PURPLE URINE BAG SYNDROME: RETHINKING THE ROLE OF URINARY AND GUT MICROBIOME IN THE PATHOGENESIS OF URINARY TRACT INFECTIONS

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Abstract: The effort of the Humane Microbiome Project has led to the awareness that many districts of the human organism, like the skin, gastrointestinal tract, and genitals harbor their normal resident microbes. For this reason, the scientific community overcame the dogma that urines are sterile. Instead, the urinary tract hosts many bacteria, the so-called urobiome, that contribute to its homeostasis and pathology. Urobiome seems to be involved in the pathogenesis of the urinary tract infections (UTIs) and its relationship with the gut microbiome is still far from being understood. We describe a case of an emergent urinary condition, the “purple urine bag syndrome” (PUBS) that displayed with a peculiar combination of pathogens: Corynebacterium urealitycum and Enterococcus faecium. Both bacteria have been described as components of the urobiome and the latter is a well-known member of the gut microbiome but also a possible uropathogen. This case report is the starting point to analyze what we know about urobiome, its role in UTIs, and its interactions with the gut microbiome in the so-called “gut-UTIs axis”.

Keywords: Urinary microbiome, Urobiome, Gut microbiome, Gut-UTIs axis, Urinary tract infections, Purple urine bag syndrome.

INTRODUCTION

For many years, urines have been thought to be sterile before reaching the urethra, at least in healthy individuals. This may be explained by the fact that standard microbiological methods are not able to identify and characterize the great variability of urinary bacterial species so that all the unidentified ones are referred as “uncultivated” bacteria while a polymicrobial growth is considered as a contamination of the specimen².

The Human Microbiome Project (http://commonfund.nih.gov/hmp/) has highlighted that, like the skin³, mouth⁴, gastrointestinal tract⁵, and vagina⁶, the healthy urinary tract hosts resident bacteria⁷,⁸.

The identification of the urinary microbiome (UM), or urobiome, has become possible by improving the sample collection and laboratory techniques used to identify bacteria that are usually overlooked with standard cultures, that commonly adopt a ≥10⁵-CFU/ml threshold to be considered positive⁹.

Pearce et al.¹⁰ reported that an increased volume of the urine specimen, diverse growth media, and atmospheric condition, and lengthened incubation time make many of these uncultivated bacteria...
cultivable. Anyway, detection and confirmation of the urobiome have become easier after introducing two non-culture, complementary, assays—the 16S ribosomal RNA (rRNA) gene sequencing and the expanded quantitative urine culture (EQUC). The first identifies bacterial DNA and the latter detects microorganisms that are usually reported as “no growth” and demonstrates that they are alive11-13.

To assess what is the most valid sample collection technique, some authors collected specimens from voided urine (VU), transurethral catheter (TUC), and suprapubic aspirate (SPA) of all the patients. VU sample composition differs from the TUC and the SPA but it is accurate for clinical care, like in the diagnosis of common urinary tract infections (UTIs). TUC and SPA microbiologic populations are similar in the same individual, that is why, despite the SPA being the gold standard for microbiome research, TUC is recommendable as it is less harmful and easier to perform14.

One last limitation for the study of the urobiome is the highly adherent nature of uropathogen to the apical cells of the urinary epithelium (umbrella cells). Standard cultures only pick a small amount of urines (typically 1 μl) from the supernatant of the sample, but the umbrella cells remain in the sediment of the sample15, remaining unanalyzed.

Urobiome in Health

Most of the information we know about urobiome comes from studies that compare specimens collected from healthy individuals vs. patients affected by a great variability of urologic conditions. Before discussing the urobiome composition in healthy individuals, it is important to point out that some studies have revealed that urinary tract bacteria differ from those inhabiting the gut and the vagina16-18.

Fouts et al19 state that the healthy urinary microbiome differs by gender with a prevalence of Lactobacillales in women and Corynebacterium in men. These data have been mostly confirmed by Siddiqui et al20, and by Pearce et al10, that identified Lactobacillus, Prevotella, and Gardnerella as the predominant species in healthy women bladder.

Hilt et al13 isolated Lactobacillus (15%), Corynebacterium (14.2%), Streptococcus (11.9%), Actinomycyes (6.9%), and Staphylococcus (6.9%) in the adult female bladder. Other commonly isolated genera include Aerococcus, Gardnerella, Bifidobacterium, and Actinobaculum13. Khashiya et al15 have obtained similar results.

Analyzing the samples from women with and without UTI-like symptoms, Price et al12, found that the genera Streptococcus and Gardnerella were prevalent in asymptomatic women.

In healthy men, the most common bladder bacteria are members of the Veillonella, Streptococcus, and Corynebacterium genera21.

The analysis of urine specimens from 19 healthy men isolated the five main bacterial phyla that constitute the male urethral microbiome: Firmicutes (52.6%), Actinobacteria (18.7%), Fusobacteria (10.0%), Proteobacteria (9.4%), and Bacteroidetes (7.4%). Firmicutes were found in all the specimens, and 50% of them belonged to the Lactobacillus, Corynebacterium, Streptococcus, and Sneathia spp. genera22. Dong et al23 obtained similar results studying men with and without sexually transmitted infections.

The little differences in the results of the cited studies may be explained by the generally small sample size, different sample collection methods, diverse specimen processing, and various laboratory techniques. These limitations strongly suggest the urgency to find a consensus in the terminology and methodology for the study of urinary microbiome.

Anyway, these data also suggest that urobiome is not a silent bystander in the urinary physiology, since it may play some role in maintaining the urinary tract homeostasis and health. For example, it might act as a barrier to uropathogens, competing for resources17 or modulating the urothelial innate immune system24. These hypotheses are consistent with solid research proving that a well-known condition like the asymptomatic bacteriuria (ABU) is harmless and maybe even protective against urinary tract infections25.

Urobiome in Urinary Tract Diseases

Many studies have reported that host and environmental factors may imbalance the composition of the UM and contribute to some of the main urologic and gynecologic disorders1.
UM dysbiosis promotes prostate inflammation leading to benign conditions, such as benign prostatic hyperplasia, acute and chronic prostatitis, and chronic pelvic pain syndrome. Besides direct damage, indirect harmful mechanisms, such as the UM ability to affect systemic estrogen and androgen levels, may promote prostate cancer. Variations in the urobiome composition relate to different urinary incontinence patterns in women (stress, urgency, mixed incontinence) and influence the sensitivity or resistance to some pharmacological treatments.

A recent study by Heidler et al has described that also the renal tissue has resident microorganisms. Moreover, there were important differences between benign and malignant tissue, suggesting that the renal microbiome may have an impact on renal physiology and tumorigenesis.

Xu et al has detected an enrichment of the genus Streptococcus in patients with bladder cancer. A more recent study did not identify significant differences in the microbiome composition between healthy individuals and patients with bladder cancer but concluded that some taxa were over-represented in patients with bladder cancer.

**Urobiome and Urinary Tract Infections**

The study of the urobiome is also giving a deeper insight into the understanding of the infective urinary disease. The acronym UTI (Urinary Tract Infection) indicates the infections that affect any part of the urinary apparatus. UTIs are the most common bacterial infection – independently from age – and one of the most common causes for antibiotic prescription and hospitalization. Among UTIs, the catheter-associated UTIs (CAUTIs) account for 40% of all nosocomial infections and are the most common complication of indwelling urinary catheters.

*E. coli* is the most common pathogen isolated in community-acquired UTIs; other commonly isolated pathogens are *Staphylococcus, Klebsiella spp.*, *Enterococcus spp.*, *Enterobacter spp.*, *Proteus mirabilis, Pseudomonas* and *Streptococcus*.

Even if the Gram-negative *E. coli* accounts for the majority of UTIs, it is known that Gram-positive bacteria can be common uropathogens, particularly among fragile individuals like the elderly. Moreover, there is growing evidence that, alongside the more familiar Gram-positive uropathogens, like *Staphylococci, Streptococci, and Enterococci*, other emerging and rare Gram-positive microorganisms, including *Aerococcus, Corynebacterium, Actinobaculum*, and *Gardnerella* may be responsible for UTIs. As stated before, some of those bacteria have been identified as part of the normal urobiome. Anyway, the diagnosis of UTIs caused by these bacteria can be easily missed since they may not be identified by standard laboratory tests and their polymicrobial growth can be mistakenly labeled as contamination.

**GUT-UTIS Axis**

The composition of the microbiome of one body district may influence the health and promote diseases even in distant organs. The gut microbiome plays both an indirect and a direct role in the pathogenesis of many urinary illnesses. Gut dysbiosis indirectly promotes hypertension, chronic renal disease, and kidney stone disease. A more direct role of the gut microbiome is described in the UTIs. It is commonly accepted that UTIs are caused by the colonization of the urethra by uropathogens residing in the gut, thanks to their attitude to adhere to the urinary epithelium. This pathogenic theory is supported by the fact that many uropathogens are part of the gut microbiota, in particular of the colonic one. Moreover, UTIs are more common in women because the female urethra is closer to the anus and shorter than the male urethra, thus facilitating the colonization and migration of gut bacteria to the bladder. Anyway, it seems reasonable that to make a UTI possible, the gut microbiome should undergo some dysbiotic modifications – different composition, increasing adherence and virulence – that may favor urinary colonization. Magruder et al have found that an increase of 1% of *Escherichia* or of *Enterococcus* in the gut is an independent risk factor for *Escherichia* or *Enterococcus* bacteriuria and UTIs, thus describing a gut microbiota-UTI axis.
The existence of a gut-UTIs axis suggests that the gut microbiota modulation may be a promising strategy in UTIs prevention and treatment\(^4\), as described in some case reports and studies about oral probiotics and fecal transplantation.

For oral probiotics, contrasting findings have been reported. A study by Wolff et al\(^45\), evaluating the influence of oral probiotics in affecting the composition of the urobiome in young women, has not found differences. Similar results have been found when evaluating the efficacy of oral probiotics administration in the prevention of recurrent UTIs in children\(^46\).

At present, the treatment of recurrent \textit{Clostridioides difficile} infection is the only indication for fecal microbiota transplantation. Anyway, there is growing interest in the application of this practice in the management of other pathologies associated with alteration of gut microbiota\(^47\), like recurrent UTIs\(^48,49\).

**Purple Urine Bag Syndrome: A Case Report**

Purple urine bag syndrome (PUBS) is described as a purple discoloration due to the mixture of two pigments, a red one, the indirubin, and a blue one, the indigo. These pigments are produced by bacteria containing sulphatase and phosphatase enzymes. The main bacteria involved in the pathogenesis of the PUBS are \textit{Providencia stuartii} and \textit{rettgeri}, \textit{Proteus mirabilis}, \textit{Pseudomonas aeruginosa}, \textit{Klebsiella pneumoniae}, \textit{Escherichia coli}, \textit{Morganella} and \textit{Citrobacter} spp, \textit{Enterococci}, and \textit{Group B Streptococci}. The enzymes expressed by these bacteria deaminate the tryptophan to form indole, pyruvic acid, and ammonia. In the liver, the indole is conjugated in indoxyl sulfate (indicin) that is converted by sulfatases and phosphatases in the indoxyl. Indicans give the urine a dark brown color but, when exposed to air, they are oxidized in indigo and indirubin. The combination of these pigments in the presence of polyvinyl chloride (PVC), which may be a constituent of the urine bag, gives the urine a characteristic purple appearance (Figure 1)\(^50\). Predisposing factors for PUBS are dementia, chronic debilitation, chronic urinary catheterization, female gender, high tryptophan intake, severe constipation, and urinary tract infection.

**Figure 1.** Pathogenesis of PUBS. Many organs participate in tryptophan metabolism. In the presence of predisposing factors and bacteria expressing phosphatases and sulfatases, the terminal products – the indigo (blue) and indirubin (red) – react with the PVC of the bag.
pation, urinary tract infections, and chronic kidney disease (CKD)\textsuperscript{51,52}. PUBS is considered rare but, in institutionalized patients with long-term indwelling urinary catheters, the prevalence is higher than 9.5\%\textsuperscript{53}. It is often described as a benign condition, by which non-pharmacological measures, like the sole catheter replacement and the control of predisposing factors, are preferred to the antibiotic therapy\textsuperscript{54,55}. Anyway, since there have been lethal cases\textsuperscript{56}, it seems reasonable to choose between non-pharmacological and pharmacological strategies, after an early risk stratification and the identification of the pathogen.

PUBS may be encountered in our clinical practice. Here we describe the case of an 85-year-old woman with chronic hypertensive and ischemic cardiomyopathy, atrial fibrillation, CKD stage IV, chronic obstructive pulmonary disease (COPD), and class I obesity, who was admitted to the emergency department for a subarachnoid hemorrhage. Her home therapy consisted of beta-blocker, antihypertensive, diuretics, allopurinol, antipsychotic, paracetamol, and warfarin. The vital parameters at admission were normal; among other medical devices, a urinary catheter was placed, and she was monitored in the emergency room. After 72 hours, the urine in the catheter tube and in the PVC-bag turned purple (Figure 2). The patient was afebrile and asymptomatic, but her last bowel movement was on the day of the onset of neurologic symptoms. Despite the absence of signs and symptoms, we noticed many risk factors, like old age, female gender, constipation, and CKD. We decided to perform a routine blood and urine testing and a urine culture. Meanwhile, the catheter was replaced, and she was treated for constipation. The urines were alkaline with leukocyte esterase; the blood analysis documented neutrophil leukocytosis, increased C-reactive protein (CRP), and decreasing of the estimated glomerular

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{Purple_Urine_Bag_Syndrome_Figure2.png}
\caption{PUBS in a patient with subarachnoid hemorrhage. The purple discoloration appeared after 72 hours from the urinary catheterization. The patient was afebrile and asymptomatic, but the last bowel movement was on the day of the onset of neurologic symptoms. Note that the purple hue can be seen both in the catheter tube and in the urine bag.}
\end{figure}
filtration rate (eGFR) from 17 to 9 mL/min/1.73 m². Given these results, while awaiting the report of the urine culture, empirical therapy with piperacillin-tazobactam was started and the patient was admitted to an internal medicine ward. The urine returned to its normal color on the same day. Urine culture detected *Enterococcus faecium* and *Corynebacterium urealyticum* resistant to many antibiotics and sensible to teicoplanin, tigecycline, and vancomycin. After ten days, the patient presented fever and leukocytosis with alkaline urine and leukocyte esterase. Chest X-ray was normal and there was no bacteremia. The urinary catheter was newly replaced, and she was given teicoplanin. Against persistent fever, the antibiotic therapy was reinforced with fluconazole and meropenem, with resolution of the infection.

**DISCUSSION**

The growing evidence that almost every part of the human body hosts its resident microbes, the microbiome, is challenging our medical knowledge in many disciplines or, at least, leading us to rethink what we know about many health and pathologic conditions from a different point of view. The peculiar clinical case reported in this article has been our starting point to review what we know about the urobiome and gut microbiome and their interaction in the pathogenesis of urinary tract infections. It is commonly accepted that UTIs are a consequence of the colonization of the urethra by uropathogens, especially Gram-negative bacteria. Anyway, this pathogenic model assumes that the urinary tract is sterile, thus not considering the existence of a normal urinary resident microbiome. Urobiome has a protective role against infections, which suggests that a urinary dysbiosis may be a predisposing factor for infections because it liberates a niche, usually occupied by normal flora, for pathogen colonization. It has been demonstrated that, to cause UTIs, also gut microbiome should undergo some dysbiosis, consisting of modifications in bacterial composition, adherence, and virulence. Beside physiopathologic events, also medical intervention like some medication or medical devices may promote dysbiosis. For example, the insertion of the indwelling catheter can be a source of bacteria from the external environment; anyway, the best practice for urinary catheterization is the sterile technique. Since the surface of the catheter is supposed to be sterile, it could be considered as a new niche, available for bacterial growth, also for those species that represent the minority of the normal flora and do not strongly proliferate in normal conditions. On top of that, it is hypothesized that the gut and the urinary tract communicate through a gut-urinary tract axis. The possible existence of this axis suggests that the gut microbiota modulation may be a promising strategy in UTIs prevention and treatment. We believe that our case of purple urine bag syndrome synthesizes many of the aspects described so far about the interaction among urobiome, gut microbiome, and the environment. Following recent evidence of bacterial synergy in experimental models of polymicrobial UTI, we described the co-existence of two bacteria in the same specimen. Moreover, while *E. faecalis* is known to be a causal agent of PUBS, *Enterococcus faecium* and *Corynebacterium urealyticum* have never been reported as responsible for PUBS before our case. Enterococci are Gram-positive bacteria that usually live as commensals in the human gastrointestinal tract. They can become nosocomial pathogens through multi-drug resistance acquisition and can be very difficult to treat, especially when they colonize indwelling medical devices. Enterococcus species *E. faecalis* and *E. faecium* are responsible for a minority of community-acquired UTIs, but the two of them cause 15 to 30% of CAUTIs and are the third leading cause of hospital-acquired UTIs. The virulence of enterococci depends on their resistance to stresses, like an alkaline environment with adaptive processes, including the regulation of genes involved in the amino acid transport and metabolism. *E. faecium* is an indole-producer bacterium, whereas *E. faecalis* expresses an alkaline phosphatase. Firmicutes, which include Enterococci, are widely represented in the gut microbiota but have been also described as part of the normal urobiome. *C. urealyticum* is a Gram-positive opportunistic pathogen of the skin and mucous membranes, to be found mainly in hospitalized patients. It shows a urease activity that enables the alkalization of the urinary pH and causes urinary infections; its treatment requires the administration of multiple antibiotics since it shows MDR factors. It also has been described as a normal component of the male and female urinary tract and, along with other bacteria like Aerococcus, Actinobaculum, and Gardnerella vaginalis, is considered a rare and emerging uropathogen. *Corynebacterium* may be missed as causes of UTI because of a lack of detection, misclassification, or dismissal of
significant growth as ‘microbiota contamination’. It seems reasonable to think that the co-existence of these two bacteria in the urine of our patient promoted the PUBS. The ureases of C. urealyticum alkalized the urines while the E. faecium produced the indoles that were then transformed in indicans by the enzymes of the same bacterium. The empiric antibiotic therapy and the catheter replacement caused only a temporary remission, since both E. faecium and C. urealyticum are multi-drug resistant bacteria. The susceptibility test allowed us to choose a more targeted antimicrobial therapy that led to the resolution of the infection. Two more factors may have promoted the PUBS by causing gut and urinary dysbiosis: constipation and urinary catheterization. Constipation has contributed to make tryptophan more available for deamination; in fact, the resolution of the constipation is one of the mainstays of PUBS treatment. Besides, the presence of a foreign body – the catheter – may have promoted not only the migration of enterococci from the gut, but also the formation of a new niche for resident Enterococci and Corynebacteria to proliferate. For this reason, the replacement of the catheter is another measure in the treatment of PUBS and can sometimes be enough to make the syndrome disappear. All these speculations suggest that probiotics and prebiotics may act as a treatment or preventive agents for urologic disorders, but further investigations are needed.

CONCLUSIONS

The interconnection between intestinal and urinary tract microbiomes represents an interesting field of study that may lead to a better understanding of some conditions, such as UTIs. A rare and unusual presentation of UTI, like our peculiar case of PUBS, is a good model to explore these interactions and find potential non-pharmacological strategies of prevention and treatment.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES


