Finegoldia magna*), which are from the phylum *Actinobacteria* [5,77]. Preoperative evaluation of the urine microbiome can help to alleviate unpleasant urinary symptoms and decrease the risk of perioperative UTI [5]. As a result, enhanced culture techniques allow researchers to develop a reference collection of bladder-specific isolates that can be utilized to better understand the microbial contributions to OAB and illness.

2.10. Bladder Cancer and Urinary Microbiome

Limited research has been conducted on the bladder microbiome involvement in urological cancers. Recent studies indicate that the human microbiome can affect cancer formation, however the function of microbes in bladder cancer pathogenesis has not been investigated. According to a study comparing urine samples from healthy people to bladder cancer patients using 16S rRNA sequencing, an abundance of the genus *Streptococcus* in bladder cancer patients was detected [78]. Bladder urothelial carcinoma (UBC) is the sixth most common kind of cancer globally [79]. UBC can be categorized as non-muscle

invasive, muscle invasive, or locally advanced/metastatic [80]. UBC is characterized by a heterogeneous tumor cell population and surrounding tumor microenvironment (TME). Given that the microbiota has been linked to the formation of cancer in a variety of tissues, urinary microbiome is also implicated in UBC. Only a few studies have examined the relevance of the microbiome in urologic malignancy.

Several studies were performed to define and compare the bladder cancer patients' urine microbiota to that of healthy controls. The potential changes in the extracellular matrix caused by the microbiota and the subsequent inflammation may play a role in carcinogenesis [81]. This study recruiting male bladder cancer patients and non-neoplastic controls collected midstream urine. Cancer patients' urine samples were enriched with some bacterial genera (e.g., *Acinetobacter*, *Anaerococcus*, and *Sphingobacterium*), but showed a decrease in others (e.g., *Serratia*, *Proteus*, and *Roseomonas*) [81]. Enrichment of *Herbaspirillum*, *Porphyrobacter*, and *Bacteroides* was identified in cancer patients with a high risk of recurrence and progression, suggesting that these genera might serve as risk stratification biomarkers [81]. Another study was performed to analyze bacterial populations using 16S sequencing in mid-stream urine specimens obtained from male patients diagnosed with bladder cancer and healthy, age-matched males [82]. Although microbial diversity and overall microbiome composition did not change substantially between groups, the study detected more abundant operational taxonomic units (OTUs) belonging to the genus *Fusobacterium* as a potential protumorigenic pathogen enriched in the bladder cancer group. OTUs from the genera *Veillonella*, *Streptococcus*, and *Corynebacterium* were less prevalent in the bladder cancer group [82]. An additional study reported that the midstream urine samples from bladder cancer patients exhibited a higher abundance of *Actinomyces* than the control group. The study suggested that the increased prevalence of *Actinomyces europaeus* in bladder cancer patient samples may be diagnostic of bladder cancer [83]. More recently, bladder cancer patients' urine microbiota was compared to that of healthy controls by utilizing 16S rRNA sequencing of voided urine samples. Bacterial populations were analyzed using 16S sequencing in urine specimens taken from bladder cancer patients and healthy, age-matched controls [82]. While microbial diversity and overall microbiome composition did not vary substantially across groups, the genus *Fusobacterium* was substantially enriched in the bladder cancer group and can be considered as a potential protumorigenic pathogen [82]. In healthy urines, the genera *Veillonella*, *Streptococcus*, and *Corynebacterium* were more prevalent [82]. However, owing to the small sample size, more research is required to establish if the urine microbiota is linked with bladder cancer.

Although these studies performed on the bladder cancer patients suggest the potential relationship between the bladder microbiome and bladder cancer, these studies collected voided urine specimens (midstream urine samples) which mischaracterized the urinary bladder microbiome for the urogenital microbiota [6,7]. A further comparative study of microbial communities in urine obtained via suprapubic aspiration or transurethral catheter should be performed in order to examine the contribution of the urinary bladder microbiome in bladder cancer. A recent study evaluated the need to carefully compare the microbiome profiles linked with the urine and bladder mucosa in bladder cancer patients. Tissue samples were obtained from patients after transurethral excision of cancer tissue [11]. Simultaneously, urine samples were collected from the same individuals by transurethral resectoscopy. As "five suspicious genera," *Akkermansia*, *Bacteroides*, *Clostridium sensustricto*, *Enterobacter*, and *Klebsiella* were overrepresented in tissue samples compared to urine [11]. This study discovered significant differences in some taxa, suggesting that the bladder tissue microbiota and the urine microbiota may differ to some extent [2,11]. Greater knowledge of the microbiome's function in the development and progression of bladder cancer may open the way for novel treatment approaches. The urine microbiota may serve as a biomarker for bladder cancer and as a therapeutic target. Finally, Table 1 contains a description of the major bacterial genera found in individuals with urinary diseases.

Table 1. A summary of the bacterial genera reported in individuals with urinary disease.

Disorder	Subjects	Specimens	More Abundant Microbiome than Control Group	References
UI/OAB	Women with MUI	Catheterized urine	No difference in *Lactobacilli*, but six bacterial community types identified	[59]
	Women undergoing POP/ SUI surgery	Catheterized urine	OAB group: *Atopobium vaginae*, *Finegoldia magna*	[77]
	Women with OAB	Midstream urine and vaginal swab	OAB group: *Proteus* (Less: *Lactobacillus*)	[76]
	Women undergoing SUI surgery	Voided or catheterized urine	Hormone-negative women: (Less *Lactobacillus*, *Gardnerella*)	[83]
	Women with OAB	Catheterized urine	OAB group: *Sneathia*, *Staphylococcus*, *Proteus*, *Helcococcus*, *Gemella*, *Mycoplasma*, *Aerococcus*	[84]
	Women with daily UUI	Catheterized urine	UUI group: *Sphingomonadales*, *Chitinophaga*, *Brevundimonas*, *Cadidatus Planktoluna*, *Alteromonadaceae*, *Elizabethkingia*, *Methylobacterium*, *Caldicellulosiruptor*, *Stenotrophomonas*(less: *Prevotella*, *Comamonadaceae*, *Nocardioides*, *Mycobacterium*)	[62]
	Women seeking UUI treatment	Catheterized urine	UUI group: *Actinobaculum*, *Actinomyces*, *Aerococcus*, *Arthrobacter*, *Corynebacterium*, *Gardnerella*, *Oligella*, *Staphylococcus*, *Streptococcus*	[8]
IC/BPS	Women with IC/BPS	Midstream urine	IC/BPS group: *Lactobacillus gasseri* (less *Corynebacterium*)	[68]
	Women with IC/BPS	Midstream urine	No difference in genus	[85]
	Women with IC/BPS	Midstream urine and vaginal swab	No difference in genus	[86]
	Women with IC/BPS	Catheterized urine	IC group: (less *Lactobacillus acidophilus*)	[69]
	Women with IC/BPS	Stool and vaginalswab	IC/BPS group: (less *Eggerthella sinensis*, *Colinsella aerofaciens*, *F. prausnitzii*, *Odoribacter splanchnicus*, *Lactonifactor longoviformis*)	[87]
	Women with IC/BPS	Midstream urine	No difference in genus	[88]
	Women with IC	Midstream urine	IC group: -more *Lactobacillus*	[89]
UTI	Women with acute cystitis or recurrent cystitis	Catheterized urine	Acute cystitis group: *Pseudomonas*, *Acinetobacter*, *Enterobacteriaceae* Recurrent cystitis group: *Sphingomonas*, *Staphylococcus*, *Streptococcus*, *Rothia* spp	[46]
	postoperative urinary tract infection patients	Catheterized urine and vaginal swab	Patient group: *Escherichia coli*, *Klebsiella pneumoniae*, *P. aeruginosa* (Less *Lactobacillus iners*)	[90]
Bladder cancer	Bladder cancer patients	Midstream urine	Bladder cancer group: *Actinomyces europaeus*	[91]
	Men with non-muscle invasive bladder cancer	Midstream urine	Bladder cancer group: *Fusobacterium*, *Actinobaculum*, *Facklamia*, *Campylobacter*	[82]
	Men with bladder cancer	Midstream urine	Bladder cancer group: *Acinetobacter*, *Anaerococcus*, *Sphingobacterium* (Less: *Serratia*, *Proteus*, *Roseomonas*)	[81]
	Urothelial carcinoma patients	Midstream urine	Bladder cancer group: *Streptococcus*, *Pseudomonas*, *Anaerococcus*	[78]

2.11. Restoration of the Urinary Microbiome

Probiotics are described by the World Health Organization as "useful living microorganisms that have a beneficial impact on a person's health and physiology when ingested in adequate numbers." [92]. Because probiotic bacteria are designed to repair the microbiota, probiotics used to prevent and cure genitourinary infections should include urinary microbiota. The recent characterization of the urinary microbiome and its relationship with disease has led to the development of urobiome-targeted therapies. The development of novel treatment methods for urinary tract diseases is becoming more important. Fecal microbiota transplantation (FMT) restores the gut microbiome and boosts microbial diversity and is linked with a reduction in the colonization of antibiotic-resistant pathogens [22]. This FMT also increases the antibiotic sensitivity of uropathogens *E. coli* and *Klebsiella* in patients with UTI and recurrent *Clostridium* difficile infection [93]. Probiotics have been known to work by acidifying the mucosal surface, inhibiting pathogen adherence, producing compounds such as vitamins and immunomodulators, and interacting synergistically with the host's immune system [94]. Because *Lactobacillus* species are designed to repair the vaginal microbiota, probiotics used to prevent and cure genitourinary infections should include *Lactobacillus* species [95]. *Lactobacillus* is the recommended probiotic agent for the prevention and treatment of urogynecologic infections due to these characteristics. *Lactobacilli* may inhibit uropathogenic bacteria' adhesion, proliferation, and colonization [96]. It has been shown that healthy *Lactobacillus* species' microbial communities have a significant inhibitory impact on *E. coli* [97].

The concept of oral probiotics is founded on the fact that the microorganisms that cause the majority of urogenital infections migrate from the rectum to the perineal area, and then to the vagina and mesentery [44]. Clinical investigations have shown that *Lactobacillus* may exert its effects once it enters the vagina [98,99]. Probiotic capsules containing *L. rhamnosus* and *L. fermentes* were given orally once or twice day at a dosage of 10^9 CFU. The authors of this research found that probiotic capsules taken orally can help control the vaginal flora and may be beneficial for recurrent UTIs. Additionally, oral probiotics can be more pleasant for patients than vaginal probiotics, and patient compliance with therapy may be improved. In a meta-analysis of *Lactobacillus* applications that was published [100], a total of 294 patients from five published trials were reviewed, and it was shown that those given *Lactobacillus* probiotic vaginally is safe and efficient in preventing recurrent UTIs in adult women. The study concluded that ovules with a combination of *Lactobacillus crispatus* CTV-05 or *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* B-54 were the most successful approach and that a larger number of randomized clinical trials was required to evaluate oral probiotic therapies. Side effects associated with *Lactobacillus* prophylaxis were examined in seven of the trials, and none were found in four of them. In the other three studies, minor headache, increased hunger, or fever were reported [101].

Several studies demonstrated that the vaginal route could be an effective approach for managing UTI. Probiotics are critical in this respect; it has been shown that intravaginal injection of probiotic Lactin-V, consisting of *Lactobacillus crispatus*, results in long-term colonization of the urinary microbiota by commensal bacteria and a decreased incidence of UTI [54]. The screening of *Lactobacilli* isolates from the vagina of healthy women revealed many promising probiotic candidates, including *Lactobacillus rhamnosus*, *Lactobacillus helveticus*, and *Lactobacillus salivarius* [102]. Additionally, oral treatment of other *Lactobacillus* species, including as *L. acidophilus* and *L. plantarum*, in combination with vitamin A and cranberry extract, has been found to decrease the occurrence of UTI [103]. Probiotics have a number of beneficial effects, including reducing pathogen colonization, regulating host immunity, stimulating cell proliferation, and preserving the integrity of the epithelial barrier [104–106]. *L. crispatus's* protective strategy is mediated by lactic acid production, which has wider implications in the vaginal tract against *Chlamydia trachomatis* and *Candida albicans* infection [107]. Perhaps microbiome transfer and restoration treatments might be improved to include synthetic communities of health-associated *Lactobacilli*, which

are known to influence host-microbe interactions and maintain mucosal immunological homeostasis in the genitourinary tract [22].

Probiotics may be given orally or vaginally, but bladder instillation has been shown to be more beneficial than the previous methods since it enables direct colonization of the urine bladder. This colonization results in a change in bacterial proportions toward a more healthy microbial makeup, which is beneficial against UTIs. The *Lactobacillus acidophilus* strain is a well-characterized probiotic bacteria that has been shown to boost animal production performance and immunological responses. Because UTIs are typically treated with antibiotics, the importance of accurate bacterial identification has increased as a result of the prevalence of broad-spectrum antibiotic-resistant uropathogens. To overcome these resistances, it is critical to employ effective medicines that are directed against each specific causal agent [44,108]. While few suggest using only probiotics in the prevention of recurrent UTI, it is believed that probiotics may be beneficial when used in conjunction with alternative therapies or multi-drug treatment and prophylaxis of urogenital infections [44,108].

3. Conclusions

The research of the urinary tract's microbial population has made significant progress. Continuing research is required to optimize the methodologies used for microbial identification and to create a core microbial population in healthy persons. Additional research is needed to evaluate the relationship between the urinary microbiome and various urinary tract diseases. Eventually, this will enable microbiomes to be used as diagnostic, prognostic, and therapeutic biomarkers [2]. To develop targeted treatments, it is critical to combine microbiome genomes, transcriptomics, and functional capability with host status and response [22]. Concentrating on the application of emerging microbiome technologies to the urinary microbiome has the potential to revolutionize the treatment of UTIs and other bladder disorders, as well as to provide insight into the development of novel therapeutics and intervention strategies.

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References

1. Human Microbiome Project, C. Structure, function and diversity of the healthy human microbiome. *Nature* **2012**, *486*, 207–214. [CrossRef] [PubMed]
2. Perez-Carrasco, V.; Soriano-Lerma, A.; Soriano, M.; Gutiérrez-Fernández, J.; Garcia-Salcedo, J.A. Urinary Microbiome: Yin and Yang of the Urinary Tract. *Front. Cell. Infect. Microbiol.* **2021**, *11*, 617002. [CrossRef]
3. Zasloff, M. Antimicrobial Peptides, Innate Immunity, and the Normally Sterile Urinary Tract. *J. Am. Soc. Nephrol.* **2007**, *18*, 2810–2816. [CrossRef] [PubMed]
4. Mueller, E.R.; Wolfe, A.J.; Brubaker, L. Female urinary microbiota. *Curr. Opin. Urol.* **2017**, *27*, 282–286. [CrossRef]
5. Wolfe, A.J.; Brubaker, L. Urobiome updates: Advances in urinary microbiome research. *Nat. Rev. Urol.* **2018**, *16*, 73–74. [CrossRef]
6. Modena, B.D.; Milam, R.; Harrison, F.; A Cheeseman, J.; 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. *Arab. Archaeol. Epigr.* **2016**, *17*, 712–723. [CrossRef] [PubMed]

7. 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. [CrossRef]
8. 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*, e01283-14. [CrossRef]
9. Karstens, L.; Asquith, M.; Caruso, V.; Rosenbaum, J.T.; Fair, D.A.; Braun, J.; Gregory, W.T.; Nardos, R.; McWeeney, S.K. Community profiling of the urinary microbiota: Considerations for low-biomass samples. *Nat. Rev. Urol.* **2018**, *15*, 735–749. [CrossRef]
10. Wolfe, A.J.; Toh, E.; Shibata, N.; Rong, R.; Kenton, K.S.; Fitzgerald, M.; Mueller, E.R.; Schreckenberger, P.C.; Dong, Q.; Nelson, D.E.; et al. Evidence of Uncultivated Bacteria in the Adult Female Bladder. *J. Clin. Microbiol.* **2012**, *50*, 1376–1383. [CrossRef]
11. Mansour, B.; Monyók, Á.; Makra, N.; Gajdács, M.; Vadnay, I.; Ligeti, B.; Juhász, J.; Szabó, D.; Ostorházi, E. Bladder cancer-related microbiota: Examining differences in urine and tissue samples. *Sci. Rep.* **2020**, *10*, 1–10. [CrossRef]
12. Wolfe, A.J.; Brubaker, L. "Sterile Urine" and the Presence of Bacteria. *Eur. Urol.* **2015**, *68*, 173–174. [CrossRef] [PubMed]
13. Ishihara, T.; Watanabe, N.; Inoue, S.; Aoki, H.; Tsuji, T.; Yamamoto, B.; Yanagi, H.; Oki, M.; Kryukov, K.; Nakagawa, S.; et al. Usefulness of next-generation DNA sequencing for the diagnosis of urinary tract infection. *Drug Discov. Ther.* **2020**, *14*, 42–49. [CrossRef] [PubMed]
14. Nienhouse, V.; Gao, X.; Dong, Q.; Nelson, D.E.; Toh, E.; McKinley, K.; Schreckenberger, P.; Shibata, N.; Fok, C.S.; Mueller, E.R.; et al. Interplay between Bladder Microbiota and Urinary Antimicrobial Peptides: Mechanisms for Human Urinary Tract Infection Risk and Symptom Severity. *PLOS ONE* **2014**, *9*, e114185. [CrossRef]
15. 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. [CrossRef]
16. Aguilera-Arreola, M.G.; Peña, M.D.M.; Hernández-Martínez, F.; Enriques, S.R.J.; Verdín, B.R.; Majalca-Martínez, C.; Castro-Escarpulli, G.; Albarrán-Fernández, E.; Serrano-López, S.C. Cultivation-independent approach for the direct detection of bacteria in human clinical specimens as a tool for analysing culture-negative samples: A prospective study. *SpringerPlus* **2016**, *5*, 332. [CrossRef]
17. Moustafa, A.; Li, W.; Singh, H.; Moncera, K.J.; Torralba, M.G.; Yu, Y.; Manuel, O.; Biggs, W.; Venter, J.C.; Nelson, K.E.; et al. Microbial metagenome of urinary tract infection. *Sci. Rep.* **2018**, *8*, 1–12. [CrossRef] [PubMed]
18. Jovel, J.; Patterson, J.; Wang, W.; Hotte, N.; O'Keefe, S.; Mitchel, T.; Perry, T.; Kao, D.; Mason, A.; Madsen, K.L.; et al. Characterization of the Gut Microbiome Using 16S or Shotgun Metagenomics. *Front. Microbiol.* **2016**, *7*, 459. [CrossRef]
19. Price, T.K.; Dune, T.; Hilt, E.E.; Thomas-White, K.J.; Kliethermes, S.; Brincat, C.; Brubaker, L.; Wolfe, A.J.; Mueller, E.R.; Schreckenberger, P.C. The Clinical Urine Culture: Enhanced Techniques Improve Detection of Clinically Relevant Microorganisms. *J. Clin. Microbiol.* **2016**, *54*, 1216–1222. [CrossRef]
20. 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: Int. J. Obstet. Gynaecol.* **2019**, *127*, 193–201. [CrossRef]
21. Whiteside, S.A.; Razvi, H.; Dave, S.; Reid, G.; Burton, J. The microbiome of the urinary tract—A role beyond infection. *Nat. Rev. Urol.* **2015**, *12*, 81–90. [CrossRef] [PubMed]
22. Jones-Freeman, B.; Chonwerawong, M.; Marcelino, V.R.; Deshpande, A.V.; Forster, S.C.; Starkey, M.R. The microbiome and host mucosal interactions in urinary tract diseases. *Mucosal Immunol.* **2021**, 1–14. [CrossRef]
23. El-Deeb, O.S.; Atef, M.M.; Hafez, Y.M. The interplay between microbiota-dependent metabolite trimethylamine N -oxide, Transforming growth factor β /SMAD signaling and inflammasome activation in chronic kidney disease patients: A new mechanistic perspective. *J. Cell. Biochem.* **2019**, *120*, 14476–14485. [CrossRef] [PubMed]
24. Mora-Bau, G.; Platt, A.M.; Van Rooijen, N.; Randolph, G.J.; Albert, M.L.; Ingersoll, M.A. Macrophages Subvert Adaptive Immunity to Urinary Tract Infection. *PLOS Pathog.* **2015**, *11*, e1005044. [CrossRef]
25. Schiwon, M.; Weisheit, C.; Franken, L.; Gutweiler, S.; Dixit, A.; Meyer-Schwesinger, C.; Pohl, J.-M.; Maurice, N.; Thiebes, S.; Lorenz, K.; et al. Crosstalk between Sentinel and Helper Macrophages Permits Neutrophil Migration into Infected Uroepithelium. *Cell* **2014**, *156*, 456–468. [CrossRef] [PubMed]
26. Roje, B.; Elek, A.; Palada, V.; Bom, J.; Iljazović, A.; Šimić, A.; Sušak, L.; Vilović, K.; Strowig, T.; Vlahoviček, K.; et al. Microbiota Alters Urinary Bladder Weight and Gene Expression. *Microorganisms* **2020**, *8*, 421. [CrossRef] [PubMed]
27. Smith, K.; McCoy, K.D.; Macpherson, A.J. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Semin. Immunol.* **2007**, *19*, 59–69. [CrossRef]
28. McCoy, K.D.; Geuking, M.B.; Ronchi, F. Gut Microbiome Standardization in Control and Experimental Mice. *Curr. Protoc. Immunol.* **2017**, *117*, 1–23. [CrossRef]
29. Thomas-White, K.; Forster, S.C.; Kumar, N.; Van Kuiken, M.; Putonti, C.; Stares, M.D.; Hilt, E.E.; Price, T.K.; Wolfe, A.J.; Lawley, T.D. Culturing of female bladder bacteria reveals an interconnected urogenital microbiota. *Nat. Commun.* **2018**, *9*, 1–7. [CrossRef]
30. Gottschick, C.; Deng, Z.-L.; Vital, M.; Masur, C.; Abels, C.; Pieper, D.H.; Wagner-Döbler, I. The urinary microbiota of men and women and its changes in women during bacterial vaginosis and antibiotic treatment. *Microbiome* **2017**, *5*, 1–15. [CrossRef]
31. Lüthje, P.; Brauner, H.; Ramos, N.L.; Ovregaard, A.; Gläser, R.; Hirschberg, A.L.; Aspenström, P.; Brauner, A. Estrogen supports urothelial defense mechanisms, Sci. *Transl. Med.* **2013**, *5*, 190ra80. [CrossRef]

32. Raz, R.; Stamm, W.E. A controlled trial of intravaginal estriol in postmenopausal women with recurrent urinary tract infec-tions. *N. Engl. J. Med.* **1993**, *329*, 753–756. [CrossRef] [PubMed]
33. Jacobs, K.M.; Thomas-White, K.J.; Hilt, E.E.; Wolfe, A.J.; Waters, T.P. Microorganisms Identified in the Maternal Bladder: Discovery of the Maternal Bladder Microbiota. *AJP Rep.* **2017**, *7*, e188–e196. [CrossRef]
34. Komesu, Y.M.; Dinwiddie, D.L.; E Richter, H.; Lukacz, E.S.; Sung, V.W.; Siddiqui, N.Y.; Zyczynski, H.M.; Ridgeway, B.; Rogers, R.G.; Arya, L.A.; et al. Defining the relationship between vaginal and urinary microbiomes. *Am. J. Obstet. Gynecol.* **2019**, *222*, 154-e1. [CrossRef]
35. Dubourg, G.; Morand, A.; Mekhalif, F.; Godefroy, R.; Corthier, A.; Yacouba, A.; Diakite, A.; Cornu, F.; Cresci, M.; Brahimi, S.; et al. Deciphering the Urinary Microbiota Repertoire by Culturomics Reveals Mostly Anaerobic Bacteria From the Gut. *Front. Microbiol.* **2020**, *11*, 513305. [CrossRef]
36. Patton, J.P.; Nash, D.B.; Abrutyn, E. Urinary Tract Infection: Economic Considerations. *Med Clin. N. Am.* **1991**, *75*, 495–513. [CrossRef]
37. Dielubanza, E.J.; Schaeffer, A.J. Urinary tract infections in women. *Med. Clin. North. Am.* **2011**, *95*, 27–41. [CrossRef] [PubMed]
38. Foxman, B. Urinary tract infection syndromes: Occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infect. Dis. Clin. North. Am.* **2014**, *28*, 1–13. [CrossRef]
39. Orndorff, P.E.; Falkow, S. Organization and expression of genes responsible for type 1 piliation in *Escherichia coli*. *J. Bacteriol.* **1984**, *159*, 736–744. [CrossRef] [PubMed]
40. Hooton, T.M. Uncomplicated Urinary Tract Infection. *N. Engl. J. Med.* **2012**, *366*, 1028–1037. [CrossRef]
41. Roberts, J.A. Management of pyelonephritis and upper urinary tract infections. *Urol. Clin. N. Am.* **1999**, *26*, 753–763. [CrossRef]
42. Nicolle, L.E.; Bradley, S.; Colgan, R.; Rice, J.C.; Schaeffer, A.; Hooton, T.M. Infectious Diseases Society of America Guidelines for the Diagnosis and Treatment of Asymptomatic Bacteriuria in Adults. *Clin. Infect. Dis.* **2005**, *40*, 643–654. [CrossRef]
43. Grüneberg, R.N. Changes in urinary pathogens and their antibiotic sensitivities, 1971–1992. *J. Antimicrob. Chemother.* **1994**, *33*, 1–8. [CrossRef] [PubMed]
44. Akgul, T.; Karakan, T. The role of probiotics in women with recurrent urinary tract infections. *Turk. J. Urol.* **2018**, *44*, 377–383. [CrossRef] [PubMed]
45. Garretto, A.; Miller-Ensminger, T.; Ene, A.; Merchant, Z.; Shah, A.; Gerodias, A.; Biancofiori, A.; Canchola, S.; Canchola, S.; Castillo, E.; et al. Genomic Survey of E. coli From the Bladders of Women With and Without Lower Urinary Tract Symptoms. *Front. Microbiol.* **2020**, *11*, 2094. [CrossRef]
46. Yoo, J.-J.; Shin, H.; Song, J.; Kim, M.; Yun, J.; Kim, Z.; Lee, Y.; Lee, S.; Lee, K.; Kim, W.; et al. Urinary Microbiome Characteristics in Female Patients with Acute Uncomplicated Cystitis and Recurrent Cystitis. *J. Clin. Med.* **2021**, *10*, 1097. [CrossRef]
47. Gasiorek, M.; Hsieh, M.H.; Forster, C.S. Utility of DNA Next-Generation Sequencing and Expanded Quantitative Urine Culture in Diagnosis and Management of Chronic or Persistent Lower Urinary Tract Symptoms. *J. Clin. Microbiol.* **2019**, *58*. [CrossRef]
48. Finucane, T.E. "Urinary Tract Infection"-Requiem for a Heavyweight. *J. Am. Geriatr. Soc.* **2017**, *65*, 1650–1655. [CrossRef]
49. Zhanel, G.G.; Hisanaga, T.L.; Laing, N.M.; DeCorby, M.R.; Nichol, K.A.; Weshnoweski, B.; Johnson, J.; Noreddin, A.; Low, D.E.; Karlowsky, J.A. Antibiotic resistance in Escherichia coli outpatient urinary isolates: Final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). *Int. J. Antimicrob. Agents* **2006**, *27*, 468–475. [CrossRef]
50. Nicolle, L.E. Urinary Tract Pathogens in Complicated Infection and in Elderly Individuals. *J. Infect. Dis.* **2001**, *183*, S5–S8. [CrossRef] [PubMed]
51. Croxall, G.; Weston, V.; Joseph, S.; Manning, G.; Cheetham, P.; McNally, A. Increased human pathogenic potential of Escherichia coli from polymicrobial urinary tract infections in comparison to isolates from monomicrobial culture samples. *J. Med. Microbiol.* **2011**, *60*, 102–109. [CrossRef]
52. Lavigne, J.-P.; Nicolas-Chanoine, M.-H.; Bourg, G.; Moreau, J.; Sotto, A. Virulent Synergistic Effect between Enterococcus faecalis and Escherichia coli Assayed by Using the Caenorhabditis elegans Model. *PLOS ONE* **2008**, *3*, e3370. [CrossRef] [PubMed]
53. Raz, R. Urinary Tract Infection in Postmenopausal Women. *Korean J. Urol.* **2011**, *52*, 801–808. [CrossRef] [PubMed]
54. Stapleton, A.E.; Au-Yeung, M.; Hooton, T.M.; Fredricks, D.N.; Roberts, P.L.; Czaja, C.A.; Yarova-Yarovaya, Y.; Fiedler, T.; Cox, M.; Stamm, W.E. Randomized, Placebo-Controlled Phase 2 Trial of a Lactobacillus crispatus Probiotic Given Intravaginally for Prevention of Recurrent Urinary Tract Infection. *Clin. Infect. Dis.* **2011**, *52*, 1212–1217. [CrossRef]
55. Sumati, A.; Saritha, N. Association of urinary tract infection in women with bacterial vaginosis. *J. Glob. Infect. Dis.* **2009**, *1*, 151–152. [CrossRef]
56. Gilbert, N.M.; O'Brien, V.P.; Lewis, A.L. Transient microbiota exposures activate dormant Escherichia coli infection in the bladder and drive severe outcomes of recurrent disease. *PLOS Pathog.* **2017**, *13*, e1006238. [CrossRef]
57. Domann, E.; Hong, G.; Imirzalioglu, C.; Turschner, S.; Kühle, J.; Watzel, C.; Hain, T.; Hossain, H.; Chakraborty, T. Culture-Independent Identification of Pathogenic Bacteria and Polymicrobial Infections in the Genitourinary Tract of Renal Transplant Recipients. *J. Clin. Microbiol.* **2003**, *41*, 5500–5510. [CrossRef]
58. Aoki, Y.; Brown, H.W.; Brubaker, L.; Cornu, J.N.; Daly, J.O.; Cartwright, R. Urinary incontinence in women. *Nat. Rev. Dis. Prim.* **2017**, *3*, 1–20. [CrossRef]
59. Komesu, Y.M.; Network, F.T.P.F.D.; E Richter, H.; Carper, B.; Dinwiddie, D.L.; Lukacz, E.S.; Siddiqui, N.Y.; Sung, V.W.; Zyczynski, H.M.; Ridgeway, B.; et al. The urinary microbiome in women with mixed urinary incontinence compared to similarly aged controls. *Int. Urogynecol. J.* **2018**, *29*, 1785–1795. [CrossRef]

60. Harwich, M.D.; Alves, J.M.; A Buck, G.; Strauss, J.F.; Patterson, J.L.; Oki, A.T.; Girerd, P.H.; Jefferson, K.K. Drawing the line between commensal and pathogenic Gardnerella vaginalis through genome analysis and virulence studies. *BMC Genom.* **2010**, *11*, 375. [CrossRef] [PubMed]
61. Abbasian, B.; Shair, A.; O'Gorman, D.B.; Pena-Diaz, A.M.; Brennan, L.; Engelbrecht, K.; Koenig, D.W.; Reid, G.; Burton, J.P. Potential Role of Extracellular ATP Released by Bacteria in Bladder Infection and Contractility. *mSphere* **2019**, *4*, e00439-19. [CrossRef] [PubMed]
62. Karstens, L.; Asquith, M.; Davin, S.; Stauffer, P.; Fair, D.; Gregory, W.T.; Rosenbaum, J.T.; McWeeney, S.K.; Nardos, R. Does the Urinary Microbiome Play a Role in Urgency Urinary Incontinence and Its Severity? *Front. Cell. Infect. Microbiol.* **2016**, *6*, 78. [CrossRef] [PubMed]
63. Hanno, P.M.; Burks, D.A.; Clemens, J.Q.; Dmochowski, R.R.; Erickson, D.; Fitzgerald, M.P.; Forrest, J.B.; Gordon, B.; Gray, M.; Mayer, R.D.; et al. AUA Guideline for the Diagnosis and Treatment of Interstitial Cystitis/Bladder Pain Syndrome. *J. Urol.* **2011**, *185*, 2162–2170. [CrossRef]
64. Giannantoni, A.; Bini, V.; Dmochowski, R.; Hanno, P.; Nickel, J.C.; Proietti, S.; Wyndaele, J.J. Contemporary Management of the Painful Bladder: A Systematic Review. *Eur. Urol.* **2012**, *61*, 29–53. [CrossRef] [PubMed]
65. Heritz, D.M.; Lacroix, J.-M.Y.; Batra, S.D.; Jarvi, K.A.; Beheshti, B.; Mittelman, M.W. Detection of eubacteria in interstitial cystitis by 16S rDNA amplification. *J. Urol.* **1997**, *158*, 2291–2295. [CrossRef]
66. Haarala, M.; Kiiholma, P.; Nurmi, M.; Uksila, J.; Alanen, A. The role of Borrelia burgdorferi in interstitial cystitis. *Eur. Urol.* **2000**, *37*, 395–399. [CrossRef]
67. Agarwal, M.; Dixon, R. A study to detect Helicobacter pylori in fresh and archival specimens from patients with interstitial cystitis, using amplification methods. *BJU Int.* **2003**, *91*, 814–816. [CrossRef]
68. Nickel, J.C.; Stephens-Shields, A.J.; Landis, J.R.; Mullins, C.; Van Bokhoven, A.; Lucia, M.S.; Henderson, J.P.; Sen, B.; Krol, J.E.; Ehrlich, G.D.; et al. A Culture-Independent Analysis of the Microbiota of Female Interstitial Cystitis/Bladder Pain Syndrome Participants in the MAPP Research Network. *J. Clin. Med.* **2019**, *8*, 415. [CrossRef]
69. Abernethy, M.G.; Rosenfeld, A.; White, J.R.; Mueller, M.G.; Lewicky-Gaupp, C.; Kenton, K. Urinary Microbiome and Cytokine Levels in Women With Interstitial Cystitis. *Obstet. Gynecol.* **2017**, *129*, 500–506. [CrossRef]
70. Li, H.; Zhang, L.; Chen, L.; Zhu, Q.; Wang, W.; Qiao, J. Lactobacillus acidophilus alleviates the inflammatory response to enterotoxigenic Escherichia coli K88 via inhibition of the NF-κB and p38 mitogen-activated protein kinase signaling pathways in piglets. *BMC Microbiol.* **2016**, *16*, 1–8. [CrossRef]
71. Qiao, J.; Li, H.; Wang, Z.; Wang, W. Effects of Lactobacillus acidophilus dietary supplementation on the performance, intestinal barrier function, rectal microflora and serum immune function in weaned piglets challenged with *Escherichia coli* lipopolysaccharide. *Antonie van Leeuwenhoek* **2015**, *107*, 883–891. [CrossRef] [PubMed]
72. Stewart, W.F.; Van Rooyen, J.B.; Cundiff, G.; Abrams, P.; Herzog, A.R.; Corey, R.; Hunt, T.L.; Wein, A.J. Prevalence and burden of overactive bladder in the United States. *World J. Urol.* **2003**, *20*, 327–336. [CrossRef] [PubMed]
73. Haylen, B.T.; de Ridder, D.; Freeman, R.M.; Swift, S.E.; Berghmans, B.; Lee, J.; Monga, A.; Petri, E.; Rizk, D.E.; Sand, P.K.; et al. An international urogynecological association (IUGA)/international continence society (ICS) joint report on the terminology for female pelvic floor dysfunction. *Neurourol. Urodynamics* **2009**, *29*, 4–20. [CrossRef]
74. Van Dijk, M.M.; Wijkstra, H.; Debruyne, F.M.; De La Rosette, J.J.; Michel, M.C. The role of nocturia in the quality of life of men with lower urinary tract symptoms. *BJU Int.* **2010**, *105*, 1141–1146. [CrossRef] [PubMed]
75. Leron, E.; Weintraub, A.Y.; Mastrolia, S.A.; Schwarzman, P. Overactive Bladder Syndrome: Evaluation and Management. *Curr. Urol.* **2018**, *11*, 117–125. [CrossRef]
76. 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. [CrossRef]
77. Fok, C.S.; Gao, X.; Lin, H.; Thomas-White, K.; Mueller, E.R.; Wolfe, A.J.; Dong, Q.; Brubaker, L. Urinary symptoms are associated with certain urinary microbes in urogynecologic surgical patients. *Int. Urogynecol. J.* **2018**, *29*, 1765–1771. [CrossRef]
78. Xu, W.; Yang, L.; Lee, P.; Huang, W.; Nossa, C.; Ma, Y.; Deng, F.-M.; Zhou, M.; Melamed, J.; Pei, Z. Mini-review: Perspective of the microbiome in the pathogenesis of urothelial carcinoma. *Am. J. Clin. Exp. Urol.* **2014**, *2*, 57–61.
79. Bray, F.; Me, J.F.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer J. Clin.* **2018**, *68*, 394–424. [CrossRef]
80. Crow, P.; Ritchie, A. National and international variation in the registration of bladder cancer. *BJU Int.* **2003**, *92*, 563–566. [CrossRef]
81. Wu, P.; Zhang, G.; Zhao, J.; Chen, J.; Chen, Y.; Huang, W.; Zhong, J.; Zeng, J. Corrigendum: Profiling the Urinary Microbiota in Male Patients With Bladder Cancer in China. *Front. Cell. Infect. Microbiol.* **2018**, *8*, 429. [CrossRef]
82. Popović, V.B.; Šitum, M.; Chow, C.-E.T.; Chan, L.S.; Roje, B.; Terzić, J. The urinary microbiome associated with bladder cancer. *Sci. Rep.* **2018**, *8*, 1–8. [CrossRef]
83. 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.* **2016**, *216*, 55-e1. [CrossRef]

84. Wu, P.; Chen, Y.; Zhao, J.; Zhang, G.; Chen, J.; Wang, J.; Zhang, H. Urinary Microbiome and Psychological Factors in Women with Overactive Bladder. *Front. Cell. Infect. Microbiol.* **2017**, *7*, 488. [CrossRef]
85. Bresler, L.; Price, T.K.; Hilt, E.E.; Joyce, C.; Fitzgerald, C.M.; Wolfe, A.J. Female lower urinary tract microbiota do not associate with IC/PBS symptoms: A case-controlled study. *Int. Urogynecol. J.* **2019**, *30*, 1835–1842. [CrossRef] [PubMed]
86. Meriwether, K.V.; Lei, Z.; Singh, R.; Gaskins, J.; Hobson, D.T.G.; Jala, V. The Vaginal and Urinary Microbiomes in Premenopausal Women With Interstitial Cystitis/Bladder Pain Syndrome as Compared to Unaffected Controls: A Pilot Cross-Sectional Study. *Front. Cell. Infect. Microbiol.* **2019**, *9*, 92. [CrossRef]
87. Braundmeier-Fleming, A.; Russell, N.T.; Yang, W.; Nas, M.Y.; Yaggie, R.E.; Berry, M.; Bachrach, L.; Flury, S.C.; Marko, D.S.; Bushell, C.B.; et al. Stool-based biomarkers of interstitial cystitis/bladder pain syndrome. *Sci. Rep.* **2016**, *6*, 26083. [CrossRef]
88. Nickel, J.C.; Stephens, A.; Landis, J.R.; Mullins, C.; Van Bokhoven, A.; Lucia, M.S.; Ehrlich, G. MAPP Research Network Assessment of the Lower Urinary Tract Microbiota during Symptom Flare in Women with Urologic Chronic Pelvic Pain Syndrome: A MAPP Network Study. *J. Urol.* **2016**, *195*, 356–362. [CrossRef] [PubMed]
89. Siddiqui, H.; Lagesen, K.; Nederbragt, A.J.; Jeansson, S.L.; Jakobsen, K.S. Alterations of microbiota in urine from women with interstitial cystitis. *BMC Microbiol.* **2012**, *12*, 205. [CrossRef]
90. Thomas-White, K.; Gao, X.; Lin, H.; Fok, C.S.; Ghanayem, K.; Mueller, E.R.; Dong, Q.; Brubaker, L.; Wolfe, A.J. Urinary microbes and postoperative urinary tract infection risk in urogynecologic surgical patients. *Int. Urogynecol. J.* **2018**, *29*, 1797–1805. [CrossRef]
91. Bi, H.; Tian, Y.; Song, C.; Li, J.; Liu, T.; Chen, Z.; Chen, C.; Huang, Y.; Zhang, Y. Urinary microbiota – a potential biomarker and therapeutic target for bladder cancer. *J. Med Microbiol.* **2019**, *68*, 1471–1478. [CrossRef] [PubMed]
92. Reid, G.; Dols, J.; Miller, W. Targeting the vaginal microbiota with probiotics as a means to counteract infections. *Curr. Opin. Clin. Nutr. Metab. Care* **2009**, *12*, 583–587. [CrossRef] [PubMed]
93. Tariq, R.; Pardi, D.S.; Tosh, P.K.; Walker, R.C.; Razonable, R.R.; Khanna, S. Fecal Microbiota Transplantation for recurrent clostridium difficile infection reduces recurrent urinary tract infection frequency. *Clin. Infect. Dis.* **2017**, *65*, 1745–1747. [CrossRef] [PubMed]
94. Iannitti, T.; Palmieri, B. Therapeutical use of probiotic formulations in clinical practice. *Clin. Nutr.* **2010**, *29*, 701–725. [CrossRef]
95. Govender, Y.; Gabriel, I.; Minassian, V.; Fichorova, R. The Current Evidence on the Association Between the Urinary Microbiome and Urinary Incontinence in Women. *Front. Cell. Infect. Microbiol.* **2019**, *9*, 133. [CrossRef]
96. Hanson, L.; VandeVusse, L.; Jermé, M.; Abad, C.L.; Safdar, N. Probiotics for Treatment and Prevention of Urogenital Infections in Women: A Systematic Review. *J. Midwifery Women's Heal.* **2016**, *61*, 339–355. [CrossRef] [PubMed]
97. Beerepoot, M.A.J.; Geerlings, S.E.; Van Haarst, E.P.; Van Charante, N.M.; ter Riet, G. Nonantibiotic Prophylaxis for Recurrent Urinary Tract Infections: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *J. Urol.* **2013**, *190*, 1981–1989. [CrossRef]
98. Reid, G.; Beuerman, D.; Heinemann, C.; Bruce, A.W. Probiotic Lactobacillusdose required to restore and maintain a normal vaginal flora. *FEMS Immunol. Med Microbiol.* **2001**, *32*, 37–41. [CrossRef]
99. Morelli, L.; Zonenenschain, D.; Del Piano, M.; Cognein, P. Utilization of the Intestinal Tract as a Delivery System for Urogenital Probiotics. *J. Clin. Gastroenterol.* **2004**, *38*, S107–S110. [CrossRef]
100. Grin, P.; Kowalewska, P.M.; Alhazzan, W.; E Fox-Robichaud, A. Lactobacillus for preventing recurrent urinary tract infections in women: Meta-analysis. *Can. J. Urol.* **2013**, *20*, 6607–6614.
101. Abad, C.; Safdar, N. The Role of Lactobacillus Probiotics in the Treatment or Prevention of Urogenital Infections – A Systematic Review. *J. Chemother.* **2009**, *21*, 243–252. [CrossRef] [PubMed]
102. Pino, A.; Bartolo, E.; Caggia, C.; Cianci, A.; Randazzo, C.L. Detection of vaginal lactobacilli as probiotic candidates. *Sci. Rep.* **2019**, *9*, 1–10. [CrossRef] [PubMed]
103. Koradia, P.; Kapadia, S.; Trivedi, Y.; Chanchu, G.; Harper, A. Probiotic and cranberry supplementation for preventing recurrent uncomplicated urinary tract infections in premenopausal women: A controlled pilot study. *Expert Rev. Anti-infective Ther.* **2019**, *17*, 733–740. [CrossRef] [PubMed]
104. van Hemert, S.; Meijerink, M.; Molenaar, D.; A Bron, P.; de Vos, P.; Kleerebezem, M.; Wells, J.M.; Marco, M.L. Identification of Lactobacillus plantarum genes modulating the cytokine response of human peripheral blood mononuclear cells. *BMC Microbiol.* **2010**, *10*, 293. [CrossRef] [PubMed]
105. Tkhruni, F.N.; Aghajanyan, A.E.; Balabekyan, T.R.; Khachatryan, T.V.; Karapetyan, K.J. Characteristic of Bacteriocins of Lactobacillus rhamnosus BTK 20-12 Potential Probiotic Strain. *Probiotics Antimicrob. Proteins* **2019**, *12*, 716–724. [CrossRef]
106. Johnson-Henry, K.C.; Hagen, K.E.; Gordonpour, M.; Tompkins, T.A.; Sherman, P.M. Surface-layer protein extracts from Lactobacillus helveticus inhibit enterohaemorrhagic Escherichia coli O157:H7 adhesion to epithelial cells. *Cell. Microbiol.* **2006**, *9*, 356–367. [CrossRef]
107. Nardini, P.; Palomino, R.A.N.; Parolin, C.; Laghi, L.; Foschi, C.; Cevenini, R.; Vitali, B.; Marangoni, A. Lactobacillus crispatus inhibits the infectivity of Chlamydia trachomatis elementary bodies, in vitro study. *Sci. Rep.* **2016**, *6*, 29024. [CrossRef] [PubMed]
108. Hiergeist, A.; Gessner, A. Clinical implications of the microbiome in urinary tract diseases. *Curr. Opin. Urol.* **2017**, *27*, 93–98. [CrossRef]