Vaginal microbiome transplantation in women with intractable bacterial vaginosis

Ahinoam Lev-Sagie1,6*, Debra Goldman-Wohl1,6, Yotam Cohen2,6, Mally Dori-Bachash2, Avner Leshem2,3, Uria Mor2, Jacob Strahilevitz4, Allon E. Moses4, Hagit Shapiro2, Simcha Yagel1 and Eran Elinav2,5*

We report the results of a first exploratory study testing the use of vaginal microbiome transplantation (VMT) from healthy donors as a therapeutic alternative for patients suffering from symptomatic, intractable and recurrent bacterial vaginosis (ClinicalTrials.gov NCT02236429). In our case series, five patients were treated, and in four of them VMT was associated with full long-term remission until the end of follow-up at 5–21 months after VMT, defined as marked improvement of symptoms, Amsel criteria, microscopic vaginal fluid appearance and reconstitution of a Lactobacillus-dominated vaginal microbiome. One patient presented with incomplete remission in clinical and laboratory features. No adverse effects were observed in any of the five women. Notably, remission in three patients necessitated repeated VMT, including a donor change in one patient, to elicit a long-standing clinical response. The therapeutic efficacy of VMT in women with intractable and recurrent bacterial vaginosis should be further determined in randomized, placebo-controlled clinical trials.

Bacterial vaginosis (BV) is a form of vaginal microbial community alteration in which the microbiome normally dominated by Lactobacillus species switches to one characterized by the emergence of anaerobes1–4. BV is prevalent in women of reproductive age, affecting from one-fourth to one-third of women1. It ranges from an asymptomatic finding in most cases to a clinically symptomatic entity characterized by an abnormal, often malodorous vaginal discharge in 16% of women diagnosed with BV, summing up to a prevalence of 4.4% for symptomatic BV in women aged 14–49 years5. BV prevalence increases with age in fertility treatments7–10) and susceptibility to sexually transmitted infections11. At the clinically severe end of the BV spectrum, treatment with antibiotics (either systemic or vaginal) is associated with a 30% relapse rate within 3 months of initial treatment and a relapse rate of up to 50–70% within 1 year12. Therapeutic options are very limited in the subpopulation of women who experience persistent or recurrent BV despite multiple antibiotic treatment attempts13–15. Maintenance antimicrobial treatment16,17 is often the treatment suggested in these cases, but it can predispose to vaginal candidiasis18 and resistant infections19,20. Importantly, probiotic treatment of symptomatic patients with oral or vaginal administration of bacterial Lactobacillus strains has produced mixed results11,22, suggesting that the microbiome as a whole, rather than a single bacterial species, may be necessary for an effective cure at the clinically severe end of the BV spectrum. Fecal microbiome transplantation (FMT), in which feces from healthy donors are introduced into recipients’ intestines to replace their disease-associated microbiome, has recently been successfully used in treating severe and recurrent Clostridium difficile infection23. Although gastrointestinal microbiome interventions may offer a different ecological scenario than those related to a dysbiotic vaginal microbiome, we hypothesized that a similar use of VMT might be beneficial in treating the most severe cases of recurrent and antibiotics-nonresponsive BV.

Five patients were recruited (aged 27–47 years and referred to as patients A–E; Extended Data Fig. 1). All suffered from intractable BV, defined as four or more symptomatic episodes of BV during the previous year11, relapsing after repeated, prolonged and diverse antibiotic attempts requiring continuous maintenance antibiotic treatment to remain symptom free. All patients reported a substantial negative impact of BV symptoms on their quality of life, including devastating consequences to their relationships, sexual intimacy and self-esteem. All five patients were otherwise healthy. Patient screening, exclusion criteria and the consent process are described in the Methods. The three donors were premenopausal, healthy volunteers, aged 35–48 years (donors 1–3; Extended Data Fig. 1), who did not report having BV in the last 5 years or any history of recurrent BV. Donor selection and screening are detailed in the Methods. Repeated communication between the lead physician and the donors ensured that the behavioral requirements (e.g., abstinence from sexual activity for 1 week before donation in the sexually active donor) were strictly followed.

Before transplantation, all patients were treated with an intravaginal antibiotic regimen16 that previously resulted in a longer symptom-free period, which consisted of 5 g clindamycin cream (2%) for 7 d (recipients B, C and E) or 5 g metronidazole gel (0.75%) for 5 d (recipients A and D). VMT was performed 1 week after completion of antibiotic treatment16. During the procedure, vaginal fluid for transplantation was collected from the donors starting from the seventh day of the menstrual cycle (Methods) and taken from the upper half of the vagina and cervical fornices, while avoiding the cervix. The collected discharge was evaluated by pH and microscopy, diluted with 1 ml of sterile saline and transferred to the recipient’s posterior fornix, without the use of a speculum (Fig. 1a). VMT was completed within no more than 60 min of sample collection and was performed at any stage during the recipient’s menstrual cycle, except during menstruation. After the first VMT, repeat VMTs were performed in cases of symptom recurrence or with reappearance of one or more positive Amsel criteria during

1Department of Obstetrics and Gynecology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel. 2Immunology Department, Weizmann Institute of Science, Rehovot, Israel. 3Department of Surgery, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel. 4Department of Clinical Microbiology and Infectious Diseases, Hadassah-Hebrew University Medical Center, Jerusalem, Israel. 5Division of Cancer–Microbiome Research, DKFZ, Heidelberg, Germany. 6These authors contributed equally: Ahinoam Lev-Sagie, Debra Goldman-Wohl, Yotam Cohen. *e-mail: levsgie@netvision.net.il; eran.elinav@weizmann.ac.il
follow-up examinations (Extended Data Fig. 2). At each follow-up appointment (weekly for the first month and monthly to bimonthly thereafter), patients were interviewed and underwent a vaginal examination (including quantification of discharge, pH measurement, whiff test and microscopy). Remission of BV was defined at each appointment as disappearance of symptoms, normalization of all Amsel criteria and appearance of a normal Lactobacillus-dominated microbiome by light microscopy.

Four patients (patients A–D) had long-lasting improvements in their Amsel scores (Fig. 1b), microscopic vaginal fluid appearance (Fig. 1c,d), whiff test, discharge and vaginal fluid pH (Fig. 1d) after 1–3 VMT sessions (Extended Data Figs. 2 and 3). The fifth patient (patient E) had partial remission, manifesting as a subjective reduction of symptoms, negative whiff test, cumulative Amsel scoring of 0–1 and an intermediate microscopic vaginal fluid appearance. Patients A and B underwent a single VMT each from donors 1
Fig. 2 | Metagenomic microbiome assessment of the vaginal microbiome following VMT. a, Metagenomic PCA performed on the donors’ and recipients’ baselines. b, Metagenomic BC distance from baseline, correlated with the Amsel criteria scores. c, Metagenomic PCA performed on samples from the donors and the baseline and last collected samples from each participant. Arrows depict the conversion of VMT recipients from baseline samples to samples after successful VMT and are color by the respective donor’s color. Dots unconnected by the arrows represent the microbiome configurations of donors. Inset, bar plot displaying the Euclidean distances of pre-VMT (full red bar) and post-VMT (empty red bar) samples to samples from each donor; **P = 0.0012, paired two-tailed t-test. Error bars are s.d. from the mean; n = 5 recipients.

d, Metagenomic BC distance from the respective donor, correlated with the Amsel criteria scores.

e, Metagenomic assessment of the change in the microbiome composition at the genus level after VMT. Arrows indicate a VMT, with color corresponding to the donor.

f, Metagenomic bar plot denoting the species contributing the most to the first PC. Arrows indicate a VMT, with colors corresponding to the donor; triangles indicate an antibiotic treatment. Note that patient D received an antibiotic treatment also prior to her second VMT procedure, but the corresponding triangular mark was not included owing to space limitations.

g,h, Metagenomic KEGG gene annotated PCA (n = 50 total recipient samples), colored by Amsel criteria (g) and by relative abundance of the Bifidobacterium genus and the Lactobacillus genus (h). A–E are the individual VMT recipients.
and 2, respectively. Both reported immediate clinical improvement, with the disappearance of odor within 1 week of transplantation, and a gradual decrease in discharge, resulting in no symptoms 1 month after VMT. In both patients, normalization of all Amsel criteria as well as normal Lactobacillus-dominated microscopic appearance was documented 1 week after transplant and persisted on follow-up examinations (11.5 months in patient A and 5.5 months in patient B). Patient C received the microbiome of donor 1. She reported an improvement of symptoms after VMT and became BV negative according to Amsel criteria, but her microscopic findings were consistent with persistence of BV. She therefore underwent a repeat VMT from the same donor (donor 1) without preceding antibiotic treatment. For 4 months, the patient reported an improvement of symptoms and BV status was negative according to Amsel criteria. However, 4.5 months after the first VMT, she experienced a recurrence of odor, positive Amsel criteria and a BV microbiome appearance on microscopy, all consistent with recurring BV. She, therefore, underwent a third VMT, this time using a sample from a different donor (donor 3) after vaginal antibiotic treatment. After this VMT, she reported complete resolution of symptoms; Amsel criteria were normalized; and microscopy showed a normal Lactobacillus-dominated appearance for 11 months of follow-up. Patients D and E likewise had a fluctuant course. After a first VMT from donor 1, patient D experienced a recurrence of symptoms, positive Amsel criteria and microscopic findings consistent with BV. She underwent a second VMT from the same donor (donor 1), after which she reported clinical improvement of symptoms and was BV negative according to Amsel criteria. However, she exhibited an intermediate vaginal microbial appearance on microscopy and therefore underwent a third VMT from the same donor (donor 1), after which she reported clinical improvement with the disappearance of odor and improvement of discharge, associated with negative Amsel criteria and a normal Lactobacillus-dominated appearance on microscopy. On evaluation 21 months after the third transplant, the patient reported no recurrences, had negative Amsel criteria and exhibited normal microscopy. After VMT from donor 2, patient E reported a partial symptomatic improvement, associated with negative Amsel criteria and a normal Lactobacillus-dominated appearance on microscopy, for 4 weeks of follow-up. She then took systemic antibiotics for pharyngitis and soon after reported a recurrence of odor, accompanied by positive Amsel criteria and BV-characteristic microscopic appearance. She underwent a repeat VMT from the same donor (donor 2), resulting in the normalization of all Amsel criteria and improvement of her microscopic vaginal appearance to an intermediate microbiome configuration, coupled with partial symptomatic improvement, for 6.5 months of follow-up.

To characterize the genus-level changes associated with VMT, we sequenced all donors’ and recipients’ vaginal microbiome samples using 16S ribosomal DNA (rDNA) sequencing (Methods). Interestingly, healthy microbiomes clustered differently from the microbiomes of BV-diagnosed patients (Extended Data Fig. 4a) after applying principal-coordinates analysis (PCoA) with UniFrac distances. Using Bray–Curtis (BC) dissimilarity, we followed recipients’ microbiomes before and after VMT and observed a rapid change in the composition of all microbiomes after VMT, correlated with recovery in all of the Amsel criteria (Extended Data Fig. 4b).

To study the effect of VMT on vaginal microbiome species-level composition and metagenomic function, all donors’ and recipients’ samples underwent shotgun metagenomic sequencing. As expected, the microbiomes of donors and recipients were found in two distinct clusters using PCA (Fig. 2a). The effect of VMT on global microbiome composition over the follow-up period was assessed by BC dissimilarity on the species level, as compared to patients’ baseline BV configuration. Four of five VMT recipients exhibited a drastically changed microbiome composition already at the first month after VMT, which correlated with a notable recovery of their Amsel criteria (Fig. 2b) as well as with every discrete clinical criterion (Extended Data Fig. 5a). Patient C experienced the same trend, only after the third and successful VMT (Fig. 2b). The post-VMT microbiome of one patient (patient E) relapsed to her baseline microbiome BV composition after failure of the first VMT (Fig. 2b). However, the repeat successful VMTs in this patient induced a distinctively different configuration, mirrored by a marked species-level BC distance from baseline, similarly to the post-VMT trend observed with the other four patients after a successful VMT (Fig. 2b).

In four of the five VMT recipients, the vaginal microbiome configuration remained distinct from the baseline BV configuration over a period of 5–21 months after a successful VMT (Fig. 2c). Notably, the post-VMT vaginal microbiome composition became significantly more similar to that of the collective donor vaginal microbiome configuration, as compared to the corresponding similarity between the pre-VMT and donor configurations (Euclidean distances, \( P = 0.0012 \); Fig. 2c and Extended Data Fig. 5b). This similarity was present after a successful VMT and through the follow-up period (Fig. 2d). Notably, the current preliminary case series is underpowered to statistically test a person-specific donor contribution to a recipient’s specific clinical features or microbiome configuration after VMT. Larger future cohorts may enable better resolution or, alternatively, demonstrate that distinctions can be made between only a ‘healthy’ versus a BV microbiome configuration.

This post-VMT compositional change was mostly dominated by an expansion in members of the Lactobacillus genus, combined with a decrease in members of the Bifidobacterium genus, closely related to the Gardnerella genus. (The reference that was used for taxonomic annotations classifies Gardnerella genus and Gardnerella vaginalis specie as Bifidobacterium and Bifidobacterium vaginalis, accordingly; Fig. 2c and Extended Data Fig. 5c.) Other genera, including Fannyhessea and Prevotella, were reduced upon successful VMT-induced remission of BV (Extended Data Fig. 5d). We further used species-level PCA in reducing the complex microbiome dimensionality (Extended Data Fig. 5e). Indeed, the PCA clustered the samples into a BV cluster, containing mostly samples with one or more Amsel-diagnosed BV features, and a healthy cluster, with mostly no diagnosed clinical features (Extended Data Fig. 5e). We applied a \( k \)-means algorithm (\( k = 2 \)), using the coordinates of the first and second PCs, to define the two clusters that were visually identified (Extended Data Fig. 5e). We then calculated the purity score for each Amsel criteria score division. Considering the purity scores, we classified our samples into two groups according to their Amsel score (i.e., the first group comprised all samples having Amsel criteria = 0 and the second group comprised all samples with Amsel criteria > 0). To see whether the groups were indeed different, we conducted a permutational analysis of variance test using the BC dissimilarity matrix (\( P < 0.05 \)). The difference between the two clusters could be explained by the relative levels of Bifidobacterium and Lactobacillus genera in each sample (Extended Data Fig. 5f). The most dominant features that contributed to the change in the first PC, which differentiated between the clusters, consisted mostly of Lactobacillus crispatus specie in the healthy cluster and Bifidobacterium vaginal in the BV cluster (Fig. 2f), demonstrating the 15 overall most PC1-influential taxa, as represented in each vaginal microbiome configuration. Interestingly, recipient E, who was the only partial clinical responder, featured a different dominant post-VMT lactobacillus strain (Lactobacillus gasseri), that was not one of the top 15 PC1-influential strains in the other four VMT recipients.

Functional microbiome changes after VMT, as assessed using the Kyoto Encyclopedia of Genes and Genomes (KEGG), revealed two distinct functional clusters that separated the BV microbiome from the healthy one (Fig. 2g), corresponding to the taxonomic differences noted between these conditions (Fig. 2h). Upon recipient follow-up, functional BC distances from the baseline microbiome correlated with a decrease in Amsel criteria (Extended Data Fig. 6a).
Changes in the functional potential of the microbiome shifted at the time of VMT (Extended Data Fig. 6b) and remained unaltered over the follow-up period (Extended Data Fig. 6c). Similarly to the taxonomic analysis, the current preliminary case series was underpowered to statistically test person-specific donor and recipient similarities in functional microbiome characteristics, and these could only cluster as BV, healthy donor and post-VMT groups. An alternative functional analysis using Gene Ontology (GO) terms demonstrated that the post-VMT BC distance from the baseline likewise correlated with the decrease in Amsel criteria in three patients (patients A–C; Extended Data Fig. 7a), whereas in two patients (patients D and E) the distances remained unchanged. Nonetheless, a clear PCA cluster could be observed (Extended Data Fig. 7b) between the BV and healthy microbiome configurations, and these could be clearly linked to the different taxonomic composition of the BV and healthy clinical states (Extended Data Fig. 7c). The most dominant GO terms remained stable throughout VMT and the follow-up period, potentially explaining the low BC changes we observed using this analytical method (Extended Data Fig. 7d), whereas the second PC exhibited a substantial change in GO term signatures over the course of the follow-up period (Extended Data Fig. 7e). Collectively, we report the feasibility of using VMT as a long-term treatment for recurrent, antibiotics-nonresponsive and intractable BV. Although we did not observe adverse effects associated with VMT in this study, we cannot completely exclude potential risks associated with any microbiome transfer procedure. The transplant of antimicrobial-resistant microbes has been reported in immunocompromised patients undergoing FMT[19], and the long-term consequences of VMT remain unknown. Gynecologic and obstetric complications, however unlikely, are also possible. Additionally, the risks of unintended pregnancy due to the transfer of sperm or the transfer of undetected pathogens with the vaginal fluid are not negligible. In our study, donor selection followed stringent criteria to minimize the risks, yet these criteria may not be applicable in other settings. The use of contraception by recipients as a mandatory criterion in future studies, associated with the development of a ‘vaginal fluid bank’ in which samples from suitable donors will be kept for a period allowing for repeated screening, and verification of the absence of HIV sero-conversion before VMT, may be recommended. Finally, BV may be asymptomatic or readily treatable with antibiotics in most women, and therefore VMT should be considered only in cases of multiple treatment failures and substantial disruption of the patient’s quality of life due to chronic and intractable symptoms. Although all patients enrolled in this exploratory study benefited from the procedure, the efficacy of VMT as a treatment in intractable BV needs to be determined in randomized, placebo-controlled trials.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41591-019-0600-6.

Received: 17 June 2019; Accepted: 28 August 2019; Published online: 7 October 2019

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Acknowledgements
We thank the members of the Hadassah-Hebrew University Medical Center Department of Obstetrics and Gynecology; the Elbar lab at the Weizmann Institute of Science and members of the DFKE Cancer–Microbiome division for insightful discussions, and thank S.M. Cohen for expert editing. All cartoons were created, under a license, using BioRender software. The study was supported by a grant from the Joint Research Fund of the Hebrew University of Jerusalem and Hadassah Medical Center. H.S. is the incumbent of the V.R. Schwartz Research Fellow Chair. E.E. is supported by Yael and Rami Unger, the Leona M. and Harry B. Helmsley Charitable Trust, the Adelis Foundation, the Pearl Wilensky Merlo Scientific Progress Research Fund, the Lowenberg and Sandra Post Family Foundation, the Daniel Morris Trust, the Park Avenue Charitable Fund, the Hanna and Dr. Ludwik Wallach Cancer Research Fund, the Howard and Nancy Marks Charitable Fund, Aliza Moussaieff, the estate of Malka Moskowitz, the estate of Myron H. Ackerman, the estate of Bernard Bishin for the WIS-Clalit Program, Donald and Susan Schwartz, and grants funded by the European Research Council, the Israel Science Foundation, the Israel Ministry of Science and Technology, the Israel Ministry of Health, the Helmholtz Foundation, the Els Knore Freisnous Foundation, the Garvan Institute, the European Council’s and Colitis Organization, the Deutsch-Israelische Projektkooperation and the Wellcome Trust. E.E. is the incumbent of the Sir Marc and Lady Tamara Feldmann Professorial Chair, a senior fellow at the Canadian Institute of Advanced Research and an international scholar at the Bill & Melinda Gates Foundation and the Howard Hughes Medical Institute.

Author contributions
A.L.-S., D.G.-W. and E.E. conceived the study. A.L.-S. recruited and supervised the participants and performed all clinical procedures. Y.C. performed all computational analyses. M.D.-B. performed sample preparation, processing and sequencing. A.L. and U.M. assisted with the computational analysis. I.S. and A.E.M. provided microbiology insights and support. S.Y. contributed to study conception and design. H.S. supervised all lab work. A.L.-S., D.G.-W., Y.C. and E.E. interpreted the experiments and wrote the manuscript.

Competing interests
E.E. is a paid consultant at DayTwo and BiomX. None of this work is related to, funded or endorsed by, shared or discussed with or licensed to any commercial entity. None of the other authors has any competing interest.

Additional information
Extended data is available for this paper at https://doi.org/10.1038/s41591-019-0600-6. Supplementary information is available for this paper at https://doi.org/10.1038/s41591-019-0606-0.

Correspondence and requests for materials should be addressed to A.L.-S. or E.E.

Peer review information Joao Monteiro was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.

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Methods

Human study cohort. This study was conducted at the Hadassah Medical Center in Jerusalem, Israel. All participants provided written informed consent. The research protocol was approved by the ethics committee at the Hadassah Medical Center (HMO-0667-13) and the Weizmann Institute of Science (603-1, 680-1) with ClinicalTrials.gov ID NCT02236429.

Bacterial vaginosis. BV was diagnosed by the Amsel criteria, requiring three of the four following symptoms or signs: homogenous, thin, white discharge; pH >4.5; a fishy odor of vaginal discharge before or after addition of 10% potassium hydroxide (i.e., the whiff test); and >20% vaginal epithelial cells studded with adherent coccobacilli (clue cells) on microscopic examination16-17. On microscopy, bacterial microbiome appearance was defined as normal (lactobacilli dominated), BV (coccid-bacillary dominated) or intermediate, as quantified by using the Hay–Ionon criteria18.

Recipients. Inclusion criteria. Recipients were included if they were aged 18–50 years with recurrent BV, defined as ≥2 symptomatic episodes of BV during the previous year, and required maintenance antibiotic treatment (twice weekly) to remain symptom free or if they experienced recurrence of BV in ≤2 months after treatment, with a documented history of recurrent BV. Exclusion criteria included pregnancy or planned pregnancy in the upcoming year or infection with hepatitis B, hepatitis C, HIV or syphilis. Study candidates underwent screening for cervicovaginal infection with Chlamydia trachomatis, Neisseria gonorrhoea, Mycoplasma genitalium or Trichomonas vaginalis, using a PCR assay. Any patient who presented a positive result for any of these infections received the standard recommended treatments documented with a documented assay result deemed mandatory for inclusion in the study. All patients underwent a cervical cytology screening test (Pap test) and PCR-based screening for human papilloma virus (HPV). In the case of an abnormal cytology test or a positive HPV test, patients were referred for colposcopy. In addition, vaginal cultures for yeast and bacteria (streptococci groups A, B, C and G), urine cultures, urinalysis, and serology analysis for HIV, hepatitis A, B and C, Treponema pallidum, herpes viruses and cytomegalovirus (CMV) were performed in all cases.

Donors. Inclusion criteria. Donors were included if they were aged 18–50 years and prernopausal. Exclusion criteria included history of BV in the last 5 years or any history of recurrent BV; absence of a cervico-vaginal sexually transmitted infection (C. trachomatis, N. gonorrhoea, M. genitalium or T. vaginalis); a positive HPV test; vaginal presence of streptococci groups A, C or G; history of recurrent candida vulvovaginitis; history of recurrent urinary tract infections; use of any antibiotics in the month preceding vaginal fluid collection; use of systemic medication; use of probiotics (orally or vaginally); consumption of herbal or homeopathic remedies; acute illness; history of cancer; history of anogenital dysplasia; history of anogenital HPV; history of anogenital herpes; vulvar or vaginal disease (acute or chronic); pregnancy; abnormal urinalysis or infection; or seropositivity to HIV, hepatitis B, hepatitis C, herpes or syphilis.

Donors’ long-term medical and sexual history was familiar to the lead clinician. Two were non-sexually active for 8 years or more; one was engaged in a 25-year monogamous relationship. Donors had a negative history of vaginal symptoms and underwent an examination to verify the absence of BV and other vaginitis, using a thorough history, gynecologic exam, vaginal fluid microscopic analysis, and PCR assays. All donors answered a questionnaire that is used at our blood bank (see below) to screen for possible risk factors for acquiring an infection that we potentially missed using PCR assays, cultures and serology. Donors were screened for potential important infections, including group B Streptococcus (GBS) and CMV, so as not to expose women who were GBS or CMV negative to potential future complications of pregnancy and delivery caused by GBS or CMV. Before vaginal fluid collection, it was explicitly verified with the donors that they did not have sexual intercourse in the week preceding the intervention.

Donor history questionnaire.

1. Are you generally healthy?
2. Do you suffer from any health problem? If you do, please specify.
3. Have you taken any medication during the past month? If so, please indicate each one.
4. Have you traveled abroad during the past 12 months? If so, please specify where and when.
5. Did you get a tattoo/ear or body piercing/accidental needlestick during the past 6 months?
6. Were you bitten by an animal in the last few months?
7. Did you suffer from hepatitis?
8. Did you live with a person who has hepatitis in the past 6 months?
9. Did you suffer from tuberculosis during the past two years?
10. Did you receive antibiotic treatment for a sexually transmitted infection (e.g., Chlamydia, gonorrhea, trichomonas) in the past year?
11. Did you live in a malaria-infected area or suffer from malaria?
12. Did you undergo any surgery? If yes, please specify which? When?
for a Tecan automated platform. For shotgun sequencing, Illumina libraries were prepared using a Nextera DNA Sample Prep kit (Illumina, FC-121-1031), according to the manufacturer’s protocol, and sequenced on the Illumina NextSeq platform with a read length of 80 bp. We then used Illumina’s bcl2fastq script to make the fastq files. Host reads were removed using KneadData with default parameters and the hg19 reference. Taxonomic assignment was performed with Kraken230, using this recommended prebuilt index database31. From the resulting table, we extracted the counts for both genus and species levels separately. We then subsampled the count tables so each sample had 100,000 reads in total. Three samples that did not reach 100,000 reads were excluded from the taxonomic analysis. On top, we filtered out all bacteria whose total abundance after rarefaction was 10^−4. In all clinical–microbiome distance correlations, only time points that included both clinical and microbiome measurements were used. Functional annotation was performed using the Humann232 pipeline, with the same input reads we used for the taxonomic analysis. The output was normalized to counts per million, combined and annotated to KEGG and GO terms using Humann’s built-in scripts (humann2_regroup_table with UniRef90ko and UniRef90go, respectively, together with humann2_rename_table). These datasets were later filtered to remove all genes and terms for which the abundance was <10^−3.

**Statistical analysis.** Paired t-tests were used to compare baseline and post-VMT distances to the centroid of the three donors. The distances were computed on all PCs. PCA was performed using the scikit-learn package in Python, after performing a log transformation in all cases. Figure 2f shows the 15 leading loadings (in absolute value) for the first and second PCs. k-means clustering was also performed using scikit-learn, for 100 iterations with random_state = iteration’s index.

Permutation tests were performed in the following manner. Under the null hypothesis that the microbiome profiles of samples originating from both groups have the same distribution, we let \( \mu_i \) denote the mean of pairwise dissimilarity between the original groups. For \( i = 1 \) to \( 10^5 \), we shuffled the labels of the groups stratifying the permutations so that labels were switched only within the same subject’s samples. We then denoted the mean of pairwise dissimilarity of the relabeled groups by \( \mu_i \). The probability of the null hypothesis is \( p = \frac{\# \text{ of iterations} \geq \mu_0}{10^5} \).

For example, we counted each iteration that had a higher mean of pairwise dissimilarity, and the \( P \) value is the sum of these divided by the number of iterations.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**
The sequencing data has been deposited at the European Nucleotide Archive with accession number PRJEB34085. All other requests for raw and analyzed data, generated as part of a clinical trial, will be promptly reviewed by the Hadassah-Hebrew University Medical Center and the Weizmann Institute of Science to verify whether the request is subject to intellectual property confidential obligations or affects patient confidentiality.

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### Clinical background of donors and recipients.

|                         | Recipients |                      | Donors |                      |                      |
|-------------------------|------------|----------------------|--------|----------------------|----------------------|
|                         | A          | B                    | C      | D                    | E                    | 1         | 2         | 3         |
| Age                     | 34         | 27                   | 47     | 43                   | 29                   | 36 ± 8.71 | 35        | 35        | 39.33 ± 7.5 |
| Duration of symptoms    | 48         | 30                   | 8      | 6                    | 16                   | 21.6 ± 17.51 | 0         | 0         |            |
| Relationship            | yes        | no                   | no     | yes                  | yes                  | no        | no        | yes       |
| Parity, Gravidity       | 3.3        | 0.0                  | 2.2    | 6.3                  | 0.0                  | 0.0       | 0.0       | 4.3       |
| Contraception method    | Mirena IUD | Condom               | Mirena IUD | None               | Condom               | None      | Oral      | Mirena contraception | IUD                  |
| Coitus (years)          | 12         | 10                   | 26     | 23                   | 8                    | 15.8 ± 8.13 | 0         | 8         | 29        | 12.33 ± 14.97 |
| Coitus frequency (per week) | 2         | 0                    | 3      | 2                    | 0                    | 1.4 ± 1.34 | 0         | 0         | 2         | 0.67 ± 1.15 |
| Oral sex                | no         | no                   | yes    | no                   | no                   | no        | no        | yes       |
| Regular menstruation   | no         | yes                  | no     | yes                  | yes                  | yes       | yes       | no        |
| Tampon use              | no         | no                   | no     | yes                  | yes                  | yes       | Cup       | Cup       |
| Menstrual duration (days) | 3         | 5                    | 0      | 4                    | 3                    | 3 ± 1.87 | 5         | 4         | 6         | 5 ± 1     |
| Smoking                 | no         | no                   | no     | yes                  | yes                  | no        | no        | no        |
| Douching                | no         | no                   | no     | no                   | no                   | no        | no        | no        |
| HPV                     | Negative   | 55,56                | 71,70,4 | 82                  | 18                   | 54        | Negative | Negative | Negative |
| Intercourse after VMT  | Often      | no                   | often  | often                | rarely               |           |           |           |

*IU D-Intrauterine device, HPV - Human Papilloma Virus

Extended Data Fig. 1 | Clinical background of donors and recipients.
| Recipient A | Symptoms     | Examination-discharge pH | Amine Test Microscopy | Amsel (out of 4) Microscopic diagnosis |
|-------------|--------------|--------------------------|-----------------------|---------------------------------------|
| Before VMT  | Discharge, Odor | Yes                      | 4.6 Positive | 100% clue cells | 4 BV                                  |
| 7 days after VMT | None          | No                       | 4.6 Negative          | No flora                             |
| 13 days after VMT | Discharge | Normal                   | 4 Negative | Lactobacilli, few | Normal                               |
| 21 days after VMT | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 36 days after VMT | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 96 days after VMT | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 138 days after VMT | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 187 days after VMT | None         | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 230 days after VMT | None         | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 293 days after VMT | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 356 days after VMT | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| Recipient B  | Discharge, Odor | Yes                      | 5 Positive    | 100% clue cells | 4 BV                                  |
| 7 days after VMT | None          | No                       | 4.6 Negative          | No flora                             |
| 14 days after VMT | Less discharge | Normal                   | 4.6 Negative          | Lactobacilli                        |
| 21 days after VMT | Less discharge | Normal                   | 4 Neutral Lactobacilli | Normal                             |
| 41 days after VMT | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 94 days after VMT | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 127 days after VMT | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| Recipient C  | Discharge, odor | Yes                      | 4.7 Positive    | 100% clue cells | 4 BV                                  |
| 7 days after first VMT | None         | Normal                   | 4.5 Negative          | No flora                             |
| 14 days after first VMT | None         | Normal                   | 4.6 Negative          | Lactobacilli, few                   |
| 21 days after first VMT, Repeated VMT | None          | Normal                   | 4 Borderline Coccobacilli, no clue cells | 2 BV                               |
| 11 days after second VMT | None          | No                       | 4 Negative         | No flora                             |
| 25 days after second VMT | None          | No                       | 5 Negative         | No flora                             |
| 43 days after second VMT | None          | No                       | 5 Negative         | No flora                             |
| 53 days after second VMT | None          | No                       | 4.8 Negative Coccobacilli, no clue cells | 1 BV                               |
| 100 days after second VMT | None          | No                       | 4.6 Borderline Coccobacilli, no clue cells | 2 Intermediate                      |
| 144 days after second VMT | Odor          | Yes                      | 5 Positive    | Coccobacilli, no clue cells | 3 BV                                  |
| 25 days after third VMT - donor 3 | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 53 days after third VMT | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 83 days after third VMT | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 119 days after third VMT | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 151 days after third VMT | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 333 days after third VMT | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| Recipient D  | Discharge, odor | Yes                      | 5 Positive    | 100% clue cells | 4 BV                                  |
| 17 days after first VMT | Discharge, odor | Yes                      | 4.7 Negative          | Mixed flora                         |
| 15 days after second VMT | None          | No                       | 4.7 Negative          | Mixed flora                         |
| 22 days after second VMT | None          | No                       | 4 Negative        | Mixed flora                         |
| 29 days after second VMT | Odor          | No                       | 4.6 Negative          | Coccobacilli, no clue cells         |
| 36 days after second VMT | Less discharge | No                      | 4 Negative         | Mixed flora                         |
| 43 days after second VMT | Less discharge | No                      | 4 Borderline        | Coccobacilli, no clue cells         |
| After Antibiotics- third VMT | None          | No                       | 4 Negative         | No flora                             |
| 30 days after third VMT | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 69 days after third VMT | Odor          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 103 days after third VMT | Odor          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 138 days after third VMT | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 159 days after third VMT | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 334 days after third VMT | Discharge      | Yes                      | 4 Negative    | Coccobacilli, candida               |
| 637 days after third VMT | None          | Normal                   | 4 Negative Lactobacilli | Normal                             |
| Recipient E  | Discharge, Odor | Discharge           | 5 Positive    | 100% clue cells | 4 BV                                  |
| 7 days after VMT | Discharge, Odor | Normal                   | 4.6 Negative          | No flora                             |
| 14 days after VMT | Discharge, Odor | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 28 days after VMT | Discharge, Odor | Normal                   | 4 Negative Lactobacilli | Normal                             |
| 36 days after VMT | Odor          | Discharge           | 4.6 Positive | Coccobacilli, no clue cells       |
| After Antibiotics- second VMT | None          | No                       | 4.6 Negative          | No flora                             |
| 21 days after second VMT | Discharge | Normal                   | 4 Negative        | Lactobacilli                         |
| 28 days after second VMT | Discharge      | Normal                   | 4 negative Lactobacilli, no clue cells | 1 Intermediate                      |
| 78 days after second VMT | Discharge      | Normal                   | 4 negative Lactobacilli, no clue cells | 0 Intermediate                      |
| 108 days after second VMT | Discharge, odor | Normal                   | 4 negative Lactobacilli, no clue cells | 0 Intermediate                      |
| 134 days after second VMT | Discharge      | Normal                   | 4 negative No flora  | Normal                              |
| 204 days after second VMT | Discharge      | Normal                   | 4 negative Lactobacilli, no clue cells | 0 Intermediate                      |

Extended Data Fig. 2 | Recipient clinical parameters over time.
**Extended Data Fig. 3** | Recipient pre-VMT and post-VMT range of clinical values throughout the follow-up period.
Extended Data Fig. 4 | Genus-level 16S recombinant DNA assessment of the vaginal microbiome following VMT. (a) 16S principal coordinates analysis using UniFrac distances colored by Amsel criteria scores, n = 50 total recipient samples; (b) Bray-Curtis distances from baseline, correlated with the Amsel criteria scores measured on the same day. A–E are the individual VMT recipients.
Extended Data Fig. 5 | Metagenomic compositional assessment of the vaginal microbiome following VMT. (a) Bray-Curtis distances from baseline, correlated with the Amsel criteria scores measured on the same day; (b) Bray-Curtis distances from respective donor, correlated with the Amsel criteria scores measured on the same day; (c) Metagenomic assessment of the change in the microbiome composition at the Genus level following VMT in absolute values; (d) Change in microbiome in the genus level following VMT; (e) PCA performed on the metagenomic taxonomic data colored by Amsel criteria scores and divided into cluster using 2-means algorithm (n = 47 total recipient samples); (f) PCA colored by relative abundance of Bifidobacterium and of Lactobacillus genus (n = 47 total recipient samples). A–E are the individual VMT recipients.
Extended Data Fig. 6 | Metagenomic functional (KEGG) assessment of the vaginal microbiome following VMT. (a) Bray-Curtis distances from baseline, correlated to Amsel criteria scores and their components, measured on the same day; (b) Change in microbiome functional KEGG gene annotated following VMT; (c) Metagenomic bar plot denoting the KEGG genes that most contributed to the first principal component. A–E are the individual VMT recipients.
Extended Data Fig. 7 | Metagenomic functional (GO) assessment of the vaginal microbiome following VMT. (a) Bray-Curtis distances from baseline, correlated to Amsel criteria scores measured on the same day; (b), (c), principal component analysis, n = 50 total recipient samples, colored by (b), Amsel criteria score, (c) relative abundance of Bifidobacterium and of Lactobacillus genus; (d) Change in microbiome functional GO terms annotated following VMT; (e) Metagenomic bar plot denoting the GO terms that most contributed to the second principal component. A–E are the individual VMT recipients.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

☐ n/a

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
  Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

No software was used.

Data analysis

Python: scipy 0.19.1, matplotlib 2.0.2, seaborn 0.9.0, scikit-learn 0.20.0, pandas 0.20.3, numpy 1.15.1.
R: vegan 2.5-5

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

ENA: PRJEB34085

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences
☐ Behavioural & social sciences
☐ Ecological, evolutionary & environmental sciences
Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | Proof of concept case control study consisting of 5 recipients and 3 donors. No sample size calculation was performed. |
|-------------|------------------------------------------------------------------------------------------------------------------|
| Data exclusions | Samples were not used if they had a low read count and were excluded during rarefaction (Shotgun metagenomic taxonomic only). This was done using a rarefaction curve, and all samples under 100K bacterial reads were dropped. |
| Replication | Replication was not relevant in this study - proof of concept case series |
| Randomization | Randomization was not relevant in this study - proof of concept case series |
| Blinding | Blinding was not relevant in this study - proof of concept case series |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|---------------------------------|---------|
| n/a | Involved in the study |
| ☒ Antibodies | ☒ ChIP-seq |
| ☒ Eukaryotic cell lines | ☒ Flow cytometry |
| ☒ Palaeontology | ☒ MRI-based neuroimaging |
| ☒ Animals and other organisms | |
| ☑ Human research participants | |
| ☑ Clinical data | |

Human research participants

Policy information about studies involving human research participants

| Population characteristics | 5 intractable bacterial vaginosis patients, three healthy donors |
|---------------------------|---------------------------------------------------------------|
| Recruitment               | VMT recipient Inclusion criteria: ages 18-50 with recurrent BV, defined as ≥4 symptomatic episodes of BV during the previous year, who required maintenance antibiotic treatment (twice weekly) in order to remain symptom-free, or if they experienced recurrence of BV in ≤2 months following treatment, with a documented history of recurrent BV. Exclusion criteria: pregnancy or a planned pregnancy in the upcoming year, infection with Hepatitis B, Hepatitis C, HIV and syphilis. Study candidates underwent screening for cervicovaginal infection with C. trachomatis, N. gonorrhoea, Mycoplasma genitalium, and T. vaginalis, using a polymerase chain reaction (PCR) assay. Any patient presenting a positive result for any of these infections received the standard recommended treatment, with a documented negative assay result deemed mandatory for inclusion in the study. All patients underwent a cervical cytology screening test (Pap test) and PCR-based screening for human papilloma virus (HPV). In case of an abnormal cytology test or positive HPV testing, patients were referred for colposcopy. In addition, vaginal cultures for yeast and bacteria (Streptococci Groups A,B,C and G), urine cultures, urinalysis, and serology analysis for HIV, Hepatitis A, B and C, Treponema pallidum, Herpes viruses, and CMV were performed in all cases. Donors Inclusion criteria: Ages 18-50, pre-menopausal. Exclusion criteria: history of recurrent BV, presence of cervico-vaginal STD (Chlamydia trachomatis, Neisseria gonorrhoea, Mycoplasma genitalium and Trichomonas vaginalis), positive HPV-testing, vaginal presence of streptococci groups A, C, G, history of recurrent candida vulvovaginitis, history of recurrent urinary tract infections, use of any antibiotics in the month proceeding vaginal fluid collection, use of systemic medication, use of probiotics (orally or vaginally), consumption of herbal or homeopathic remedies, acute illness, history of cancer, history of anogenital dysplasia, history of anogenital HPV, history of anogenital herpes, vulvar or vaginal disease (acute or chronic), pregnancy, abnormal urinalysis or infection, or seropositivity to HIV, Hepatitis B, C, and G, Herpes or syphilis. Donors long-term medical and sexual history was familiar to the lead clinician. As such, this present exploratory case series may be influenced by self-selection of healthy high-compliance donors. Two were non-sexually active for 8 years or more, while one was engaged in a 25-year monogenous relationship. Donors featured a negative history of vaginal symptoms and underwent an examination to verify the absence of BV and other vaginitis, using a thorough history, gynecological exam, cultures and PCR assays. All donors answered a questionnaire used at our blood bank screening (see below), to screen for possible risk factors for acquiring an infection that we have potentially missed using PCR assays, cultures and serology. Donors were screened for all potentially important infections, including GBS and CMV in order not to expose GBS or CMV negative woman to potential future complications of pregnancy and delivery caused by GBS and CMV. Before vaginal fluid collection, it was explicitly verified with... |
the donors that they did not have intercourse in the week preceding the intervention.

**Ethics oversight**

Hadassah-Hebrew University Medical Center; Weizmann Institute of Science

Note that full information on the approval of the study protocol must also be provided in the manuscript.

### Clinical data

**Policy information** about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

| Clinical trial registration | NCT02236429 |
|----------------------------|-------------|
| Study protocol             | in the manuscript |
| Data collection            | All clinical data was documented and decoded by the leading clinician. Microbiome samples were decoded and sequenced at the Weizmann Institute of Science. |
| Outcomes                   | Degree of bacterial vaginosis- clinical Amsel score, whiff test, vaginal fluid PH, vaginal discharge, microscopy, microbiome characteristics. |