Send Orders for Reprints to reprints@benthamscience.ae

The Open Microbiology Journal, 2016, *10*, 23-26 23

BENTHAM OPEN

CrossMark

The Open Microbiology Journal

Content list available at: www.benthamopen.com/TOMICROJ/

DOI: 10.2174/1874285801610010023

Letter to the Editor: Diagnostic Criteria in Urological Diseases do not Always Match with Findings by Extended Culture Techniques and Metagenomic Sequencing of 16S rDNA

Vitaly Smelov[1], Kurt Naber[2] and Truls E. Bjerklund Johansen[3,*]

[1] *Screening Group, International Agency for Research on Cancer, World Health Organization, 150 Cours Albert Thomas, 69372 Lyon, France*

[2] *Technical University of Munich, Karl-Bickleder-Str. 44c, 94315 Straubing, Germany*

[3] *Department of Urology, Oslo University Hospital, PO Box 4959, Nydalen, Oslo, Norway*

Abstract: Some diseases of the urinary tract are defined by the presence of microorganisms while others are defined by their absence. The underlying idea has always been that urine from healthy subjects is sterile and a negative urine culture has usually been taken as discriminative for an infection to be absent. Several disorders with symptoms that resemble infections are regarded as separate entities based on the exclusion of bacterial growth such as overactive neurogenic bladder and pelvic pain syndromes. During the recent years two paradigmata related to the role of bacteria in urological disease classification have changed completely. Firstly, bacteriuria does not necessarily mean an infection, and secondly, if extended sets of culture media for identification of fastidious and anaerobic bacteria or culture-independent metagenomic sequencing (MGS) is applied, a broad range of even non-culturable bacteria has been detected in the ”sterile” bladder urine in healthy individuals. The aim of this editorial is to initiate a discussion to redefine the criteria for urinary tract infections and non-infectious urological disorders with similar symptoms. Clinical studies, in which extended sets of culture media and MGS are integrated, are needed to clarify the pathogenesis of urological disorders where bacteria may play a role. The pure detection of bacteria in the urine does not by itself prove an infectious etiology of a specific disorder. It is important to avoid that results of new technologies lead to unnecessary antibiotic consumption with unwanted collateral damage and adverse events.

Keywords: Asymptomatic bacteriuria, metagenomic sequencing, non-infectious urological disorders, urinary tract infection.

INTRODUCTION

In a recent paper in the Open Microbiology Journal Siddiqui *et al.* report 16S rDNA findings in the urine of a woman diagnosed with overactive bladder disease (OAB). A complex bacterial profile, with fastidious and anaerobic bacteria, was observed in contrast to the findings in routine urine culture. Thus the authors question the role of microorganisms in disease classification [1]. Their question is relevant as some diseases of the urinary tract are defined by the presence of microorganisms while others are defined by their absence. The aim of the present paper is to discuss how findings by extended culture techniques and contemporary metagenomic sequencing of 16S rDNA challenge classic diagnostic criteria for numerous urological diseases.

Diagnoses based on the presence of microorganisms

Urinary tract infections (UTI) is a big group of disorders usually classified as uncomplicated and complicated, but more recently also by risk factors and severity grading depending on the clinical presentation [2]. The diagnosis requires clinical symptoms and evidence of living bacteria in the urine, usually quantified by numbers of colony

* Address correspondence to this author at the Department of Urology, Oslo University Hospital, Po.box 4959, Nydalen, Oslo, Norway; Tel: +47 91841063; E-mail: tebj@medisin.uio.no

forming units per milliliter (CFU/ml). Leucocytes and blood in urine are signs of the host response. Leucocytes infiltrate the mucosa and release immunoactive proteins. As a result urothelial cells may burst and be shed into the urine; sometimes bleeding occurs [3].

Culture tests and urine microscopy have been the gold standard for diagnosing UTI. When sexually transmitted pathogens are suspected an amplification system is also used [4, 5]. The concept of significant bacteriuria was introduced by Kass in order to differentiate between infection and contamination and was based on monoculture of a dominant pathogen [6]. However, no fixed bacterial count has been considered conclusive for significant bacteriuria in all kinds of UTIs and under all circumstances [7]. Currently, the critical numbers of uropathogens in a midstream sample of urine (MSU) should exceed 10^4 CFU/ml in men or vary from $\geq 10^3$ CFU/ml in acute uncomplicated cystitis till $\geq 10^5$ CFU/ml in women with complicated UTIs. The lower the colony counts in MSU the higher the likelihood of contamination. In a suprapubic bladder puncture specimen, any count of bacteria is considered diagnostic [5]. The currently used routine urine culture method is limited to detect easily culturable aerobic bacteria only and not fastidious and anaerobic bacteria. The underlying idea has always been that urine from healthy subjects is sterile and a negative or positive urine culture has usually been taken as discriminative for an infection to be absent or present, respectively.

Diagnoses based on the absence of microorganisms

Several urological disorders with symptoms that resemble infections are regarded as separate entities based on the exclusion of bacterial growth with conventional techniques. Examples are overactive neurogenic bladder, female urethral syndrome, bladder pain syndrome/interstitial cystitis and chronic pelvic pain syndrome/chronic inflammatory prostatitis. Interestingly, the differentiation of chronic abacterial prostatitis in inflammatory and non-inflammatory depends on the presence of white cells in expressed prostatic secretion which may be a sign of a host reaction to infection. Biopsy has a role in the diagnostic work-up in interstitial cystitis to detect mast cells in the mucosa, which is regarded a specific host reaction.

Change of paradigmata

During the recent years two pretended paradigmata related to the role of bacteria in urological disease classification have changed completely [8, 9]:

i. We have learned that asymptomatic bacteriuria, although "significant" according to the bacterial load, should not be considered an infection anymore. It rather represents colonization or, under certain circumstances a risk factor, and treatment is generally not necessary and sometimes even harmful [5, 10]. Furthermore, it has been shown that bladder instillation with certain *E.coli* strains may even prevent recurrent infections by bacterial interference [11]. Hence, bacteriuria does not necessarily mean an infection.
ii. There is increasing evidence that if extended sets of culture media for identification of fastidious and anaerobic bacteria or culture-independent metagenomic sequencing (MGS) is applied, a broad range of even non-culturable bacteria can be detected in the "sterile" bladder urine in healthy individuals [9, 12 - 17] in female patients with different urological disorders [1, 17 - 20] and in expressed prostatic secretions in men [21]. The terms urinary microbiota and urinary microbiome have been adopted to define the microorganisms that normally exist within the bladder [15].

Thus, sterile urine seems to be a myth and the recent MGS-based findings on the urine microbiome encourage a discussion to redefine the criteria for urinary tract infections and non-infectious urological disorders with similar symptoms.

The role of MGS in diagnosing urological disorders

In their case report Siddiqui and colleagues [1] presented a female patient with a 10 years history of overactive bladder (OAB) syndrome diagnosed after exclusion of other urological disorders, such as interstitial cystitis and bladder cancer. The patient had been treated with various anticholinergic agents with temporary relief only. When urine culture revealed a "significant" bacteriuria of $>10^5$ cfu/ml with α-hemolytic streptococci (*Streptococcus viridans* group) she was treated for one week with trimethoprim 160 mg b.i.d. About a month later the routine culture was negative, but the urinary symptoms persisted.

By comparison of midstream and catheter urine samples, Hooton *et al.* [7] demonstrated that streptococci are rarely uropathogens but rather contaminants. It was also demonstrated that very low counts of *E. coli* could be relevant, while

other Gram-negative rods or *S. saprophyticus* were not [7]. Although trimethoprim is not recommended for the treatment of streptococcal infections, it would most likely be an adequate treatment for a low count UTI caused by *E. coli.* Therefore, according to the classical approach the OAB diagnosis made by Siddiqui *et al*. can still be regarded as confirmed, especially since an intermittent electric stimulation treatment was satisfactory, a treatment which the patient wanted to continue.

On two occasions, when the streptococcal bacteriuria was registered and one year later, respectively, the authors analyzed the patient's urine with a culture-independent 16S ribosomal DNA pyrosequencing analysis. Findings in the 1st urine sample were consistent with the routine culture of α-hemolytic streptococci, but also for several fastidious bacteria, such as Atropium*, Prevotella*, and *Ureaplasma spp*. The latter were also confirmed in the routine culture negative 2nd urine sample one year later. Based on these findings the authors speculate whether or not these bacteria, especially the *Ureaplasma sp*., may be the infectious agents causing the patient`s chronic symptoms. Unfortunately, no adequate antibiotic therapy for *Ureaplasma sp*. with macrolides or tetracyclines was administered and without the results of adequate treatment no conclusions can be drawn about the possible importance of these "difficult-to-culture" bacteria for the patient`s symptoms. Thus, despite highly sophisticated investigations the gain of new knowledge from this case report is rather limited.

CONCLUSION

Extended sets of culture media and culture-independent metagenomic sequencing (MGS) enable us to detect a wide range of fastidious and anaerobic and even non-culturable bacteria in the "sterile" bladder urine in healthy individuals as well as in patients with different urological disorders. We must of course take advantage of these new technologies and it is high noon to redefine UTIs and various urological disorders hitherto based on findings by routine culture only. The new technologies will shed new light on the poorly understood etiological relationship of microorganisms and malignant tumors of the urinary tract, as found for example with Herpes virus and Schistosomiasis in bladder cancer. It is expected that the application of novel MGS technologies will further improve findings, as the significance of sequencing data is based on the number of sequencing reads [22]. However, for cost reasons it will probably take time before novel assays become clinically applicable, but while the MGS methods remain expensive, the sequencing related costs have decreased rapidly. It is also expected that further MGS studies will extend our knowledge about the microbiome of the urogenital tract in both men and women, and the technology opens intriguing new perspectives regarding the role of microorganisms in diseases of the urinary tract.

However, as we recently learned in the case of asymptomatic bacteriuria, the pure detection of bacteria in the urine does not by itself prove an infectious etiology in a specific disorder. In order to clarify the pathogenesis of urological disorders where bacteria may play a role we need to integrate extended sets of culture media and MGS in well-designed clinical studies. Having said this, it is important to avoid that implementation of new technologies leads to increased consumption of antibiotics with unwanted adverse events and collateral damage.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Siddiqui H, Lagesen K, Nederbragt AJ, Eri LM, Jeansson SL, Jakobsen KS. Pathogens in urine from a female patient with overactive bladder syndrome detected by culture-independent high throughput sequencing: A case report. Open Microbiol J 2014; 8: 148-53. [PMID: 25685246]

[2] Johansen TE, Botto H, Cek M, *et al.* Critical review of current definitions of urinary tract infections and proposal of an EAU/ESIU classification system. Int J Antimicrob Agents 2011; 38(Suppl.): 64-70. [http://dx.doi.org/10.1016/j.ijantimicag.2011.09.009] [PMID: 22018988]

[3] Wullt B, Sunden F. Asymptomatic bacteriuria with the model strain Escherichia coli 83972 protects against symptomatic urinary tract infections. Urogenit Infect Eur Assoc Urol Consult Urol Dis Arnh 2010; pp. 314-8.

[4] Litwin MS, McNaughton-Collins M, Fowler FJ Jr, *et al.* The National Institutes of Health chronic prostatitis symptom index: development and validation of a new outcome measure. J Urol 1999; 162(2): 369-75. [http://dx.doi.org/10.1016/S0022-5347(05)68562-X] [PMID: 10411041]

[5] Grabe M, Bartoletti R, Bjerklund Johansen TE, *et al.* EAU Guidelines on urological infections. In: EAU Guidelines [Internet]. european association of urology; 2015 [cited 2015 Jun 18]. Available from: http: //uroweb.org/wp-content/uploads/19-Urological-infections_LR2.pdf 2015.

[6] Kass EH. Bacteriuria and pyelonephritis of pregnancy. Arch Intern Med 1960; 105: 194-8. [http://dx.doi.org/10.1001/archinte.1960.00270140016003] [PMID: 14404662]

[7] Hooton TM, Roberts PL, Cox ME, Stapleton AE. Voided midstream urine culture and acute cystitis in premenopausal women. N Engl J Med 2013; 369(20): 1883-91. [http://dx.doi.org/10.1056/NEJMoa1302186] [PMID: 24224622]

[8] Wagenlehner FM, Naber KG. Editorial commentary: asymptomatic bacteriuria--shift of paradigm. Clin Infect Dis 2012; 55(6): 778-80. [http://dx.doi.org/10.1093/cid/cis541] [PMID: 22677709]

[9] Kogan MI, Naboka YL, Ibishev KS, Gudima IA, Naber KG. Human urine is not sterile - shift of paradigm. Urol Int 2015; 94(4): 445-52. [http://dx.doi.org/10.1159/000369631] [PMID: 25766599]

[10] Cai T, Mazzoli S, Mondaini N, *et al.* The role of asymptomatic bacteriuria in young women with recurrent urinary tract infections: to treat or not to treat? Clin Infect Dis 2012; 55(6): 771-7. [http://dx.doi.org/10.1093/cid/cis534] [PMID: 22677710]

[11] Wullt B, Ragnarsdottir B, Fischer H, Gronberg-Hernandez J, Lutay N, Svanborg C. Urogenital Infections In: Kurt G. Naber, Anthony J. Scaeffer, Chris F. Heyns, Tetsuro Matsumoto, Daniel A. Shoskes, Truls E. Bjerklund Johansen, Eds. Immunity, genetics and susceptibility to urinary tract infection. 1st ed. Grafos, Spain: Urogenit Infect 2010; pp. 21-30.

[12] Siddiqui H, Nederbragt AJ, Lagesen K, Jeansson SL, Jakobsen KS. Assessing diversity of the female urine microbiota by high throughput sequencing of 16S rDNA amplicons. BMC Microbiol 2011; 11: 244. [http://dx.doi.org/10.1186/1471-2180-11-244] [PMID: 22047020]

[13] Hilt EE, McKinley K, Pearce MM, *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 2014; 52(3): 871-6. [http://dx.doi.org/10.1128/JCM.02876-13] [PMID: 24371246]

[14] Fouts DE, Pieper R, Szpakowski S, *et al.* Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder associated with spinal cord injury. J Transl Med 2012; 10: 174. [http://dx.doi.org/10.1186/1479-5876-10-174] [PMID: 22929533]

[15] Wolfe AJ, Brubaker L. "Sterile Urine" and the Presence of Bacteria. Eur Urol 2015; 68(2): 173-4. [http://dx.doi.org/10.1016/j.eururo.2015.02.041] [PMID: 25774008]

[16] Lewis DA, Brown R, Williams J, *et al.* The human urinary microbiome; bacterial DNA in voided urine of asymptomatic adults. Front Cell Infect Microbiol 2013; 3: 41. [http://dx.doi.org/10.3389/fcimb.2013.00041] [PMID: 23967406]

[17] Pearce MM, Hilt EE, Rosenfeld AB, *et al.* The female urinary microbiome: a comparison of women with and without urgency urinary incontinence. MBio 2014; 5(4): e01283-14. [http://dx.doi.org/10.1128/mBio.01283-14] [PMID: 25006228]

[18] Khasriya R, Sathiananthamoorthy S, Ismail S, *et al.* Spectrum of bacterial colonization associated with urothelial cells from patients with chronic lower urinary tract symptoms. J Clin Microbiol 2013; 51(7): 2054-62. [http://dx.doi.org/10.1128/JCM.03314-12] [PMID: 23596238]

[19] Brubaker L, Nager CW, Richter HE, *et al.* Urinary bacteria in adult women with urgency urinary incontinence. Int Urogynecol J Pelvic Floor Dysfunct 2014; 25(9): 1179-84. [http://dx.doi.org/10.1007/s00192-013-2325-2] [PMID: 24515544]

[20] Nienhouse V, Gao X, Dong Q, *et al.* Interplay between bladder microbiota and urinary antimicrobial peptides: mechanisms for human urinary tract infection risk and symptom severity. PLoS One 2014; 9(12): e114185. [http://dx.doi.org/10.1371/journal.pone.0114185] [PMID: 25486068]

[21] Smelov V, Arroyo Mühr LS, Bzhalava D, Brown LJ, Komyakov B, Dillner J. Metagenomic sequencing of expressed prostate secretions. J Med Virol 2014; 86(12): 2042-8. [http://dx.doi.org/10.1002/jmv.23900] [PMID: 24532541]

[22] Beal MA, Glenn TC, Somers CM. Whole genome sequencing for quantifying germline mutation frequency in humans and model species: cautious optimism. Mutat Res 2012; 750(2): 96-106. [http://dx.doi.org/10.1016/j.mrrev.2011.11.002] [PMID: 22178956]

Received: July 10, 2015 Revised: August 10, 2015 Accepted: August 13, 2015