The microbiome of the urinary tract —a role beyond infection

Samantha A. Whiteside, Hassan Razvi, Sumit Dave, Gregor Reid and Jeremy P. Burton

Abstract | Urologists rarely need to consider bacteria beyond their role in infectious disease. However, emerging evidence shows that the microorganisms inhabiting many sites of the body, including the urinary tract—which has long been assumed sterile in healthy individuals—might have a role in maintaining urinary health. Studies of the urinary microbiota have identified remarkable differences between healthy populations and those with urologic diseases. Microorganisms at sites distal to the kidney, bladder and urethra are likely to have a profound effect on urologic health, both positive and negative, owing to their metabolic output and other contributions. Connections between the gut microbiota and renal stone formation have already been discovered. In addition, bacteria are also used in the prevention of bladder cancer recurrence. In the future, urologists will need to consider possible influences of the microbiome in diagnosis and treatment of certain urological conditions. New insights might provide an opportunity to predict the risk of developing certain urological diseases and could enable the development of innovative therapeutic strategies.

Whiteside, S. A. *et al. Nat. Rev. Urol.* **12**, 81–90 (2015); published online 20 January 2015; doi:10.1038/nrurol.2014.361

Introduction

The microbiota is defined as the microorganisms in a particular environment.[1,2] More specifically, the term refers to the microbial taxa that are associated with an environment and are revealed using molecular techniques such as 16S ribosomal RNA (rRNA) sequencing. Conversely, the term microbiome is less firmly defined. Some groups limit the use of microbiome to the catalogue of microbes and their genes only,[2] whereas our group and others prefer to refer to this as the metagenome (all genetic material of a population including plasmids). The term microbiome is used as a reference to the habitat as a whole,[1] thus, incorporating the biotic and abiotic factors, encompassing host and microorganism genomes and environmental conditions (Figure 1). The populations are composed of bacteria, archaea, viruses and fungi, which are predominantly found in the gastrointestinal tract, but also in other exposed tissues, such as the skin, upper respiratory and urogenital tracts.[3–5] However, other tissues that were once considered sterile, such as the brain, breast, placenta and the urinary tract, also harbour unique bacterial communities.[6–9] At present, our knowledge of the microbiome is restricted to bacterial information, owing to technical limitations, but future advancements will enable a better understanding of the interactions between the different populations and their roles in the microbiome.

The recent identification of a microbiota in the bladder has important implications for the urologist. Studies of other body systems suggest that the microbiota is critical in the maintenance of health and/or development of disease. For example in the gastrointestinal tract, links between gut dysbiosis, chronic *Clostridium difficile* infection and colorectal cancer have been reported.[10–12] For the urinary tract, researchers have only begun to assess the relationship between the urinary microbiota and urologic disease.

It is now widely appreciated that the gut microbiota has a key role in homeostasis, regulating health and disease at distal sites throughout the body. Gastrointestinal dysbiosis has been linked to abnormalities within the brain,[13] heart,[14] musculoskeletal system[15] and of metabolic processes.[16] Notably, a recent metagenomic analysis of intestinal microbes highlights the power of the microbiome, which was a better predictor of the development of type 2 diabetes than the individual's genomic composition.[17]

However, the relationship between these actively metabolizing organisms and urogenital health has yet to be completely elucidated. Given the role of the kidneys and bladder in filtration and storage of waste, respectively, microbial profiles and microbial metabolites of the gut and other organs might influence the urinary microbiota, and alterations might affect urinary homeostasis. Whether the microbiomes of these sites are predictive of the risk of urological disease or malfunction is unclear.

This Review seeks to characterize the microbiome–host relationship with regards to urological health and examines the potential of probiotic intervention to modulate the risk of disease.

The microbiota of the urinary tract

A number of recent studies suggest that the urinary tract harbours a unique urinary microbiota,[7,18–29] which is substantially different from the populations of the gut and

Department of Microbiology and Immunology (**S.A.W.**), Division of Urology, Department of Surgery (**H.R.**, **S.D.**, **J.P.B.**), The University of Western Ontario, 1151 Richmond Street, London, ON N6A 3K7, Canada. Canadian Centre for Human Microbiome and Probiotic Research, Lawson Health Research Institute, 268 Grosvenor Street, London, ON N6A 4V2, Canada (**G.R.**).

Correspondence to: J.P.B. jeremy.burton@lawsonresearch.com

Competing interests
The authors declare no competing interests.

Key points

- Contrary to doctrine, the urinary tract is inhabited by a unique urinary microbiota; further research is needed to characterize this microbial community in health and disease
- Alterations in the urinary microbiota have been linked to urologic disease, such as neurogenic bladder dysfunction, interstitial cystitis and urgency urinary incontinence
- The microbiome, particularly that of the gut, has a key role in the development and progression of disease within the urinary tract
- Although early studies of probiotics in patients with nephrolithiasis or bladder cancer have demonstrated variable effectiveness, such alternative treatment strategies focused on reconstituting the microbiome should be further explored

vagina. These findings challenge the long-held doctrine of the urinary tract proximal of the urethra as a sterile environment and raise the question of why these bacteria had not been discovered previously.

Discovery

Traditionally, bacteriological culture of urine is used to isolate and identify pathogens involved in the development of UTIs; for example, aerobic, fast-growing organisms, such as *Escherichia coli* and *Enterococcus faecalis*.[30] Conversely, slow-growing, anaerobic or fastidious organisms, such as *Corynebacterium, Lactobacillus* and *Ureaplasma*, are rarely isolated from the urinary tract, as routine culture techniques are not designed to support the growth of these genera.[18,31–33] However, using advanced detection technologies, these bacteria have been identified as members of the urinary microbiota in multiple studies.[19–23] Similarly, owing to such advanced culture and molecular techniques, *Lactobacillus iners* replaced *Lactobacillus acidophilus* as the generally accepted dominant species in the microbiota of the healthy vagina.[34,35] Hence, improvements in PCR and 16S rRNA sequencing technology have made it possible to ascertain the bacterial communities of the body,[36] leading to the discovery of the microbiota of the urinary tract.

This revelation raises many questions. How can so many species, including some known to cause UTI, survive and multiply within the urinary tract without causing chronic infection? The required nutrients seem to be available, and presumably many organisms carry the necessary virulence factors for infection. Why would genera, such as *Jonquetella, Parvimonas, Proteiniphilum* and *Saccharofermentans*, previously unfamiliar to urologists, seemingly arbitrarily colonize the urinary tract of individuals aged >70 years and what are these organisms doing?[27]

Stapleton[37] has suggested that nonmodifiable host factors have a role in the colonization by these organisms. If host factors indeed 'select' the species that become established, what are the receptors and the basis for selection in evolutionary terms for a given individual? Did bacteria and their hosts coevolve? This process might encompass human urinary tract adaptation to accommodate certain bacterial species, for example, through expression of specific receptors, as well as mutations in bacteria enabling adherence to the uroepithelium and survival in the variable urinary conditions. Clinically, it is known that children of women with a history of recurrent UTIs often also have recurrent infections.[38] Is this increased susceptibility to UTIs caused by genetic inheritance or the transfer of bacterial species during childhood or, more specifically, at birth? If the latter is the case, should some babies be delivered by Caesarean section and artificially colonized by selected bacterial species? Indeed, artificial colonization of children delivered by Caesarean section using probiotics to prevent the development of IgE-associated allergy has previously been successful.[39]

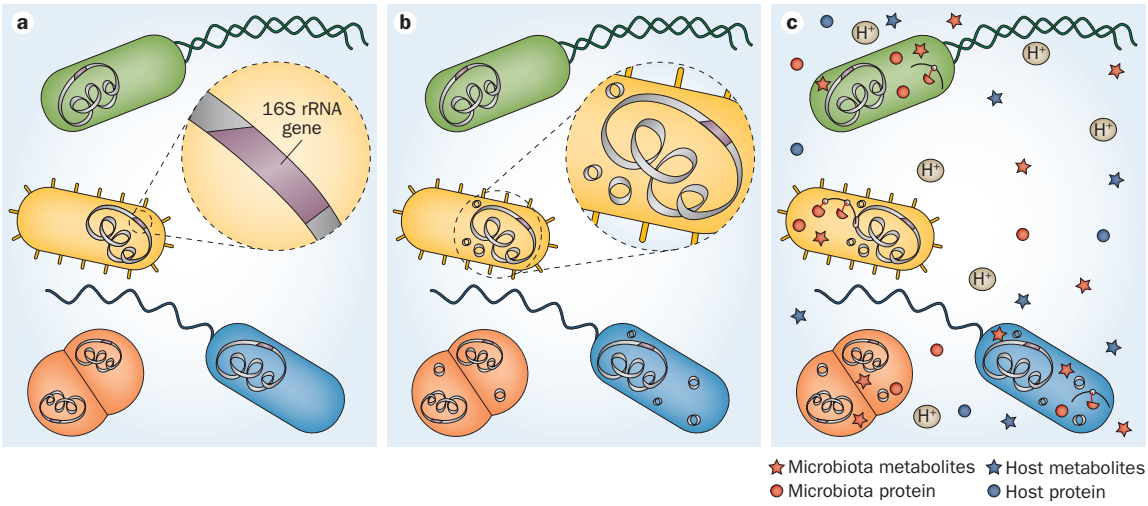

Figure 1 | Definition of the microbiota, metagenome and microbiome as used in this Review. Each image represents the same population; however, different approaches to define the population provide different information. **a** | Microbiota: 16S rRNA surveys are used to taxonomically identify the microorganisms in the environment. **b** | Metagenome: the genes and genomes of the microbiota, including plasmids, highlighting the genetic potential of the population. **c** | Microbiome: the genes and genomes of the microbiota, as well as the products of the microbiota and the host environment. Abbreviation: rRNA, ribosomal RNA.

Table 1 | Studies characterizing the urine microbiota

Study	Patients (*n*)	Notable taxa*	Sample collection method
Nelson *et al.* (2010)[25]	Men with STI (10) Men without STI (9)	*Lactobacillus, Sneathia, Gemella, Aerococcus, Corynebacterium, Streptococcus, Veillonella, Prevotella, Anaerococcus, Propionibacterium, Atopobium, Staphylococcus*	First-void urine
Dong *et al.* (2011)[26]	Men with STI (10) Men without STI (22)	*Lactobacillus, Sneathia, Veillonella, Corynebacterium, Prevotella, Streptococcus, Ureaplasma, Mycoplasma, Anaerococcus, Atopobium, Aerococcus, Staphylococcus, Gemella, Enterococcus, Finegoldia, Neisseria, Propionibacterium, Ralstonia*	First-void urine
Siddiqui *et al.* (2011)[22]	Healthy women (8)	*Lactobacillus, Prevotella, Gardnerella, Peptoniphilus, Dialister, Finegoldia, Anaerococcus, Allisonella, Streptococcus, Staphylococcus*	Clean-catch midstream urine
Fouts *et al.* (2012)[24]	Healthy controls (26; 58% women) Patients with NBD (27; 48% women)	Orders: Lactobacillales, Enterobacteriales, Actinomycetales, Bacillales, Clostridiales, Bacteroidales, Burkholderiales, Pseudomonadales, Bifidobacteriales, Coriobacteriales	Midstream urine, intermittent catheterization, Foley catheter
Nelson *et al.* (2012)[21]	Healthy adolescent men (18)	*Lactobacillus, Streptococcus, Sneathia, Mycoplasma, Ureaplasma*	First-void urine
Siddiqui *et al.* (2012)[23]	Women with IC (8)	*Lactobacillus, Gardnerella, Corynebacterium, Prevotella, Ureaplasma, Enterococcus, Atopobium, Proteus, Cronobacter*	Clean-catch midstream urine
Wolfe *et al.* (2012)[7]	Healthy women (12) Women with POP or UI (11)	*Lactobacillus, Actinobaculum, Aerococcus, Anaerococcus, Atopobium, Burkholderia, Corynebacterium, Gardnerella, Prevotella, Ralstonia, Sneathia, Staphylococcus, Streptococcus, Veillonella*	Clean-catch midstream urine, suprapubic aspirate, transurethral catheter
Lewis *et al.* (2013)[27]	Healthy men (6) Healthy women (10)	*Jonquetella, Parvimonas, Proteiniphilum, Saccharofermentans* Phyla: Actinobacteria, Bacteroidetes	Clean-catch midstream urine
Fricke *et al.* (2014)[20]	Patients receiving first renal transplant (60; 37% women)	*Lactobacillus, Enterococcus, Pseudomonas, Streptococcus* Families: Bifidobacteriaceae, Corynebacterineae	Not described
Hilt *et al.* (2014)[18]	Healthy women (24) Women with OAB (41)	*Lactobacillus, Corynebacterium, Streptococcus, Actinomyces, Staphylococcus, Aerococcus, Gardnerella, Bifidobacterium, Actinobaculum*	Transurethral catheterization
Pearce *et al.* (2014)[19]	Healthy women (58) Women with urgency UI (60)	*Gardnerella, Lactobacillus, Actinobaculum, Actinomyces, Aerococcus, Arthrobacter, Corynebacterium, Oligella, Staphylococcus, Streptococcus*	Transurethral catheterization
Willner *et al.* (2014)[28]	Patients with acute uncomplicated UTI (50; 76% women)	*Anaerococcus, Peptoniphilus, Streptococcus, Lactobacillus, Staphylococcus, Escherichia, Pseudomonas*	Midstream urine

*Identified by the authors of the original studies as predominant or of significantly more prevalent than other populations; listed as genera, unless otherwise noted. Abbreviations: IC, interstitial cystitis; NBD, neurogenic bladder dysfunction; OAB, overactive bladder; POP, pelvic organ prolapse; STI, sexually transmitted infection; UI, urinary incontinence.

Alterations and disease

Investigations of bacterial populations in the urinary tract of healthy volunteers and patients with different diseases have revealed an altered microbiota in individuals with neurogenic bladder dysfunction (NBD), interstitial cystitis, urgency urinary incontinence and sexually transmitted infections (Table 1).[19,23–26] In patients with NBD, the alterations to the microbiota seem to positively correlate with the duration of the condition and the type of catheter used in bladder emptying.[24] The latter is intriguing and supports the 'receptivity' concept, whereby the presence of a new surface 'attracts' certain organisms, changing the dynamics of the microbiota. Urine from patients with interstitial cystitis had lower bacterial diversity than urine from healthy volunteers compounded by an increase in the relative abundance of the genus *Lactobacillus* (92% versus 57%).[23] This finding raises two questions. First, is the loss of diversity a cause of or caused by the condition? Second, why is the increase specific for *Lactobacillus*, a genus that is considered beneficial in both the intestine and vagina, in a diseased state?[10,40,41] Interestingly, reduced bacterial diversity has also been correlated with other chronic inflammatory states, including obesity and inflammatory bowel disease.[42,43] Evidently, urinary microbiota studies are providing a whole range of novel ideas about urinary health and disease, yet still demonstrate that the microbiota of patients with symptomatic UTIs are dominated by organisms that have previously also been identified via traditional culture methods.[28]

Microbiota of different populations

Although the numbers of studies are limited, some found significant differences between the urinary microbiota of men and women.[24,27] This finding is not surprising given the differences in anatomical structure, hormones and local defences, but it is worthy of study in relation to disease susceptibility. For example, analysis of the EUROCARE-4 cancer survival data highlighted the disparity in bladder cancer survival between the sexes:

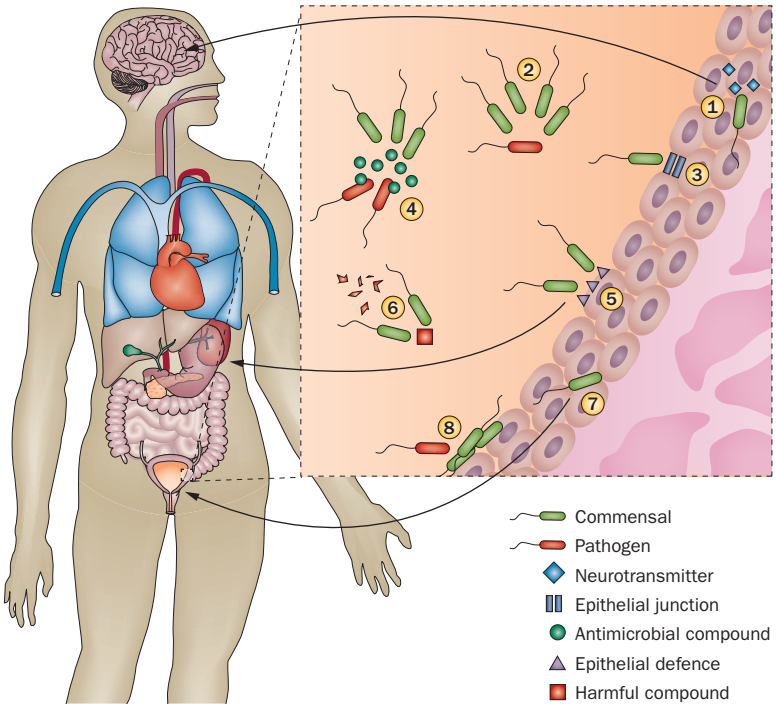

Figure 2 | Potential roles of the urinary microbiota in homeostasis of the urinary tract. Bacteria might produce neurotransmitters that interact with the nervous system (1). Commensal bacteria might outcompete pathogens for common resources (2). Bacteria might have a role in the regulation and maintenance of epithelial junctions (3). Commensals might produce antimicrobial compounds that kill pathogens (4). Bacteria might prime epithelial defences, including immune defences (5). Commensal bacteria might degrade harmful compounds (6). Bacteria might be necessary for proper development of the urinary tract, including the uroepithelium, immune system and peripheral nervous system within the bladder and surrounding tissues (7). Commensals might create a barrier, blocking pathogen access to the uroepithelium (8).

the age-adjusted 5-year relative survival was 4.2% lower for women compared with men.[44] By contrast, for all cancers combined, the report noted a significant survival advantage for women. As an example regarding noncancerous urinary tract diseases, one study demonstrated that men have a higher incidence of renal calculi compared with women.[45] It is possible that differences in the urinary microbiota of men and women have a role in these disparities.

To date, the variations in detected organisms between samples have been too high to indicate that comparable bacterial communities in individuals of the same age or gender exist.[22,25–27] However, Lewis *et al.*[27] propose the presence of a 'core' bladder microbiota—a subset of bacteria that exist at variable abundances within the urinary tract regardless of age. This hypothesis needs to be confirmed by large studies accompanied by investigations to identify why these organisms have evolved with the human urinary tract. This evolution could encompass coevolution, but also changes in the urinary microbiota with an individual's age: the microbiota of children are likely to differ from adults, and the microbiota of adults are likely to differ within age groups, for example, owing to changes in urinary metabolites, personal hygiene and voiding habits.

Maintenance of homeostasis

Assuming a mutualistic relationship, in which the bacteria benefit from the host's nutrient supply, pH, oxygen concentration and other survival factors, it remains unclear what advantage the microbiota provide to the host. The human microbiome is critical in the maintenance of health and development at different sites throughout the body, but whether the urinary microbiota have a role similar to that of bacterial communities at other mucosal sites requires further investigation (Figure 2). In addition, it is known that microbes are important in establishing the immune system after birth and maintaining its effectiveness throughout life.[46] The presence of dedicated immune cells within the urinary tract raises the question of whether the urinary microbiota has a similar role in priming the immune system.

Bacteria are also able to interact with many environmental toxins, such as heavy metals, polycyclic aromatic hydrocarbons, pesticides, ochratoxins, plastic monomers and organic compounds.[47–50] After certain toxins have been removed from the blood stream through renal filtration, their storage within the bladder provides ample time for the urinary microbiota to interact with and alter these compounds. This 'metabolism' can increase or decrease the risk of diseases, including cognitive dysfunction, renal pathologies and urinary cancers, that can be caused by these toxins.[51]

The gut microbiota has been linked to the development of both the enteric and central nervous system after birth.[52] However, little is known about bacterial interactions with the peripheral nervous system. The urinary microbiota might be required for correct development of and signalling within this system, potentially through the production of neurotransmitters. Loss of these functions might be the cause of diseases, such as overactive bladder and interstitial cystitis. Indeed, studies on germ-free mice show that the absence of microbes correlates with a compromised immune system, leaky gut, as well as behavioural and neurological disorders.[13] Studies of urinary tract function in germ-free animals are rare, so it is currently unclear exactly how this system is influenced by the microbiota. Finally, the urinary microbiota might act as a barrier to uropathogens, for example, through competition for resources, similar to other body sites.[53] In the vagina, secretions can have inhibitory activity against *E. coli*, particularly in communities dominated by *Lactobacillus crispatus*.[54]

One of the limitations of 16S rRNA sequencing is its inability to differentiate between live and dead bacteria or bacterial DNA fragments. Hilt *et al.*[18] addressed this issue by expanding culture conditions beyond the ones that are used routinely to isolate bacteria from urine. Using these modified culture criteria, 80% of 65 samples, of which 92% were culture negative ($<10^3$ colony forming units (CFU)/mL) using traditional culture techniques, grew bacteria. Comparison of these findings to their high-throughput sequencing results suggested that a majority of the bacteria found within the urinary microbiota via 16s rRNA sequencing were indeed alive.

Factors influencing the microbiota

It is well known that dietary factors can influence the risk of urinary infections and calculi. Consistent high water intake is important, but it is perhaps a little simplistic to suggest that it can help cure UTIs. Indeed, urine from individuals with increased water intake significantly increased the initial deposition rates and numbers of adherent *E. coli* and *E. faecalis* to silicone rubber.[55] This finding is of particular importance in patients with catheter-associated UTIs and suggests that high water intake leads to the dilution of a urinary factor that inhibits microbial deposition, rendering it ineffective. Notably, a proportion of UTI cases resolve without intervention for unexplained reasons.[56–58] Furthermore, one pilot study found that the use of ibuprofen for the treatment of acute uncomplicated UTI is comparable to that of ciprofloxacin.[59] Unfortunately, results from a large, randomized, controlled, double-blind study have not yet been published.[60]

These findings raise a number of questions. Does the urinary microbiota simply need time to reconstitute following an acute infection? If so, why do not all infections resolve without intervention? Are there other triggers of infection, for example, alterations in the gut microbiota? Many microbial metabolites are found in the urine, which can be used as an indicator of gut dysbiosis. For example, in rats, in which the microbiota was suppressed by antibiotic treatment, the urinary metabolite profiles between the treated and control rats were remarkably different.[61] Clearly, many waste products, including microbial metabolites, are excreted via the urinary tract, but whether these metabolites are harmful to the urinary microbiota and their role in the development of UTIs is unclear. Multiple groups have explored the use of NMR to differentiate the metabolites in the urine from healthy patients and those with suspected UTI,[62] as well as to identify biomarkers of morbidity.[63]

Consumption of cranberry juice has been proposed to reduce the incidence of recurrent UTIs; however, in 2012, a randomized controlled trial in women with a history of recurrent UTI found no protective effects.[64] Other studies have suggested that D-mannose, a component of cranberry juice and other juices, especially pineapple juice, inhibits the attachment of bacterial type 1 fimbriae to cell surfaces, presumably reducing the pathogen's ability to remain in the urinary tract and infect the host.[65] Although more studies are needed—which should also examine the effect on the total microbiota—urinary components indeed seem likely to have an important role in determining which organisms inhabit the urinary tract.

In addition, the virome is likely to be important in maintaining the urinary microbiota. The virome is defined as the set of viruses infecting eukaryotic, bacterial, and archaic microorganisms found within the host, as well as the virus-derived genetic elements that have integrated chromosomally.[66] Similar to the bacterial contingent of the microbiome, the virome can be found throughout the entire human body, including the urinary tract, and is not limited to the mucosal sites. To date, little is known about the influence of the virome on the urinary microbiota and this topic is likely to receive much attention in the coming years. However, as viruses can be DNA or RNA encoded and owing to high genomic diversity, characterization of the virome is complicated by the limitations of current technology and the lack of a universal phylogenetic marker, such as the 16S rRNA in bacteria.[67]

Importantly, the ways in which antibiotics and antimicrobial agents prescribed to patients, as well as trace amounts found in drinking water and food, affect the human microbiome are only now being realized.[68] The fact that bacterial resistance to antibiotics is increasing is well known; however, the use of low-dose, prophylactic antibiotics to prevent recurrent infection remains an accepted therapeutic option. These practices might be creating bacterial persister cells that are genetically homogenous to previous generations, but exhibit a greater fitness, enabling them to be more capable of invading the uroepithelium and acting as a source of sepsis and infection, but potentially also of becoming part of the urinary microbiota.[69] In children with anatomical congenital anomalies that predispose them to recurrent UTI, infection is often managed with low-dose antibiotic prophylaxis—the development of persister cells due to such medication might be contributing to the recurrent nature of the UTI.

Changes in bacterial diversity over the course of an individual's life have been described for the intestinal microbiota and are also likely to occur to some extent in the urinary microbiota,[70] given the influence of the former on the latter. Hormonal changes associated with puberty and menopause,[71] as well as constipation and urinary incontinence, are known to influence the microbiome.[72] In women, such changes primarily affect the composition of lactobacilli populations in the vagina.[73] In addition, in women, sexual activity can alter the microbiota of both the urethra and the vagina.[4] Furthermore, in male adolescents (aged 14–17 years) who have engaged in vaginal sexual intercourse, bacteria commonly associated with bacterial vaginosis can colonize the coronal sulcus and distal urethra.[21] These genera were not found in sexually inexperienced individuals, suggesting that colonization was related to sexual activity. The same study also found significant differences in the types of bacteria colonizing the coronal sulcus between circumcised and uncircumcised adolescents. After puberty, the prostate has developed sufficiently to produce prostatic fluid that contains various antimicrobial substances,[74] which are capable of influencing the urinary microbiota. In addition, spermicidal agents might disrupt both the male and the female urinary microbiota, similar to disruptions observed in the vagina.[75,76] Furthermore, anal and oral penetration during sexual intercourse exposes men to two atypical microbial environments.

As has been proposed for the gut,[77] the balance between certain microbial groups or organisms that contribute to a conserved metagenome might be more important than the overall composition of the urinary

microbiota. For example, two populations might contain completely different sets of bacterial species, yet perform the same function, as the genetic potential is present in both populations. This property adds an aspect of redundancy to the microbiome, acting as a safeguard in the maintenance of health and development of disease. It is important to note that the metagenome represents only the genetic potential of the microbiota, not the actual activity of the population.

The microbiota in specific diseases

Urolithiasis

Several studies have described an inverse relationship between the occurrence of *Oxalobacter formigenes* in the intestinal microbiota and the development of renal calcium oxalate stones. In the colon, *O. formigenes* utilizes oxalate as its primary substrate,[78] thus, *O. formigenes* is believed to be essential for the degradation of dietary oxalate in the human body. Studies indicated that individuals lacking this bacterium have an increased urinary oxalate concentration.[79–81] Further, colonization with the bacterium is associated with a 70% reduction in risk of recurrent calcium oxalate stone formation and the colonization rate of healthy subjects is almost double that of individuals with nephrolithiasis.[81,82]

Multiple studies have sought to characterize the mechanisms behind the relationship between *O. formigenes* colonization and the development of oxalate stones. Treatment of hyperoxaluric animals with live *O. formigenes* or isolated oxalate-degrading enzymes decreased urinary oxalate levels.[83,84] This decrease might, in part, be due to stimulated oxalate secretion into the gut caused by new colonization of the gut with *O. formigenes* or promotion of bacterial survival within the colon owing to the ingestion of bacterial lysates.[85]

O. formigenes acquisition is not well understood, but is assumed to occur during childhood and, according to animal studies, bacteria might be picked up through environmental exposure.[86] In children (aged 4–18 years) in Poland, similar colonization rates were found between paediatric stone formers and apparently healthy children (27.6% versus 26% of children, respectively).[87] Conversely, a Ukrainian study demonstrated age-dependent colonization, with no detection of the organism during the first year after birth followed by detection in all participants by the age of 8 years. Colonization then seems to decrease to 70–80% by the age of 12 years and further declines into adulthood, with reported colonization rates as low at 60%.[88] The discordance is noteworthy, yet might be caused by limited antibiotic access in the Ukraine at the time of the study and arguable overprescription of antibiotics in Poland.[87] Moreover, patients receiving long-term antibiotic therapy, such as cystic fibrosis patients, have a lower prevalence of *O. formigenes* in comparison with healthy individuals,[89] concordant with a greater incidence of calcium oxalate nephrolithiasis.[90] In adults, this eradication might be permanent, as researchers have had minimal success in recolonizing adult patients.[88,91,92]

Given the limited preventative options for calcium oxalate nephrolithiasis, alternative treatments are highly sought after. Recolonization or replacement of *O. formigenes* within the gastrointestinal tract represents a valid option; however, studies exploring this strategy are contradictory.[93] In humans, administration of *O. formigenes* HC1 (5×10^{10} CFU) reduced both urinary oxalate excretion and oxalate:creatinine ratios.[93] Further, the use of live *O. formigenes* against primary hyperoxaluria type 1 in patients decreased oxalate excretion by 20–50% in the urine of patients with functioning kidneys, yet oxalate excretion again increased following cessation of treatment, presumably because the bacterium was unable to colonize the gastrointestinal tract.[94] In 2013, Siener *et al.*[79] identified that *O. formigenes* lowers the concentration of oxalate available for absorption in the intestine, which might explain the notable decrease in urinary oxalate excretion. Indeed, consumption of probiotics significantly decreased oxaluria in patients with enteric hyperoxaluria or after administration of oral oxalate,[95–97] but led to unsatisfactory results in mildly hyperoxaluric patients.[98] Thus, probiotic studies with negative results that include patients on low-oxalate diets should not be considered as true probiotic failures.[90] Borghi *et al.*[90] argue that, based on available studies, probiotics should not be assumed ineffective in the treatment of calcium nephrolithiasis. Rather, researchers should focus on identifying the patient population in which this treatment strategy is effective. Such patient selection might be more difficult than it sounds, especially considering the Human Microbiome Project's findings that no single phylum is found at the same body site in every individual; indeed, each individual sample was unique in terms of phylum and genus composition.[99]

To date, therapeutic administration of *O. formigenes* has been hampered by its complicated growth requirements and subsequent lack in understanding of the optimal dietary oxalate levels required to maintain intestinal colonization—not to mention the regulatory issues of using non-food-grade bacteria in humans. Thus, other options have been explored. In rats, oxalate-degrading enzymes from *O. formigenes* have been combined with a 2% oxalate diet in an enzyme therapy that decreased urinary oxalate levels by 65% compared with controls.[91]

Other oxalate-degrading bacteria using different enzymatic pathways have also been explored as probiotic alternatives to *O. formigenes*. Indeed, certain lactic acid bacteria are capable of oxalate degradation *in vitro*, and when given orally reduce urinary oxalate excretion.[95,100] The administration of a mixture of *L. acidophilus, Lactobacillus plantarum, Lactobacillus brevis, Streptococcus thermophilus* and *Bifidobacterium infantis* strains over a period of 4 weeks in patients with idiopathic calcium oxalate urolithiasis and hyperoxaluria significantly reduced urinary excretion of oxalate.[100] Conversely, a double-blind, placebo-controlled study found that neither 2×10^{11} CFU of *L. acidophilus, L. brevis, S. thermophilus* and *B. infantis* at a ratio of

1:1:4:4 nor a mixture of 115 mg Fructooligosaccharide, 4.5×10^9 CFU *Enterococcus faecium*, 3×10^6 CFU *Saccharomyces boulardii* and 2×10^6 CFU *S. cerevisiae* significantly increased intestinal oxalate metabolism or decreased oxalate absorption from the gut beyond the effects of a controlled diet.[95] It is still not established whether the mechanism behind the reduction in oxalate absorption is related to the bacterial ability to utilize the molecule or to modulate the host's epithelial barrier functions, resulting in decreased oxalate absorption.

Urolithiasis is also one of many diseases, including renal cell cancer in men, that have been associated with obesity.[101,102] This relationship is of particular interest as obesity is correlated with dysbiosis of the oral cavity and the intestinal tract.[43] Urolithiasis has also been linked to metabolic syndrome, which is associated with increased urinary levels of uric acid, oxalate and calcium, as well as decreased citrate levels.[103] Future studies should clarify whether dysbiosis of the gut, beyond the loss of *O. formigenes* colonization, might be a cause of renal stone formation.

Although dysbiosis of a distal microbiota might not be the trigger of disease development, it might have a role in disease progression. For example, chronic kidney disease (CKD) has clearly been linked to an aberrant gut microbiota,[104–106] particularly through the production of uraemic retention molecules (URMs) by the microbiota.[107,108] Accumulation of these organic waste products leads to the development of uraemic illness and has been implicated in progression of CKD to end-stage renal failure.[107]

Bladder cancer

The microbiome might have a role in the prevention of recurrent superficial bladder cancer. In patients with this disease, recurrence rates can be as high as 66% at 5 years and 88% at 15 years.[109] Transurethral resection of the bladder tumour followed by weekly intravesical instillation of BCG (attenuated *Mycobacterium tuberculosis* vaccine) is currently considered the most effective management for intermediate and high-risk noninvasive tumours. Despite a significant reduction in recurrence and progression rates following BCG, almost half of patients will not respond and the disease might even progress.[110]

The mechanism by which the bacteria in the BCG vaccine prevent cancer recurrence has yet to be fully elucidated. Successful BCG immunotherapy requires an interaction with the bladder wall, which is dependent on fibronectin[111] and α5β1 integrins.[112] BCG binds to fibronectin with specific binding proteins, leading to the induction of $CD8^+$ T cells and natural killer cells. BCG is thought to initiate crosslinking between α5β1 integrins, which leads to cell cycle arrest.[113] The α5β1 integrins are also necessary for BCG internalization[112] and host cell death.[114] BCG soluble factors have been linked to cell death through the production of reactive oxygen species in bladder cancer cells *in vitro*, correlating with DNA damage, the extent of which was related to the duration of BCG exposure.[115]

At other exposed mucosal sites, such as in the gut or oral activity, the commensal bacteria are essential in the maintenance of host homeostasis and it seems rational to presume that a similar relationship exists in the bladder. Interestingly, *Lactobacillus* and, most notably, *L. iners* has been found in the urinary microbiota.[7,18,20–28] In 2013, our group demonstrated that *L. iners* binds fibronectin with a greater affinity than other lactobacilli derived from vaginal samples or probiotics.[116] In addition, numerous indigenous commensal and probiotic bacterial strains demonstrated the ability to attenuate mucosal inflammation via inhibition of the NF-κB pathway, as well as IL-6 and IL-8.[117] This interaction might be enhancing or reducing the efficacy of BCG therapy. BCG therapy might be further affected by the indigenous microbiota, potentially influencing clinical outcomes by competing with BCG to bind fibronectin, thereby altering the host's immunological response. Strategies to promote or deplete the indigenous bladder microbiome might be used in the future to improve the efficacy of BCG treatment.

Although rare, life-threatening adverse effects are associated with BCG. For example, 0.4% of patients develop BCG sepsis,[118] thus, research into alternative therapies is warranted. In mice, *Lactobacillus casei* decreased the growth of transplantable bladder tumours and pulmonary metastases.[119] In a rat bladder cancer model, similar results were observed when treating animals orally or intravesically with *L. casei*.[120] In addition, these effects were reproduced with dead bacteria.[121] Notably, such beneficial responses are not limited to *L. casei*: *Lactobacillus rhamnosus* GG recruited natural killer cells to the bladder and draining lymph nodes of healthy mice receiving weekly instillations of the bacterium at rates comparable to BCG.[122] *In vitro* studies also demonstrate cytotoxic effects of *L. rhamnosus* GG in bladder cancer cells.[123]

In a double-blind, placebo-controlled randomized trial, orally administered *L. casei* Shirota decreased superficial bladder cancer recurrence.[124] Although controversial, these findings have been confirmed in multiple other studies.[109] However, the mechanism of action and whether the treatment is superior or complementary to BCG remain unclear. Although the interactions of probiotics with the immune system have been well established, questions are also raised about the probiotic interaction with the gut microbiota of patients. Is intestinal microbial dysbiosis present in patients with superficial bladder cancer and does application of probiotics ameliorate some of the accompanying changes to the gut microbiota?

In colorectal cancer, a dysbiotic gut microbiota is thought to contribute to the development of the genetic mutations, epigenetic changes and aberrant immunological signalling pathways linked to disease development.[11,12] Indeed, bacteria have been linked to the development of multiple cancers through various mechanisms: gastric cancer, gastric and skin mucosa-associated lymphoid tissue lymphoma, immunoproliferative small intestinal disease, ocular adnexal lymphoma, colorectal

cancer and breast cancer.[8,125–128] Bladder cancer has been associated with a number of risk factors, including smoking and *Schistosoma haematobium*,[125] and asymptomatic bacteriuria has also been clinically noted.[29] In addition, a preliminary study found alterations in the urinary microbiota of patients with urothelial carcinoma in comparison with healthy individuals.[29]

Many questions currently remain unanswered. Does the gut microbiota have a role in the development of bladder cancer? Many carcinogens are produced in the gut, absorbed by the blood and filtered and stored by the urinary tract prior to excretion. Which effects do these compounds have on the urinary microbiota? Certainly, some members of the commensal microbiome are able to sequester heavy metals and other toxic substances thought to be risk factors for bladder cancer.[47]

Future directions

Apart from probiotic applications for the treatment of renal stones and bladder cancer, oral and vaginal probiotic therapies have been successful in decreasing the recurrence rates of UTI.[129] Pending further exploration, the use of such treatment strategies is likely to lead to a reduction in antibiotic use and subsequent resistance. The effectiveness of intravaginal and intravesical probiotic application in the treatment of UTI and bladder cancer opens the door for the administration of probiotics via these routes also for other urological diseases.

Alternative treatment strategies involving interventions targeting the colonic microbiome, such as the use of faecal transplants, have also been experimentally explored and are worth further research. In 2012, one study successfully transferred the intestinal microbiota of lean donors to recipients with metabolic syndrome, resulting in a notable improvement in human insulin sensitivity.[130] Faecal transplants have been successfully used in the treatment of chronic *C. difficile* infections,[131] but are controversial owing to potential inadvertent transfer of other infectious agents. Another alternative to faecal transplants is synthetic stool therapy.[132] In this strategy, a relatively well defined, but diverse, collection of >30 bacterial strains is propagated for use as a stool alternative with the aim of creating a safe, reproducible and consistent treatment. Such therapies might be useful for diseases, in which the gut microbiome has a role in the progression of the disease. Although their application is unlikely to cure disease, it might represent a tool for management of symptoms. In the field of urology, faecal transplants might be beneficial in the management of, for example, urolithiasis.

Alternatively, the development of a synthetic urinary microbiota for transplantation might lead to an effective treatment for patients with recurrent UTI, as the cause of this condition is probably similar to recurrent infection caused by *C. difficile*, in which the infection is likely to be associated with an inability to reconstitute the normal microbiota. Recurrent UTI is unlikely to be compounded by many genomic factors, although hereditary links have been suggested,[37] making it a good candidate for microbiota transfer. Finally, an altered microbiome might potentially be useful as an early diagnostic indicator of disease, although further studies in large populations are required to determine the disease-specific profiles that might form the basis of future diagnostic tests.

Conclusions

Undoubtedly, the microbiome is linked to urological health and disease, but the extent of this relationship is still unclear. Over the next few years defining the influence of the microbiome on urological homeostasis will be key in understanding the development and progression of urological disease. Concurrently, exploration and incorporation of more probiotic and microbiome-targeted treatment strategies within urology is warranted.

1. Cho, I. & Blaser, M. J. The human microbiome: at the interface of health and disease. *Nat. Rev. Genet.* **13**, 260–270 (2012).
2. Ursell, L. K., Metcalf, J. L., Parfrey, L. W. & Knight, R. Defining the human microbiome. *Nutr. Rev.* **70** (Suppl. 1), S38–S44 (2012).
3. Lazarevic, V. *et al.* Metagenomic study of the oral microbiota by Illumina high-throughput sequencing. *J. Microbiol. Methods* **79**, 266–271 (2009).
4. Gajer, P. *et al.* Temporal dynamics of the human vaginal microbiota. *Sci. Transl. Med.* **4**, 132ra52 (2012).
5. Grice, E. A. *et al.* Topographical and temporal diversity of the human skin microbiome. *Science* **324**, 1190–1192 (2009).
6. Branton, W. G. *et al.* Brain microbial populations in HIV/AIDS: α-proteobacteria predominate independent of host immune status. *PLoS ONE* **8**, e54673 (2013).
7. Wolfe, A. J. *et al.* Evidence of uncultivated bacteria in the adult female bladder. *J. Clin. Microbiol.* **50**, 1376–1383 (2012).
8. Urbaniak, C. *et al.* Microbiota of human breast tissue. *Appl. Environ. Microbiol.* **80**, 3007–3014 (2014).
9. Aagaard, K. *et al.* The placenta harbors a unique microbiome. *Sci. Transl. Med.* **6**, 237ra65 (2014).
10. Sekirov, I., Russell, S. L., Antunes, C. M. & Finlay, B. B. Gut microbiota in health and disease. *Physiol. Rev.* **90**, 859–904 (2010).
11. Arends, M. J. Pathways of colorectal carcinogenesis. *Appl. Immunohistochem. Mol. Morphol.* **21**, 97–102 (2013).
12. Yang, T., Owen, J. L., Lightfoot, Y. L., Kladde, M. P. & Mohamadzadeh, M. Microbiota impact on the epigenetic regulation of colorectal cancer. *Trends Mol. Med.* **19**, 714–725 (2013).
13. Cryan, J. F. & Dinan, T. G. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat. Rev. Neurosci.* **13**, 701–712 (2012).
14. Gan, X. T. *et al.* Probiotic administration attenuates myocardial hypertrophy and heart failure following myocardial infarction in the rat. *Circ. Heart Fail.* **7**, 491–499 (2014).
15. Scher, J. U. *et al.* Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *eLife* **2**, e01202 (2013).
16. Larsen, N. *et al.* Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS ONE* **5**, e9085 (2010).
17. de Vos, W. M. & Nieuwdorp, M. Genomics: A gut prediction. *Nature* **498**, 48–49 (2013).
18. Hilt, E. E. *et al.* Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. *J. Clin. Microbiol.* **52**, 871–876 (2014).
19. Pearce, M. M. *et al.* The female urinary microbiome: A comparison of women with and without urgency urinary incontinence. *MBio* **5**, e01283-14 (2014).
20. Fricke, W. F., Maddox, C., Song, Y. & Bromberg, J. S. Human microbiota characterization in the course of renal transplantation. *Am. J. Transplant.* **14**, 416–427 (2014).
21. Nelson, D. E. *et al.* Bacterial communities of the coronal sulcus and distal urethra of adolescent males. *PLoS ONE* **7**, e36298 (2012).
22. 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.* **11**, 244 (2011).

23. 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.* **12**, 205 (2012).
24. Fouts, D. E. *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.* **10**, 174 (2012).
25. Nelson, D. E. *et al.* Characteristic male urine microbiomes associate with asymptomatic sexually transmitted infection. *PLoS ONE* **5**, e14116 (2010).
26. Dong, Q. *et al.* The microbial communities in male first catch urine are highly similar to those in paired urethral swab specimens. *PLoS ONE* **6**, e19709 (2011).
27. Lewis, D. A. *et al.* The human urinary microbiome; bacterial DNA in voided urine of asymptomatic adults. *Front. Cell. Infect. Microbiol.* **3**, 41 (2013).
28. Willner, D. *et al.* Single clinical isolates from acute uncomplicated urinary tract infections are representative of dominant *in situ* populations. *MBio* **5**, e01064-13 (2014).
29. Xu, W. *et al.* Mini-review: perspective of the microbiome in the pathogenesis of urothelial carcinoma. *Am. J. Clin. Exp. Urol.* **2**, 57–61 (2014).
30. Ronald, A. The etiology of urinary tract infection: traditional and emerging pathogens. *Am. J. Med.* **113** (Suppl. 1A), 14S–19S (2002).
31. Soriano, F. & Tauch, A. Microbiological and clinical features of *Corynebacterium urealyticum*: urinary tract stones and genomics as the Rosetta Stone. *Clin. Microbiol. Infect.* **14**, 632–643 (2008).
32. Lee, J. W., Shim, Y. H. & Lee, S. J. *Lactobacillus* colonization in infants with urinary tract infection. *Pediatr. Nephrol.* **24**, 135–139 (2009).
33. Latthe, P. M., Toozs-Hobson, P. & Gray, J. *Mycoplasma* and *Ureaplasma* colonisation in women with lower urinary tract symptoms. *J. Obstet. Gynaecol.* **28**, 519–521 (2008).
34. Burton, J. P. & Reid, G. Evaluation of the bacterial vaginal flora of 20 postmenopausal women by direct (Nugent score) and molecular (polymerase chain reaction and denaturing gradient gel electrophoresis) techniques. *J. Infect. Dis.* **186**, 1770–1780 (2002).
35. Burton, J. P., McCormick, J. K., Cadieux, P. A. & Reid, G. Digoxigenin-labelled peptide nucleic acid to detect lactobacilli PCR amplicons immobilized on membranes from denaturing gradient gel electrophoresis. *Lett. Appl. Microbiol.* **36**, 145–149 (2003).
36. Di Bella, J. M., Bao, Y., Gloor, G. B., Burton, J. P. & Reid, G. High throughput sequencing methods and analysis for microbiome research. *J. Microbiol. Methods* **95**, 401–414 (2013).
37. Stapleton, A. E. Urinary tract infection pathogenesis: host factors. *Infect. Dis. Clin. North Am.* **28**, 149–159 (2014).
38. Ragnarsdóttir, B., Lutay, N., Grönberg-Hernandez, J., Köves, B. & Svanborg, C. Genetics of innate immunity and UTI susceptibility. *Nat. Rev. Urol.* **8**, 449–468 (2011).
39. Kuitunen, M. *et al.* Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. *J. Allergy Clin. Immunol.* **123**, 335–341 (2009).
40. Boris, S. & Barbés, C. Role played by lactobacilli in controlling the population of vaginal pathogens. *Microbes Infect.* **2**, 543–546 (2000).
41. Liévin-Le Moal, V. & Servin, A. L. Anti-Infective Activities of *Lactobacillus* strains in the human intestinal microbiota: from probiotics to gastrointestinal anti-infectious biotherapeutic agents. *Clin. Microbiol. Rev.* **27**, 167–199 (2014).
42. Ott, S. J. *et al.* Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. *Gut* **53**, 685–693 (2004).
43. Turnbaugh, P. J. *et al.* A core gut microbiome in obese and lean twins. *Nature* **457**, 480–484 (2009).
44. Micheli, A. *et al.* The advantage of women in cancer survival: an analysis of EUROCARE-4 data. *Eur. J. Cancer* **45**, 1017–1027 (2009).
45. Ghani, K. R. *et al.* Emergency department visits in the United States for upper urinary tract stones: trends in hospitalization and charges. *J. Urol.* **191**, 90–96 (2014).
46. Kau, A. L., Ahern, P. P., Griffin, N. W., Goodman, A. L. & Gordon, J. I. Human nutrition, the gut microbiome and the immune system. *Nature* **474**, 327–336 (2011).
47. Monachese, M., Burton, J. P. & Reid, G. Bioremediation and tolerance of humans to heavy metals through microbial processes: a potential role for probiotics? *Appl. Environ. Microbiol.* **78**, 6397–6404 (2012).
48. Nee, L. E. *et al.* Environmental-occupational risk factors and familial associations in multiple system atrophy: a preliminary investigation. *Clin. Auton. Res.* **1**, 9–13 (1991).
49. Friesen, M. C., Costello, S., Thurston, S. W. & Eisen, E. A. Distinguishing the common components of oil- and water-based metalworking fluids for assessment of cancer incidence risk in autoworkers. *Am. J. Ind. Med.* **54**, 450–460 (2011).
50. Zlăvog, A. V. *et al.* Estimation of ochratoxin A in the human blood of Romanian population. *Rev. Med. Chir. Soc. Med. Nat. Iasi* **117**, 1009–1013 (2013).
51. Mirvish, S. S. Role of N-nitroso compounds (NOC) and N-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC. *Cancer Lett.* **93**, 17–48 (1995).
52. Cryan, J. F. & O'Mahoney, S. M. The microbiome–gut–brain axis: from bowel to behavior. *Neurogastroenterol. Motil.* **23**, 187–192 (2011).
53. Hooper, L. V. & Gordon, J. I. Commensal host–bacterial relationships in the gut. *Science* **292**, 1115–1118 (2001).
54. Ghartey, J. P. *et al. Lactobacillus crispatus* dominant vaginal microbiome is associated with inhibitory activity of female genital tract secretions against *Escherichia coli*. *PLoS ONE* **9**, e96659 (2014).
55. Habash, M. B., Van der Mei, H. C., Busscher, H. J. & Reid, G. The effect of water, ascorbic acid, and cranberry derived supplementation on human urine and uropathogen adhesion to silicone rubber. *Can. J. Microbiol.* **45**, 691–694 (1999).
56. Ferry, S. A., Holm, S. E., Stenlund, H., Lundholm, R. & Monsen, T. J. The natural course of uncomplicated lower urinary tract infection in women illustrated by a randomized placebo controlled study. *Scand. J. Infect. Dis.* **36**, 296–301 (2004).
57. Nicolle, L. E., Zhanel, G. G. & Harding, G. K. Microbiological outcomes in women with diabetes and untreated asymptomatic bacteriuria. *World J. Urol.* **24**, 61–65 (2006).
58. Reid, G. *et al.* Microbiota restoration: natural and supplemented recovery of human microbial communities. *Nat. Rev. Microbiol.* **9**, 27–38 (2011).
59. Bleidorn, J., Gágyor, I., Kochen, M. M., Wegscheider, K. & Hummers-Pradier, E. Symptomatic treatment (ibuprofen) or antibiotics (ciprofloxacin) for uncomplicated urinary tract infection?—Results of a randomized controlled pilot trial. *BMC Med.* **8**, 30 (2010).
60. Gágyor, I. *et al.* Immediate versus conditional treatment of uncomplicated urinary tract infection - a randomized-controlled comparative effectiveness study in general practices. *BMC Infect. Dis.* **12**, 146 (2012).
61. Swann, J. R. *et al.* Variation in antibiotic-induced microbial recolonization impacts on the host metabolic phenotypes of rats. *J. Proteome Res.* **10**, 3590–3603 (2011).
62. Gupta, A., Dwivedi, M., Mahdi, A. A., Khetrapal, C. L. & Bhandari, M. Broad identification of bacterial type in urinary tract infection using ^{1}H NMR spectroscopy. *J. Proteome Res.* **11**, 1844–1854 (2012).
63. Nevedomskaya, E. *et al.* ^{1}H NMR-based metabolic profiling of urinary tract infection: combining multiple statistical models and clinical data. *Metabolomics* **8**, 1227–1235 (2012).
64. Stapleton, A. E. *et al.* Recurrent urinary tract infection and urinary *Escherichia coli* in women ingesting cranberry juice daily: a randomized controlled trial. *Mayo Clin. Proc.* **87**, 143–150 (2012).
65. Scharenberg, M., Schwardt, O., Rabbani, S. & Ernst, B. Target selectivity of FimH Antagonists. *J. Med. Chem.* **55**, 9810–9816 (2012).
66. Virgin, H. W. & Todd, J. A. Metagenomics and personalized medicine. *Cell* **147**, 44–56 (2011).
67. Virgin, H. W. The virome in mammalian physiology and disease. *Cell* **157**, 142–150 (2014).
68. Gibs, J. *et al.* Occurrence and partitioning of antibiotic compounds found in the water column and bottom sediments from a stream receiving two wastewater treatment plant effluents in northern New Jersey, 2008. *Sci. Total Environ.* **458–460**, 107–116 (2013).
69. Goneau, L. W. *et al.* Selective target inactivation rather than global metabolic dormancy causes antibiotic tolerance in uropathogens. *Antimicrob. Agents Chemother.* **58**, 2089–2097 (2014).
70. Yatsunenko, T. *et al.* Human gut microbiome viewed across age and geography. *Nature* **486**, 222–227 (2012).
71. Hummelen, R. *et al.* Vaginal microbiome and epithelial gene array in post-menopausal women with moderate to severe dryness. *PLoS ONE* **6**, e26602 (2011).
72. Zhu, L. *et al.* Structural changes in the gut microbiome of constipated patients. *Physiol. Genomics* **46**, 679–686 (2014).
73. Heinemann, C. & Reid, G. Vaginal microbial diversity among postmenopausal women with and without hormone replacement therapy. *Can. J. Microbiol.* **51**, 777–781 (2005).
74. Com, E. *et al.* Expression of antimicrobial defensins in the male reproductive tract of rats, mice, and humans. *Biol. Reprod.* **68**, 95–104 (2003).
75. Gupta, K., Hillier, S. L., Hooton, T. M., Roberts, P. L. & Stamm, W. E. Effects of contraceptive method on the vaginal flora: a prospective evaluation. *J. Infect. Dis.* **181**, 595–601 (2000).
76. McGroarty, J. A., Tomeczek, L., Pond, D. G., Reid, G. & Bruce, A. W. Hydrogen peroxide production by *Lactobacillus* species: correlation with susceptibility to the spermicidal compound nonoxynol-9. *J. Infect. Dis.* **165**, 1142–1144 (1992).
77. Keeney, K. M., Yurist-Doutsch, S., Arrieta, M. C. & Finlay, B. B. Effects of antibiotics on human microbiota and subsequent disease. *Annu. Rev. Microbiol.* **68**, 217–235 (2014).
78. Cornick, N. A. & Allison, M. J. Anabolic incorporation of oxalate by *Oxalobacter formigenes*. *Appl. Environ. Microbiol.* **62**, 3011–3013 (1996).

79. Siener, R. *et al.* The role of *Oxalobacter formigenes* colonization in calcium oxalate stone disease. *Kidney Int.* **83**, 1144–1149 (2013).
80. Jiang, J. *et al.* Impact of dietary calcium and oxalate, and *Oxalobacter formigenes* colonization on urinary oxalate excretion. *J. Urol.* **186**, 135–139 (2011).
81. Kaufman, D. W. *et al. Oxalobacter formigenes* may reduce the risk of calcium oxalate kidney stones. *J. Am. Soc. Nephrol.* **19**, 1197–1203 (2008).
82. Kelly, J. P., Curhan, G. C., Cave, D. R., Anderson, T. E. & Kaufman, D. W. Factors related to colonization with *Oxalobacter formigenes* in U.S. adults. *J. Endourol.* **25**, 673–679 (2011).
83. Hatch, M., Gjymishka, A., Saliido, E. C., Allison, M. J. & Freel, R. W. Enteric oxalate elimination is induced and oxalate is normalized in a mouse model of primary hyperoxaluria following intestinal colonization with *Oxalobacter*. *Am. J. Physiol. Gastrointest. Liver Physiol.* **300**, G461–G469 (2011).
84. Sidhu, H., Allison, M. J., Chow, J. M., Clark, A. & Peck, A. B. Rapid reversal of hyperoxaluria in a rat model after probiotic administration of *Oxalobacter formigenes*. *J. Urol.* **166**, 1487–1491 (2001).
85. Freel, R. W., Hatch, M., Green, M. & Soleimani, M. Ileal oxalate absorption and urinary oxalate excretion are enhanced in *Slc26a6* null mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* **209**, G719–G728 (2006).
86. Cornelius, J. G. & Peck, A. B. Colonization of the neonatal rat intestinal tract from environmental exposure to the anaerobic bacterium *Oxalobacter formigenes*. *J. Med. Microbiol.* **53**, 249–254 (2004).
87. Sikora, P. *et al.* Intestinal colonization with *Oxalobacter formigenes* and its relation to urinary oxalate excretion in pediatric patients with idiopathic calcium urolithiasis. *Arch. Med. Res.* **40**, 369–373 (2009).
88. Sidhu, H. *et al.* Evaluating children in the Ukraine for colonization with the intestinal bacterium *Oxalobacter formigenes*, using a polymerase chain reaction-based detection system. *Mol. Diagn.* **2**, 89–97 (1997).
89. Sidhu, H. *et al.* Absence of *Oxalobacter formigenes* in cystic fibrosis patients: a risk factor for hyperoxaluria. *Lancet* **352**, 1026–1029 (1998).
90. Borghi, L., Nouvenne, A. & Meschi, T. Probiotics and dietary manipulations in calcium oxalate nephrolithiasis: two sides of the same coin? *Kidney Int.* **78**, 1063–1065 (2010).
91. Sidhu, H. *et al.* Direct correlation between hyperoxaluria/oxalate stone disease and the absence of the gastrointestinal tract-dwelling bacterium *Oxalobacter formigenes*: possible prevention by gut recolonization or enzyme replacement therapy. *J. Am. Soc. Nephrol.* **10** (Suppl. 14), S334–S340 (1999).
92. Knight, J., Deora, R., Assimos, D. G. & Holmes, R. P. The genetic composition of *Oxalobacter formigenes* and its relationship to colonization and calcium oxalate stone disease. *Urolithiasis* **41**, 187–196 (2013).
93. Duncan, S. H. *et al. Oxalobacter formigenes* and its potential role in human health. *Appl. Environ. Microbiol.* **68**, 3841–3847 (2002).
94. Hoppe, B. *et al. Oxalobacter formigenes*: a potential tool for the treatment of primary hyperoxaluria type 1. *Kidney Int.* **70**, 1305–1311 (2006).
95. Lieske, J. C. *et al.* Diet, but not oral probiotics, effectively reduces urinary oxalate excretion and calcium oxalate supersaturation. *Kidney Int.* **78**, 1178–1185 (2010).
96. Okombo, J. & Liebman, M. Probiotic-induced reduction of gastrointestinal oxalate absorption in healthy subjects. *Urol. Res.* **38**, 169–178 (2010).
97. Ferraz, R. R. *et al.* Effects of *Lactobacillus casei* and *Bifidobacterium breve* on urinary oxalate excretion in nephrolithiasis patients. *Urol. Res.* **37**, 95–100 (2009).
98. Goldfarb, D. S., Modersitzki, F. & Asplin, J. R. A randomized, controlled trial of lactic acid bacteria for idiopathic hyperoxaluria. *Clin. J. Am. Soc. Nephrol.* **2**, 745–749 (2007).
99. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature* **486**, 207–214 (2012).
100. Campieri, C. *et al.* Reduction of oxaluria after an oral course of lactic acid bacteria at high concentration. *Kidney Int.* **60**, 1097–1105 (2001).
101. Cho, E., Adami, H. O. & Lindblad, P. Epidemiology of renal cell cancer. *Hematol. Oncol. Clin. North Am.* **25**, 651–665 (2011).
102. Stamatelou, K. K., Francis, M. E., Jones, C. A., Nyberg, L. M. & Curhan, G. C. Time trends in reported prevalence of kidney stones in the United States: 1976–1994. *Kidney Int.* **63**, 1817–1823 (2003).
103. Gorbachinsky, I., Akpinar, H. & Assimos, D. G. Metabolic syndrome and urologic diseases. *Rev. Urol.* **12**, e157–e180 (2010).
104. Niwa, T. *et al.* The protein metabolite hypothesis, a model for the progression of renal failure: an oral adsorbent lowers indoxyl sulfate levels in undialyzed uremic patients. *Kidney Int. Suppl.* **62**, S23–S28 (1997).
105. Schepers, E., Glorieux, G. & Vanholder, R. The gut: the forgotten organ in uremia? *Blood Purif.* **29**, 130–136 (2010).
106. Poesen, R., Meijers, B. & Evenepoel, P. The colon: an overlooked site for therapeutics in dialysis patients. *Semin. Dial.* **26**, 323–332 (2013).
107. Satoh, M. *et al.* Uremic toxins overload accelerates renal damage in a rat model of chronic renal failure. *Nephron Exp. Nephrol.* **95**, e111–e118 (2003).
108. Evenepoel, P., Meijers, B. K., Bammens, B. R. & Verbeke, K. Uremic toxins originating from colonic microbial metabolism. *Kidney Int. Suppl.* **76**, S12–S19 (2009).
109. Hoesl, C. E. & Altwein, J. E. The probiotic approach: an alternative treatment option in urology. *Eur. Urol.* **47**, 288–296 (2005).
110. Fahmy, N., Lazo-Langner, A., Iansavichene, A. E. & Pautler, S. E. Effect of anticoagulants and antiplatelet agents on the efficacy of intravesical BCG treatment of bladder cancer: a systematic review. *Can. Urol. Assoc. J.* **7**, E740–E749 (2013).
111. Ratliff, T. L., Palmer, J. O., McGarr, J. A. & Brown, E. J. Intravesical bacillus Calmette-Guérin therapy for murine bladder tumors: initiation of the response by fibronectin-mediate attachment of bacillus Calmette-Guérin. *Cancer Res.* **47**, 1762–1766 (1987).
112. Kuroda, K., Brown, E. J., Telle, W. B., Russell, D. G. & Ratliff, T. L. Characterization of the bacillus Calmette-Guérin by human bladder tumor cells. *J. Clin. Invest.* **91**, 69–76 (1993).
113. Chen, F., Zhang, G., Iwamoto, Y. & See, W. A. BCG directly induces cell cycle arrest in human transitional carcinoma cell lines as a consequence of integrin cross-linking. *BMC Urol.* **5**, 8 (2005).
114. Pook, S. H., Rahmat, J. N., Esuvaranathan, K. & Mahendran, R. Internalization of *Mycobacterium bovis,* bacillus Calmette-Guérin, by bladder cells is cytotoxic. *Oncol. Rep.* **18**, 1315–1320 (2007).
115. Rahmat, J. N., Esuvaranathan, K. & Mahendran, R. Bacillus Calmette-Guérin induces cellular reactive oxygen species and lipid peroxidation in cancer cells. *Urology* **79**, 1411.e15–1411.e20 (2012).
116. McMillan, A., Macklaim, J. M., Burton, J. P. & Reid, G. Adhesion of *Lactobacillus iners* AB-1 to human fibronectin: a key mediator for persistence in the vagina? *Reprod. Sci.* **20**, 791–796 (2013).
117. Cosseau, C. *et al.* The commensal *Streptococcus salivarius* K12 downregulates the innate immune responses of human epithelial cells and promotes host–microbe homeostasis. *Infect. Immun.* **76**, 4163–4175 (2008).
118. Lamm, D. L. Efficacy and safety of bacille Calmette-Guérin immunotherapy in superficial bladder cancer. *Clin. Infect. Dis.* **31** (Suppl. 3), S86–S90 (2000).
119. Kato, I., Kobayashi, S., Yokokura, T. & Mutai, M. Antitumor activity of *Lactobacillus casei* in mice. *Gan* **72**, 517–523 (1981).
120. Tomita, K. *et al.* Influence of *Lactobacillus casei* on rat bladder carcinogenesis [Japanese]. *Nihon Hinyokika Gakkai Zasshi* **85**, 655–663 (1994).
121. Takahashi, T. *et al.* 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.* **166**, 2506–2511 (2001).
122. Seow, S. W., Rahmat, J. N., Bay, B. H., Lee, Y. K. & Mahendran, R. Expression of chemokine/cytokine genes and immune cell recruitment following the instillation of *Mycobacterium bovis,* bacillus Calmette-Guérin or *Lactobacillus rhamnosus* strain GG in the healthy murine bladder. *Immunology* **124**, 419–427 (2008).
123. Seow, S. W. *et al.* Lactobacillus species is more cytotoxic to human bladder cancer cells than *Mycobacterium bovis* (bacillus Calmette-Guérin). *J. Urol.* **168**, 2236–2239 (2002).
124. Aso, Y. & Akazan, H. Prophylactic effect of a *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer. BLP Study Group. *Urol. Int.* **49**, 125–129 (1992).
125. de Martel, C. *et al.* Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. *Lancet Oncol.* **13**, 607–615 (2012).
126. Schwabe, R. F. & Jobin, C. The microbiome and cancer. *Nat. Rev. Cancer* **13**, 800–812 (2013).
127. Gagliani, N., Hu, B., Huber, S., Elinav, E. & Flavell, R. A. The fire within: microbes inflame tumors. *Cell* **157**, 776–783 (2014).
128. Irrazábal, T., Belcheva, A., Girardin, S. E., Martin, A. & Philpott, D. J. The multifaceted role of the intestinal microbiota in colon cancer. *Mol. Cell* **54**, 309–320 (2014).
129. Stapleton, A. E. *et al.* Randomized, placebo-controlled phase 2 trial of a *Lactobacillus crispatus* probiotic given intravaginally for prevention of recurrent urinary tract infection. *Clin. Infect. Dis.* **52**, 1212–1217 (2011).
130. Vrieze, A. *et al.* Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* **143**, 913–916.e7 (2012).
131. Youngster, I. *et al.* Fecal microbiota transplant for relapsing *Clostridium difficile* infection using a frozen inoculum from unrelated donors: a randomized, open-label, controlled pilot study. *Clin. Infect. Dis.* **58**, 1515–1522 (2014).
132. Allen-Vercoe, E. Bringing the gut microbiota into focus through microbial culture: recent progress and future perspective. *Curr. Opin. Microbiol.* **16**, 625–629 (2013).

Acknowledgements

The authors' research work was supported by The W. Garfield Weston Foundation.

Author contributions

All authors researched the data for the article, provided substantial contributions to discussions of its content, wrote the article and undertook review and/or editing of the manuscript before submission.