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Characterisation of the human uterine microbiome in non-pregnant women through deep sequencing of the V1-2 region of the16S rRNA gene.

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Background. It is widely assumed that the uterine cavity in non-pregnant women is a sterile body environment under physiological conditions. We have recently shown through fluorescence-in-situ-hybridization however, that in women with overt dysbiosis of the vaginal microbiome, a polymicrobial *Gardnerella vaginalis*-dominated biofilm adheres to the endometrium in some half of these patients, casting doubt over the alleged sterility of the human uterus. In a small exploratory study, we therefore aimed to assess the putative presence of a uterine microbiome in a series of non-pregnant women through deep sequencing of the V1-2 hypervariable region of the 16S ribosomal RNA (rRNA) gene.

Methods. We sampled the endometrial surface by use of a transcervical device designed to avoid contamination from the vagina and endocervix in nineteen non-pregnant women with reproductive failure (recurrent implantation failure, recurrent pregnancy loss, or both) in the absence of uterine anomalies on hysteroscopy. Following DNA extraction, the V1-2 region of the 16S rRNA gene was targeted using the 27F and 338R primers. By use of the Illumina MiSeq platform, 16S rRNA gene amplicon sequences were identified and annotated to the highest taxonomical level by comparison with published sequences available through the Ribosomal Database Project.

Results. Out of 183 unique 16S rRNA gene amplicon sequences, sixty operational taxonomic units or phylotypes constituted at least 1% of all reads in at least one subject, of which 15 phylotypes were present in all samples, possibly representing the uterine core microbiome, dominated by *Bacteroides xylanivorans*, *Bacteroides thetaiotaomicron*, *Bacteroides fragilis*, and *Pelomonas*. Accordingly, three bacterial phyla, *Proteobacteria, Firmicutes and Bacteroidetes*, were consistently present, *Bacteroidetes* dominating the endometrial community in most women. Twelve out of the 19 bacterial communities showed an average mutual similarity of approximately 75%. In some women, the endometrial community was also characterized by a single abundant species co-occurring with the core microbiota, in particular *Lactobacillus crispatus*, *Lactobacillus iners*, and *Prevotella amnii*, while in two women the community largely diverged from the remainder.

Discussion. Our findings are, albeit not necessarily generalizable, consistent with the presence of a unique microbiome residing on the endometrium of the human non-pregnant uterus in women of reproductive age, primarily dominated by *Bacteroidetes*, though in some women by *Firmicutes* co-occurring with the former. A majority of women showed a rather

similar endometrial community, dominated by only a few *Bacteroides* and *Pelomonas* phylotypes. Consistent with our current understanding of the human microbiome, the uterine microbiome is likely to have a previously unrecognized role in uterine physiology and human reproduction. Further study is therefore warranted to document community ecology and dynamics of the uterine microbiota, as well as the role of the uterine microbiome in health and disease.

INTRODUCTION

It has been acknowledged for over a century that the lower female genital tract harbours a unique microbiota. Over the past decades, a growing body of epidemiological evidence has substantiated the pivotal role of this microbiota in health and disease (Martin, 2012). As a result, and due to the increased availability of affordable, high throughput molecular identification techniques, characterisation of the vaginal microbiome has become a growing area of interest, with over 50 studies having been published on the subject in the past five years (van de Wijgert et al., 2014)

In stark contrast to the vaginal niche, the upper female genital tract is conventionally considered a sterile body environment under physiological conditions. Albeit difficult to conceive that such anatomically closely related organs like the vagina and the uterus are strictly delineated in terms of microbial colonisation, the endocervix is generally believed to provide a highly effective barrier against the ascent of vaginal microorganisms. Though the barrier properties of the endocervix have not been fully elucidated, endocervical barrier function is generally attributed to the physical barrier provided by the viscoelastic endocervical mucus (Linden et al., 2008) and to unique innate and adaptive mucosal immunity features (Quayle, 2002; Wira et al., 2005; Hickey et al., 2011).

During the second half of the 20th century, several researches have challenged the paradigm of the sterility of the uterus through culture-based techniques (Butler, 1958; Bollinger, 1964; Mishell et al., 1966; Ansbacher, Boyson & Morris, 1967; Spore et al., 1970; Grossman et al., 1978; Pezzlo et al., 1979; Sparks et al., 1981; Knuppel et al., 1981; Heinonen et al., 1985; Nelson & Nichols, 1986; Eschenbach et al., 1986; Teisala, 1987; Hemsell et al., 1989; Cowling et al., 1992; Møller et al., 1995). Endometrial samples in these studies have basically been obtained through three different approaches, i.e. transcervical sampling with special devices that aim at minimizing the risk of cervicovaginal contamination, perioperative transfundal aspiration, and direct sampling of the endometrial cavity after the fundus has been opened by sterile techniques immediately after hysterectomy. With the exception of one small study (Teisala, 1987), endometrial bacteria were found in all studies, though the prevalence of bacterial colonisation of the uterine cavity ranged widely from 3.8% (Butler, 1958) up to 89.0% (Hemsell et al., 1989), leaving the postulate of a uterine microbiota unresolved.

It is now well established that only a minority of bacteria on Earth can be readily cultivated *in vitro* (Vartoukian, Palmer & Wade, 2010), which also implies that the putative presence of an endometrial microbiota has not been thoroughly explored. We have previously shown through a culture-independent, 16S rRNA gene-based approach that in pregnant and non-pregnant women with bacterial vaginosis, a common manifestation of vaginal dysbiosis, half of the patients present with a polymicrobial biofilm that spreads into the uterus thereby covering the endometrium (Swidsinski et al., 2013). Hence, the view of the endometrial cavity as a sterile body compartment may not be longer tenable. In the present study, we aimed to explore the putative presence of a uterine microbiome in a cohort of non-pregnant women of reproductive age using a barcoded Illumina paired-end sequencing method targeting the V1-2 hypervariable region of the 16S ribosomal RNA (rRNA) gene.

METHODS

Patient recruitment and ethical considerations

Between March to June 2013, consecutive patients with reproductive failure attending our outpatient hysteroscopy facility were invited to participate in the study. All study participants gave their oral and written informed consent for endometrial sample collection and subsequent microbiological analysis. All experiments were performed in accordance with relevant guidelines and regulations. Ethical approval was obtained from the Ghent University Hospital Institutional Review Board under reference EC2013/053.

Patient characteristics

All patients included (n=19) were Belgian or Dutch residents of white Caucasian origin who were referred to the Ghent University Hospital Department of Reproductive Medicine for recurrent implantation failure (n=11), recurrent pregnancy loss (n=7), or both (n=1), and who underwent a hysteroscopic examination as part of the diagnostic work up. Study participants had a median age of 32 years with a range of 25 to 39 years. Among patients with recurrent implantation failure six were nulligravid, while five had a history of at least one biochemical pregnancy (median 1, range 1 to 3). Patients with recurrent pregnancy loss had a history of multiple previous pregnancies (median 4, range 3 to 6). One patient was referred for both recurrent implantation failure and recurrent pregnancy loss and had five early pregnancy losses. In all patients it was verified that no pregnancy or intra-uterine procedure was documented for at least six months preceding their inclusion in the study. In none of the patients uterine anomalies could be documented during hysteroscopy.

Endometrial sampling procedure

Patients assumed a classic lithotomy position for the endometrial sampling procedure and the subsequent hysteroscopy. A non-lubricated, sterile, stainless steel Collin speculum was inserted into the vagina to allow for proper visualisation of the ectocervix and the external os of the cervix in particular. Subsequently, the cervical surface and external os were rinsed with an aqueous 0.5% chlorhexidine gluconate solution.

As the endometrial sampling device we used the Tao Brush™ IUMC Endometrial Sampler (Cook OB-GYN, Bloomington, Ind., USA). This particular device has been developed at the Indiana University Medical Center and is primarily intended for the early detection of endometrial carcinoma, though it has also been found suitable for the procurement of endometrial samples without contamination from endocervix and vagina for microbiologic study (Tao, 1997). Briefly, the endometrial sampling device is equipped with a brush that is protected by a covering sheath laterally and by a small sphere on top to protect the brush from any contamination during passage through the vaginal lumen and endocervical canal.

In the present study, the Tao Brush™ IUMC Endometrial Sampler was carefully inserted into the vagina thereby avoiding contact with the vulva and the vaginal introitus. During passage through the vagina the device was allowed to make contact with the sterile speculum, but not with the vaginal walls. After insertion of the sheathed brush into cervical canal the brush was further moved upwards into the uterine cavity. Once the brush is in place, the sheath is removed, and the small, flexible brush is then rotated five times thereby sampling the entire inside of the uterus. The brush is then re-sheathed before it is withdrawn from the uterine cavity. Hence, due to the specific design of the endometrial sampler, and when correctly used, the brush does not make contact at any time during the procedure with the vulva, the vagina,

the cervical os or the endocervical cervical, thereby virtually eliminating any risk of cervicovaginal contamination.

Following the above procedure, the brush was separated in a sterile manner from all other parts of the device. The brush was then placed in a sterile Falcon tube and stored at -80 °C until transport to the laboratory for further processing.

DNA extraction and Illumina sequencing

Genomic DNA extraction was performed mechanically as previously described (Vilchez-Vargas et al., 2013). Libraries for Illumina sequencing were constructed with three-nested PCR as previously described (Camarinha-Silva et al., 2014). Libraries were paired-end sequenced using the Illumina MiSeq platform. All reads were conservatively trimmed to 140 nucleotides. The paired ends were subsequently matched yielding 280 nucleotides. A total of 782,683 reads were obtained from the 19 samples and after quality filtering (Camarinha-Silva et al., 2014) a total of 676,206 reads were available for downstream analysis, including clustering according to Camarinha Silva *et al* (Camarinha-Silva et al., 2014). Through this approach a total of 183 unique sequences were obtained. The minimum sequencing depth was 25,756 reads per sample and consequently all samples were randomly normalized to this number, using the phyloseq package (McMurdie & Holmes, 2013) and R program (R Core Team, 2012) . The unique sequences served to define *operational taxonomic units* (OTUs) through similarity clustering (Camarinha-Silva et al., 2014), whereby OTUs are further referred to as *phylotypes* (Phy).

177 ***Data-analysis and reporting***

178 Phylotypes were manually assigned to the highest taxonomical level after automatic
179 annotation by aligning these sequences with the rRNA gene sequence data available through
180 the Ribosomal Database Project (RDP) as previously described (Camarinha-Silva et al.,
181 2014). Dissimilarity between the distinct bacterial communities based on the total number of
182 unique sequences per sample was calculated with the vegan package in R (Oksanen et al.,
183 2007), using Bray-Curtis dissimilarity (Beals, 1984). Heat maps were generated using the
184 gplots (Warnes et al., 2012) and RColorBrewer (Brewer, 2015) packages by using absolute
185 numbers of phylotypes following normalization to the minimum sequencing depth.

186

187 Since this was an exploratory study on the putative presence of an endometrial microbiome,
188 we aimed for patients with reproductive failure from a mere pragmatic approach, considering
189 these patients had to undergo a hysteroscopy, which also allowed us to confirm the absence of
190 visible uterine anomalies. However, apart from having reproductive failure in common, our
191 limited patient series was highly diverse with regard to a number of clinical characteristics
192 and we therefore refrained from any attempt in correlating clinical and microbiological data.

193

194 **RESULTS**

195

196 ***Sampling depth***

197 Rarefaction curves were constructed to estimate whether the sampling depth in each
198 endometrial sample was sufficient to cover the overall bacterial diversity. The curves show
199 that saturation was reached at >15,000 reads per sample (Fig. S1), and hence sufficient for all
200 samples.

201

202 ***Species diversity***

203 Sequencing of the V1-2 region of the 16S rRNA genes present in the complete endometrial
204 bacterial communities of the 19 subjects with a minimum of 25,756 sequence reads, resulted
205 in a total of 183 bacterial phylotypes, which could be identified and annotated at the
206 phylogenetic levels of Order (93.4% of phylotypes), Family (91.3% of phylotypes), Genus
207 (84.2% of phylotypes) and Species (33.3% of phylotypes). An overview of all 183 bacterial
208 phylotypes along with their relative abundances can be found in Table S1. Out of the 183
209 phylotypes, 123 phylotypes had a relative abundance of less than 1% of sequence reads per
210 sample in all samples and these phylotypes are therefore provisionally considered as minor
211 components of the uterine microbiome. The highest taxonomical level to which the 60 more
212 abundant phylotypes could be assigned, were Species for 23 phylotypes, Genus for 30
213 phylotypes, Family for three phylotypes, Order for one phylotype, and Class for three
214 phylotypes, respectively. The endometrial bacterial community structure of the 19 subjects by
215 accounting for the 60 phylotypes with an abundance of at least 1%, is shown as a heat map in
216 Fig. 1.

217

218 ***Interindividual variability in community structure***

219 An overview of the degree of similarity in bacterial community structure of the 19
220 endometrial samples is shown in Fig. 2. Twelve out of the 19 bacterial communities (S6, S7,
221 S9, S10, S11, S12, S14, S15, S16, S17, S18, and S19) showed an average mutual similarity of
222 approximately 75% (average Bray-Curtis dissimilarity index 24.6%, range 13.2% to 34.3%)
223 and were characterised by the consistent presence of several phylotypes present with
224 comparable abundances, including a high relative abundance of *Bacteroides fragilis* (Phy7)
225 and *Bacteroides thetaiotaomicron* (Phy2), and some previously largely unrecognized
226 phylotypes including *Bacteroides xylanivorans* (Phy1) and *Pelomonas* (Phy3). Remarkably,

the bacterial communities of subjects S15 and S17 were, despite the otherwise overall high similarity, also characterised by the abundance of *Lactobacillus crispatus* (Phy5), accounting for 25.3% (S15) and 17.1% (S17) of the overall number of reads, respectively.

While resembling the aforementioned series of bacterial communities (Bray-Curtis dissimilarity index of 43.6% relative to all other samples), the microbiome in subject S8 was also characterized by *Lactobacillus iners* (Phy4) as an abundant species (18.4% of the overall number of reads). Subject S5 was dissimilar (Bray-Curtis dissimilarity index of 45.9% relative to all other samples) primarily due to the abundant presence of *Prevotella amnii* (Phy28) as a unique species to this patient group, accounting for 19.1% of the overall number of reads in this sample. The uterine microbiome in subject S5 was further characterised by other phylotypes unusual to this niche in our patient series, such as *Saccharofermentans* (Phy44) and *Prevotella timonensis* (Phy10). The uterine microbiome in subjects S3 and S4 diverged from the remainder (Bray-Curtis dissimilarity index of 50.8 and 53.5% relative to all other samples, respectively), mainly due to the abundance of *Lactobacillus crispatus* (Phy5) constituting 35.5% (S3) and 52.1% (S4) of the endometrial bacterial communities in these subjects. *Lactobacillus jensenii* was also relatively abundant in subject S3. In subject S2 the bacterial community differed to a considerable extent (Bray-Curtis dissimilarity index of 60.1%), which can be mainly attributed to the abundant presence of *Lactobacillus iners* (Phy4) making up 55.4% of the overall number of reads in this sample.

Finally, in two subjects, S1 and S13, the endometrial bacterial community largely diverged from all other communities, with a Bray-Curtis dissimilarity index of 79.0 and 90.7%, respectively. In subject S1, the similarity with the remainder of women was limited to the low abundance of the species present in all or most samples, however with *Lactobacillus crispatus*

(Phy5) absolutely dominating the bacterial community with a relative abundance of 79.1%. In subject S13, the endometrial bacterial community was highly different from all other community structures, and dominated by *Prevotella timonensis* (Phy10), *Prevotella* (Phy15), and *Prevotella disiens* (Phy56), together accounting for 48.8% of the overall number of reads in this sample. The endometrial microbiome of subject S13 was further characterised by a number of other phylotypes uncommon or even unique to the niche under study in this patient series, such as *Atopobium vaginae* (Phy17), *Porphyromonas uenonis* (Phy38), *Mobiluncus curtisii* (Phy47), *Dialister* (Phy55), *Peptostreptococcus anaerobius* (Phy72), *Peptoniphilus* (Phy60), *Moryella* (Phy67), and *Saccharofermentans* (Phy44).

Uterine core microbiome

Albeit a large overall bacterial diversity was observed in the intra-uterine environment with 183 phylotypes detected through 16S rRNA sequencing, a defined set of 15 phylotypes with an abundance of at least 1% was observed in all subjects (Fig. 1), suggesting that these phylotypes may constitute the uterine core microbiome. This core microbiome can basically be clustered in two groups of phylotypes according to their abundances. *Bacteroides xylanivorans* (Phy1), *Bacteroides thetaiotaomicron* (Phy2), *Bacteroides fragilis* (Phy7), and *Pelomonas* (Phy3) were abundant in all samples, though much less so in the two largely different samples S1 and S13. Other phylotypes, including bacteria affiliated to *Escherichia/Shigella* (Phy11 and Phy8), *Bacteroidetes vulgatus* (Phy12), *Chitinophagaceae* (Phy9), *Betaproteobacteria* (Phy6) and *Pseudomonas* (Phy13) were detected in all samples, but for the most part at lower abundances. Several phylotypes also occurred with low, but highly variable abundances and included bacteria affiliated to *Caulobacter* (Phy34), *Betaproteobacteria* (Phy19), *Acidovorax* (Phy21), *Bacteroides ovatus* (Phy20), and *Pelomonas* (Phy14) (Fig. 2). The presumed uterine core microbiome therefore basically

consists of three bacterial phyla, in particular *Proteobacteria, Firmicutes and Bacteroidetes*, with *Bacteroidetes* dominating the endometrial community in almost 90% of the women included.

It was further observed that a limited number of phylotypes, though not consistently present across all endometrial bacterial communities, were more abundant than the core phylotypes in some women. *Lactobacillus crispatus* was present in 12 out of the 19 samples, and the most abundant phylotype compared to the remainder of individual phylotypes in subjects S17, S15, S3, S4, and S1 (17.1%, 25.3%, 35.5%, 52.1%, and 79.1% of the total number of sequence reads respectively). Similarly, *L. iners* was present in 7 out of the 19 samples, and the predominant phylotype in two subjects (18.4 and 55.4% in subjects S8 and S2, respectively). *Prevotella* species in turn, including *Prevotella amnii* (Phy28), *Prevotella timonensis* (Phy10), *Prevotella* (Phy15), and *Prevotella disiens* (Phy56) were predominant in subjects S5 and S13.

Noteworthy, *Gardnerella vaginalis* (Phy79) was present in six samples, but always as a minor component (less than 1% of the total sequence reads per sample) of the uterine microbiome. *Atopobium vaginae* (Phy17) was also present in six samples, but only a dominant phylotype in subject S13.

DISCUSSION

We sought to demystify the longstanding contention that the non-pregnant human uterus is sterile and revealed through 16S rRNA gene sequencing that in a cohort of selected, non-pregnant women of reproductive age, the endometrial cavity harbours a unique microbiome. Although a large number of, mostly low-abundant, phylotypes were identified, it is also apparent that the endometrial bacterial community is characterised in most women by a

limited number of particular phylotypes that are consistently present in a rather similar distribution, presumptively considered as the uterine core microbiome. It is further remarkable that in several women a distinct phylotype was actually the single most dominant one, co-occurring with, rather than displacing the uterine core microbiota. In two women the uterine core microbiome was however largely replaced by other phylotypes, one of which having a uterine microbiota involving a number of phylotypes that are associated with vaginal dysbiosis. The limited sample size of our study, does not allow us however to make any statements on community state types or dysbiosis of the uterine environment.

Mitchell *et al* very recently also described endometrial colonisation in hysterectomy patients, not by a microbiome-wide approach, but by targeting a series of 12 vaginal bacterial species through qPCR, including three keystone *Lactobacillus* species and nine bacterial vaginosis indicator species, and found that in 52 out of the 58 women included, at least one of the selected vaginal species was present in the uterine cavity (Mitchell et al., 2015). One previous study involving 11 women and aiming at the study of female genital tract bacterial ecology associated with levonorgestrel intrauterine system use included deep sequencing of the V1-V3 regions of 16S rRNA genes in endometrial samples (Jacobson et al., 2014). The latter authors discarded their uterine microbiome data however as having most likely resulted from contamination during sampling. This is quite illustrative of the historical reluctance to the idea of a non-sterile intra-uterine environment, the endocervix having assumed mythic proportions and described as the *Colossus of Rhodes* of the female genital tract (Quayle, 2002). It has recently also been recognized that the previously considered impregnable endocervical mucus plug does inhibit though not block the passage of ascending bacteria from the vagina during pregnancy (Hansen et al., 2014).

The implications of the discovery of the uterine microbiome for human health and disease are paramount. Viniker suggested more than a decade ago – even before the term 'microbiome' was coined – that unrecognised endometrial bacterial colonisation might help us to elucidate a number of common gynaecological and obstetric conditions (Viniker, 1999). As exemplified by our increasing knowledge on the gut as the most extensively studied human microbiome site, host-microbe interactions are now found to be essential to many aspects of human physiology (Dethlefsen, McFall-Ngai M & Relman, 2007). Accordingly, the upper female genital tract microbiota can reasonably be expected to have a role in uterine physiology and in human reproduction, as recently also suggested by others (Reid et al., 2015). We have previously documented that subfertile women are considerably more prone to present with dysbiosis of the vaginal microbiome as compared to the background population (van Oostrum et al., 2013). We have further shown that bacterial vaginosis involves the presence of an adherent vaginal polymicrobial biofilm (Verstraelen & Swidsinski, 2013) and that this dysbiotic biofilm also adheres to the endometrium in half of the patients presenting with bacterial vaginosis (Swidsinski et al, 2013). Hence, albeit the vaginal and uterine microbiomes appear to be quite different bacterial communities residing in completely different physicochemical and immune environments, dysbiosis of the vagina may still predispose to dysbiosis of the uterine microbiome. This would explain for instance the consistent association between dysbiosis of the vaginal microbiome and unfavourable outcomes of human reproduction, such as subfertility (van Oostrum et al., 2013; Siro I, Zarek & Segars, 2014), assisted reproductive technology failure (van Oostrum et al., 2013; Siro I, Zarek & Segars, 2014) and preterm birth (Espinoza, Erez & Romero, 2006; Mysorekar & Cao, 2014; Payne & Bayatibojakhi, 2014). Further study is therefore warranted to document the origins and the dynamics of the uterine microbiota. Though the ascent from the vagina appears the most plausible route, it is noteworthy that alternative routes have been suggested

with regard to colonisation of the intra-uterine environment in pregnancy. Aagaard et al recently reported a comprehensive study of the placental microbiome and pointed at the similarity between the placental and oral microbiota (Aagaard et al., 2014), feeding the concept of a haematogenous oral-placental route (Mendz, Kaakoush & Quinlivan, 2013). Jiménez *et a*l in turn suggested that entero-placental bacterial trafficking may be involved in establishing the fetal intra-uterine environment (Jiménez et al., 2008) .

We do acknowledge that the results of our exploratory study should be taken with caution. Since we specifically collected samples from white Caucasian patients with reproductive failure, our microbiome data are not necessarily generalizable to all reproductive-aged women. We explicitly aimed for non-pregnant women in whom samples were obtained distant from pregnancy, to avoid any potential influence of gestation on genital tract colonisation. We also chose for the approach of including women that underwent a hysteroscopy immediately after endometrial sampling as this allowed us to verify that none of the study subjects presented with intra-uterine anomalies. Secondly, although we maximized efforts to avoid any source of contamination during clinical sampling and subsequent sample processing, contamination bias cannot be entirely ruled out. Nonetheless, it should be borne in mind that due to the sequencing depth, next-generation sequencing might reveal the presence of a number of bacterial taxa that were unexpected in a given body site. Illustrative to the latter is that in the aforementioned study by Jacobson *et al*, the uterine microbiome data were discarded due to abundance of *Burkholderia* which the authors described as a common environmental contaminant (Jacobson et al., 2014). The *Burkholderia* genus has been identified in various body sites however, and interestingly, found an important component indeed of the intra-uterine environment in pregnant women (Aagaard et al., 2014), becoming more predominant in women with preterm labour (Antony et al., 2015)

We conclude that the present study along with other recent studies are consistent with the presence of distinct microbiota residing in the upper female genital tract (Pelzer et al, 2011; Pelzer et al, 2012; Pelzer et al, 2013a; Pelzer et al, 2013b; Swidsinski et al, 2013; Jacobson et al., 2014) as part of the human microbiome in women of childbearing age, and hence that further study is warranted to establish the role of the female genital tract microbiome in women's health.

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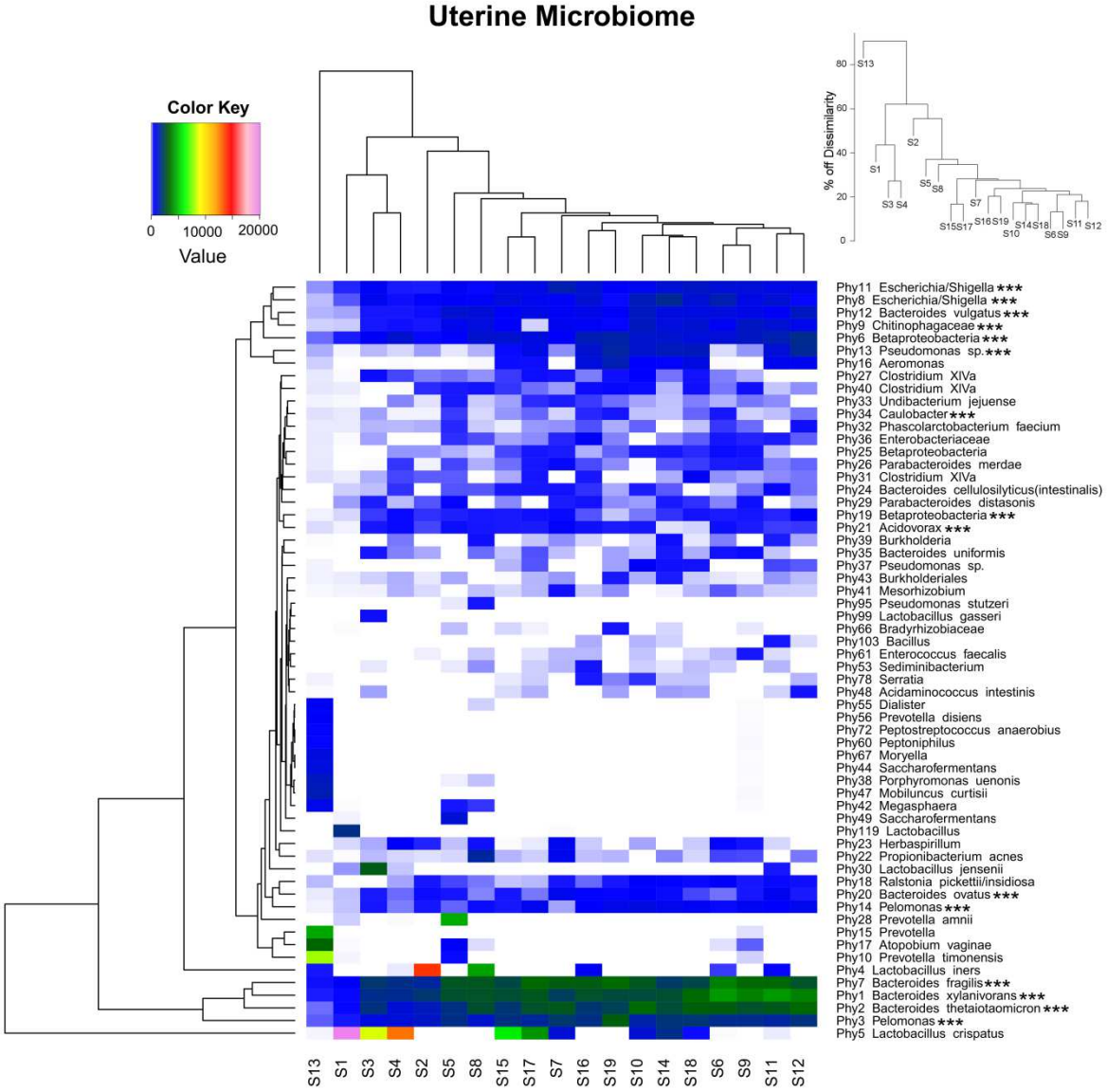
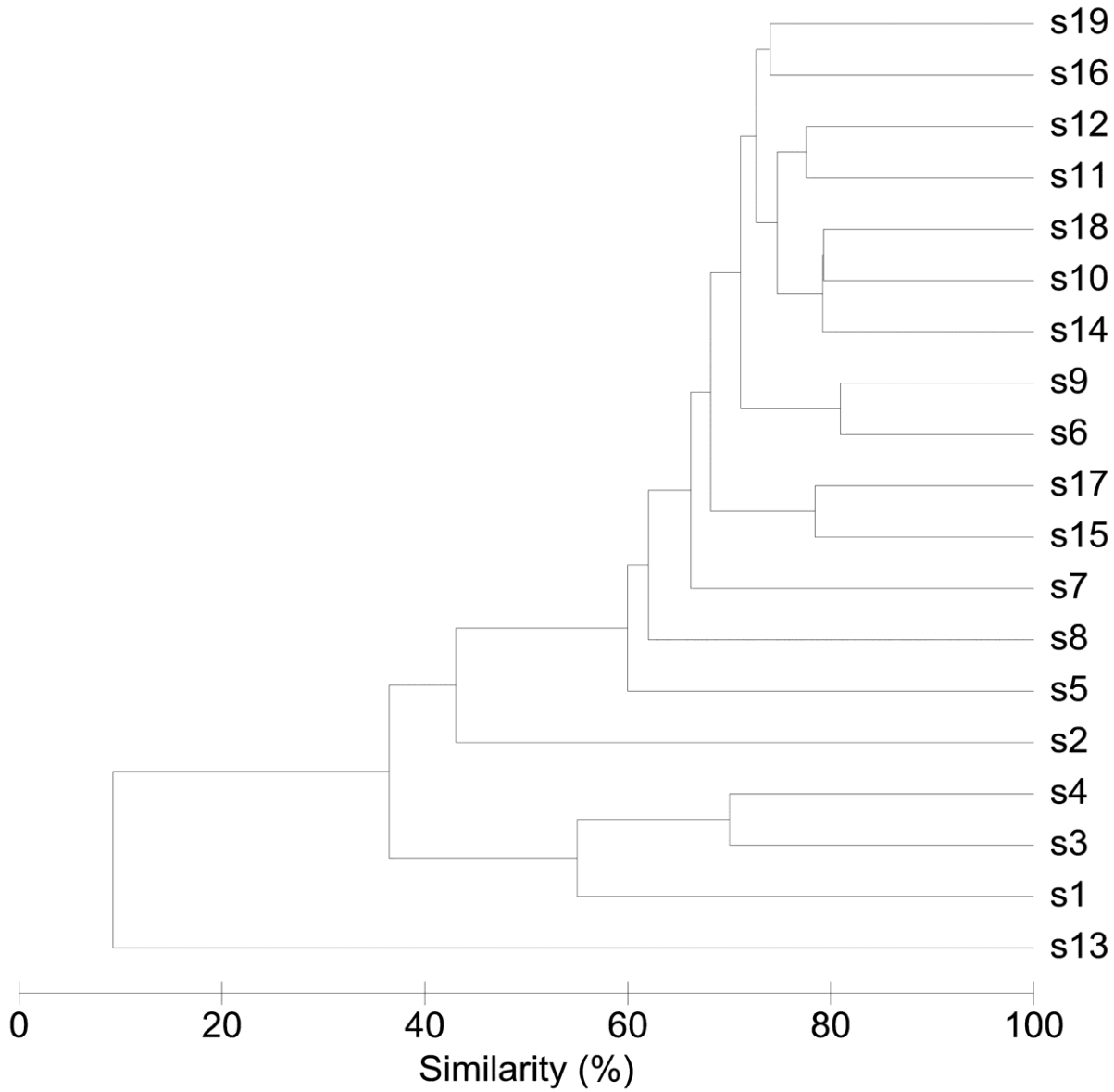
Figure 1. Endometrial bacterial community structure (n=19) by accounting for the 60 phylotypes with an abundance of at least 1%.



The heat map provides an overview of the bacterial phylotypes that had an abundance of at least 1.0% of the total number of reads in at least one endometrial sample. The colour codes correspond to: blue, 1-10% of the community; dark green, 11-20%; light green, 21-30%; yellow, 31-40%; orange, 41-50%; red, 51-60% and pink, 61-100%. Phylotypes present in all samples are labelled with asterisks.

Figure 2. Similarity between bacterial communities in endometrial samples (n=19).



The dendrogram was constructed by agglomerative hierarchical clustering (group-average) based on a relative abundance matrix of phylotypes. The percentage of similarity between the communities was calculated using the Bray-Curtis similarity algorithm.