Urinary microbiota analyzed by extended microbiology in subjects with and without urological disorders

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Abstract

A healthy person's urine is not a sterile environment, and its microbiota contain a wide range of Gram-positive and Gram-negative aerobic and anaerobic bacteria, with Gram-positive aerobic microorganisms predominating. There are significant differences between the urinary microbiota of healthy men and women. The composition of healthy women's urinary microbiota depends on age and sexual activity. Non-clostridial anaerobic bacteria (NAB) predominate Enterobacteriaceae in case of acute obstructive pyelonephritis with bacteriuria of 10^4 CFU/ml and higher. Experiments have shown that obstructive pyelonephritis can be caused by various species of NAB (*Peptococcus* spp., *Bacteroides* spp., *Eubacterium* spp.). In every third case of recurrent symptomatic uncomplicated lower urinary tract infection (UTI) Enterobacteriaceae with low level bacteriuria of $\leq 10^3$ CFU/ml are found and every third case of acute cystitis is not associated with typical uropathogens. Low levels of bacteriuria (10^2–10^3 CFU/ml) correlate with symptoms of upper and lower UTI and thus cannot be considered contamination. Asymptomatic bacteriuria is normal in healthy individuals.

Keywords: urinary tract infection, bacteriuria, urinary microbiota, urine culture, urine examination, culture medium, complicated urinary tract infection, uncompli-cated urinary tract infection

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Summary of recommendations

1. A sample of urine for bacteriological examination should be collected minimizing contamination of the material, and culture of urine should be conducted on an expanded set of culture media. To identify the facultative anaerobic bacteria it is advisable to use MacConkey Agar, HiCrome Candida Differential Agar, HiCrome Enterococci Agar, HiCrome Aureus Agar Base, Blood Agar prepared according to Mueller, Hinton agar added with sheep erythrocytes, and in order to identify non-clostridial anaerobic bacteria to use Anaerobic Agar, Broth Shaedler, Shaedler Agar, Bacteroides Bile Esculinum Agar, MRS Agar.
2. Culture medium for the cultivation of anaerobic and microaerophilic bacteria should be used for bacteriological urine culture in acute and chronic infections of the upper and lower urinary tracts.
3. In acute obstructive pyelonephritis, a level of bacteriuria for Enterobacteriaceae equal to 10^3–10^4 CFU/ml should be considered clinically significant.
4. In the experimental obstruction of the ureter, several taxons of non-clostridial anaerobic bacteria (*Peptococcus* spp., *Bacteroides* spp., *Eubacterium* spp.) 10^5 CFU/ml lead to the development of acute suppurative lesions of the kidney. In this regard, non-clostridial anaerobic bacteria should be considered as an etiological factor of acute obstructive pyelonephritis.
5. Difficult to cultivate microorganisms at a level of bacteriuria $<10^4$ CFU/ml should be considered etiologic for acute obstructive pyelonephritis if typical uropathogens are absent.
6. If non-clostridial anaerobic bacteria are isolated at a level of $\leq 10^3$ CFU/ml and typical uropathogens are absent in the urine of patients with uncomplicated infection of the lower urinary tract, their causative role cannot be ruled out.
7. Gram-positive microorganisms and non-clostridial anaerobic microorganisms in voided or catheterized urine samples at levels of 10^2–10^3 CFU/ml, should not be considered contamination.
8. Asymptomatic bacteriuria is normal in healthy individuals.

1 Introduction

Standard microbiological protocols for investigation of urine include microscopic examination with Gram stain and urine culture using media such as Blood and MacConkey Agar incubated at aerobic conditions (35°Ð¡, 24 hours) [1], [2], [3], [4], [5], [6], [7], [8], [9], [10], [11]. Such investigations aim to identify microorganisms in the urine known to cause urinary tract infection (UTI). These are mainly species of Enterobacteriaceae (*Escherichia coli*, *Klebsiella* spp., *Proteus* spp., et al.), *Pseudomonas* spp. and some selective Gram-positive species (*Staphylococcus aureus*, *S. saprophyticus*, *Enterococcus* spp., et al.). These standard protocols, however, narrow down the possible spectrum of uropathogens. Based on the ensuing results new antibacterial agents are developed and manufactured. Common knowledge about uropathogens remained unchanged until recently, when some studies showed, that some microorganisms, so far unknown as uropathogens, may also cause UTI [12], [13], [14], [15], [16], [17], [18].

As early as 1939 Schulte et al. [19] isolated *Bacteroides* spp. and anaerobic streptococci in patients with unilateral pyelonephritis. Later in 1976 Japanese authors [20] created an experimental model of acute pyelonephritis caused by *B. fragilis*, proving morphologically the etiologic role of this organism in inflammatory kidney lesions. Sapico et al. [21] isolated some taxons of non-clostridial anaerobic bacteria (NAB), such as *Bifidobacterium* spp. and *Veillonella* spp., from the bladder urine of patients with permanent indwelling urethral catheter. Brook [22] isolated *Bacteroides* spp., *Peptococcus* spp., and *Bifidobacterium* spp. from the urine of children with acute pyelonephritis and cystitis and as early as 1980 indicated the necessity of using anaerobic bacteriological studies. Apostolopoulo et al. [23] also noticed the relationship between NAB and development of kidney infections, because NAB were identified in 24.4% of kidney pathology samples after nephrectomy and 22.2% of the kidney bioptates and bladder urine with the predominance of *Bacteroides* spp. DuPrey et al. [24] described a case of acute pyelonephritis caused by *Lactobacillus delbrueckii* with a level of bacteriuria of $>10^{10}$ CFU/mL. The patient had a history of type II diabetes mellitus and hypothyreoidism.

In the 1990s our investigators' group extended the spectrum of culture media, including anaerobic culturing techniques in order to increase the isolation of a wider spectrum of aerobic and anaerobic bacteria. Those media were previously used to study vaginal and bowel microbiota [1], [2], [3], [10]. We could show [25], [26], [27], [28], [29], [30] that urine of healthy men, women and children and urine of patients with UTI contains a wide spectrum of aerobic and anaerobic bacteria and also virus-bacteria associations.

Finally the realization of the Project to Study the Human Microbiome (2008–2012) became a serious breakthrough in proving that urine of healthy individuals and of patients with UTI is colonized by many different microorganisms with different levels of bacteriuria [4], [31], [32], [33], [34], [35], [36], [37], [38], [39], [40]. New impulses for a modern view on urinary microbiota were generated by Brubaker [7]. He stated that "the standard urine culture protocol was not designed to detect bacteria that require special nutrients, grow slowly, cannot tolerate oxygen, or are present in small numbers ($<10^3$ CFU/mL). Some of these organisms may be involved in urinary disorders". Aagaard [41] critised standards even sharper: "Even if a single microbe is the etiologic agent of infection, the pathogenesis and pathophysiology of infection can be viewed within the context of the microbiome and human biology", and further "Connections among microbiomes in different body sites may help us to understand patterns of human infections".

Now, we have entered a new era to investigate the urinary microbiota of healthy subjects and patients suffering from UTI. This will lead us to a new understanding of etiology, pathophysiology and management of UTI in human.

2 Methods

A systematic literature search was performed for the last 10 years in MEDLINE, PubMed, ClinicalKey, Cochrane for the following key words: microbiota urine, urine culture, culture urine, culture medium for the examination of the urine, urinary tract infection and the following limitations: age, children, pregnant women, clinical studies, English, abstract available, only peer reviewed.

A total of 1,189 publications was found and screened by title and abstract. After exclusion of duplicates a total of 50 publications was included into the review.

3 Results

3.1 Urinary microbiota of healthy women

The paradigm on the sterility of a healthy person's urine was based for more than half a century only on the bacteriological methods to investigate urine samples. We have changed this approach by using both, the conventional culture media (blood and MacConkey agar) and also a wide range of additional culture media to detect also facultative anaerobic bacteria (FAB) and non-clostridial anaerobic bacteria (NAB). These media are commonly used for identification of microorganisms derived from colon and vagina. For the FAB cultivation we used MacConkey Agar, HiCrome Candida Differential Agar, HiCrome Enterococci Agar, HiCrome Aureus Agar Base, Blood Agar, prepared according to Mueller Hinton agar with addition of sheep red blood cells. For the NAB cultivation we used Anaerobic Agar, Shaedler Broth, Shaedler Agar, Bacteroides Bile Esculinum Agar, MRS Agar. Nutritional media were purchasedfrom HiMedia (India). The culture media were incubated with the test material under aerobic (24–48 hours) and anaerobic (48–72 hours) conditions (10% $Đ_iĐž_2$, 10% $Đ_2$, 80% N_2).

To study the microbiota of normal urine, 66 healthy women were divided into three groups:

- group I – 22 not sexually active women (18–25 years)
- group II – 24 sexually active women (18–25 years)
- group III – 20 sexually active women in the postmenopause (52–65 years)

All women were healthy according to medical history, general physical examination, and vital signs. Especially they had no urological and gynecological disorders including ultrasound examination of the internal genitalia. They had no infectious diseases during the last year, and were without medication for the last 2 months. In group III the menopause occurred before 5 years or earlier, without any pelvic organ prolapse, with the uterus still present and with at least two deliveries without abortion in the history. Complete blood count and urinalysis were normal in all women.

From each subject midstream samples of morning urine were collected for bacteriological examination, which was repeated three times with an interval of 3 days. The urine samples were collected in a way to minimize the risk of contamination with microorganisms from the skin, periurethral area and vagina. A total of 198 urine samples was studied.

After a threefold bacteriological examination of urine samples from healthy women of the groups I, II, III, no sterile sample was found in any case. Microorganisms were detected in all urine samples with different species at different proportions between FAB and NAB (Table 1).

Extracted Table: Table 1

For urine of healthy women of all age groups, the dominance of *coagulase-negative staphylococci* (CNS), *Corynebacterium* spp., *Peptococcus* spp., *Propionibacterium* spp., *Eubacterium* spp., and *Peptostreptococcus* spp. was typical. In the urine of postmenopausal women, *Enterococcus* spp. are more frequent ($p<0.05$). The dominance of *Lactobacillus* spp. in urine of young women (group I and II) is opposed by their rare (10%) detection in urine of postmenopausal women. *E. coli*, *Veillonella* spp., *Prevotella* spp. appeared more frequently in the urine of sexually active women and postmenopausal women than in the urine of sexually not active young women. Some FAB species were not found in the urine of healthy postmenopausal women. The lowest detectable degree of FAB bacteriuria (10^2 CFU/ml) was found for almost all microorganisms, except for *E. coli* in postmenopausal women (10^4 CFU/ml). On the contrary, the level of bacteriuria for most of NAB for young women was between 10^3 and 10^5 CFU/ml and for postmenopausal women this level was reduced to the lowest detectable level (10^2 CFU/ml).

In an unpublished study we conducted a study comparing three midstream urine samples obtained at 8.00, 12.00, 16.00 hours in a single day from 20 healthy, sexually active women aged 18–20 years. In general insignificant daily fluctuations of frequencies and levels of bacteriuria were found (Figure 1) with the exception of certain genera, as confirmed by cluster analysis.

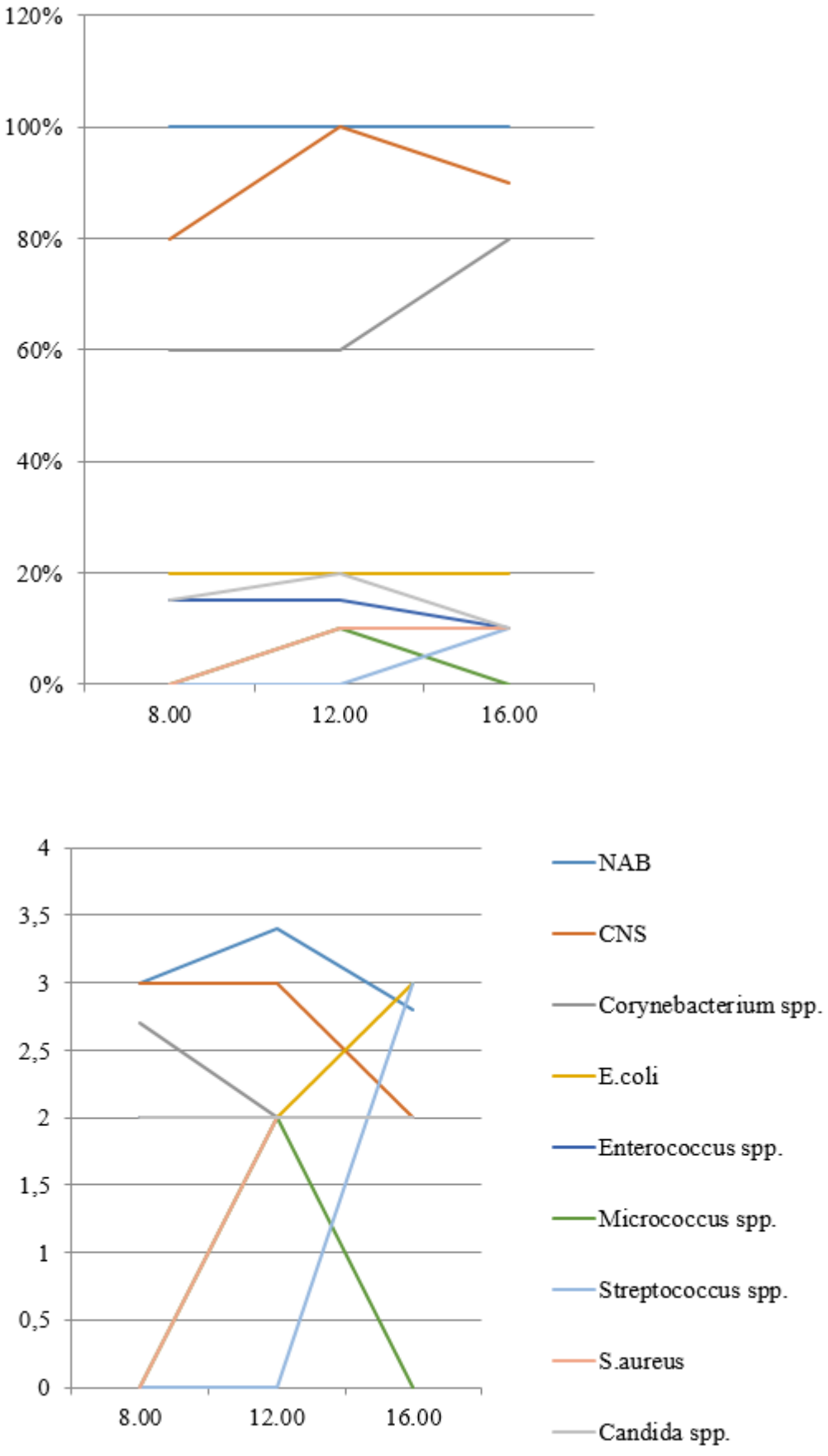

Figure 1: Frequency of detection (%) and average number (lg CFU/ml) of microorganisms detected from healthy women's urine.

In conclusion, the urine of healthy women of all age groups is not sterile and is dominated by Gram-positive and not Gram-negative species of FAB and NAB.

3.2 Urinary microbiota of healthy men

Midstream morning urine samples were collected three times with an interval of 3 days from 20 sexually active, healthy (criteria see above) men aged 20–25 years and cultivated on the expanded set of culture media as mentioned above (Table 2).

Table 2: Urinary microbiota of healthy men

Microorganisms	**Frequency of detection (%)**	**The average level of bacteriuria (CFU/ml)**
Facultative anaerobic bacteria (FAB)		
CNS	89.3	10^2
Corynebacterium spp.	78.6	10^2
Enterococcus spp.	50.0	10^2
E. coli	10.7	10^2
S. aureus	10.7	10^2
Non-clostridial anaerobic bacteria (NAB)		
Eubacterium spp.	78.6	10^3
Peptostreptococcus spp.	50.5	10^2
Bacteroides spp.	21.4	10^3
Peptococcus spp.	21.4	10^2
Megasphaera	21.4	10^2
Propionibacterium spp.	10.7	10^2
Veillonella spp.	10.7	10^2
Mobiluncus spp.	10.7	10^3
Fusobacterium spp.	10.7	10^2

In the urine of healthy men there is a prevalence of CNS, *Corynebacterium* spp., and *Enterococcus* spp. among the FAB, and *Eubacterium* spp., and *Peptostreptococcus* spp. among the NAB. The NAB patterns ranged twice as wide as compared with the FAB patterns. The levels of bacteriuria for FAB and NAB were at the lower level of detection.

3.3 Comparison between urinary microbiota of healthy women and men

The comparison was conducted for the young sexually active groups. The urinary microbiota of men and women were similar in the dominant frequency of CNS and *Corynebacterium* spp. However, the following differences were found: The FAB range found in men was twice as narrow as that found in women. The spectra of NAB in men and women differed: in men *Lactobacillus* spp., *Actinomyces* spp. were absent, but *Megasphaera* spp., *Mobiluncus* spp., and *Fusobacterium* spp. could be detected in contrast to women.

The cluster analysis of the microbiological results revealed typical “female” and “male” urinary microbiota spectra (Figure 2). In mixed clusters men were more similar to men than men to women. The majority of female urinary microbiota (66.6 per cent) were found in mixed clusters, which means higher differences in the urinary microbiota of women than of men.

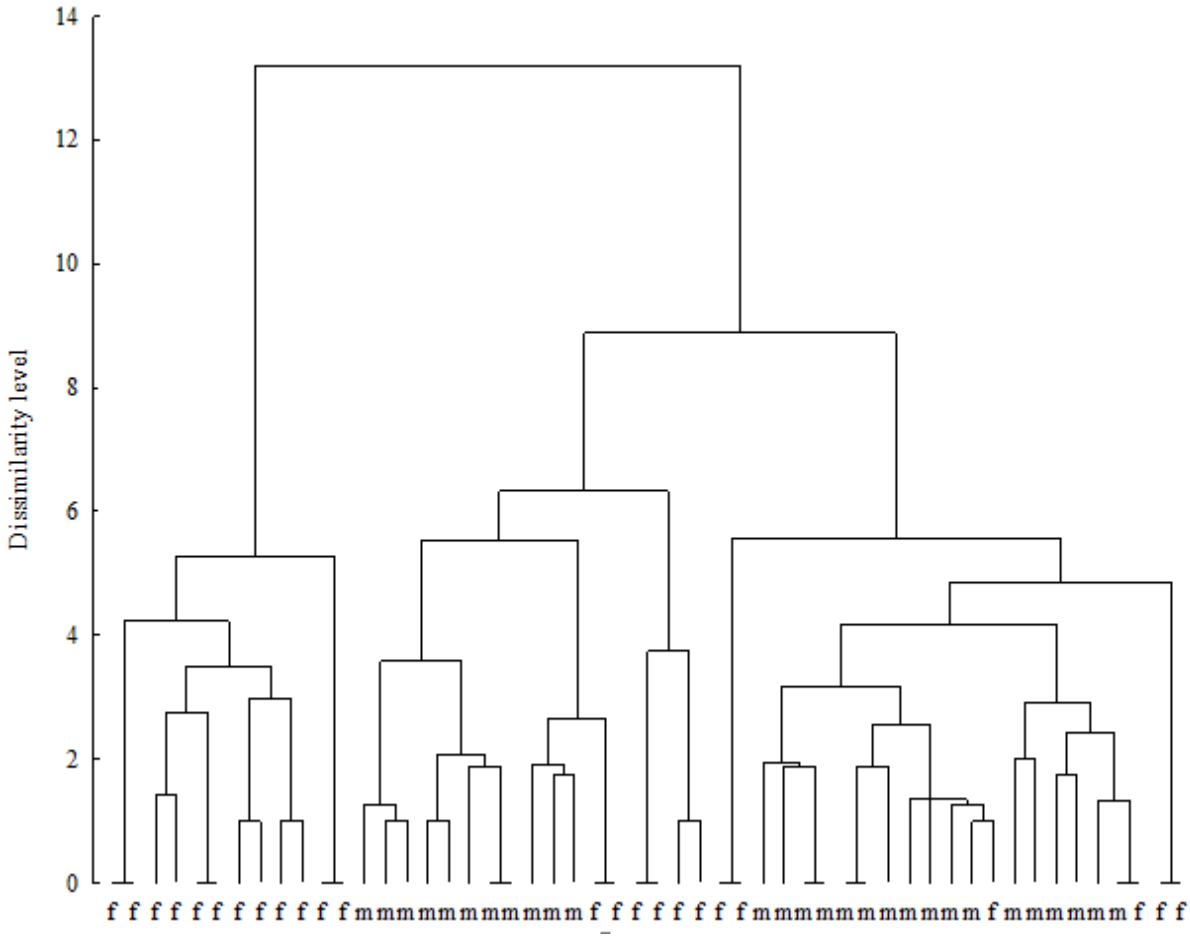

Figure 2: Hierarchical clustering of urinary microbiota in healthy men and womenNote: m - male; f - female

Our bacteriological results of urinary microbiota in healthy individuals, using an expanded set of culture media, correspond with other studies. Siddiqui et al. [42] found by high throughput sequencing of 16S RNA amplicons 45 different species of microorganisms in the female urine microbiota with predominance of *Lactobacillus* spp., *Prevotella* spp., and *Gardnerella* spp.

Fouts et al. [43] revealed the dominant role of *Corynebacterium* spp. in the urinary microbiome of healthy men. *Lactobacillus* spp., *Corynebacterium* spp., *Gardnerella* spp., *Prevotella* spp., and *Enterococcus* spp. showed differences between both genders. These results suggest that the state of healthy urine is, in fact, one of "asymptomatic bacteriuria". In our studies presented above urine of healthy sexually active men revealed also a prevalence of FAB with CNS (89.3%) and *Corynebacterium* spp. (78.6%).

In a study by Wolfe et al. [33] the bacterial spectra of urine collected during normal urination, through a transurethral catheter (TUC) and taken by suprapubic aspiration (SPA) were compared. The presence of bacteria in these samples was assessed by bacterial culture, light microscopy, and 16S rRNA gene sequencing. Fourteen genera out of 321 total (5%) in samples obtained by TUC and SPA were present in different relative abundances. Of those 14 genera, only Lactobacillus represented more than 1% of the total sequences present in either the TUC (26.96%) or SPA (6.94%) samples. The most abundant genera found for TUC samples were similar to those found for SPA urine samples.

From their results the authors concluded that standard clinical microbiological procedures favor detection of fast-growing bacteria only in the presence of oxygen. They consistently underestimate slow-growing bacteria and can detect neither anaerobic bacteria nor those whose preferred growth conditions remain unknown. As an example the authors discussed a clinical observation. that urine, obtained from a participant and determined by standard cultivation procedures to be positive for *E. coli*, was rich in DNA associated with the fastidious genera *Aerococcus* (40% and 50% in SPA and TUC, respectively) and *Actinobaculum* (25% and 17%) yet poor in DNA identified with *E. coli* (3% and 1%). The disparity between the culture and molecular assays raised the question: are this woman's clinical symptoms due to the relatively small number of the "typical" uropathogenic *E. coli*, the numerically superior fastidious bacteria, or a combination of both?

In this context the authors stated further, that "the likelihood that diverse bacteria can exist within the female urinary tract has important implications for urinary tract disorder researchers. These findings should stimulate work to advance our understanding of the roles played by these bacterial communities in the development of UTI and other urinary tract disorders that remain poorly understood. Such studies could make it possible to identify at-risk UTI populations and allow hypothesis-based research of improved targeted treatments and/or prevention efforts".

These studies, including ours, may provide a new prospect on the concept of "normal (asymptomatic) bacteriuria", and on additional infectious agents causing inflammatory diseases of the urinary tract.

3.4 Acute complicated (obstructive) pyelonephritis

The majority (>90%) of patients with acute obstructive pyelonephritis present with bacteriuria of $\geq 10^5$ CFU/ml of *Enterobacteriaceae* or some Gram-positive species (*S. aureus*, *S. saprophyticus*, *Enterococcus* spp.) as the main etiological pathogens. Since according to our studies urine of healthy individuals does not only contain species of Enterobacteriaceae, but also a wide spectrum of CNS, *Corynebacterium* spp., and NAB, the involvement of these microorganisms in the development and clinical course of acute obstructive pyelonephritis (AOP) may be of interest. Consequently we studied the urinary microbiota of the renal pelvis immediately after release of the ureter obstruction.

Our research [44] included 72 patients (18–74 years) with AOP, caused by ureteral stone with obstruction lasting 1–14 days. For culture urine from the bladder was obtained by catheterization and from the renal pelvis by ureteral catheter or percutaneous nephrostomy immediately after release of the ureteral obstruction. Bacteriological examination of urine was performed using an expanded set of culture media as mentioned above.

Study results of the microbiota found in the bladder and renal pelvis urine are presented in Table 3.

Extracted Table: Table 2

The study showed a high frequency (81.8%) of Gram-negative bacteria in the bladder urine at AOP, which are common uropathogens. To a lesser extent (63.8%) a wide range of Gram-positive bacteria was identified in the urine. The highest frequency (94.4%) was demonstrated for NAB. It is remarkable that the level of bacteriuria for Enterobacteriaceae had a wide range (10^2–10^8 CFU/mL) and only in 30.5% of cases a level of ≥10^5 CFU/ml, and in 49.2% a level of ≥10^4 CFU/ml was found. At the same time a high level of bacteriuria (≥10^4 CFU/ml) was found in 8.8% for Gram-positive bacteria (CNS, *Corynebacterium* spp., and *Enterococcus* spp.) and 31.3% for NAB.

The microbiota of the bladder and renal pelvis urine were comparable concerning spectrum, frequency and level of bacteriuria for Enterobacteriaceae. The Gram-positive microflora and NAB, however, showed a significantly reduced frequency in the renal pelvis urine, but concerning the five main NAB species the average level of bacteriuria in the renal pelvis was higher than the level of the bladder bacteriuria. The bacteriuria levels of Gram-positive bacteria were similar for bladder and renal pelvis urine.

Thus, the study confirms the validity of the bacteriological evaluation of bladder urine for the detection of pathogens, related to the development of acute pyelonephritis after ureteral obstruction.

In all of the 72 cases the urinary microbiota included three or more taxons of microorganisms. In 94.4% of the cases an aerobic-anaerobic composition was found, but in only 5.6% a purely aerobic one. In 29.2% of the cases, the level of bacteriuria was ≥10^4 CFU/ml for at least 2 taxons. Considering the wide range of microorganisms found in the urine of the renal pelvis, the question arises, which of the taxons finally initiated or potentiated the pyelonephritis?

3.5 Experimental modeling of acute obstructive pyelonephritis

Using the AOP animal model published by Giamarellos-Bourboulis et al. [45] 60 male rabbits of the New Zealand breed were randomly divided into six groups of 10 animals each. Bacterial suspension of NAB (volume 1 ml containing 10^5 CFU/ml) obtained from AOP patients were injected into the renal pelvis of the animals:

- Group 1 – *E. coli*
- Group 2 – *Peptococcus* spp.
- Group 3 – *Eubacterium* spp.
- Group 4 – *Propionibacterium* spp.
- Group 5 – *Bacteroides* spp.
- Group 6 – *E. coli* + *Peptococcus* spp.

The animals were killed on day 1, 3, 7, 14 and 21 of the experiment. Pathomorphologic studies of the kidney samples were performed using tissue preparations stained with hematoxylin-eosin.

Experimental AOP caused by *E. coli* showed typically morphological alterations [46], [47] and served as control.

All animals in groups 2–6 developed morphological features of acute pyelonephritis. As a result of ureteral obstruction in all animals an acute kidney inflammatory reaction took place already on the first day of the experiment. In group 2 on the first day pyelitis and peripyelitis were observed. On days 2–3 a septic phlebitis and arteritis developed, and on days 3–7 foci of papillomatosis and infarctions of the cortex and medulla were observed.

In group 3 formation of microabscesses was detected on the first day, and on the third day heart attacks, papillary necrosis and contralateral kidney damage occured. In group 4, serofibrinous pelvis inflammation was registered from the 1st day, from 1st–3rd day pyelitis and peri-pyelitis, and from the 3rd–7th day minimum inflammatory reactions of the renal medulla were seen. In group 5, acute inflammation of the renal interstitial tissue (medulla) developed on the 1st day, and on the 3rd–7th day microabscesses of the renal parenchyma were seen. In group 6 from the 1st day onwards, pyelitis, peri-pyelitis, a serious suppurative inflammation of the medulla, and septic thrombophlebitis were noted, on the 3rd–7th days hemorrhagic necroses of the renal parenchyma developed, and all animals died up to the 14th day.

Thus, in experimental animals with ureteral obstruction, NAB inoculated into the renal pelvis reproduced morphological features of acute purulent inflammation of the renal pelvis and renal parenchyma. Strains of *Propionibacterium* spp. basically provoked pyelitis and peri-pyelitis with a minimal involvement of kidney parenchyma, but *Peptococcus* spp. provoked the development of inflammatory reactions similar to *E.coli*. Monovariant *Eubacterium* spp. and *Bacteroides* spp. cause more severe alterations of kidney destruction compared to alterations caused by *E. coli*. Mixed infection *E. coli* + *Peptococcus* spp. was responsible for the most severe kidney destruction in form of abscesses, heart attacks, hemorrhagic necrosis and finally animals' death.

The severity of (ascending) renal damage in the animal experiments could be ranked as shown in Figure 3:

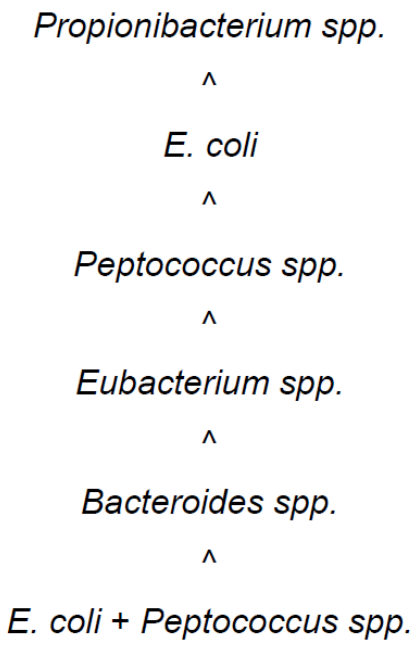

Figure 3: The severity of (ascending) renal damage in the animal experiment

3.6 Urine microbiota in women with recurrent uncomplicated lower urinary tract infections (ULUTI)

In 25–30% of cases acute cystitis is caused by Enterobacteriaceae and by some Gram-positive species with a level of bacteriuria $<10^5$ CFU/ml [48], [49], [50]. According to the recommendations of the EAU bacteriuria $\geq 10^4$ CFU/ml is considered diagnostically significant in symptomatic recurrent infections of the lower urinary tract [51]. The same uropathogenic bacteria are considered to cause pyelonephritis.

We performed bacteriological examination of the morning midstream urine from 144 women (20–50 years) with an acute cystitis of recurrent ULUTI (Table 4). Duration of recurrent ULUTI: <1 year (14.3%), 2–4 years (35.4%) and >5 years (50.3 %). All patients had been previously treated repeatedly with antibiotics.

Table 4: Urine microbiota of women with ULUTI

Microorganisms	**The frequency of detection (%)**	**Range (CFU/ml)**	**Meanlevel of bacteriuria (CFU/ml)**
Enterobacteriaceae			
E. coli	35.5	10^2–10^6	$10^{4.2}$
Klebsiella spp.	9.7	10^2–10^6	$10^{4.0}$
Enterobacter spp.	9.1	10^2–10^5	$10^{3.4}$
Citrobacter spp.	6.9	10^2–10^7	$10^{3.8}$
Proteus spp.	4.8	10^2–10^7	$10^{5.7}$
Providencia rettgeri	2.1	10^6	$10^{6.0}$
Nonfermentative gram-negative bacteria			
Pseudomonas spp.	4.2	10^6	$10^{6.0}$
Gram-positive flora			
CNS	65.2	10^2–10^4	$10^{2.7}$
Corynebacterium spp.	61.1	10^2–10^5	$10^{2.5}$
Enterococcus spp.	28.5	10^2–10^3	$10^{2.4}$
Streptococcus spp.	12.5	10^2	$10^{2.0}$
S. aureus	10.4	10^2–10^5	$10^{3.3}$
Candida spp.	26.4	10^2–10^5	$10^{3.0}$
NAB			
Propionibacterium spp.	50.7	10^2–10^4	$10^{2.6}$
Peptococcus spp.	48.6	10^2–10^6	$10^{3.2}$
Eubacterium spp.	42.4	10^2–10^5	$10^{3.1}$
Lactobacillus spp.	40.9	10^2–10^5	$10^{3.4}$
Peptostreptococcus spp.	29.2	10^2–10^5	$10^{3.2}$
Bacteroides spp.	11.1	10^2–10^4	$10^{2.7}$
Actinomyces spp.	7.6	10^2–10^4	$10^{2.5}$
Fusobacterium spp.	6.2	10^2–10^3	$10^{2.3}$
Prevotella spp.	5.5	10^3	$10^{3.0}$

The proportion of *Enterobacteriaceae* in the microbiota was 68.1%, while *E. coli* was cultured only in 35.5% of the cases. The isolation frequency of *Klebsiella* spp. and *Enterobacter* spp. from urine was higher in this than in other studies. Overall, the level of bacteriuria $\leq 10^3$CFU/ml with *Enterobacteriaceae* was detected in 36.7% of cases, and that $\geq 10^4$ CFU/ml in 63.3%. The spectrum of Gram-positive bacteria and NAB were identical with the urine microbiota of healthy women. However, their frequency was lower and the level of bacteriuria significantly higher than in healthy subjects.

Thus, the qualitative composition of the urine microbiota in female patients with acute cystitis was practically identical to that in healthy women with increasing frequency of detection and levels of bacteriuria with Enterobacteriaceae. However, it is not known whether such a low number ($\leq 10^3$ CFU/mL) of Enterobacteriaceae can cause acute cystitis, or whether the infection is caused by organisms of all other taxa in the presence of *Enterobacteriaceae*. After all in 27.7% of cases with ULUTI such uropathogens (Enterobacteriaceae, *Pseudomonas* spp., *Enterococcus* spp., *S. aureus*, *S. saprophyticus*) were not found in the urine of these patients. It seems likely that on standard culture media hardly cultivated and/or non-cultivated microorganisms may have been involved in the development of such an acute cystitis episode. With an expanded set of culture media in all cases of ULUTI aerobic-anaerobic bacterial compositions could be identified assuming that such "unproven" bacteria were the pathogens of the recurrent cystitis.

4 Further Research

Further studies are needed to elucidate the role of hardly cultivated microorganisms found in the urine of healthy individuals, in the development of symptomatic infections of upper and lower urinary tract. The levels of bacteriuria of dominant bacterial taxons should be determined and their ability to cause symptomatic infections of the upper and lower urinary tract. It is advisable to improve the standard of the classic bacteriological examination of the urine.

5 Conclusion

Polymicrobial bacteriuria should be considered normal condition of healthy individuals. The concepts of significant bacteriuria ($\geq 10^5$ CFU/ml) as proposed by Kass E. [52], pathogenic bacteriuria, “asymptomatic bacteriuria”, and contamination need to be reconsidered. A number of NAB species are symbiotes in the urine of healthy individuals, but can also cause acute septic renal infection, similarly to *E. coli*. The proof of two or more bacterial species in the urine of patients with OAP at a concentration of $\geq 10^{4–5}$ CFU/ml allows to consider them as causative in the development of a renal inflammatory process.

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