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Invited review

Microbiota of male genital tract: Impact on the health of man and his partner

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ABSTRACT

This manuscript describes the male genital tract microbiota and the significance of it on the host's and his partner's health. Microbiota exists in male lower genital tract, mostly in urethra and coronal sulcus while high inter-subject variability exists. Differences appear between sexually transmitted disease positive and negative men as well as circumcised and uncircumcised men. Upper genital tract is generally germ-free, except in case of infections. Prostatitis patients have frequently abundant polymicrobial communities in their semen, expressed prostatic secretion and/or post-massage urine. Coryneform bacteria have ambivalent role in male urogenital tract being frequently commensals but sometimes associated with prostatitis and urethritis. Interactions between male and female genital tract microbiota are highly likely yet there are very scarce studies on the couples' genital tract microbiota. Increase of bacterial vaginosis-type microbiota and coliforms are the most typical findings in men while the adverse effect of male genital tract bacteria on in vitro fertilization and pregnancy outcome has also been indicated.

Contents

Abbreviations: BPH, benign prostate hyperplasia; BV, bacterial vaginosis; CDC, Centers for Disease Control and Prevention; CFU, colony forming units; CP/CPPS, chronic prostatitis/chronic pelvic pain syndrome; CS, coronal sulcus; DGGE, denaturing gradient gel electrophoresis; EPS, expressed prostatic secretion; GU, gonococcal urethritis; NCNGU, nonchlamydial, nongonococcal urethritis; NGU, nongonococcal urethritis; NIH, National Institutes of Health; OxS, oxidative stress; PCR, polymerase chain reaction; Post-M, post-massage urine; PPROM, preterm, prelabor, rupture of membranes; Pre-M, pre-massage urine; STI, sexually transmitted diseases; VB1, voided bladder 1, initial-stream urine; VB2, voided bladder 2, midstream urine; VB3, voided bladder 3, post-massage urine.

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1. Introduction

It is said that we are born 100% human but we die 90% microbial. It means that there are ten times more microbial than human cells in our body, and that each of us contains 150 times more microbial than human genes. Our microorganisms are collectively known as microbiota. The genomes of these microbiota act together as a living system known as the microbiome (i.e., the collection of genes in the microbiota).

Recognition that the human microbiome is an integral component of the human body, and, on the other hand, majority (up to 80%) of the bacterial species found in the human body are uncultured or even unculturable, has initiated MetaHit and Human Microbiome Project that were launched in 2008. Therefore understanding of the human microbiome continues to grow rapidly. A recent PubMed search (October 2012) of the term "microbiome" revealed more than 2500 publications. Majority of these publications have been devoted to intestinal tract microbiome but the microbiomes of mouth, skin and female genital tract have been researched as well.

Characterization of the male genital tract microbiota has always lagged behind investigations in other body sites, including female genital tract. The term "penis microbiome" revealed 5 publications, "urethra microbiome" 3 publications and "male genital tract microbiome" 1 publication while "semen microbiome" and "coronal sulcus microbiome" gave no result. This is somewhat surprising because commensal bacteria might mediate male reproductive tract homoeostasis.

In this review we attempt to uncover the microbiota of male genital tract, including urethra, coronal sulcus, expressed prostatic secretion and semen, as well as the impact of this microbiota on man's health and also his partner's health. This review does not deal neither with the causative agents of sexually transmitted diseases nor mycoplasmas. Due to very low number of studies applying novel mass sequencing method, we describe here also the studies that have applied cultures and the "old" molecular methods.

2. Microbiota of male genital tract

2.1. Urethra and coronal sulcus

It is generally accepted that microbiota exists in lower male genital tract. The penis itself provides distinct anatomical environments in the urethra and the coronal sulcus (CS). Studies of CS region have mostly concentrated on the etiology of balanoposthitis and the effect of circumcision on the sexual transmission of pathogens (including HIV, HSV and HPV) while studies of urethra are usually carried out for detecting sexually transmitted pathogens and mycoplasmas. Both sites are exposed to foreign microbial communities during sexual activity but at the same time both sites provide also a suitable microbiotope for aerobic, microaerophilic and anaerobic bacteria that form their microbiota.

Nearly 35 years ago Bowie et al. [1] performed a study on 69 Caucasian males with nongonococcal urethritis (NGU) and 39 controls where aerobic and anaerobic cultures from the urethra were done. *Staphylococcus epidermidis*, *Corynebacterium* spp., lactobacilli, anaerobic Gram positive cocci and *Bacteroides* spp. were the most frequently found bacteria. The controls had significantly more aerobic lactobacilli, *Haemophilus vaginalis* (today known as *Gardnerella vaginalis*), alpha-hemolytic streptococci and anaerobes, predominantly *Bacteroides* species as compared to NGU patients. Microflora of the two NGU groups (*Chlamydia trachomatis* positive and negative) were similar.

At the same time Davidson [2] detected yeasts in circumcised and uncircumcised men sampling coronal sulcus and meatus of the penis. Yeasts were isolated nearly at similar rates (14–17%) in both groups but the circumcised men had significantly fewer symptoms. Among the female contacts the majority (80%) of partners of yeast-positive men while 32% of the partners of yeast-negative men had yeast infection.

Chambers et al. [3] performed urethral cultures in 90 adolescent youth and reported that the profile of anaerobic, but not aerobic, bacteria isolated from the urethra was related to the presence or absence of previous sexual activity while myco- and ureaplasmas were isolated from sexually active patients only.

The study by Willén et al. [4] was an in-depth analysis of normal urogenital microflora – specimens from six locations in 97 healthy men scheduled for vasectomy were analyzed applying aerobic and anaerobic cultures. The authors found 71% of the strains colonizing the coronal sulcus being present also in the urethra indicating that the distal part of the urethra is colonized by a bacterial flora similar to that in the sulcus. Coagulase negative staphylococci and streptococci were the dominant microbial groups.

Mazuecos et al. [5] performed aerobic and anaerobic cultures of urethral samples from 110 men: 35 with no evidence of urethritis (control group), and 75 with urethritis (17 gonococcal urethritis [GU] and 58 nongonococcal urethritis [NGU]). *Staphylococcus* spp. were isolated less frequently in patients with GU than in the controls and those with NGU. Anaerobic bacteria were isolated in 62% of patients, with similar isolation rates in each group. Gram-negative anaerobes were more frequently isolated in men with urethritis, especially NGU, compared to controls. *Peptostreptococcus magnus* was the most frequent anaerobic species found in the control group, while *Peptostreptococcus prevotii* was most frequently seen in the urethritis group. *Prevotella* spp., *Bacteroides* spp. and *Fusobacterium* spp. were significantly more frequently isolated in patients with NGU.

Montagnini Spaine et al. [6] collected samples from the external urethral orifice, navicular fossa, and penile urethra from uncircumcised male patients without any inflammatory and/or infectious urethral processes and performed aerobic cultures. Of the 30 patients, 40% had bacteria in all three sampled segments, 33.3% had bacterial colonization in two segments 27% had colonization in only one segment (external urethral orifice). Gram positive bacteria including coagulase negative staphylococci, viridans streptococci, corynebacteria and enterococci were the most frequently isolated.

Schneider et al. [7] sampled coronal sulcus and urethra of a diverse group of circumcised (37.1%) and uncircumcised (62.9%) men ($n = 315$) in South India applying aerobic and anaerobic cultures. Nearly half (48%) of the subjects were HIV infected, 36% were tuberculosis infected and 16% were controls. Gram-negative pathogens were more prevalent among study participants in HIV infected and tuberculosis infected men as compared with controls. Uncircumcised men were more likely to be colonized with gram positives (*Staphylococcus aureus*, *Enterococcus* spp.), gram negatives (*Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*), or any pathogen (gram positives, gram negatives, *Clostridium* spp., *Candida albicans*).

Price et al. [8] have investigated the effect of circumcision on the coronal sulcus microbiome in 12 HIV-negative Ugandan men applying sequence-tagged 16S rRNA gene pyrosequencing targeting the V3–V4 hypervariable regions. Among the 42 unique bacterial families identified, *Pseudomonadaceae* and *Oxalobacteraceae* were the most abundant irrespective of circumcision status. Circumcision was associated with a significant decrease in putative anaerobic bacterial families, especially *Clostridiales* Family XI and *Prevotellaceae* whereas aerobes and skin taxa increased following circumcision.

In another African study where circumcised and uncircumcised men were compared, Mehta et al. [9] analyzed the genital ulcer specimens through multitag pyrosequencing of the

bacterial small subunit rRNA genes. In this study 23 out of 59 cases (39%) had unknown etiology. Bacterial diversity was greater in the latter in comparison with men with STI etiology. Anaerobic bacteria were more common in genital ulcers of uncircumcised men. The authors indicated that the specific anaerobic bacteria associated with ulcers of unknown etiology (*Fusobacterium*, *Sneathia*, *Oxobacter and Anaerovorax*) have cytotoxic properties that can exacerbate epithelial disruptions leading to ulcer-like appearance and therefore circumcision may reduce genital ulcer disease through a reduction in these anaerobic bacteria.

Urine is the CDC-recommended sample type for nucleic acid based diagnostics of *Neisseria gonorrhoeae* and *C. trachomatis* [10]. Despite the utility of urine specimens for diagnostic purposes, it has been unclear whether this specimen type will be equally useful for studies of the male urethral microbiome. Dong et al. [11] collected paired urine and swab specimens from 32 men and compared their microbiomes using multiplex 16S rRNA gene PCR and deep pyrosequencing. The results showed that the microbiomes in male first-catch urine and urethral swab specimens are nearly identical, independent of STI or urethral inflammation status. Riemersma et al. [12] and Nelson et al. [13,14] have used first void urine samples to reveal urethral microbiome.

Riemersma et al. [12] applied 16S rRNA gene allele restriction fragment length polymorphisms (RFLP) analysis to compare first void urine samples of 5 nonchlamydial, nongonococcal urethritis (NCNGU) patients and 5 controls. The study identified diverse microbial communities and substantial intra- and interperson variability. In two patients a previously suggested pathogen (*Mycoplasma genitalium* or *Haemophilus parainfluenzae*) was found. Relatively often *Pseudomonas* spp. and *Pseudomonas*-like bacteria were detected.

Nelson et al. [13] applied large-scale Sanger sequencing of 16S rRNA gene to characterize microbial communities in first catch urine collected from a cohort of sexually active adult men. Seven phyla were identified in total, *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Proteobacteria* and *Bacteroidetes* were frequently detected, whereas *Tenericutes* and *TM7* were less abundant. *Lactobacillus* (especially *Lactobacillus iners*), *Corynebacterium*, *Streptococcus* and *Sneathia* spp. accounted for approximately 50% of the total urine sequences and the 10 most common genera represented almost 75% of urine sequences, although 72 genera were detected in total. A high degree of inter-subject variability was evident but clustering analyses indicated striking differences between the STI positive and negative individuals – urine microbiomes from STI positive men were dominated by fastidious, anaerobic and uncultivated bacteria while the same taxa were rare in STI negative individuals.

The same research group [14] used 16S rRNA sequencing to characterize the microbiota of the CS and urine of 18 adolescent men over three consecutive months. Three genera were in most specimens – *Corynebacterium*, *Staphylococcus* and *Anaerococcus*. Other abundant genera included *Peptoniphilus*, *Prevotella*, *Finegoldia*, *Porphyromonas*, *Propionibacterium* and *Delftia*. BV-associated taxa like *Atopobium*, *Megasphaera*, *Mobiluncus*, *Prevotella* and *Gemella* were detected in CS specimens from both sexually experienced and inexperienced participants. Urine primarily contained taxa that were not abundant in CS specimens like *Lactobacillus* and *Streptococcus*. Some genera like *Sneathia*, *Mycoplasma* and *Ureaplasma* were only found from sexually active participants. CS microbiotas appeared to be more stable than their urine microbiotas and the composition of CS microbiotas were strongly influenced by circumcision. In this study group *Pseudomonas* were notably less abundant in CS specimens than they were in a group of adult African men described by Price et al. [8].

2.2. Prostatitis as a stimulus for male upper genital tract microbiota studies

The above-described studies describing the microbiota of male urethra or coronal sulcus form a minority of male genital tract microbiota studies. More studies have been performed using the prostate-specific specimens – expressed prostatic secretion (EPS), post-massage urine (VB3, post-M) or semen. The reason for that is a frequent but badly understood condition – chronic prostatitis. Aiming to improve the diagnosis and treatment of prostatitis, the National Institutes of Health (NIH) established an International Prostatitis Collaborative Network. This group convened two consensus conferences (1995 and 1998) to establish a new definition and classification of prostatitis syndromes [15,16]. Bacterial etiology has been shown for two categories – acute bacterial or NIH I and chronic bacterial or NIH II that are caused by known urinary tract pathogens – *E. coli* and other *Enterobacteriaceae*, enterococci and staphylococci.

The etiology of remaining categories (NIH III and NIH IV) is largely unknown. The most widespread category of prostatitis – Chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS) or NIH III is divided into inflammatory (NIH IIIA) and noninflammatory (NIH IIIB) subtypes, according to presence or absence of white blood cells in prostate-specific materials. Asymptomatic inflammatory prostatitis or NIH IV is the newest category of prostatitis that is usually found by chance [17].

NIH III prostatitis lacks 'traditional' uropathogens in prostate-specific materials (semen, VB3 or EPS). To this date, no single etiopathogenetic mechanism has been proved yet, but an interrelated and multifactorial cascade has been proposed by [18] where an initiating event (infection, trauma, etc.) may lead to immunologic stimulation, inflammation, neurogenic stimulation, neuropathic damage with afferent nerve upregulation and ultimately, pain.

Opinions about the plausibility of infectious etiology have ranged from one extreme to another: one group declared evidence non-existent [19]; another suggested that the major cause was a single overlooked pathogen [20]; third was wary of extant but encouraging about future evidence [21]. Whether and when the usual commensals of male genital tract may act as pathogens in certain conditions is a good question without good answer.

We hereby present some studies that are more relevant to question about microbiota in male upper genital tract and therefore this overview has no ambition to be a comprehensive review of prostatitis microbiology.

2.3. Expressed prostatic secretion and post-massage urine

Traditionally, a 'four-glass test' (also called Meares–Stamey test) had been used for diagnosis of chronic prostatitis. This quantitative and localizing technique includes sequential samples from initial-stream urine (voided bladder 1, VB1), midstream urine (VB2), prostatic secretions obtained by prostate massage (EPS) and post-massage urine (VB3) [22]. However, later surveys [23] indicated that most urologists never employ this test during lower urinary tract evaluation for prostatitis, therefore a simpler and more cost-effective version of this test (also called pre- and post-massage test or 'two-glass test') was developed by Nickel [24] that involved the culture and microscopic examination of urine before (pre-M) and after (post-M) prostatic massage. The diagnosis of bacterial prostatitis is made when the bacterial colony count in the EPS, VB3 or post-M is at least 10-fold greater than in pre-massage urine specimen(s).

Bartoletti et al. [25] used 152 infertile men as controls for 2006 prostatitis patients. Meares–Stamey four-glass test was positive in 2.9% of infertility patients and 13.3% of prostatitis patients. The

latter is nearly the proportion of samples that usually gives positive result when etiological factor of chronic prostatitis is looked for.

Shahed and Shoskes [26] have considered any Gram-positive aerobic bacteria as pathogens if those were expressed specifically in EPS or were expressed in 100 times higher concentration in VB3 than in VB1. The most common Gram-positive microorganisms in their study were staphylococci, enterococci and corynebacteria.

Anaerobic bacteria have been seldom searched from prostate-specific specimens. Szöke et al. [27] searched for anaerobic species in EPS of 50 patients with chronic prostatitis using a cut-off value of 10^6 CFU/ml and 6-day incubation. As a result, they observed that 18 of 50 patients were positive for anaerobes only and six of were positive for both aerobic and anaerobic species. Nearly half of the patients (26 men) remained microbiologically negative at this cut-off value. *Peptostreptococcus* sp. was the most common, followed by *Propionibacterium* sp., *Bacteroides ureolyticus*, *Prevotella* sp., *Bifidobacterium* sp., *Eubacterium* sp., *Prevotella* sp. and *Veillonella* sp. Disappearance of anaerobes from EPS was associated with therapeutic success.

Hou et al. [28] characterized the bacterial communities in EPSs of men with CP/CPPS, infertile men and controls by amplification of the V3 regions of the 16S rRNA genes. The results showed that the gene-positive rate in the CP/CPPS and infertile patients was much higher than in the normal men. According to DGGE analysis, the EPS bacterial community structure in the CP/CPPS patients differed from that observed in infertile men, as well as between CP/CPPS patients with and without inflammation. The authors concluded that the ecological balance of the EPS microenvironment might play an important role in the manifestation of CP/CPPS with and without inflammation.

2.4. Semen

Microbiological studies of semen have been mostly conducted in the context of infertility. At the same time, infertility has been frequently associated with leukocytospermia, the same is also the basis for diagnosing asymptomatic inflammatory prostatitis (NIH IV). For prostatitis studies, semen is a clinical sample that is relatively easy to obtain, especially in younger men, and is relatively less time-consuming for physician than EPS. It has been approved for prostatitis diagnostics by NIH workshop on chronic prostatitis in Bethesda, MD, USA, 1995 [16]. Although some investigators have found the sensitivity of microbiological semen analysis inferior to that of EPS for diagnosing prostatitis [29], the others have claimed the contrary results [30,31].

Studies of our research group have supported the latter opinion indicating that from the microbiological viewpoint, semen is a suitable specimen differentiating well between prostatitis patients and controls [32–34]. In these studies, quantitative cultures of wide spectrum of microorganisms, including anaerobic and microaerophilic species were employed. Numerous species and genera were found, including anaerobic bacteria in three fourth of specimens. The most frequent microorganisms were coagulase negative staphylococci, corynebacteria, anaerobic Gram positive cocci and anaerobic Gram-negative rods. The main difference between inflammatory prostatitis patients (both symptomatic [NIH IIIA] and asymptomatic [NIH IV]) and controls was quantitative – inflammatory prostatitis patients harbored significantly higher total concentration of bacteria (mean 10^5 CFU/ml) as well as higher number of different species (mean 5) in their semen than controls (mean 10^3 CFU/ml and 3 species). This finding was somewhat surprising as we assumed that prostatitis patients could have rather monoinfection. Instead, we found abundant polymicrobial communities that may be comparable to bacterial vaginosis in women suggesting that prostatitis might be viewed as an unfavorable shift in the balance of genital tract microbiota.

Our finding fits to the hypothesis of Liu et al. [35] that dysbacteriosis in male genital tract may be an underlying, primary cause of chronic prostatitis and that the wide use of antibiotics may be an initiating risk factor for prostatitis.

Rehewy et al. [36] performed aerobic and anaerobic cultures of semen samples from men who had sired children within the past 6 mo and asymptomatic infertile men. Overall, 54% of the samples from first group and 73% from the second group were culture positive. Mixed bacterial flora was more varied and present in a higher colony counts in infertile men. *S. epidermidis*, *S. aureus*, *Corynebacterium* species, *Mycoplasma hominis* and *Ureaplasma urealyticum* were isolated from both groups, and in addition, from infertile men also *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Enterococcus feacalis*, *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *P. aeruginosa*, *C. albicans*, *Bacteroides*, *Peptostreptococcus* and *Eubacterium* were found. The antibacterial effect of seminal plasma from this group was greater than that from infertile group.

Balmelli et al. [37] have also investigated semen samples of men consulting for infertility. Huge number, 3196 semen analyses were performed and in 9.8% of them *Bacteroides ureolyticus* was found. The presence of this micro-organism was coupled to an increased presence of *Enterococcus* sp., to an increased number of short-tailed spermatozoa and epithelial cells, and to a decreased total fructose concentration. The latter suggest that this anaerobic micro-organism might specifically colonize the seminal vesicles, while the normal zinc values recorded suggest a normal prostatic function.

Willén et al. [4] performed aerobic and anaerobic semen cultures of 97 healthy men scheduled for vasectomy and found that 83% of them contained bacteria, both aerobic and anaerobic. Presence of multiple bacterial species in semen was not associated with abnormal sperm function. Forty-four percentage of the strains found in semen and 58% of those in prostatic secretion were also identified in the urethra. In this study also 61 intraoperatively obtained vas deferens samples were cultured and only one culture was found to be positive that gives important information about absence of normal microbiota in this part of genital tract.

By our best knowledge, there are only two studies that have used molecular methods to detect wide spectrum of microorganisms in semen [38,39].

Jarvi et al. [38] applied polymerase chain reaction with a universal eubacterial primer, cloning and sequence analysis. They investigated healthy culture-negative semen donors as well as infertile men and found that an equal proportion, two thirds of both groups had at least 10^4 bacteria per ml of semen that was significantly more than by cultures. The most pertinent bacteria were *Prevotella* sp. Other bacteria included several anaerobic and aerobic species like *Peptostreptococcus*, *Veillonella*, *Eubacterium*, *Corynebacterium* group, *Rubrivirax*, *Actinobacillus*, *Streptococcus*, *and Burkholderia*.

Kiessling et al. [39] detected bacteria in semen by sequencing PCR-amplified ribosomal RNA gene regions (rDNAs) in men undergoing fertility evaluation or vasectomy. Conditions of PCR were adjusted to detect only abundant organisms (>20,000 bacteria/ml). Similarly to the study of Jarvi et al. [38] 65% of the men were positive. The most frequently found genera were *Peptoniphilus*, *Anaerococcus*, *Finegoldia*, *Peptostreptococcus*, *Corynebacterium*, *Staphylococcus*, *Lactobacillus* and *Streptococcus*. The authors concluded that abundant bacteria in semen are not commensals but arise from infection in the male genitourinary tract.

In some studies the properties of prostatitis-related bacteria have been investigated. Mazzoli et al. [40] have detected biofilm producing ability of these bacteria and found that the

majority of *E. coli*, other Gram negative bacteria, staphylococci and enterococci strains were strong or medium producers. They also noted that prostatic calcifications are biofilm-related. Ivanov et al. [41] found that the most common isolates from semen in healthy men and men with chronic prostatitis were coryneforms, lactobacilli, coagulase-negative staphylococci, micrococci and streptococci while *Enterobacteriaceae*, enterococci and *S. aureus* were isolated only from the chronic prostatitis group. They also detected the anti-complement activity of semen microorganisms and found that microorganisms of prostatitis patients exhibited greater anti-complement phenotype than that of controls. They suggested that characterizing prostatitis microbiota should be focused upon functional parameters (resistance to host defense mechanisms) rather than upon classical taxonomy.

In case of any prostate-specific material, there is an inherent possibility of urethral contamination. Our study group has compared semen with first-catch urine that reveals urethral microflora [32]. The microorganisms' concentration and number of species were significantly higher in patients' semen than in patients' urine, and these specimens shared only one third of species showing that most of the semen microorganisms originate from upper genital tract.

2.5. Prostate tissue

Prostate tissue has mainly been researched in association with prostate cancer diagnostics, and there are also a few studies which have used samples from organ donors. Transrectal, transperineal and transurethral biopsy have been used. All these methods may be associated with contamination of specimen, especially transrectal, although double needle techniques can help to diminish it. Doble et al. [42] found bacteria from only 15% of patients' biopsy samples while Lee et al. [43] found that this number would be approximately 37% in both patients and controls, and both groups used transperineal biopsy that minimized bacterial contamination. Doble et al. [42] treated their patients with antibiotics respective to these bacteria. Treatment failure led the authors to the idea that these bacteria were contaminants rather than causative agents. Takahashi et al. [44] found 26% of positive samples applying real-time PCR method and also Matsumoto et al. [45] reported that biopsy culture is seldom positive compared with EPS or semen. At the same time Krieger et al. [46] who also applied transperineal biopsy found 16S rRNA gene in a substantial proportion (77%) of samples. They found also sexually transmitted pathogens in prostate tissue of 8% of CP/CPPS patients (*C. trachomatis*, *M. genitalium* or *Trichomonas vaginalis*). Berger et al. [47] demonstrated that in case of inflammation in EPS the prostate tissue contained more anaerobic bacteria, higher bacterial counts and more different species than the patients whose EPS was noninflammatory. The latter is in good correlation with our studies although we used semen as specimen [32–34].

Hochreiter et al. [48] and Xie et al. [49] have investigated the autopsy material from apparently healthy men for the presence of prokaryotic nucleic acids. Xie et al. [49] found that fifth of the normal and half of the inflammatory samples had traces of bacteria inside. Hochreiter et al. [48] found traces of bacteria in association with inflammatory changes due to BPH or cancer, while healthy prostates were void of such traces.

Leskinen et al. [50] investigated prostatic tissue samples obtained in radical prostatectomy from 10 patients with CPPS symptoms and 10 nonsymptomatic patients with localized prostate cancer. Only one sample was positive for bacterial DNA therefore bacterial etiology for CPPS symptoms could not be demonstrated in prostate cancer patients. At the same time the results also suggest that prostate is unlikely to harbor bacterial normal flora that confirms the results of Hochreiter et al. [48].

Hence, the prostatie tissue studies provide somewhat contradictory results. There are some probable reasons for that. In case of biopsy studies, contamination is highly likely. In case of prostatitis symptoms, the inflammation may be focal and not diffuse, therefore the sampling may give different results. Autopsy samples from apparently healthy men may give positive result due to very high prevalence of (asymptomatic) prostatitis in elderly men.

2.6. Coryneform bacteria in male genital tract

In many studies coryneform bacteria have been revealed from male urogenital tract. Mostly these bacteria tend to be often overlooked as commensals but some authors have associated these microorganisms with prostatitis [51–54]. Coryneform bacteria are aerobic, asporogenous, irregular Gram-positive rods. They belong to the phylum *Actinobacteria*. Their classification has undergone dramatic changes – genus *Corynebacterium* has been defined more narrowly and many species now belong to other genera like *Arthrobacter*, *Cellulomonas* and *Rhodococcus*, instead. With a notorious exception of *Corynebacterium diphtheriae*, the coryneform bacteria have been considered as part of the normal human flora or environmental contaminants, but were recognized increasingly as a cause of life-threatening diseases later [55,56].

The list of coryneforms found in male urogenital tract includes *Corynebacterium singulare*, *Corynebacterium freneyi*, *Corynebacterium afermentans*, *Corynebacterium xerosis*, *Corynebacterium* group ANF, *Corynebacterium striatum*, *Corynebacterium amycolatum*, *Corynebacterium macginley*, *Corynebacterium jeikeium*, *Dermabacter hominis*, *Corynebacterium minutissimum* and even *C. diphtheriae* [52,54,57–59]. A new coryneform species *C. seminale* (also known as *C. glucuronolyticum*) was discovered first from prostatitis patients [60]. Later it has been associated also with urethritis [61].

In addition to cuturable ones, also the unculturable or fastidious coryneforms may appear in male genital tract that remain undetected during routine cultures [53,54].

Nucleotide-based studies [54,62] showed that *Corynebacterium* sp. were the most common bacteria in the EPS or urine, respectively, among prostatitis patients. Tanner et al. [54] found with 16S rRNA gene probe an unexpectedly diverse list of *Corynebacterium* species, up to nine species from one patient were found, and some unidentified species were characteristic to men with prostatitis only. Interestingly, 7 of 11 men who had bacteria in EPS were susceptible to treatment with antibiotics. It has been also speculated (although not proved) that coryneforms could grow in the prostate as a biofilm that would enhance antibiotic resistance [54].

Our study group has investigated coryneform bacteria in inflammatory prostatitis patients and healthy controls [63,64]. These bacteria were present in 76% of inflammatory prostatitis patients (NIH IIIA and NIH IV categories) and 83% controls. Half of men harbored corynebacteria in both semen and urine, 22% of men harbored them in semen only and 3% in urine only. Their total concentration was greater in semen than in urine (median 5000 vs 100 CFU/ml). The subjects had up to 6 (mean 1.3) different coryneforms present. The most frequent species was *Corynebacterium seminale*. Two coryneform bacteria were significantly more frequently found from prostatitis patients – *Corynebacterium* group G and *Arthrobacter* sp. We subsequently set a threshold limit of $\geq 10^4$ CFU/ml to bacterial concentration in order to reveal possible differences between patients and controls at quantitative level. In controls, only four bacterial groups managed to outnumber this threshold: *C. seminale*, *C. jeikeium*, *Corynebacterium* sp. and catalase-negative coryneforms. In prostatitis patients also *Arthrobacter* sp., *Brevibacterium* sp., *Cellulomonas*/*Microbacterium*, *Corynebacterium* group F1 and *Corynebacterium* group G exceeded

that threshold. These data indicate that coryneform bacteria may appear a major component of male genital tract microbiota.

3. Impact of male genital tract microbiota on man's health

3.1. Susceptibility to sexually transmitted diseases

There is strong evidence that the composition of the reproductive tract microbiota is linked to reproductive health and resistance to STI in women. *Lactobacillus* spp. regulate the balance of pro-inflammatory cytokines in vaginal secretions, block colonization and invasion of some pathogens and produce lactic acid, hydrogen peroxide and bacteriocins that inhibit other vaginal microorganisms. Reduction of vaginal *Lactobacillus* spp. is associated with the overgrowth of anaerobic bacteria that occurs in BV, and increased susceptibility to bacterial and viral STI [14].

Much less is known about the effect of genital tract microbiota on male health yet it has been suggested that the bacterial colonization of male coronal sulcus and urethra might also impact the risk of STI [1,5,12,65]. Lactobacilli are not so prevalent in male genital tract than in vagina yet they have been identified in urine and urethral swabs [11,13,14,39] and therefore they may have protective role against foreign microorganisms. Nelson et al. [13] found that urine microbiomes from STI positive men were dominated by fastidious, anaerobic and uncultivated bacteria while the same taxa were rare in STI negative individuals, and noted that similar BV-like communities in female genital tract are associated with increased risk for STI.

As concerns the CS microbiota then it is believed to mediate effects of circumcision on risk of HIV and other STI since the biotope for anaerobic and Gram negative communities disappear after circumcision [8,14]. Price et al. [8] have proposed that the anoxic microenvironment of the subpreputial space may support pro-inflammatory anaerobes that can activate Langerhans cells to present HIV to CD4 cells in draining lymph nodes and that the reduction in putative anaerobic bacteria after circumcision may play a role in protection from HIV and other sexually transmitted diseases. However, the present data are clearly insufficient to conclude whether the STI-associated communities precede, are co-transmitted with or are established subsequent to STI.

3.2. Prostate pathologies and infertility

Prostate gland is the most commonly diseased internal organ of the human body. Human prostate pathologies (including prostatitis, benign prostate hyperplasia and prostate cancer) are one of the clinical problems with the greatest impact in the third millennium with important impacts in terms of social, health-related and individual costs, including impact on fertility and patient quality of life [40,66]. Recent studies have shown that diseases of this organ are in more close association than supposed in the past. Prostatitis that begins usually in younger age can be considered central that significantly interferes complaints of benign prostatic hyperplasia (BPH) and that is an important risk factor for prostate cancer [67]. Also, the border between BPH and prostatitis is blurred [68], and some believe that they may be the same disease [69,70]. One of the factors stimulating the growth of prostate may be endotoxin of *E. coli* [71]. Microorganisms have been found in prostate tissue in 21–44% of BPH patients while signs of inflammation in 90–100% of patients [72–76].

Prostatitis-associated inflammation may cause obstruction of male genital tract and impair spermatogenesis. High-grade oxidative stress in case of prostatitis is associated with alterations in metabolism, motility and DNA damage of spermatozoa [77,78]. In a large WHO-conducted study, prostatitis has been found to comprise an important proportion (12%) and holding an outstanding 3rd place among principal causes of male infertility [79].

Prostate cancer is the second leading cause of male cancer deaths in the Western world. In prostatitis cancer patients the bacteria have been found from prostate tissue in 81–89% of cases, among others *E. coli*, *Bacteroides* sp. [80], *Propionibacterium acnes* [81].

3.3. General health

Microbiota of each biotope of human body is tightly integrated into host–microbiota ecosystem and complicated cross-talk takes place between its components. In addition to colonization resistance, our microbiota participates in metabolism, immune system stimulation and trophic function of epithelium. Male genital tract microbiota is not an exception in that respect although its microbial mass is undoubtedly lower than in intestinal tract where 2 kg of pure bacterial mass is considered to exist.

Microorganisms may translocate from each mucosal site, especially in case of immune deficiency, underlying diseases or dysbiosis, and consequently the infections outside of genital tract are possible.

Genital tract dysbiosis-related prostatitis is a condition that is characterized by oxidative stress (OxS) – an imbalance between the production and detoxification of reactive oxygen species that can cause tissue damage. This condition is present also in case of prostate cancer, reviewed by Khandrika et al. [82] and BPH [83,84]. OxS in the male genital tract is associated with infertility and deterioration of semen quality. At the same time the prostatitis-associated local OxS is accompanied by systemic one (that can be revealed in blood and urine) as shown by our studies [84–86]. Since the OxS is thought to be involved in the pathogenesis of many diseases including cancers, cardiovascular diseases and even mental diseases, the prostatitis-associated OxS may pose an increased risk for development of these diseases. It is worth mentioning that the patients with BPH have a considerably higher prevalence of cardiovascular diseases than the general population [87] that might be at least partially explained by systemic OxS in their organism.

4. Impact of male genital tract microbiota on partner's health

The mucosal surface of the female genital tract is a complex biosystem that provides a barrier against the outside world and participates in both innate and acquired immune defense systems. This mucosal compartment has adapted to a dynamic, nonsterile environment challenged by a variety of antigenic/inflammatory stimuli associated with sexual intercourse and endogenous vaginal microbiota [88].

At the same time vaginal microbiota is an open ecosystem that can be significantly affected by sexual intercourse. Semen contains several factors like male reproductive proteins, markers of inflammation and microorganisms. Alkalinization of the vaginal niche during intercourse may enhance a shift from lactobacilli-dominated microbiota to a BV-like type. Therefore the fluctuations in vaginal ecosystem are highly likely while significant dysbalance of this system may lead to several maladies including urinary tract infections and also infertility.

Several studies have shown that frequent sexual intercourse, multiple sex partners, frequent episodes of receptive oral sex, receptive anal sex before vaginal intercourse, and sex with an uncircumcised male partner may cause fluctuations of vaginal microbial communities and contribute BV episodes [89–94]. It has

also been shown that sexual partners harbor the same strains of BV-associated *G. vaginalis* [95].

Leppäluoto [96] has proposed a hypothesis that in addition to plymicrobial BV there could be a monobacterial form of BV, *G. vaginalis* vaginitis, which may be a physiological post-coital condition for protection of ejaculated spermatozoa, characterized by 'pure' *Gardnerella* flora and elevated pH as an immediate result of an incidental unprotected coital act through neutralization of vaginal acid and replacement of *Lactobacillus* by *Gardnerella* flora. The author indicates that this hypothesis arose from their previous studies where in most of the women the dominant *Lactobacillus* morphotype flora seen in pre-coital smears was replaced by *G. vaginalis* morphotype dominant flora in post-coital smears [97,98].

At the same time Verstraelen et al. [99] suggested that BV may be considered a sexually enhanced disease (SED), with frequency of intercourse being a critical factor. This may relate to two distinct pathogenetic mechanisms: (1) in case of unprotected intercourse alkalinization of the vaginal niche enhances a shift from lactobacilli-dominated microflora to a BV-like type of microflora and (2) in case of unprotected and protected intercourse mechanical transfer of perineal enteric bacteria is enhanced by coitus. A similar mechanism of mechanical transfer may explain the consistent link between non-coital sexual acts and BV. Similar observations supporting the SED pathogenetic model have been made for vaginal candidiasis and for urinary tract infection [100,101].

At the same time some studies have not confirmed the link between sexual activity and bacterial vaginosis. For example, Morison et al. [102] examined the occurrence of bacterial vaginosis (BV) in a rural African setting ($n = 30$) using self-collected swabs on alternate days through four menstrual cycles. They had no association between BV and intercourse reported in the previous 4 days or intercourse frequency.

Some below-described studies have revealed fluctuations in other vaginal bacteria and not so much BV-associated communities.

Hooton et al. [103] followed 40 women over a median period of 28 weeks to ascertain the effects on vaginal microflora of sexual intercourse alone compared with sexual intercourse associated with use of a diaphragm with a spermicide. Compared with no sex, the intercourse with use of a diaphragm/spermicide in the preceding 3 days was strongly associated with increases in rates of vaginal colonization with *Candida* sp. and uropathogenic flora, including *E. coli*, other gram-negatives, group B and D streptococci. At the same time decrease in rates of lactobacillus colonization was noted. Except for *E. coli* colonization, no such increases in rates of vaginal colonization were seen after sexual intercourse without diaphragm/spermicide. This study indicates mostly the serious adverse effect of this contraception method on vaginal microbiota.

In a very thorough study Eschenbach et al. [104] investigated 42 women before (1 month and 1–2 days) and after (8–12 h, 2–3 days, and 6–8 days) an index episode of sexual intercourse. The 22 subjects who used no condoms had significantly more *E. coli* in the vagina after intercourse. Also the 20 subjects who used condoms had a trend toward more vaginal *E. coli* and other enteric gram-negative rods after intercourse. A parallel increase in *E. coli* and enteric Gram negatives occurred in the urine of both groups. In this study intercourse with or without a condom had no effect on vaginal lactobacilli and also on pH as measured 8–12 h later. The results of this study are compatible with findings of other researchers [105,106] that intercourse is associated with a transient increase of *E. coli* colonization in the vagina and urine. Foxman et al. [107] showed that uropathogenic *E. coli* were nine times more likely than other *E. coli* to be shared between sex partners.

Newton et al. [108] enrolled 617 women in a 1-year longitudinal study (baseline, 6 and 12 months) assessing the effect of sexual behaviors on the vaginal microflora. They found quite mild impact of sexual behaviors on the vaginal microbiota, yet *Streptococcus agalactiae* was associated with multiple partners and cunnilingus while *Candida* sp. with fellatio and frequent sex.

Santiago et al. [109] performed longitudinal study of 17 women in which swabs and Gram stains were available for each day of two consecutive menstrual cycles. The swabs were cultured every 7th day and the bacteria were identified using tDNA-PCR and 16S rRNA gene sequencing. Due to low number of sexual intercourse events without condom the authors avoided conclusions about the influence of sex on vaginal microbiota.

Although male genital tract microbiota directly influences the partner's one, there are very few studies about the couples' genital tract microbiota because specimen collection from both partners in parallel is complicated.

Wittemer et al. [110] cultured endocervical, vaginal and seminal microbiota before in vitro fertilization in 951 couples. The implantation rate was significantly diminished in case of endocervical bacterial growth. Positive cultures from both vagina and semen decreased clinical pregnancy rate and increased spontaneous miscarriage rate significantly more than vaginal infection alone.

Kjaergaard et al. [111] investigated 11 couples with preterm, prelabor, rupture of membranes (PPROM) and 18 couples with normal pregnancies. Urine and semen samples were collected from men while samples from vagina, cervix, urine and placenta from women. Pyospermia was found in three men of PPROM group (two of these couples were *C. trachomatis* positive) while in none of control group. The authors suggested that male genital tract microbiota is associated with preterm, prelabor rupture of membranes in their spouses. However, *C. trachomatis* as sexually transmitted pathogen does not belong to microbiota.

Disturbed microbial communities that appear in male genital tract in case of prostatitis are very likely an important cause of changes in vaginal microbiota, however, there are no studies on this topic. Our research group has compared vaginal microbiota just before and 8–12 h after intercourse on the 6th–8th days of the menstrual cycle in 17 women who presented with infertility of the couple. Semen samples from men were collected during menstruation of the partner, 3–5 days before the vaginal microbiota samples. In five men of this group inflammatory prostatitis was diagnosed according to leukocytospermia [112]. In total, 67 different species or genera were isolated from aerobic, microaerobic and anaerobic quantitative cultures; 36 microorganisms were isolated from men and 54 from women. In women, the most frequently isolated bacteria were lactobacilli, coagulase-negative staphylococci, corynebacteria, anaerobic Gram-positives, and streptococci, while in men, corynebacteria, streptococci, coagulase-negative staphylococci, and anaerobic Gram-negative rods were most frequently found. We noted the increase in Nugent scores in 6 out of 17 women after intercourse although no cases of BV (score ≥ 7) emerged after intercourse. The Nugent score increase after intercourse was accompanied by shifts in cultured microbiota – some species disappeared while others emerged. These shifts were more prominent in partners of prostatitis patients, indicating the significant influence of prostatitis-associated microbial communities on vaginal microbiota. At the same time these shifts were less expressed in case of lower Nugent score indicating protective role of *Lactobacillus*-dominant microbiota.

In the second stage of this study the vaginal microbiome in 15 of these women was profiled using sequencing of the V6 region of 16S rRNA gene by applying Illumina paired-end protocol on HiSeq 2000 platform. In 8 women lactobacilli predominated (*L. iners*, *Lactobacillus crispatus*, *Lactobacillus jensenii*, *Lactobacillus gasseri*), in 4 women *G. vaginalis* while in 3 women other bacteria predominated (*Streptococcus*, *Enterobacteriaceae*, *Veillonella*, *Pseudomonas*, *Atopobium* and

others). Principal Coordinate Analysis revealed larger shifts of bacterial community structure in case of 4 women that may interfere with fertilization due to unfavorable environment of reproductive tract. Illumina sequencing confirmed the culture-based results that initial Nugent score was significantly higher in the women with larger microbiota shift than in the others (unpublished data). Hence, we have been first to reveal the immediate effect of unprotected sexual intercourse on vaginal microbiota applying Illumina sequencing as well as the guarding virtue of normal lactoflora against the post-intercourse shifts.

5. Conclusions

Rapidly developing sequencing methods and analytical techniques are enhancing our ability to understand the human microbiome, however, characterization of the male genital tract microbiota has always lagged behind investigations in other body sites. At the same time all of our microorganisms are tightly integrated into host–microbiota ecosystem that is usually well balanced though sometimes contributes several local and systemic maladies.

It is generally accepted that microbiota exists in lower male genital tract, mostly in urethra and coronal sulcus. According to newer mass sequencing studies the most frequent phyla in urethra are *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Proteobacteria* and *Bacteroidetes* while high inter-subject variability exists. Differences appear between sexually transmitted disease positive and negative men (the latter have less fastidious, anaerobic and uncultivated bacteria) as well as circumcised and uncircumcised men (circumcision causes decrease of Gram negative and anaerobic bacteria). Therefore association of both urethral and coronal sulcus microbiota with acquisition of sexually transmitted diseases can be assumed yet present data are insufficient to conclude whether the different communities precede, are co-transmitted with or are established subsequent to diseases.

Upper genital tract (including prostate tissue and vas deferens) is generally germ-free, except in case of infections (including prostatitis and also other prostate diseases). The studies of prostate-specific specimens have given contradictory results yet several investigations indicate that prostatitis patients have frequently abundant polymicrobial communities in their semen, expressed prostatic secretion and/or post-massage urine. Again, whether this microbiota type is prerequisite, epiphenomenon of consequence of upper genital tract inflammation needs additional studies. Coryneform bacteria (both culturable and unculturable) have been frequently found from male urogenital tract. They tend to be often overlooked as commensals but some authors have associated them with prostatitis and also urethritis.

Impact of male genital tract microbiota on female one is highly likely and it has been revealed in most of studies although some opposite results have been obtained as well. Increase of vaginosis-type microbiota and coliforms are the most typical findings. There are very scarce studies on the couples' genital tract microbiota yet adverse effect of male genital tract bacteria on in vitro fertilization and pregnancy outcome has been revealed while also the guarding virtue of normal female lactoflora against the post-intercourse shift has been shown.

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