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Commentary

The urine microbiome – Contamination or a novel paradigm?

Niels Frimodt-Møller

Dept. of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark

Grine et al. [1] in this article of *EBioMedicine* report an interesting finding by quantitative PCR of *Methanobrevibacter smithii,* an archaeal methanogen, in the urine of 34 patients comprising 9% of 383 urines submitted for culture for suspected urinary tract infection (UTI). In 31 of the samples the bacteria could be cultured by a special culturing method for methanogens. In all cases standard urinary pathogens were identified in co-culture. In 19 patients symptomatic UTI was confirmed, including pyelonephritis and prostatitis. All these cases did well on antibiotics for the facultative anaerobes, although it was shown, that *M. smithii* is resistant towards standard urinary antibiotics, but susceptible to metronidazole.

This paper adds to a growing list of studies, where DNA-methods including PCR, 16S-analysis or whole genome sequencing detect in urine samples a wide range of bacterial species, which are not found by standard aerobic culture [2–5]. The term "urine microbiome" is being proposed as a term denouncing the old paradigm, that urine in the bladder is usually sterile. The bacterial species include lactobacilli, obligate anaerobes, *Gardnerella vaginalis*, non-hemolytic streptococci and myco- and ureaplasms amongst others.

Bacteria in the bladder urine can arrive from the kidneys, which in some cases of systemic infections (e.g. Salmonella spp., *Staphylococcus aureus* or Candida spp. as the best known) let bacteria through and into the urine, or by ascending from outside via the urethra.

Bacterial contamination of the clean catch midstream urine (MSU) has been known since Kass defined the criteria for discerning between asymptomatic bacteriuria and pyelonephritis [6]. Later, Hooton and coworkers demonstrated the importance of correct urine sampling by intermittent catheter e.g. enterococcal species and group B streptococci were mostly contaminants found in samples taken by MSU only [7]. In the Grine study, urines were sampled by MSU [1]; this represents a possible weakness, as urine sampling for studying the real urine microbiome should be obtained via a sterile urethral catheter or by suprapubic puncture in order to avoid contamination – at least in women [4].

The presence of obligate anaerobes in human urine was reported already in the 1960´ies, where anaerobic culture techniques began improvement in microbiological laboratories [8,9]. Although occasional case stories illustrated that serious infections with obligate anaerobes could ensue from a urinary primary focus [10], it was generally acknowledged that anaerobes in the urine seemed to be occasional occurrences. It did not change the standard urinary culture techniques, i.e. to search for so-called typical urinary pathogens, which grow on standard agar media under aerobic conditions – most typically *E. coli*. Slight improvement in microbiological culture conditions such as extending incubation time to 48 h in an atmosphere with added CO_2 expanded the urinary pathogen "family" with "new" organisms such as *A. schaalii* and *Aerococcus urinae*. Using DNA methods as mentioned above and lowering the limits for quantitative counts to 10 CFU/ml – which demanded larger volumes of urine analyzed – it is now possible and has been shown to find bacteria in almost all individuals, both patients with symptoms of UTI and asymptomatic, normal controls [4]. And in most cases three or more bacterial or fungal species can be detected.

In case of *Methanobrevibacter smithii* it appears to be easier and faster to apply a PCR or other DNA method instead of culture, which takes days to perform, and this counts for most of the members of the "urine microbiome" [1]. But before we discard the standard urine culture and analyze all samples in the molecular biology laboratory, we must know more about the importance of this microbiome. How many patients with symptoms of UTI and standard-culture negative have infection with other bacteria? Is recurrent UTI caused by lack of antibiotic coverage of these "other" bacterial species? What is "asymptomatic bacteriuria", or should this term be discarded, if bacteria are present in all individuals? Do members of the urine microbiome protect against standard pathogens and should antibiotic treatment be designed better to avoid removing the normal microbiome if possible? How does the microbiome interplay with the host's immune system in the urinary tract?

Future research in these issues needs strict adherence to correct sampling of urine to avoid contamination from the microbiome of the skin and the vagina, confirmation by culture or microscopy of bacteria detected by DNA-methods, better definition or criteria of UTI including analysis of host immune system reaction to the microbes in question and strict criteria for effect of treatment. This research will however help us to understand one of the most frequent bacterial infections causing huge suffering and costs for humans.

Author contribution

Niels Frimodt-Møller wrote the Commentary.

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E-mail address: niels.frimodt-moeller@regionh.dk.

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Declaration of interests

Niels Frimodt-Møller has nothing to disclose.

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