Length-Heterogeneity Polymerase Chain Reaction as a Diagnostic Tool for Bacterial Vaginosis

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To the Editor: Bacterial vaginosis (BV) is the most prevalent form of vaginitis among women of reproductive age, affecting 8–23% of women globally.[1] Clinically, BV is typically diagnosed using Amsel's criteria and the Nugent scoring system.[2,3] However, these methods are inaccurate in many cases. In this study, we explored the feasibility of using length-heterogeneity-polymerase chain reaction (LH-PCR) for diagnosis of BV.

Sixty-five women with BV were recruited at Peking University First Hospital from September 2012 to July 2013, as described previously.[4] The women were 18–53 years old and had regular menstruation. They were treated with a single 5-day regimen of intravaginal metronidazole gel (37.5 mg daily) and were asked to return after both 6–8 days and 30 days for a test-of-cure examination. Vaginal samples were collected on D0 (the initial visit), D7 (6–8 days after the initial visit), and D30 (the 30-day follow-up visit). Genomic DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). PCR amplification of the 16S rRNA gene was performed using universal primers 27F and 338R, as described by Ritchie *et al*.[5] The 27F primer was labeled with 6-carboxyfluorescein at the 5′ end. PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany). The purified PCR products were analyzed using an ABI PRISM 3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) in GeneScan mode, with the GS500 Liz internal size standards (Applied Biosystems), at SinoGenoMax Co., Ltd. (Beijing, China). LH-PCR electropherograms were examined using GeneMapper software version 3.7 (Thermo Fisher Scientific, US). The minimum noise threshold was set at 50 fluorescent units of peak height after normalization of the sum of total fluorescence in each profile. Data were processed to create binary data (presence, 1; absence, 0) matrices and analyzed with the additive main effects and multiplicative interaction model using T-REX online software.[6] We used primers 27F and 338R to extract target sequences from previously sequenced 454 pyrosequencing library data. The length of each extracted sequence was calculated and matched to a corresponding LH-PCR peak. Assuming that the primers extracted the same sequences from a 454 pyrosequencing library as from a genomic DNA sample, we assigned the taxonomic identifiers of the extracted 454 sequences (previously obtained by searching against the RDP database) to the corresponding LH-PCR peaks.

Sixty-five women were included in our study. Based on their Nugent scores on D30, 48 patients were successfully cured and 17 patients were not cured. The samples were divided into five groups: Group 0, D0 represents the disease condition; Group 1, cured group at D7; Group 2, failed group at D7; Group 3, cured group at D30; and Group 4, failed group at D30.

A total of 195 DNA samples were analyzed by LH-PCR. Peaks 340 to 375 bp long were the most informative, in all the LH-PCR profiles. PCoA based on LH-PCR profiles distinguished between intravaginal microbiota collected from BV and healthy women in Group 3 [Supplementary Figure 1]. Groups 0 and 4 were indistinguishable from each other in the PCoA plot, and both distinct from Group 3, as seen in our previous high-throughput sequencing study.[4] Clinically, the intravaginal microbiota at D7 returned to normal after metronidazole treatment. The PCoA distribution of Group 1 samples (cured at D7) was distinct from that of Group 0 (samples taken at D0) and overlapped with that of Group 3 (cured at D30). The distributions of Groups 1 and 2 were distinct from Group 0 but partially overlapped with Group 3.

The presence or absence of 14 LH-PCR amplicons differed between the five groups, based on the results of a Kruskal-Wallis test. Fragments of 340 bp, 341 bp, 343 bp, 344 bp, 345 bp, 347 bp, 349 bp, 353 bp, 354 bp, 356 bp, 361 bp, 363 bp, 368 bp, and 375 bp were classified, respectively, as coming from genera *Mycobacterium*, *Ureaplasma* or *Mycoplasma*, *Sneathia*, *Corynebacterium*, *Atopobium*, *Gardnerella*, *Mobiluncus*, *Prevotella*, *Actinomyces*, *Staphylococcus*, *Anaerococcus*, *Peptoniphilus*, *Megasphaera,* and *Lactobacillus*. Relative peak areas of these fragments were plotted

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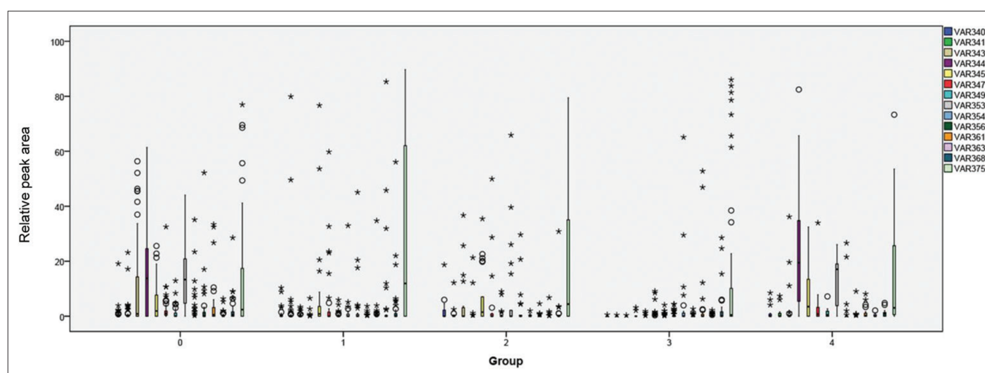

Figure 1: Length-heterogeneity profiles of the relative diversity and abundance of the predominant bacterial fragments at three time points (days 0, 7, and 30). Group 0 is the disease condition at day 0; Group 1 is the cured group at day 7; Group 2 is the failed group at day 7; Group 3 is the cured group at day 30; and Group 4 is the failed group at day 30. LH-PCR profiles show two distinct patterns: the untreated/failed groups and the cured groups. Groups 0 and 4 showed greater relative peak areas for several fragments than Groups 1, 2, and 3, suggesting that bacterial diversity is richer in bacterial vaginosis infection than in normal conditions. LH-PCR: Length-heterogeneity polymerase chain reaction.

for each group of samples to show whether microbial patterns could predict the prognosis [Figure 1]. Groups 0 and 4 showed greater relative peak areas for several fragments, than Groups 1, 2, and 3, suggesting that bacterial diversity is richer in BV infection than under normal conditions. Ten of the 14 fragments (341 bp, 343 bp, 345 bp, 347 bp, 349 bp, 353 bp, 354 bp, 361 bp, 368 bp, and 375 bp) come from genera (i.e., respectively, *Ureaplasma* or *Mycoplasma*, *Sneathia*, *Atopobium*, *Gardnerella*, *Mobiluncus*, *Prevotella*, *Actinomyces*, *Anaerococcus,* and *Megasphaera*) that include pathogenic bacteria that cause the clinical symptoms of BV. *Lactobacillus* (the 375 bp fragment) is the predominant genus of vaginal microbiota in healthy women.[7] Groups 0 and 4 (from women with clinical symptoms of BV) show relative increases of pathogenic bacteria and decreases of *Lactobacillus*. This suggests that the LH-PCR profiles here are sufficient to diagnose BV.

In conclusion, LH-PCR can provide useful information on patients' vaginal microbiome, allowing physicians to adjust therapeutic regimes and improve the rate of successful treatment of BV. The LH-PCR peaks at 341 bp, 343 bp, 345 bp, 347 bp, 349 bp, 353 bp, 354 bp, 361 bp, 368 bp, and 375 bp are potential indicators of BV and may be used in future as diagnostic and prognostic markers.

Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.

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Conflicts of interest

There are no conflicts of interest.

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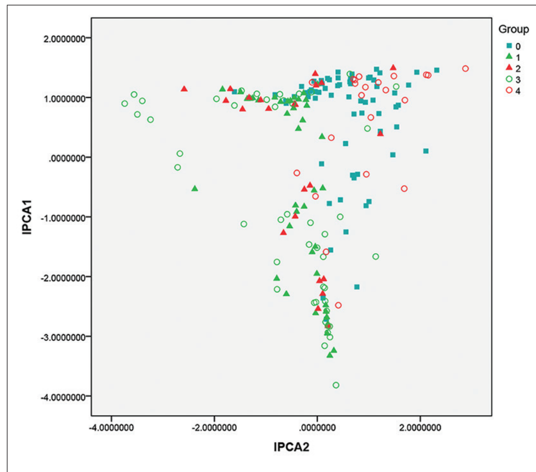

Supplementary Figure 1: Principal component analysis of the Bray-Curtis dissimilarity among all samples at day 0, day 7, and day 30. Group 0 is the disease condition at day 0; Group 1 is the cured group at day 7; Group 2 is the failed group at day 7; Group 3 is the cured group at day 30; and Group 4 is the failed group at day 30. The vaginal microbial community showed two discrete patterns: the untreated/failed groups and the cured groups. IPCA: Independent principal component analysis.