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Identification of bacterial vaginosis-associated bacteria in male urethra: Co-occurrence of *Atopobium vaginae* and *Gardnerella vaginalis*

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ABSTRACT

Aims: Bacterial vaginosis (BV) is characterized by a transition in vaginal microflora from lactobacilli to anaerobic bacteria. *Gardnerella vaginalis* and *Atopobium vaginae* are considered the most responsible pathogens for the etiology of BV. Colonization of male urethra with BV-associated bacteria has been rarely investigated. The aim of this study was to investigate the differences in the presence of BV-associated bacteria in the healthy male urethra in regard to sexual exposure.
Methodology and results: The first-catch urine specimens, representative of urethral swabs, from 114 healthy male volunteers, were included in this study. *Lactobacillus* spp., *L. crispatus*, *L. jensenii*, *L. gasseri*, *L. iners*, *G. vaginalis*, *A. vaginae*, *Peptoniphilus* spp., *P. lacrimalis*, BVAB2, *Mageeibacillus indolicus*, *Megasphaera* type I, *Mobiluncus mulieris*, *Leptotrichia*/*Sneathia*, *Corynebacterium* spp., and *Prevotella* spp. were investigated using a PCR assay. The most frequently identified BV-associated bacteria were *Lactobacillus* spp., *Peptoniphilus* spp., and *G. vaginalis*. There was no association between any BV-associated bacteria and sexual exposure. There was statistically significant co-occurrence of *A. vaginae* and *G. vaginalis* in the MU of subjects independently of sexual exposure ($p = 0.025$). Also, there was a significant association between *G. vaginalis* and smoking ($p = 0.023$).
Conclusion, significance and impact of study: To the best of our knowledge, this is the first study reporting the co-occurrence of *G. vaginalis* and *A. vaginae* in the male urethra independently of sexual exposure.

Keywords: Male urethra, bacterial vaginosis, *Atopobium vaginae*, *Gardnerella vaginalis*, sexual exposure

INTRODUCTION

Unlike the female urogenital tract, few studies on the male urogenital tract microbiota have been reported to date (Nelson *et al.*, 2010; 2012). Since much previous research has relied on cultivation-dependent methods, our knowledge about commensal microorganisms inhabiting the male urogenital tract has been limited to culturable bacteria (Chambers *et al.*,1987). Furthermore, the majority of the studies on the male urethral microbiota are restricted to the sexually transmitted bacterial pathogens (Nelson *et al.*, 2012). Recently, novel molecular techniques have increased our understanding about the microbiota of male urogenital tract previously esteemed to be sterile.

Bacterial vaginosis (BV) is the most common genital infection among women of reproductive age. It has been numerously shown that it is strongly linked to sexual behavior. Yet, an endless controversy over the sexual transmission of BV remains elusive (Fredricks *et al.*, 2007). BV is characterized by a transition in vaginal microbiota from lactobacilli to other anaerobic bacteria (Fredricks *et al.*, 2007). *G. vaginalis* and *A. vaginae* are considered the most responsible pathogens for the vague etiology of BV (Hardy *et al.*, 2016). However, potential reservoirs of putative anaerobic bacteria associated with BV have not been thoroughly studied.

Male urethra (MU) is a duct allowing the passage of both urine and semen. Therefore, it is spatially impossible to separate the urinary and genital tract in men. As a result, the bidirectional transmission of microorganisms between genital tracts of male and female is possible during sexual activity. Therefore, MU may provide a suitable environment for reservoir and transmission of microorganisms during sexual intercourse (Nelson *et al.*, 2012). *G. vaginalis* carriage in MU has been previously reported (Zozoya *et al.*, 2016). On the other hand, colonization of MU with other BV-associated bacteria has been rarely investigated in healthy men (Riemersma *et al.*, 2003). In the present study, we aimed to explore the

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differences in the presence of BV-associated bacteria in the urethra of sexually active and inactive healthy adult men by the genus- or species-specific PCR assay.

MATERIALS AND METHODS

Study design and sample collection

The present research is designed as a prevalence study to investigate the rates of BV-associated bacteria in MU. First-catch urine (FCU) specimens were substituted for the urethral swabs to use a non-invasive sampling technique instead of an invasive one. Also, FCU was formerly used as the representative of urethral swabs (Dong *et al*., 2011). A subset of the healthy adult men (n=150) of reproductive age is recruited from university students, workers, and officials in Konya province of Turkey between January-June 2014. All participants were aged 18 years or over. The participants provided a written informed consent prior to the enrollment. The participants filled in a questionnaire form comprising age, gender, occupation, contact number, marital status, male virginity, smoking, circumcision, and presence of signs and symptoms of urinary tract infection. The exclusion criteria were as follows: A history of antibiotic use during the past four weeks, a recent history of urinary tract infection, the presence of urethral discharge, urolithiasis, and urogenital malignancies. The study was approved by the local ethics committee of Selcuk University, Faculty of Medicine (decision no: 2013/277).

DNA extraction and PCR assay

The urine samples were examined microscopically for the absence of leukocytes. Then, the quantitative culture of each urine specimen was carried out to ensure the absence of growth of bacterial or fungal pathogens. The urine specimen of 15 mL was centrifuged at 3045 × *g* for 15 min by NF 800R (Nüve, Ankara, Turkey). The supernatant was discharged, and the sediment was resuspended in 2 mL of urine. The urine specimens were stored at -80 °C until DNA isolation.

Table 1: The primers with their sequence data and annealing temperatures.

Target	Primer	Sequence (5′-3′)	Annealing, °C	Reference
Universal bacterial 16s rRNA	Bac27F EUB338R-I	AGAGTTTGATCCTGGCTCAG GCTGCCTCCCGTAGGAGT	61	Ling *et al.*, 2010
Lactobacillus spp.	LactoF LactoR	TGGAAACAGRTGCTAATACCG GTCCATTGTGGAAGATTCCC	61	Byun *et al.*, 2004
L. crispatus	L.crisp-452F L.crisp-1023R	CTTTGTATCTCTACAAATGGCACTA ACAGGGGTAGTAACTGACCTTTG	61	Fredricks *et al.*, 2007
L. iners	L.iners-453F L.iners-1022R	ACAGGGGTAGTAACTGACCTTTG ATCTAATCTCTTAGACTGGCTATG	55	Fredricks *et al.*, 2007
L. jensenii	LjensF LjensR	AAGTCGAGCGAGCTTGCCTATAGA CTTCTTTCATGCGAAAGTAGC	61	Tamrakar *et al.*, 2007
L. gasseri	LgassF LgassR	AGCGAGCTTGCCTAGATGAATTTG TCTTTTAAACTCTAGACATGCGTC	61	Tamrakar *et al.*, 2007
G. vaginalis	G.vag 644F G.vag 851R	GGGCGGGCTAGAGTGCA GAACCCGTGGAATGGGCC	62	Fredricks *et al.*, 2007
A. vaginae	Atop-442F Atop-1017R	GCAGGGACGAGGCCGCAA GTGTTTCCACTGCTTCACCTAA	55	Fredricks *et al.*, 2007
BVAB2	BVAB2-619F BVAB2-1024R	TTAACCTTGGGGTTCATTACAA AATTCAGTCTCCTGAATCGTCAGA	55	Fredricks *et al.*, 2007
M. indolicus	BVAB3 -999F BVAB3-1278R	TGCTTCGCCTCGCGACGTC AACTGCTTGGCTCGAGATTATC	55	Fredricks *et al.*, 2007
Leptotrichia/Sneathia	Lepto-395F Lepto-646R	CAATTCTGTGTGTGTGAAGAAG ACAGTTTTGTAGGCAAGCCTAT	55	Fredricks *et al.*, 2007
Megasphaera tip1	MegaE-456F MegaE-667R	GATGCCAACAGTATCCGTCCG CCTCTCCGACACTCAAGTTCGA	55	Fredricks *et al.*, 2007
M. mulieris	Mobil-577F M.mulie-1026R	GCTCGTAGGTGGTTCGTCGC CCACACCATCTCTGGCATG	62	Fredricks *et al.*, 2007
Peptoniphilus spp.	Pepton-1003F Pepton-1184R	GACCGGTATAGAGATATACCCT CACCTTCCTCCGATTTATCATC	55	Fredricks *et al.*, 2007
P. lacrimalis	P.lacri-999F Pepton-1184R	AAGAGACGAACTTAGAGATAAGTTTT CACCTTCCTCCGATTTATCATC	55	Fredricks *et al.*, 2007
Prevotella spp.	PrevG1-468F PrevG1-857R	GTCCCTTATTGCATGTACCATAC GCCGCTAACACTAGGTGCTA	55	Fredricks *et al.*, 2007
Corynebacterium spp.	Cory52F Cory1479R	GAACGCTGSCGGCGTGCTTAAC TTGTTACRRCTTCGTCCCAATCGCC	61	Tanner *et al.*, 1999

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Table 2: The GenBank accession numbers and lengths of sequences used as positive controls.

Acc no	Bacterium	(bp)
KU375103	*Lactobacillus crispatus* strain SU1	258
KU587713	Uncultured *Eggerthella* spp. clone BV283-1	205
KU587714	Uncultured *Lactobacillus* spp. clone NF284	447
KU587715	Uncultured *Eggerthella* spp. clone BV278-1	203
KU587716	Uncultured *Lactobacillus* spp. clone NF285: (*L. crispatus*)	205
KU587717	Uncultured *Mobiluncus* spp. clone BV279: (*M. mulieris*)	207
KU587718	Uncultured *Lactobacillus* spp. clone BV278-2: (*L. iners*)	459
KU587719	Uncultured *Prevotella* spp. clone BV278-3	329
KU513750	Uncultured *Leptotrichia* spp. isolate BV206-2	217
KU513751	Uncultured bacterium isolate BVAB2-204-1 (BVAB2)	324
KU513752	*Atopobium vaginae* isolate BV202	501
KU513753	Uncultured bacterium isolate BVAB2-204-2	332
KU513754	*Atopobium vaginae* isolate BV206-3	471
KU513755	Uncultured bacterium isolate BVAB2-206-4	328
KU513756	*Mageeibacillus indolicus* isolate BV233: (BVAB3)	235

Table 3: The rates of bacteria detected in urine specimens in accordance with sexual exposure.

The number of bacterial species or genera identified	Sexually-inexperienced men		Sexually-experienced men		Total	
	n	(%)	n	(%)	n	(%)
1	11	(22.4)	5	(7.7)	16	(14.0)
2	24	(49.0)	25	(38.5)	49	(43.0)
3	9	(18.4)	25	(38.5)	34	(29.8)
4	5	(10.2)	5	(7.7)	10	(8.8)
5	0	(0)	2	(3.0)	2	(1.8)
6	0	(0)	3	(4.6)	3	(2.6)
Total	49	(43.0)	65	(57.0)	114	(100)
The mean quantity of bacteria identified	2.2		2.7		p = 0.008	

Before the extraction, the urine sediment was incubated with 200 µL (20%) lysozyme solution at 37 °C for one hour. DNA was extracted using QIAamp mini kit (Qiagen Inc., Germany) from the sediment of urine specimen after re-centrifugation at 30.000 × *g* for 15 min (Hermle Z216MK, Wehingen, Germany) according to the manufacturer's instructions.

Eleven bacterial species and six genera were selected to be screened for their presence. *Lactobacillus* spp., *L. crispatus*, *L. jensenii*, *L. gasseri*, *L. iners*, *G. vaginalis*, *A. vaginae*, *Peptoniphilus* spp., *P. lacrimalis*, BVAB1, BVAB2, *Mageeibacillus indolicus* (formerly known as BVAB3), *Megasphaera* type I, *Mobiluncus mulieris*, *Leptotrichia*/*Sneathia*, *Corynebacterium* spp. and *Prevotella* spp. were all separately investigated using a genus- or species-specific PCR assay (Nanohelix, South Korea) according to the PCR conditions reported previously (Tanner *et al*., 1999; Byun *et al*., 2004; Fredricks *et al*., 2007; Tamrakar *et al*., 2007; Ling *et al*., 2010). Primers and annealing temperatures were presented in Table 1. A universal bacterial 16S rRNA primer set (Bac27F, EUB338R-I) was used to confirm bacterial DNA prior to the genus- or species-specific PCR amplification. BVAB1 was excluded from the study due to the lack of precise sequencing results in spite of repeated PCR reactions (even with the additional *de novo* primers for BVAB1), purification, and sequencing procedures.

G. vaginalis ATCC 14018, *Corynebacterium* spp., and four *Lactobacillus* species isolated from clinical specimens were used as positive controls. The bacteria previously identified from the vaginal samples were sequenced and used as positive controls for the unculturable bacteria (See Table 2 for the Genbank accession numbers of the sequences with ≥ 200 base pair (bp) in length). A PCR mixture tube containing all the reagents with no DNA template was used as the negative control.

Statistical analyses

The Chi-square independence test was used to analyze the association between the co-occurrence of the most frequently detected four bacteria (*Peptoniphilus* spp., *G. vaginalis*, *L. iners*, and *A. vaginae*). The association between these four bacteria and sexual-exposure and smoking status were investigated by the Chi-square independence test. The relationship between the mean quantity of the bacteria detected in urine samples and the status of sexual intercourse was analyzed by Mann-Whitney U test, a non-parametric statistical method. The statistical significance level was accepted at $p < 0.05$.

Malays. J. Microbiol. Vol 15(1) 2019, pp. 69-75
DOI: http://dx.doi.org/10.21161/mjm.002518

RESULTS

Out of 150, 36 male participants with the exclusion criteria were excluded from the study. Urine specimens of 114 healthy male volunteers were further investigated. The mean age of the participants was 29.38 ± 0.753 years (range 19-60, median 28) with 57% married or partnered, 43% reported being the male virgin, 44.7% smoking, 70.2% being working, and 100% circumcised.

Using bacterium-specific PCR assay, 1-6 bacterial species were identified per sample (Table 3). The mean quantities of the bacterial species in the urine samples of the sexually-experienced and -inexperienced participants were 2.7 and 2.2, respectively (Table 3). There was a significant difference in the mean quantities of the bacterial species in the urine samples of sexually-experienced and -inexperienced subjects (p = 0.008).

The most frequent bacteria were: *Lactobacillus* spp. (88.6%), *Peptoniphilus* spp. (53.5%), *L. iners* (51.8%), *G. vaginalis* (50.8%), *A. vaginae* (20.2%), and *Corynebacterium* spp. (17.5%). Rarely identified bacteria were: *L. jensenii*, *Prevotella*, BVAB2, *L. crispatus*, *L. gasseri*, *Megasphaera* type1, *M. indolicus*, *Leptotrichia/Sneathia*, and *M. mulieris* were infrequently detected (6.1%-0.9%). BVAB2, *M. indolicus*, *Megasphaera* type 1, *Leptotrichia*/*Sneathia* and *M. mulieris* were all detected only in the urine samples of sexually active participants (Table 4). *P. lacrimalis* was not detected in any urine sample.

Table 4: The rates of bacterial genera/species in the urine specimens.

Bacteria	n	(%)
Lactobacillus spp.	101	(88.6)
Peptoniphilus spp.	61	(53.5)
L. iners	59	(51.8)
G. vaginalis	58	(50.9)
A. vaginae	23	(20.2)
Corynebacterium spp.	20	(17.5)
L. jensenii	7	(6.1)
Prevotella	7	(6.1)
BVAB2	4	(3.5)
L. crispatus	2	(1.8)
L. gasseri	2	(1.8)
Megasphaera type 1	2	(1.8)
M. indolicus	1	(0.9)
Leptotrichia/*Sneathia*	1	(0.9)
M. mulieris	1	(0.9)
P. lacrimalis	0	(0)

There was no association between the most frequently identified four BV-associated bacteria (*L. iners*, *A. vaginae*, *G. vaginalis*, and *Peptoniphilus* spp.) and sexual exposure. The co-occurrence of *A. vaginae* and *G. vaginalis* in MU was statistically significant independently of sexual exposure (p = 0.025). Also, there was a significant association between *G. vaginalis* and smoking (p = 0.023).

DISCUSSION

The present study is one of the rare studies specifically investigating BV-associated bacteria in the MU of healthy subjects using species-, and genus-specific PCR. In fact, there are few studies concerning urogenital tract microbiome (Nelson *et al*., 2010; Nelson *et al*., 2012; Manhart *et al*., 2013. Recently, next-generation sequencing of 16S rRNA gene amplicons has been used for identification of bacteria most of which are unculturable or difficult to be isolated by standard culture methods (Siddiqui *et al*., 2011). On the other hand, choosing different hypervariable sub-regions of 16S rRNA, such as V1-V3 or V4-V6, may end up with variable sensitivities, in turn under-representing various taxa and may have poor discriminatory power for some genera (Janda and Abbott, 2007; Siddiqui *et al*., 2011; Yang *et al*., 2016). Indeed, using bacterium-specific PCR (genus and species specific) may be a more reliable approach to identify some bacterial species, in case we are looking for a specific bacterium and we need to confirm their presence.

To the best of our knowledge, the co-occurrence of *G. vaginalis* and *A. vaginae* in the MU has not been reported to date. The co-existence of these two bacteria has been previously reported as significant in vaginal samples of women with BV (Trama *et al*., 2008). Although recent studies have reached an agreement on a polymicrobial etiology of BV, *G. vaginalis* and *A. vaginae* are the most responsible agents because of their capability to launch a biofilm adherent to the vaginal epithelium (Fredricks *et al*., 2007; Malaguti *et al*., 2015; Hardy *et al*., 2015; Hardy *et al*., 2016). The biofilm acts as a backbone incorporating other anaerobic bacteria into its layers, thus furthering BV (Hardy *et al*., 2016). Our findings suggest that the construction of a clue cell may be possible even in the urethra of sexually-inexperienced men. In other words, the MU may be a reservoir for BV due to the co-occurrence of *G. vaginalis* and *A. vaginae*. Although *G. vaginalis* has been implicated in urethritis in men, its pathogenic role is still indefinite (Babics and Roussellier, 2015). Yet, *A. vaginae* has been infrequently shown in the MU (Thorasin *et al*., 2015). However, it should be taken into account that *Actinobacteria*, the phylum *A. vaginae* and *G. vaginalis* belongs to, could be under-represented in studies based on PCR assays due to the distinct cell wall structure of these micro-organisms and the high G+C content of their DNA (Thorasin *et al*., 2015).

In the present study, *Peptoniphilus* was one of the most frequent genera in the urine samples of male subjects along with lactobacilli. *Peptoniphilus* is a Gram-positive anaerobic coccus in the phylum *Firmicutes* and considered as part of the vaginal and gut microbiota (Thursby and Juge, 2017). Although *Lactobacillus* has been reported without exception in urine specimens of both male and female subjects, *Peptoniphilus* has been reported several times in midstream and transurethral urine samples of healthy females by 16S rRNA sequencing techniques, but not in urine specimens of healthy men (Siddiqui *et al*., 2011; Siddiqui *et al*., 2012;

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Nelson *et al*., 2012; Pearce *et al*., 2014; Aragon *et al*., 2018). There are studies demonstrating the healthy male and female urine specimens have different bacterial compositions (Fouts *et al*., 2012; Lewis *et al*., 2013). The role of *Peptoniphilus* in human infections remains highly elusive since it could not be routinely isolated in most laboratories due to the limited culture-based techniques (Brown *et al*., 2014). *Peptoniphilus* seems to be part of urethral microbiome being reported from the urine specimens with the bacterial growth of no clinical significance (< 10^4 CFU/mL) by 16S rRNA sequencing (Sabat *et al*., 2017). Additionally, despite we identified the genus *Peptoniphilus* in a higher rate, we could not identify *P. lacrimalis*, one of the BV-associated species, from the urine specimens of the subjects. Unlike Fouts *et al*. (2012) we detected *Corynebacterium* spp. at a much lower rate in the MU (17.5%). One could expect higher detection rates of *Corynebacterium* spp. in the urine specimens due to the skin colonization. The possible causes of the lower detection rate of *Corynebacterium* spp. may be the higher circumcision status in our study and the differences in the methods used.

As expected, we found that *L. iners* was the most frequent species among four lactobacilli. On the other hand, we rarely identified *Prevotella* spp., BVAB2, *M. indolicus*, *Megasphaera* type 1, *Leptotrichia*/*Sneathia*, and *M. mulieris* from the urine specimens of the participants. Interestingly, we did identify these bacteria except for *Prevotella* only from the urine samples of the sexually active subjects. Several studies have previously reported that some of the BV-associated bacteria were identified only in sexually active individuals (Nelson *et al*., 2012; Manhart *et al*., 2013). Furthermore, it has been reported that sexual intercourse may lead significant alterations in the microbial composition of both MU and vagina (Gallo *et al*., 2011; Liu *et al*., 2015; Zozoya *et al*., 2016). It is also noteworthy to mention that BV is diagnosed mostly in sexually-experienced women (Fethers *et al*., 2009). The clue cells, pathognomonic for the diagnosis of BV, have been also observed in semen and prostatic fluids of male partners of women with BV (Ni *et al*., 2005). Transmission of BV to a woman from her sexual partner subjected to the radical prostatectomy was already reported (Muzny and Schwebke, 2014) suggesting that the route of the BV transmission may be through clue cell-like desquamated epithelial cells from the MU.

Table 5. The frequency of *G. vaginalis* in the urine samples of smokers and non-smokers.

	Non-smokers	Smokers (<1 packs per day)	Smokers (>1 packs per day)
G. vaginalis	26/63	26/43	6/8

Another interesting finding of the present study is that the presence of *G. vaginalis* was significantly associated with smoking (p = 0.023) (Table 5). Similarly, higher rates of *G. vaginalis* have been found to be associated with smoking in females (Brotman *et al*., 2014). Also, we found that there was a significant difference in the total number of bacteria identified in the urine samples of sexually-experienced and -inexperienced subjects (p = 0.008). The bacterial diversity of urogenital tract between individuals was previously reported both in men and women (Siddiqui *et al*., 2011; Frølund *et al*., 2018). We have some limitations such as a small sample size and the lack of vaginal samples of the female partners. Therefore, we could not apply statistics for the bacteria identified rarely from the MU and could not interpret the smaller findings.

CONCLUSION

To the best of our knowledge, this is the first study reporting the co-occurrence of *G. vaginalis* and *A. vaginae* in the male urethra independently of sexual exposure. Comprehensive studies are needed to reveal the significance of *G. vaginalis* and *A. vaginae* in the male urethra and in BV pathogenesis. Furthermore, higher bacterial diversity was observed in sexually active participants, indicating that sexual exposure may affect microbiota of the male urethra. Also, further studies should be conducted to determine the impact of sexual exposure on the male urethral microbial composition and its influence on the health status.

COMPETING INTERESTS

The authors declare no conflict of interests.

AUTHORS CONTRIBUTIONS

Conceived and designed the experiments: ARU EIT DF. Performed the experiments: ARU. Analyzed the data: ARU EIT. Critically revised the manuscript: DF EIT. Wrote the paper: ARU.

REFERENCES

Aragón, I. M., Herrera-Imbroda, B., Queipo-Ortuño, M. I., Castillo, E., Del Moral, J. S., Gómez-Millán, J., Yucel, G. and Lara, M. F. (2018). The urinary tract microbiome in health and disease. *European Urology Focus* **4(1), 128-138.**

Babics, A., and Roussellier, P. (2015). Gardnerella vaginalis: An overlooked pathogen in male patients? *Médecine et Maladies Infectieuses* **45(10), 423-4.**

Brotman, R. M., He, X., Gajer, P., Fadrosh, D., Sharma, E., Mongodin, E. F., Ravel, J., Glover, E. D. and Rath, J. M. (2014). Association between cigarette smoking and the vaginal microbiota: A pilot study. *BMC Infectious Diseases* **14, 471.**

Brown, K., Church, D., Lynch, T. and Gregson, D. (2014). Bloodstream infections due to *Peptoniphilus* spp.: Report of 15 cases. *Clinical Microbiology and Infection* **20(11), O857-O860.**

Byun, R., Nadkarni, M. A., Chhour, K.-L., Martin, F. E., Jacques, N. A. and Hunter, N. (2004). Quantitative analysis of diverse *Lactobacillus* species present in

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advanced dental caries. *Journal of Clinical Microbiology* **42(7), 3128-3136.**

Chambers, C. V., Shafer, M. A., Adger, H., Ohm-Smith, M., Millstein, S. G., Irwin, C. E. Jr., Schachter, J. and Sweet, R. (1987). Microflora of the urethra in adolescent boys: Relationships to sexual activity and nongonococcal urethritis. *The Journal of Pediatrics* **110(2), 314-321.**

Dong, Q., Nelson, D. E., Toh, E., Diao, L., Gao, X., Fortenberry, J. D. and Van Der Pol, B. (2011). The microbial communities in male first catch urine are highly similar to those in paired urethral swab specimens. *PLoS ONE* **6(5), e19709.**

Fethers, K. A., Fairley, C. K., Morton, A., Hocking, J. S., Hopkins, C., Kennedy, L. J., Fehler, G. and Bradshaw, C. S. (2009). Early sexual experiences and risk factors for bacterial vaginosis. *The Journal of Infectious Diseases* **200(11), 1662-1670.**

Fouts, D. E., Pieper, R., Szpakowski, S., Pohl, H., Knoblach, S., Suh, M.-J., Huang, S. T., Ljungberg, I., Sprague, B. M., Lucas, S. K., Torralba, M., Nelson, K. E. and Groah, S. L. (2012). 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. *Journal of Translational Medicine* **10(174), 1-17.**

Frølund, M., Wikström, A., Lidbrink, P., Abu Al-Soud, W., Larsen, N., Harder, C. B., Sørensen, S. J., Jensen, J. S., and Ahrens, P. (2018). The bacterial microbiota in first-void urine from men with and without idiopathic urethritis. *PLoS ONE* **13(7), e0201380.**

Fredricks, D. N., Fiedler, T. L., Thomas, K. K., Oakley, B. B. and Marrazzo, J. M. (2007). Targeted PCR for detection of vaginal bacteria associated with bacterial vaginosis. *Journal of Clinical Microbiology* **45(10), 3270-3276.**

Gallo, M. F., Warner, L., King, C. C., Sobel, J. D., Klein, R. S., Cu-Uvin, S., Rompalo, A. M. and Jamieson, D. J. (2011). Association between semen exposure and incident bacterial vaginosis. *Infectious Diseases in Obstetrics and Gynecology* **Article ID 842652.**

Hardy, L., Jespers, V., Abdellati, S., De Baetselier, I., Mwambarangwe, L., Musengamana, V., van de Wijgert, J., Vaneechoutte, M. and Crucitti, T. (2016). A fruitful alliance: The synergy between *Atopobium vaginae* and *Gardnerella vaginalis* in bacterial vaginosis-associated biofilm. *Sexually Transmitted Infections* **92(7), 487-491.**

Hardy, L., Jespers, V., Dahchour, N., Mwambarangwe, L., Musengamana, V., Vaneechoutte, M. and Crucitti, T. (2015). Unravelling the bacterial vaginosis-associated biofilm: A multiplex *Gardnerella vaginalis* and *Atopobium vaginae* fluorescence *in-situ* hybridization assay using peptide nucleic acid probes. *PLoS ONE* **10(8), e0136658.**

Janda, J. M. and Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. *Journal of Clinical Microbiology* **45(9), 2761-2764.**

Lewis, D. A., Brown, R., Williams, J., White, P., Jacobson, S. K., Marchesi, J. R. and Drake, M. J. (2013). The human urinary microbiome; Bacterial DNA in voided urine of asymptomatic adults. *Frontiers in Cellular and Infection Microbiology* **3(41), 1-14.**

Ling, Z., Kong, J., Liu, F., Zhu, H., Chen, X., Wang, Y., Li, L., Nelson, K. E., Xia, Y. and Xiang, C. (2010). Molecular analysis of the diversity of vaginal microbiota associated with bacterial vaginosis. *BMC Genomics* **11(488), 1-16.**

Liu, C. M., Hungate, B. A., Tobian, A. A. R., Ravel, J., Prodger, J. L., Serwadda, D., Kigozi, G., Galiwango, R. M., Nalugoda, F., Keim, P., Wawer, M. J., Price, L. B. and Gray, R. H. (2015). Penile microbiota and female partner bacterial vaginosis in Rakai, Uganda. *mBio* **6(3), e00589-15.**

Malaguti, N., Bahls, L. D., Uchimura, N. S., Gimenes, F. and Consolaro, M. E. L. (2015). Sensitive detection of thirteen bacterial vaginosis-associated agents using multiplex polymerase chain reaction. *BioMed Research International* **Article ID 645853.**

Manhart, L. E., Khosropour, C. M., Liu, C., Gillespie, C. W., Depner, K., Fiedler, T., Marrazzo, J. M. and Fredricks, D. N. (2013). Bacterial vaginosis-associated bacteria in men: Association of *Leptotrichia/Sneathia* spp. with nongonococcal urethritis. *Sexually Transmitted Diseases* **40(12), 944-949.**

Muzny, C. A. and Schwebke, J. R. (2014). Suspected heterosexual transmission of bacterial vaginosis without seminal fluid exposure. *Sexually Transmitted Diseases* **41(1), 58-60.**

Nelson, D. E., Dong, Q., Van Der Pol, B., Toh, E., Fan, B., Katz, B. P., Mi, D., Rong, R., Weinstock, G. M., Sodergren, E. and Fortenberry, J. D. (2012). Bacterial communities of the coronal sulcus and distal urethra of adolescent males. *PLoS ONE* **7(5), e36298.**

Nelson, D. E., Van Der Pol, B., Dong, Q., Revanna, K. V., Fan, B., Easwaran, S., Sodergren, E., Weinstock, G. M., Diao, L. and Fortenberry, J. D. (2010). Characteristic male urine microbiomes associate with asymptomatic sexually transmitted infection. *PLoS ONE* **5(11), e14116.**

Ni, S. J., Huang, L., She, S. Y. and Li, Y. F. (2005). Significance of clue cells in the diagnosis of male urogenital infection. *Zhonghua Nan Ke Xue* **11(8), 598-600.**

Pearce, M. M., Hilt, E. E., Rosenfeld, A. B., Zilliox, M. J., Thomas-White, K., Fok, C., Kliethermes, S., Schreckenberger, P. C., Brubaker, L., Gai, X. and Wolfe, A. J. (2014). The female urinary microbiome: A comparison of women with and without urgency urinary incontinence. *mBio* ***5*(4), e01283-14.**

Riemersma, W. A., Van der Schee, C. J. C., Van der Meijden, W. I., Verbrugh, H. A. and Van Belkum, A. (2003). Microbial population diversity in the urethras of healthy males and males suffering from

nonchlamydial, nongonococcal urethritis. *Journal of Clinical Microbiolgy* **41(5), 1977-1986.**

Sabat, A. J., van Zanten, E., Akkerboom, V., Wisselink, G., van Slochteren, K., de Boer, R. F., Hendrix, R., Friedrich, A. W., Rossen, J. W. A. and Kooistra-Smid, A. M. D. M. (2017). Targeted next-generation sequencing of the 16S-23S rRNA region for culture-independent bacterial identification - increased discrimination of closely related species. *Scientific Reports* **7(3434) 1-12.**

Siddiqui, H., Nederbragt, A. J., Lagesen, K., Jeansson, S. L. and Jakobsen, K. S. (2011). Assessing diversity of the female urine microbiota by high throughput sequencing of 16S rDNA amplicons. *BMC Microbiology* **11(244), 1-14.**

Tamrakar, R., Yamada, T., Furuta, I., Cho, K., Morikawa, M., Yamada, H., Sakuragi, N. and Minakami, H. (2007). Association between *Lactobacillus* species and bacterial vaginosis-related bacteria, and bacterial vaginosis scores in pregnant Japanese women. *BMC Infectious Diseases* **7(128), 1-8.**

Tanner, M. A., Shoskes, D., Shahed, A. and Pace, N. R. (1999). Prevalence of Corynebacterial 16S rRNA sequences in patients with bacterial and "nonbacterial" Prostatitis. *Journal of Clinical Microbiology* **37(6), 1863-1870.**

Thorasin, T., Hoyles, L. and McCartney, A. L. (2015). Dynamics and diversity of the '*Atopobium cluste*r' in the human faecal microbiota, and phenotypic characterization of '*Atopobium cluster*' isolates. *Microbiology* **161(Pt 3), 565-579.**

Thursby, E. and Juge, N. (2017). Introduction to the human gut microbiota. *The Biochemical Journal* **474(11), 1823-1836.**

Trama, J. P., Pascal, K. E., Zimmerman, J., Self, M. J., Mordechai, E. and Adelson, M. E. (2008). Rapid detection of *Atopobium vaginae* and association with organisms implicated in bacterial vaginosis. *Molecular and Cellular Probes* **22(2), 96-102.**

Yang, B., Wang, Y. and Qian, P. Y. (2016). Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. *BMC Bioinformatics* **17(135) 1-8.**

Zozaya, M., Ferris, M. J., Siren, J. D., Lillis, R., Myers, L., Nsuami, M. J., Eren, A. M., Brown, J., Taylor, C. M. and Martin, D. H. (2016). Bacterial communities in penile skin, male urethra, and vaginas of heterosexual couples with and without bacterial vaginosis. *Microbiome* **4(16), 1-10.**

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