

Structure of Vaginal Microbiome Community After Perineal Disinfection and Its Effects on Neonatal Oral Microbiome

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Research article

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Abstract

Background: Vaginal microbiota is not only an important source of bacterial colonization for neonates, but also plays a key role in maternal and neonatal health. In China, povidone iodine is used to disinfect vaginal during delivery. To date there has been no comprehensive study to investigate the vaginal microbiome composition after disinfection.

Results: In this study, 27 women were recruited from Bao an Maternal and Child Health Hospital (Shenzhen, China). Vaginal samples before and after delivery were collected. Neonatal oral samples were also collected right after birth. Bacterial compositions of these study subjects were investigated using 16S rRNA sequencing of V3-V4 hyper-regions based on Hiseq 2500 platform. The results showed that vaginal microbiome during pregnancy were dominated by *Lactobacillus spp.* The identified microbiomes were separated into three community state types (CSTs), and a new CST (dominated by *L. helveticus*) was observed in this study. After disinfection, the relative abundance of *Lactobacillus* decreased and alpha diversity increased significantly. Moreover, most CST III and CST VI during pregnancy, both them dominated by *Lactobacillus*, shifted to CST IV in vaginal samples after disinfection. Additionally, the similar change pattern was observed in neonatal oral microbiome, and they overlapped with vaginal samples after disinfection in NMDS analysis.

Conclusions: Perineal disinfection resulted in the decrease of genus *Lactobacillus* and increase of alpha diversity both in maternal vaginal microbiome and neonatal oral microbiome. In further, it is vital to understand the influence on maternal and neonatal health of vaginal microbiome community structure change after disinfection.

Background

Accumulating evidences prove that resident vaginal microbiota is related to bacterial vaginosis, vulvo-vaginal, urinary tract infections through interfering the proliferation of some organisms (Brotman, 2011; Ryckman et al., 2009). Importantly in pregnancy, dysbiosis of vaginal microbiome community is associated with an increased risk of postpartum endometritis (Jacobsson et al., 2002). Not only as an important factor in women's reproductive health, vaginal microbiome in pregnancy also plays a key role in neonatal health (Fettweis et al., 2019; Stiemsma and Michels, 2018). It is understood that the bacterial community structure of the pregnant vagina is dominated by *Lactobacillus* species (Aagaard et al., 2012; Freitas et al., 2017; DiGiulio et al., 2015), many of which can produce biosurfactants, and bacteriocins antagonistic to pathogens (O'Hanlon et al., 2011; Matu et al., 2010).

Understanding the structure of the vaginal microbiome community in pregnancy is important for maternal and neonatal health, which draws more and more attention. Several cross-sectional and longitudinal cohorts had been examined in studying the vaginal microbiome during pregnancy (Walther-António et al., 2014; Romero et al., 2014; DiGiulio et al., 2015). Collectively, the results of these studies suggested that pregnancy leads to great stability, increases *Lactobacillus* proportional abundance and

reduces richness and diversity of vaginal microbiome. Compared to more attention focused on the vaginal microbiome during pregnancy, only a few studies investigated postpartum vaginal microbiome (Freitas et al., 2017; DiGiulio et al., 2015). They showed that vaginal communities with less *Lactobacillus* species and characterized by more rich and diverse during the post-partum period. However, all the vaginal samples included in the postpartum period were a few weeks after delivery, a comprehensive characterization of vaginal microbiome signature right after delivery has not yet been undertaken.

In China, almost all hospitals in China use povidone iodine to disinfect the vulva before transvaginal examination during maternal delivery according to the latest edition of the medical education book "Obstetrics and Gynecology" (Xie and Guo, 2013). Yet relatively little is known about the community structure of vaginal microbiome after perineal disinfection and its affection on maternal and neonatal health. In this study, we aimed to characterize the composition of the vaginal microbiome after disinfection and compared the microbial profiles to those of pregnant period.

The neonate has been exposed to the maternal vaginal microbial ecosystem during the delivery process. Further microbiota colonized at the vaginal birth canal will be introduced to the neonate (Dogra et al., 2015; Chu et al., 2017; Rutayisire et al., 2016). These pioneer microbial colonizers, especially oral microbiome, play critical roles in neonatal health and development, including nutrient acquisition, immune programming and protection from pathogens (Chu et al., 2017; Huurre et al., 2008). Since the oral microbial structure of infant is affected by multiple environmental factors, such as maternal vaginal canal, skin to skin contact, maternal breast milk feeding and so on (Lif Holgerson et al., 2011; He et al., 2015). As an important source of pioneer bacteria for neonatal oral microbiome, it is important to discern the relative potential contribution of the maternal vaginal community to the neonate oral microbiome. Our previous study also demonstrated that relative abundance of genus *Lactobacillus* decreased in neonatal oral microbiome after maternal perineal disinfection (Li et al., 2019). It is therefore crucial to understand how the vaginal microbial community restructured after perineal disinfection and its association with neonatal oral microbiome.

Results

Maternal and neonatal clinical data

Demographic and clinical characteristics of the women and newborns were provided in Table 1. A total of 27 healthy and asymptomatic women were recruited into this study. All of the subjects were Han ethnicity with ages ranging from 19 to 34 years old (mean ages, 28.1 years). The average BMI was 25.2 (range 21.1–34.1). All women gave birth between 37th and 42th gestational week with 14.26 kg (range 9.0–26.30 kg) average gestational weight gain. Most women (21/27) was at labor in 24 hours after hospitalization. The average birth weight of 27 newborns was 3205.9 g (range 2600–3850 g), including 15 boys and 12 girls.

Community structure of vaginal and oral microbiome

To compare the overall vaginal community structures before disinfection and after disinfection, nonmetric multidimensional scaling (NMDS) analysis was implemented on the bacterial abundances. Figure 1A revealed that vaginal samples before disinfection (group BD) separated well from the subjects after disinfection (group AD, $p < 0.01$, PERMANOVA analysis), whereas vaginal samples after disinfection overlapped with neonatal oral samples (group NO).

Moreover, the vaginal microbiota after disinfection and neonatal oral microbiota was associated with a higher observed species index, evenness index and alpha diversity (Figure 1B). Compared to the vaginal samples before disinfection, the mean observed OTUs numbers increased significantly in neonatal oral samples (179.89 ± 113.37 versus 13.30 ± 14.83 , $p < 0.01$) and in vaginal samples after disinfection (194.33 ± 85.69 versus 13.30 ± 14.83 , $p < 0.01$) (Figure 1B (a)). Accompanied with significantly increased mean pi index value of group AD (0.55 ± 0.16 , $p < 0.01$) and NO (0.56 ± 0.24 , $p < 0.01$) versus 0.25 ± 0.22 of group BD (Figure 1B (b)). Notably, the mean Shannon index value was 0.92 ± 1.06 of BD group, significantly lower than that of group AD (4.22 ± 1.50 , $p < 0.01$) and NO (4.19 ± 2.22 , $p < 0.01$) (Figure 1B (c)). Consistent with these observations, indices of alpha-diversity and richness were the smallest in samples obtained during pregnancy, with a significant increase in diversity detected in vaginal samples after disinfection and in neonatal oral samples. Further, the weighted UniFrac value of group BD was 0.35 ± 0.29 , significant lower than group AD (0.71 ± 0.29 , $p < 0.01$) and NO (0.69 ± 0.27 , $p < 0.01$), which indicated that vaginal microbial communities were more similar within each other in group BD than AD (Figure 1B (d)).

Vaginal and oral microbiome profiling

Microbiome of study participants was characterized using high-throughput sequencing of the 16S rRNA high-variable regions. A total of 3,788,402 reads were included in the analysis. The average sequence read count was 46,770 per sample, with a median of 46,158 (range 13,434–71,182), and the mean and median read lengths were 420 and 423 bp, respectively. The relative abundance of each participant at phylum and genus levels was showed in Figure 2. It showed that the top 10 phyla were *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Cyanobacteria*, *Firmicutes*, *OD1*, *Planctomycetes*, *Proteobacteria* and *Tenericutes* (Figure 2A). Meanwhile, the top 10 genera were *Atopobium*, *Enterococcus*, *Gardnerella*, *Lactobacillus*, *Prevotella*, *Pseudomonas*, *Ralstonia*, *Staphylococcus*, *Streptococcus* and *Thiobacillus* (Figure 2B).

The results also demonstrated that pregnancy was associated with a microbiome largely dominated by phyla *Firmicutes* ($78.65\% \pm 35.50\%$) and *Actinobacteria* ($19.83\% \pm 35.42\%$), they accounted averagely for more than 98% of the microbial population (Figure 3A). While significant shifts in bacterial phylum structure were observed at neonatal mucous and vaginal samples after disinfection (Figure 3A), with significantly decreased proportions of *Firmicutes* ($37.20\% \pm 31.99\%$ and $36.75\% \pm 31.15\%$) and *Actinobacteria* ($5.44\% \pm 8.0\%$ and $7.92\% \pm 15.32\%$), significantly increased abundance of *Proteobacteria* ($41.94\% \pm 27.66\%$ and $40.93\% \pm 26.58\%$ versus $0.35\% \pm 0.77\%$) and *Bacteroidetes* ($6.64\% \pm 8.04\%$ and

4.52% \pm 3.97% versus 0.97% \pm 2.03%). The differences at the phylum level among the three groups all presented in Figure 3B.

A similar pattern was observed at the genus level (Figure 3C). The mean proportion of *Lactobacillus* decreased from 73.71% (SD, 38.0%) during pregnancy to 26.42% (SD, 29.88%) after disinfection and 23.94% (SD, 33.03%) in neonatal oral. Similarly, genus *Gardnerella* accounted averagely 16.43% (SD, 32.24%) before disinfection, decreased to 5.26% (SD, 15.58%) after disinfection and 2.04% (SD, 6.88%) of neonatal oral microbiome. In addition, compared to the pregnancy phase, disinfection phase was also accompanied by increases in genera *Streptococcus* (3.04% \pm 14.68% versus 1.56% \pm 6.27%), *Ralstonia* (16.19% \pm 11.50% versus 0.04% \pm 0.07%), *Pseudomonas* (13.45% \pm 16.99% versus 0.09% \pm 0.27%) and *Thiobacillus* (1.74% \pm 2.94% versus 0%).

Different genus between vaginal and oral microbiome

Next, the STAMP tool was used to analyze bacterial communities in vaginal samples and to detect potential significant differences in relative abundances of genus. Figure 4A included a list of genera that significantly different between vaginal samples during pregnancy and neonatal oral samples. Among them, genera *Acinetobacter*, *Burkholderia*, *Delftia*, *Mesorhizobium*, *Paracoccus*, *Ralstonia*, *Reyranella*, *Salinispora* and *Shewanella* increased significantly in the neonatal microbiome, whereas genus *Lactobacillus* decreased significantly. In addition, the greatest differences in genus between vaginal samples before disinfection and after disinfection were presented in Figure 4B. Compared to vaginal samples during pregnancy, the relative abundance of genera *Acinetobacter*, *Bradyrhizobium*, *Burkholderia*, *Comamonas*, *Delftia*, *Mesorhizobium*, *Mycobacterium*, *Ralstonia*, *Reyranella* and *Salinispora* were higher, while genus *Lactobacillus* was dramatically lower in vaginal microbiome after disinfection. Notably, there was no difference between vaginal samples after disinfection and neonatal oral samples. Collectively, these observations suggested that the microbial composition of the vaginal samples significantly differed between pregnancy and after disinfection according to the relative abundance of sequences.

Community state types analysis and its changes after delivery

Categorizing microbiome profiles based on the taxon with the largest proportion of reads, and hierarchical clustering analysis of bacterial species from the pregnant vaginal microbiome profiles revealed 3 major community state types (CSTs), which showed in Figure 5A. Among all samples, 8 samples were dominated by species *L. iners* (CST III). Six samples were assigned to CST IV, which were dominated by genus *Gardnerella*, and also typified by higher proportions of *Aerococcus*, *Atopobium*, *Bifidobacterium*, *Corynebacterium*, *Dialister*, *Finegoldia*, *Megasphaera*, *Mobiluncus*, *Peptoniphilus*, *Prevotella*, *Ralstonia*, *Staphylococcus*, *Streptococcus* and *Sneathia* than other CSTs. The rest 13 samples were dominated by species *L. helveticus* or *L. delbrueckii*, which were clustered into a new observed community type and names as CST VI in this study.

Dominance of CST IV was observed in neonatal sample and postpartum vaginal samples (n=20 and n=17 in group NO and AD respectively) (Figure 5B). Most subjects of group BD belonged to CST III and CST VI (15/21) switched to CST IV of group NO. Similarly, they (11/21) shifted towards CST IV in vaginal microbiome after disinfection. An interesting change pattern was observed in sample S6, it changed from CST IV to CST VI in the neonatal oral microbiome.

Table 1
Demographic and clinical data for study participants

Characteristics	Value
Maternal conditions	28.1 ± 3.3
Mother's age, yrs mean (SD)	
Mother's BMI, kg mean (SD)	25.2 ± 2.6
Gestational weight gain, kg mean (SD)	14.26 ± 0.37
Gestational week, wks mean (SD)	39.5 ± 1.0
Hospital stay before labor, hours mean (SD)	16.8 ± 14.8
First degree of perineal laceration, n (%)	18 (66.7%)
Neonatal conditions	
Children sex (male), n (%)	15 (55.6%)
Birth weight, g mean (SD)	3205.9 ± 289.2
Apgar score, mean	10

Discussion

In this study, the composition of the vaginal microbiota of women after disinfection was determined and compared to the profiles before disinfection. Increased microbial richness and diversity was observed in the postpartum samples, which was consistent with previous studies (Huang et al., 2015; MacIntyre et al., 2015). In addition, differences in the microbiota between the two phases regarding bacterial abundance and prevalence were also identified. The results indicated that the composition of the vaginal microbiome was dynamically restructured after disinfection, where phyla *Firmicutes* decreased and *Bacteroidetes*, *Proteobacteria* increased significantly. Additionally, in concordance with previous observations (Fettweis et al., 2019; Serrano et al., 2019; DiGiulio et al., 2015), genus *Lactobacillus* was the predominate bacterium in most of the samples during pregnancy, with *Atopobium*, *Gardnerella*, *Prevotella*, and *Streptococcus* presented in a low proportion. Whereas after disinfection, as the most common genus in phylum *Firmicutes*, *Lactobacillus* decreased significantly, with genera *Acinetobacter*, *Bradyrhizobium*, *Burkholderia*, *Comamonas*, *Delftia*, *Mesorhizobium*, *Mycobacterium*, *Ralstonia*, *Reyranella* and *Salinispora* increased significantly. It had been reported that *Lactobacillus* was the most prevalent and dominant bacterium in female vaginal, which appeared to ensure normal vaginal microbiota and

effectively inhibit the colonization of pathogens (Borges et al., 2014). Furthermore, *Lactobacillus* of vaginal microbiota played a major role in preventing the occurrence of many diseases, including bacterial vaginosis, yeast vaginitis, urinary tract infection and sexually transmitted diseases including HIV (Reid and Bocking, 2003; Borges et al., 2014). Previous study demonstrated that reduced abundance of *Lactobacillus* resulted in increased pH and reduced H₂O₂ concentration (Witkin et al., 2007). H₂O₂ protected host against different pathogens (Petrova et al., 2013), and high pH value promoted the reproduction of pathogenic bacteria (Farage et al., 2010). Particularly, bacterial vaginosis was associated with an abnormal growth of some opportunistic pathogens and a low proportion of *Lactobacillus* species (Onderdonk et al., 2016). In some clinical studies, *Lactobacillus* species were taken as probiotics for the treatment of bacterial vaginosis and prevention of HIV transmission (Tamrakar et al., 2007; Petrova et al., 2013). So the significant decrease of *Lactobacillus* in vaginal after disinfection might be an important risk for maternal health. Alternatively, genera *Acinetobacter*, *Bradyrhizobium*, *Burkholderia*, *Comamonas*, *Delftia*, *Mesorhizobium*, *Mycobacterium*, *Ralstonia*, *Reyranella* and *Salinispora* increased significantly after disinfection. Where some *Acinetobacter* and *Ralstonia* species caused opportunistic infection in immunocompromised people (Long et al., 2018; Ryan and Adley, 2014). *Burkholderia* had some species responsible for infection in persons with compromised immune status and cystic fibrosis (Coenye and Vandamme, 2003), and *Comamonas*, *Delftia* were common opportunistic pathogens in hospital environment (Bilgin et al., 2015; Farshad et al., 2012). These opportunistic pathogens would also influence maternal health. To this end, the restructure of vaginal microbiome after disinfection might play harmful effects on maternal health. However, for the longitudinal clinical data about mother was not followed in this study, the exact impact of the vaginal microbiome structure changes after delivery on maternal health was not clear. Follow-up investigation will be taken in our next study.

Vaginal microbiota of women could generally be clustered into five community state types (CSTs) (DiGiulio et al., 2015). Four of the CSTs were characterized by high levels of *L. crispatus* (CST I), *L. gasseri* (CST II), *L. iners* (CST III) and *L. jensenii* (CST V). On the other hand, CST IV lacked *Lactobacillus* and was enriched in a combination of various anaerobic bacteria (DiGiulio et al., 2015; Albert et al., 2015). In this study, analysis of the microbiota community using hierarchical cluster analysis showed that the vaginal microbiome clustered into 3 major groups. CST III and CST IV were previously described in North American (Zhou et al., 2007; Ravel et al., 2011) and Europe people (Kiss et al., 2007; MacIntyre et al., 2015), while a new CST (named CST VI) dominated by *L. helveticus* showed in this study. In order to avoid the taxonomic bias caused by bioinformatics method, the sequences were aligned to 16S rRNA database in NCBI using BLAST. And the sequence data was reanalyzed using Mothur software (Schloss et al., 2009). Both of them confirmed that a number of samples dominated by *L. helveticus*. Previous studies demonstrated that community state types seemed to be dependent on ethnic and bio-geographical background (MacIntyre et al., 2015; Ravel et al., 2011), while these studies almost focused on North American and Europe people. The new CST dominated by *L. helveticus* might closely relate to our study samples for they all were recruited from Han ethnicity in this study. In the further study, large cohort studies should be taken into account to confirm this observation. Beyond this, V3-V4 region of 16S rRNA was used to profile the vaginal microbiome community in this study, which showed differences with a

previous study based on *cpn60* gene. To cull out the discrepancy caused by the amplified region, lower taxonomic levels should be investigated with whole genome sequencing in the next study. Additionally, the results showed that most samples during pregnancy belonged to CST III and CST VI, dominated with *Lactobacillus spp.* While after disinfection, the majority of CST III and CST VI during pregnancy switched to CST IV, in concordance with previous studies (Doyle et al., 2018; MacIntyre et al., 2015; DiGiulio et al., 2015). Increased rate of CST IV was also reported in neonatal oral samples. CST IV was observed associated with high Nugent scores and an increased risk of bacterial vaginosis, which were linked to preterm birth and chorioamnionitis (Raverl et al., 2011; MacIntyre et al., 2015; Onderdonk et al., 2016). So much more attentions should be paid on the maternal health after delivery for the povidone iodine used.

At labor onset, the vaginal microbiome was still dominated by family *Lactobacillaceae* and *Bifidobacteriaceae* (Avershina et al., 2017). Moreover, the microbiota of cesarean-born infants partially restored after exposed to maternal vaginal fluids at birth with sterile gauze, and became more similar to the microbiota of vaginal delivery infants (Dominguez-Bello et al., 2016). The previously published results indicated that lactic-acid producing bacteria still dominated at labor or right after delivery. However, significant declines in abundances of *Lactobacillus* species and increase in alpha-diversity right after delivery was observed in this study. That largely was resulted from the disinfecting operation at labor of the study cohorts. In China, as vaginal examination of normal delivery in the textbook "Obstetrics and Gynecology" (Eighth Edition) required, soapy water and povidone iodine was used to clean and disinfect the vulva during delivery in clinics (Xie and Guo, 2013). In the clinical practice, all gynecologists use cotton ball stained or hand impregnated with povidone iodine to sterilize the vulva (Li et al., 2019). And povidone iodine is a broad spectrum antiseptic in the prevention of skin infection, and its efficacy, particularly on resistant microorganisms has been shown (Durani and Leaper, 2008). Its clinical use could restructure the vaginal microbial community at a large extent.

Both the NMDS analysis and community state type results consistently indicated that the neonatal oral microbiome was similar to vaginal microbiome after disinfection, while they both notably different to vaginal microbiome during pregnancy. Significant variations in the communities of the neonatal oral microbiota were observed at different taxonomic levels. Consistent with previous study (Li et al., 2019), the relative proportion of *Lactobacillus* was also significant lower in neonatal oral microbiome. Particularly, characteristic bacteria of CST IV were significantly overrepresented in neonatal oral compared to vaginal microbiome before disinfection. Exposure to vaginal microbiome by the time of delivery, the neonate was introduced as the bacteria colonized at the vaginal birth canal (Haahr et al., 2018; Mueller et al., 2015). The mechanism of passage through the vaginal canal to neonatal might be largely impacted by the disinfecting conduction. It would be helpful to understand the process that establishes the first microbial colonizers of newborns, which was essential for gaining a comprehensive understanding of neonatal development.

The absence of longitudinal data set of maternal and neonatal health revealed some insights that need to be determined in the further study. First, during pregnancy, some vaginal microbiota profiles belonged to CST III, some belonged to CST IV, and some were clustered into CST VI, their longitude impact on

maternal and neonatal health should be followed in the next study. Second, after disinfection, some vaginal microbiome communities were still dominated by *Lactobacillus*, where others with a small proportion of *Lactobacillus*, and similar phenomena was observed in neonatal oral microbiome, but its effects on maternal and neonatal health were not clear. Third, some pregnant women might need not to be disinfected at labor, and some neonates might need to be supplemented with probiotics during growth and developmental process, the clinical operations should be changed according to the result of large cohort study.

Conclusions

In summary, it was observed in this study that perineal disinfection restructured the vaginal microbiome and affected the colonization of neonatal oral microbiome. With the relative abundance of *Lactobacillus* decreased and some opportunistic bacteria increased significantly. These observations will be helpful to uncover the impact of perineal disinfection on maternal and neonatal growth and developmental health.

Methods

Study Subjects

Twenty-seven healthy and reproductive-ages women with gestational age (GA) > 37 weeks were recruited in this study. They were asymptomatic and showed no clinical signs of vaginal disease upon examination by obstetrician with evidence of vaginal discharge and amine or fishy odor. And they also were with an uncomplicated singleton pregnancy, and without medical problems or adverse outcomes during pregnancy, without known fetal anomalies or complications, without antibiotics or other antimicrobial therapy during pregnancy. The study received ethics approval from Bao an Maternal and Child Health Hospital (Shenzhen, China). Written informed consents were obtained from all participants, and parents/guardians of the recruited newborns. Women who were incapable of understanding the informed consent or assent forms, or incarcerated were not recruited in this study. After obtaining written informed consent, demographic and clinical characteristics were collected from all participants via interview and by reviewing medical charts, including gestational age, height, weight, blood pressure, body mass index, ethnicity, age, and so on. Relevant clinical information was also obtained from neonates at birth.

Vaginal samples before delivery were collected at the first examination of hospital admissions of all participants (group BD, before disinfection). And vaginal samples after disinfection were collected before mothers left delivery room with incision stitched (group AD, after disinfection). Where sterile swabs were placed carefully on the vaginal sidewall about halfway between the introitus and the cervix, follow the instructions reported previously (Serrano et al., 2019), pressed firmly into the sidewall to a depth of roughly the diameter of the swab, rolled dorsally-ventrally back and forth four times to coat the swab. Neonatal oral samples were taken as soon as the newborns delivered and before feeding by carefully swabbing the oral mucosa (group NO, neonatal oral), which reported in the previous study (Li et al., 2019).

Three sterile swabs were obtained for every sample by trained nurses to avoid insufficient DNA concentration. All samples were stored at 4°C and transferred to -80°C storage within 30 minutes after collection until DNA extraction.

Microbiome profiling

DNA was extracted from vaginal and oral swabs using QIAamp DNA Mini kit (Qiagen, Germany). The concentrations and purity were measured using the NanoDrop One (Thermo Fisher Scientific, MA, USA). The average DNA concentration was 41.52 ng/μl per sample. And DNA concentration of vaginal samples before disinfection (69.68 ± 85.19 ng/μl) was higher than the samples after disinfection (33.15 ± 49.63 ng/μl), and higher than that of the neonatal samples (21.71 ± 45.50 ng/μl).

V3-V4 hypervariable regions of 16S rRNA genes were amplified with forward primer 338F (5'-ACTCCTACGGGAGGCAGCAG-3'), reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3'). PCR reactions, containing 25 μl 2x Premix Taq (Takara Biotechnology, Dalian Co. Ltd., China), 2 μl of each 10 mM primer, and 3 μl DNA template in a volume of 50 μl, were amplified under the following thermal profile: 94 °C for 5 min, then 30 cycles of 94 °C for 30 seconds, 52 °C for 30 seconds, 72 °C for 30 seconds, followed by 72 °C for 10 min. Amplification products were visualized with 1% agarose gel electrophoresis. And these PCR products were then pooled equimolar and sequenced on an Illumina Hiseq 2500 machine (Illumina, Inc. San Diego, California) with a 2 x 250 flow cell.

Sequencing reads were assigned to each sample, and filtered reads containing ambiguous bases or mismatches in the primer regions using custom perl scripts. Then the 16S rRNA gene sequences generated were analyzed using the bioinformatics software package QIIME2 (version 2019.4) (Bolyen et al., 2019). Paired-end reads were firstly denoised via DADA2 (Callahan et al., 2016) offered by QIIME2 with command "qiime dada2 denoise-paired", to merge paired-end reads, quality filtering and to exclude chimeric and phiX sequences. Further sequences were classified against Greengenes (13_8 revision) database (DeSantis et al., 2006) at the species level using command "qiime feature-classifier classify-sklearn". Meanwhile, an array of alpha- and beta-diversity measures was generated using command "qiime phylogeny align-to-tree-mafft-fasttree" and "qiime diversity core-metrics-phylogenetic" at a sampling depth of 1000. Where alpha diversity was calculated by Shannon's diversity index, observed OTUs (operational taxonomic units) numbers, Pielou's measure of species evenness. And beta diversity was calculated by weighted UniFrac distance.

Moreover, sequences were separately analyzed using Mothur software against Greengenes (13_8 revision) database as described previously (Schloss et al., 2009; Li et al., 2019). The sequences were clustered into OTUs based on the similarity threshold of 0.97.

Statistical analysis

The continuous demographic variables were presented as the mean \pm standard deviation (SD), including alpha (Shannon diversity and observed OTUs number estimated species richness) and beta diversity

(weighted UniFrac matrices). And the categorical characteristics were reported as numbers (percentages, %). All comparisons of this study were performed in R software at 0.05 level of significance using chi-square and *t*-tests for categorical and continuous variables, respectively. Diversity of microbiome profiles were also analyzed based on a nonmetric multidimensional scaling (NMDS) analysis, which was performed on R software using the metaMDS function in the vegan package, and accompanied with permutational multivariate analysis of variance (PERMANOVA, 999 permutations).

To determine statistical differences between the vaginal microbiome throughout pregnancy and after disinfection, the Statistical Analysis of Metagenomic Profiles (STAMP) software package (Parks and Beiko, 2010) was used. *P* values were calculated using Welch's *t*-test with Bonferroni correction. A corrected *p*-value < 0.05 was considered significant.

Assignments of vaginal microbial profiles to community state types (CSTs) were according to community compositions, and the clustering of communities based on vaginal microbial profiles was performed using mcquitty linkage hierarchical clustering analysis with the R package, essentially as previously reported (Ravel et al., 2011; Serrano et al., 2019).

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Bao an Maternal and Child Health Hospital (Shenzhen, No. QKTLL-2017-05-04) and complied with the Helsinki Declaration. Informed consents were obtained from all participants included in this study. All experiments were performed in accordance with the approved guidelines.

Consent for publication

Not applicable.

Availability of data and materials

The 16S rRNA sequence data generated and analysed during the current study is in the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA BioProject ID PRJNA596821).

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

Y. Zhu and H. Li conceived of the presented idea and planned the experiments. C. Nie and S. Chen carried out the experiments. J. Yu, and B. Xiao designed the computational framework and analyzed the data. H. Li, C. Nie, J. Yu and Y. Zhu wrote the manuscript. All authors discussed the results and contributed to the final manuscript.

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Figures

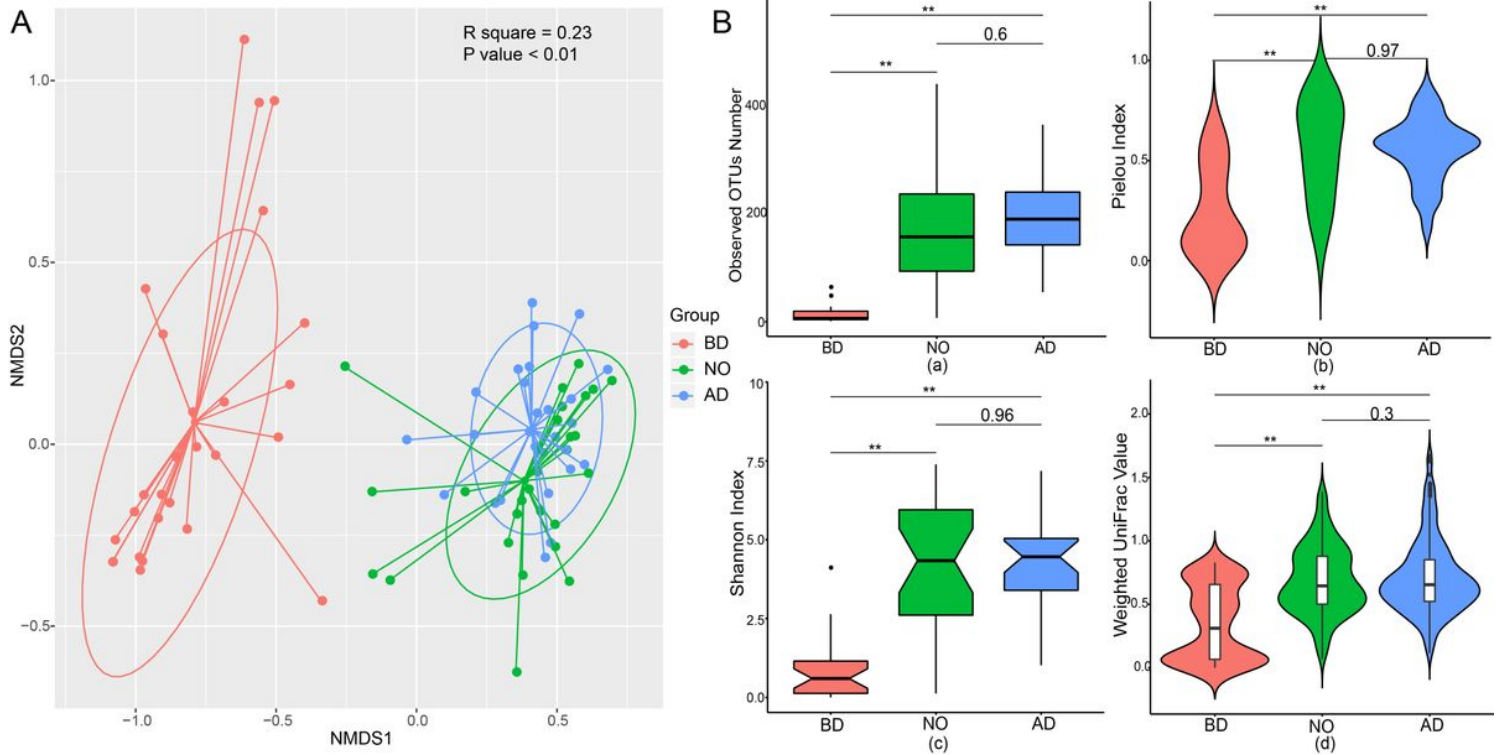


Figure 1

Nonmetric multidimensional scaling analysis and alpha diversity plots in microbiota community structure (group BD: before disinfection; group NO: neonatal oral; group AD: after disinfection). (A) Non-metric multidimensional scaling plot based on Bray-Curtis dissimilarity between samples, each point represented a subject. (B) Comparison of alpha and beta diversity among the three groups, (a) observed OTUs number, (b) Pielou index, (c) Shannon index, (d) weighted UniFrac value.

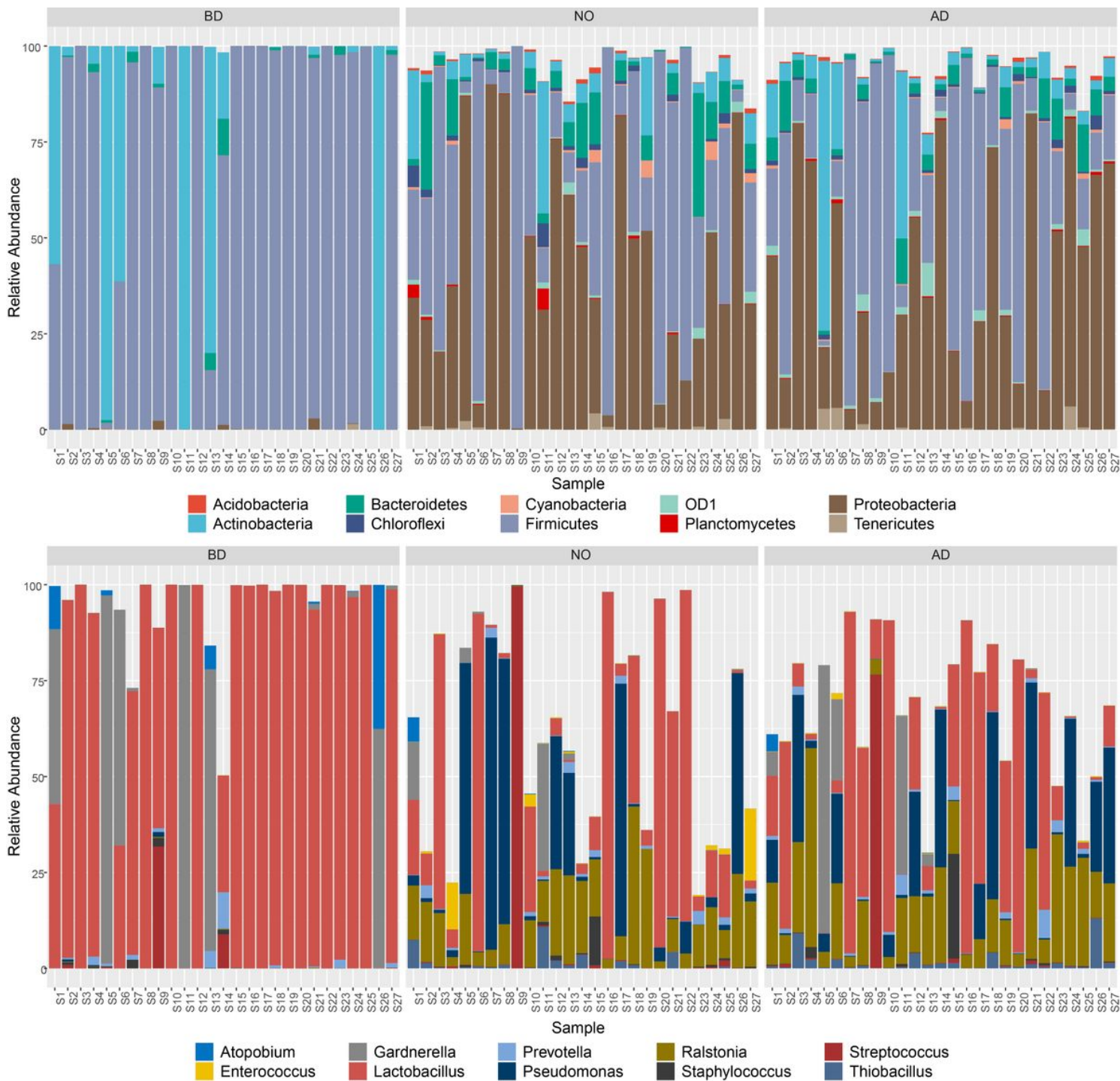


Figure 2

Relative proportions of the dominant bacterium at the phylum and genus levels of each study subject. (A) Relative abundance at the phylum level. (B) Relative abundance at the genus level.

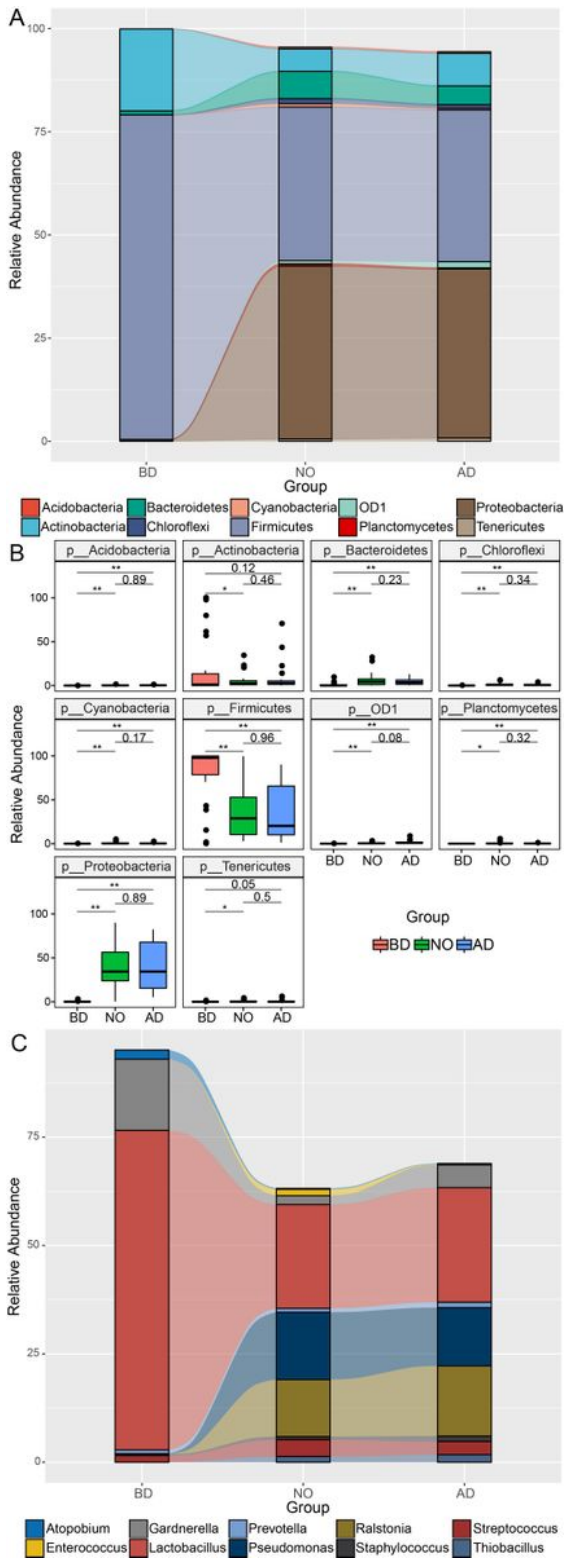


Figure 3

Shifts and differences of the microbiota among the three groups showed at the phylum and genus level. (A) Change pattern at the phylum level. (B) Difference of phylum among the three groups. (C) Change pattern at the genus level.

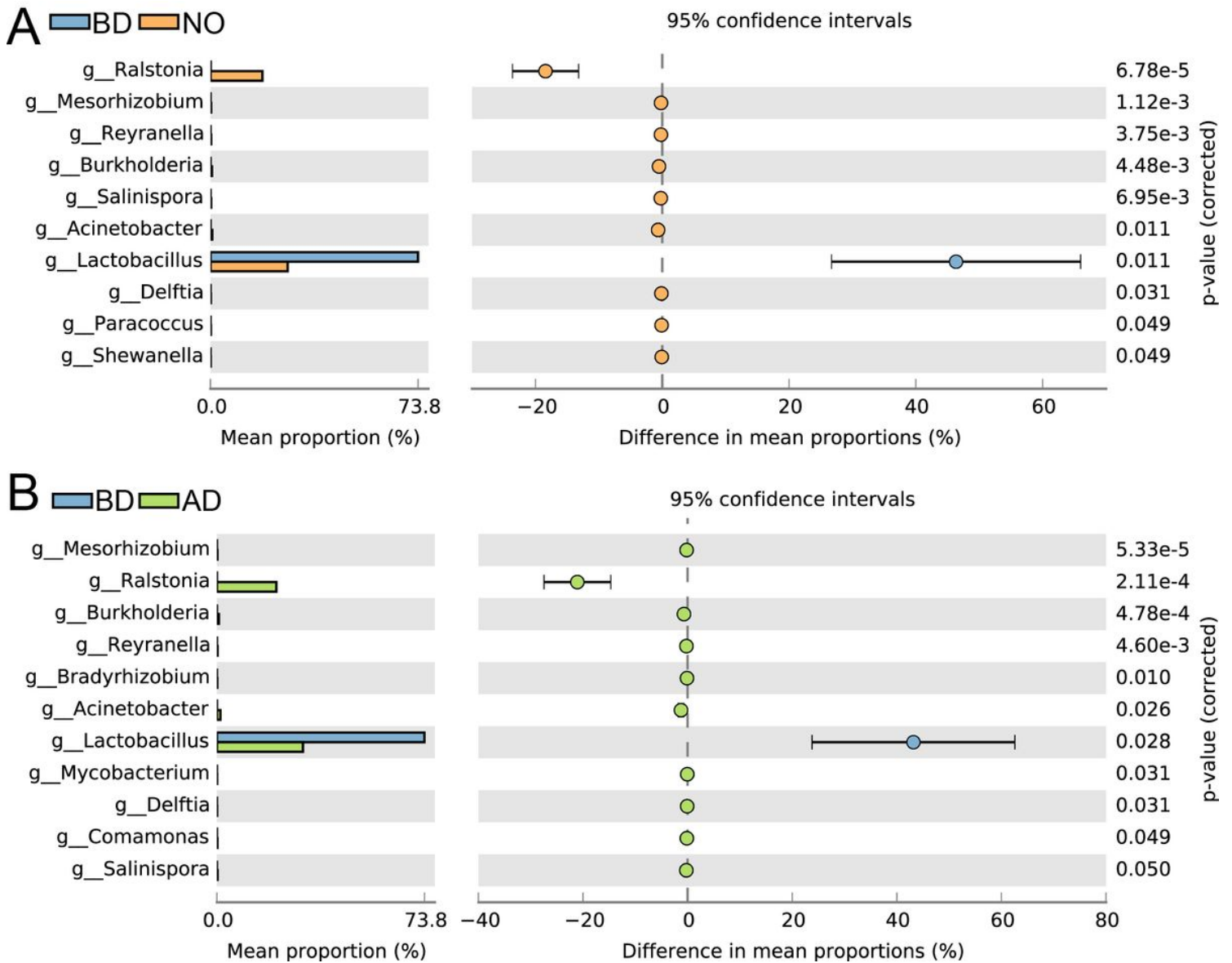


Figure 4

Differential microbial abundance among microbial communities detected in study samples. P-values were obtained by the two-sided Welch's t-test followed by Bonferonni correction. Only statistically significant differences were shown. (A) Different genera between vaginal samples during pregnancy and neonatal oral samples. (B) Different genera between vaginal samples before and after disinfection.

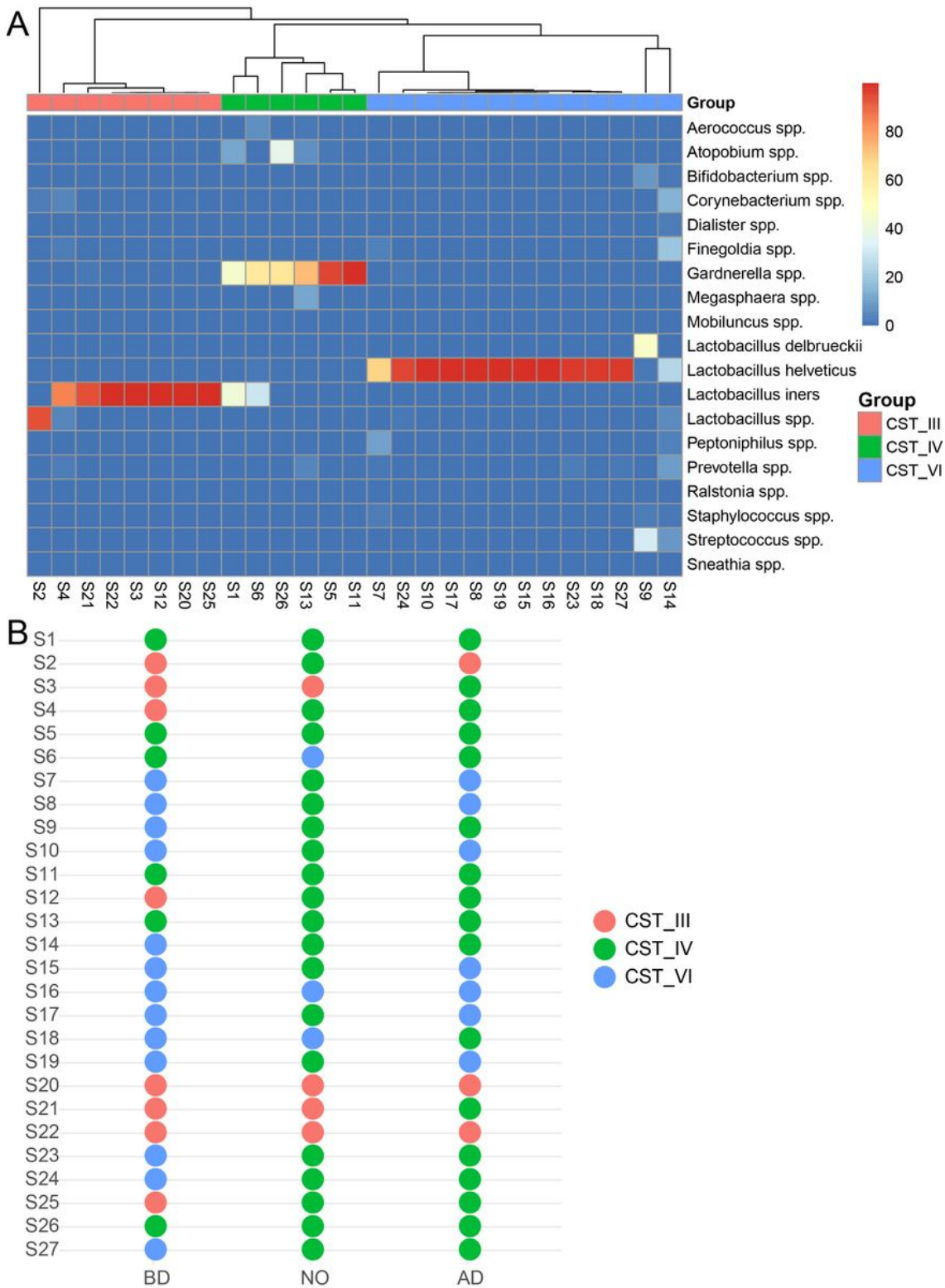


Figure 5

Vaginal community state types presented throughout pregnancy and after disinfection. (A) Hierarchical clustering analysis using mcquitty linkage of microbial species abundance showed that these subjects were clustered into 3 major groups. (B) The switch of community state types among different sampling points.