Treatment of Bacterial Vaginosis: A Comparison of Metronidazole and Clindamycin on Human Anaerobic Bacteria and Lactobacilli

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Abstract

Background: Bacterial vaginosis (BV) is a disturbance of vaginal microflora that affects up to one-third of women of reproductive age. To compare the frequencies, clinical characteristics, and antimicrobial susceptibilities of vaginal microbes before and after metronidazole and clindamycin therapy.

Results: A total of 140 premenopausal women with BV and 10 healthy women who underwent routine gynecological examination and were examined by the Vaginal Microecology Evaluation System at Beijing Obstetrics and Gynecology Hospital between October 2018 and February 2019 were recruited for this study. *Gardnerella* (*G.*) *vaginalis* and *Lactobacillus* isolates were isolated and cultured. Clinical isolates were then evaluated for antimicrobial susceptibilities in vitro to metronidazole and clindamycin. Nested PCR and denaturing gradient gel electrophoresis were used to characterize the quantitative structure of bacterial signatures. Facultatively anaerobic bacteria including *G. vaginalis* (40.31 %), *Prevotella* isolates (14.89 %) and *Atopobium* (*A.*) *vaginae* (4.65 %) were among the most frequently isolated species among the 129 samples. The clinical isolates had a significantly higher susceptibility rate to clindamycin than to metronidazole (80.00% vs 32.14%; $P = 0.002$) *in vitro*.

Conclusions: Given metronidazole sparing *Lactobacillus*, metronidazole has better vaginal acidification than clindamycin *in vivo*. The CDC-recommended regimens for BV management with metronidazole and clindamycin were demonstrated to have roughly equivalent clinical efficacy. We recommend administering *Lactobacillus* probiotics 5 to 7 days after the last antibiotic dose when choosing clindamycin therapy.

Background

Bacterial vaginosis (BV) is one of the most common reasons for abnormal vaginal discharge in women. It affects nearly 1 in 3 women of childbearing age worldwide [1]. It is characterized by an imbalance in the vaginal microflora caused by a reduction in *Lactobacillus*, which is usually the dominant organism in the vaginal microbiome, and an increase in facultatively anaerobic bacteria such as *Gardnerella* (*G.*) *vaginalis*, *Atopobium* (*A.*) *vaginae*, and *Prevotella* spp., rather than a true infectious or inflammatory state [2, 3]. *G. vaginalis* has been suggested as the principal cause of BV and it has been widely studied; however, its isolation rate varies across different studies due to the broad diversity in subject selection, detection methods, and diagnostic criteria [4]. BV enhances the acquisition and transmission of a range of sexually transmitted diseases. BV is also associated with various adverse outcomes among pregnant women [5]. Moreover, some BV agents may serve as co-factors in human papillomavirus-mediated cervical carcinogenesis [6]. The precise etiology of BV is not clear; thus, it has even been referred to as “one of the most prevalent enigmas in the field of medicine” [7].

Currently, the Centers for Disease Control (CDC) and the American College of Obstetricians and Gynecologists (ACOG) recommend either oral and intravaginal metronidazole or clindamycin to treat BV [3, 8]. In the 2015 guidelines on sexually transmitted disease management, the CDC recommended BV

treatment with a 7-day regimen of 500 mg of oral metronidazole twice a day, a 5-day regimen of 0.75% metronidazole gel administered intravaginally, and a 7-day regimen of 2% clindamycin cream administered intravaginally [8]. All the recommended regimens to treat BV have been shown to have roughly equivalent efficacy, but differences in cure definitions and follow-up times limit comparisons across trials [9, 10]. As a matter of fact, these have not been well reported by Chinese obstetricians, nurses, and gynecologists. Even with the variety of antimicrobial agents available for the treatment of BV, recurrence occurs after 12 months for almost 60% of women [11].

This study aimed to evaluate and compare the susceptibility of *G. vaginalis* and *Lactobacillus* vaginal isolates to metronidazole and clindamycin, compare the therapeutic response of these agents, and provide a detailed microbiological analysis for a clinical trial of BV.

Methods

Population and sample collection

Women in this study provided written informed consents. The form specified participants' approval to store vaginal specimens for future research. This study was approved by the Ethics Committee of Beijing Obstetrics and Gynecology Hospital (2019-KY-030-01), and was conducted in accordance with the Declaration of Helsinki principles.

The study population consisted of 140 premenopausal nonpregnant women from 18–50 years of age who were enrolled at Beijing Obstetrics and Gynecology Hospital between May 2019 and October 2019. Women who were considered positive for BV were diagnosed by scoring specimens based on the Nugent method, which is a reference standard for the laboratory diagnosis for BV recommended by the CDC [8]. Briefly, vaginal specimens were subjected to gram staining and Nugent scores of ≥7 were considered as BV (Table 1) [12]. At all study visits, participants received regular gynecological examinations and vaginal specimen collection for Nugent scoring, and participants' information were captured through interviews.

Identification of *G. vaginalis*

For strain isolation and detection, vaginal swabs collected from 140 enrolled women were plated on to Columbia blood agar base (Sigma-Aldrich, US), and cultured in an anaerobic chamber for 24–48 h at 37°C according their characteristic colony morphology. Thereafter, pinpoint colonies were subcultured and subjected to polymerase chain reaction (PCR) with the universal primer pairs 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTAGAC TT-3′). Sequence data of clinical *G. vaginalis* isolates were confirmed by comparing 16S rDNA sequences with those available in the GenBank database using BLASTn at NCBI (https://blast.ncbi.nlm.nih.gov/ Blast.cgi). Purified isolates were stock-frozen in DeMan, Rogosa, and Sharpe (MRS) broth containing 30% glycerol at −80°C until susceptibility testing was performed.

G. vaginalis isolates and cultures

Clinical *G. vaginalis* strains isolated from vaginal specimens were included in this susceptibility study. The strains were cultured anaerobically on MRS agar plates in an anaerobic glove box (Coy Laboratory Products, Inc., Grass Lake, MI) at 37℃ under anaerobic conditions; the anaerobic chamber contained 5% H_2, 5% CO_2, and 90% N_2. Different isolates were plated onto the agar medium and then suspended in modified brain heart infusion broth (Difco, Sparks, MD) until they reached a McFarland density of 0.5 (1.0×10^8 CFU/mL), as previously described [13].

Lactobacillus isolates and cultures

Ten *Lactobacillus* strains were isolated from the human vagina of 10 healthy Chinese women from May 2019 to October 2019. The human subject protocols used in this study were approved by the Ethics Committee of Beijing Obstetrics and Gynecology Hospital (2019-KY-030-01). Lactobacilli were identified as gram-positive rods on gram staining, and culture on MRS agar exhibited characteristic ground glass colony morphology. Vaginal cultures were performed as previously described [14]. The isolates were routinely cultured at 37℃ on MRS agar plates (Becton Dickinson, Rockville, MD) at 37℃, 5% CO_2, 48–72 h, from which fresh cultures were prepared as inoculation for the broth microdilution test, using a 0.5 McFarland standard to obtain a total concentration of 1.5×10^8 CFU/mL. *Lactobacillus* isolates were identified using their characteristic colony morphology and gram-staining characteristic.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed *in vitro* using the agar dilution method. We evaluated the susceptibility of 28 clinical *G. vaginalis* isolates and 10 *Lactobacillus* isolates to metronidazole vaginal effervescent tablets (Fuzhou Haiwang Fuyao Pharmaceutical Co., Fujian, China; 0.0625–64 μg/mL) and clindamycin palmitate hydrochloride dispersible tablets (GuangZhou Yipinhong Pharmaceutical Co., Guangdong, China) in accordance with the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) [15]. A sterile, 96-well microplate (Corning, Inc, Corning, NY, USA) was prepared by adding serial dilutions of antimicrobial agents horizontally from the highest concentration to the lowest concentration tested. Twofold serial dilutions of each antimicrobial agent were made. The control wells contained no antimicrobial agents and blank wells contained neither bacteria nor antimicrobials.

A 100 μL inoculum (10^6 CFU/mL) of the prepared bacterial suspension was mixed with different concentrations of metronidazole and clindamycin at 37°C and incubated in an anaerobic chamber. After 48 h, bacterial growth was evaluated by taking an endpoint reading at OD_{595} with a microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). The lowest antibiotic concentration yielding a marked reduction in growth to no growth was read as the minimum inhibitory concentration (MIC). The control strain *Bacteroides fragilis* ATCC®25285 was tested to ensure quality of testing under the same conditions. MIC breakpoints were adapted and interpreted according to the CLSI criteria for metronidazole (sensitive: ≤8 μg/mL, intermediate: 16 μg/mL, resistant: ≥32 μg/mL) and clindamycin (sensitive: ≤2 μg/mL, intermediate: 4 μg/mL, resistant: ≥8 μg/mL)[9].

Treatment

To further verify the results of the antimicrobial susceptibility testing, we recruited 24 patients with symptomatic BV from November 2019 to January 2020; these patients were diagnosed based on Nugent scores between 7–10. Upon enrollment, the patients were randomized to receive one of two treatments, 500 mg oral metronidazole twice-daily for 7 days or 2% intravaginal clindamycin cream for 7 days. After randomization, the two groups showed no significant differences in terms of age, other demographic characteristics, pH, or Nugent scores. After enrollment and treatment, the women were followed up for 7 days after the last dose of medication. Patients with a normal Nugent score as well as the absence of one or more of the clinical signs of BV (no milky white discharge, pH < 4.5, no amine odor) was defined as a clinical cure [16].

Vaginal swabs from each subject were obtained at the baseline (pretreatment) and follow-up (post-treatment) visits. The swabs were subjected to vaginal microecological evaluation and stored at –40°C for bacterial DNA extraction and subsequent experiments.

Evaluation of vaginal microbiota

Bacterial identification and evaluation for vaginal microecological indicators were performed by the Vaginal Microecology Evaluation System (VMES) in two groups [17]. Microbiological evaluations for all the samples were performed at the Department of Microecological Laboratory, Beijing Obstetrics and Gynecology Hospital. Microecological evaluation was done as follows. Vaginal discharge smears were fixed in a slide heater (Yude Company, Shenyang, Liaoning) for 1 min and gram stained. Table 2 presents the system used to determine the *Lactobacillus* morphotype grade (gram-positive rods) [18]. A higher score is indicative of relatively abnormally gram-stained lactobacilli. At least 10 high-power (100×) oil-immersion fields were examined per evaluation.

Nested PCR and denaturing gradient gel electrophoresis (DGGE)

Two culture-independent methods, nested PCR and DGGE, were successfully used to identify the bacterial composition of the vaginal ecosystem and to quantitatively characterize the bacterial signatures in 24 BV patients. Briefly, swabs were vigorously agitated, washed in phosphate-buffered saline to resuspend cells, and then centrifuged. The sediments were resuspended in 200 μL of lysis matrix, incubated at 55°C for 1–2 h, and then boiled at 100 °C for 8 min. The supernatant, which contained DNA template, was stored at −20°C or used directly for the PCR. The DNA samples were subjected to PCR in a thermocycler (Bio-Rad Laboratories) using primers W-1 (5′-AGA GTT TGA TC[AC] TGG CTC-3′) and primer W-2 (5′-TAC GCA TTT CAC C[GT]C TAC A-3′) (Beijing Biomed, Life Technologies). The PCR protocol involved an initial denaturation step, followed by annealing, elongation, and a final extension.

Preparation of gel gradients and electrophoresis were performed according to the manufacturer's instructions for the D-Code™ Universal Mutation Detection System (Bio-Rad Laboratories) as reported previously [19].

DGGE fragment bands were excised from denaturing gradient gels. The re-amplified PCR product were purified using EasyPure PCR Purification Kit protocol (TransGen Biotech, Beijing, China). The resulting PCR products were subsequently sequenced with an Applied Biosystems 3100 Capillary DNA Sequencer (Applied Biosystems). Analysis of the partial 16S rRNA sequences was conducted using the basic local alignment search tool (BLAST; http://www.ncbi.nlm.nih.gov/blast/), and the nearest-neighbor bacterial sequences were aligned with sample sequences by using the BigDye Terminator cycle sequencing kit V3.1 (Applied Biosystems). The relative proportion of each species to the whole microbial population in each swab was determined using a gel imaging and analysis system (chemID™, Saizhi Co, Beijing, China).

Statistical analysis

Data were statistically analyzed using Statistical Package for the Social Sciences v13.0 (SPSS Inc., USA). Results are presented as mean ± standard deviation (SD) of the mean of values obtained in at least triplicate. Differences in the mean or proportion between the two groups were assessed using the *t* test for independent or paired samples. Intergroup frequencies were compared using the χ^2 test or Fisher's exact probability. Mean Nugent scores and pH values were compared between groups using the *t* test for independent samples. Tests of significance were two-sided, and $P \leq 0.05$ was considered to indicate statistical significance. The Mann-Whitney *U* test was used to evaluate differences in the percentage of microbes between the treatment groups at each visit.

Results

Characterization of vaginal samples

Of the 148 samples, 125 (84.46%) were diagnosed as BV with Nugent scores of 7–10; 5 (3.38%) were considered intermediate with Nugent scores ranging from 4–6; and 11 (7.43%) were diagnosed as having other infections concomitant with BV and had Nugent scores ranging from 7–10. Based on the results of the 16S rRNA sequencing, 129 of the 148 samples from 83 enrolled BV patients were available for PCR analysis. Seven samples with no bacterial growth and 12 samples that were not readable due to base deletions/insertions or cases where complete sequence splicing and comparison could not be obtained were excluded from the analysis. The study population had a mean age of 37.36 ± 9.81 years, mean Nugent scores 7.75 ± 1.05, and mean pH values of 4.66 ± 0.42. All the 129 samples revealed clear dysbiosis in microbial composition, evidenced by low levels of *Lactobacillus* and apparent presence of facultatively anaerobic bacteria. *G. vaginalis*, *Prevotella* spp., and *A. vaginae* were the most frequently isolated species. A total of 52 *G vaginalis* isolates were present in 40.31% of the samples (52/129), 21 *Prevotella* spp. were present in 14.89% of the samples (21/129), and 6 *A. vaginae* isolates were found in 4.65% of the samples (6/129). Of the 52 *G. vaginalis* isolates, 48 (92.31%) were isolated from BV patients; 1 (1.92%), from a patient with intermediate BV; and 3 (5.77%), from cases with mixed infections. Statistically, the isolation of *G. vaginalis* did not significantly differ among the BV, intermediate BV, and mixed infection groups ($P = 0.224$).

Antimicrobial susceptibilities of *G. vaginalis*

A total of 30 clinical *G. vaginalis* isolates were recovered from blood cultures, of which 28 were available for the antimicrobial susceptibility assay. The remaining two isolates did not exhibit any bacterial growth upon culture. Nine of the isolates (32.14%) were found to be susceptible to metronidazole based on the CLSI susceptibility breakpoint of ≤8 μg/mL, 18 isolates (64.29%) showed resistance (MIC ≥ 32 μg/mL), and 1 (3.57%) showed intermediary resistance (MIC = 16 μg/mL) to metronidazole (Table 3). Of the 28 isolates, 3 did not grow well on clindamycin susceptibility test media; of the remaining 25, 20 were susceptible (MIC ≤ 2 μg/mL, 80.00%) and 5 were resistant (MIC ≥ 8 μg/mL, 20.00%) to clindamycin. The susceptibility rate of clinical isolates to metronidazole was significantly lower than that to clindamycin (P = 0.002). Clindamycin-susceptible isolates showed varying degrees of resistance to metronidazole.

Comparison of the clinical characteristics and changes in the vaginal flora

Complete data were available for all the 24 patients at the follow-up visit (7 days after the last dose). Table 4 presents a graphical analysis comparing the changes observed among the vaginal microorganisms associated with BV isolated at the baseline and 7 days after finishing the last dose of metronidazole or clindamycin. The cure rate was 75.00% (9/12) in the metronidazole group and 41.67% (5/12) in the clindamycin group. There were no statistically significant differences between the two treatment groups (P = 0.665). Speculum examination of all the study participants had shown milky white vaginal discharge before the treatment. On follow-up, 58.33% (n = 7) of the patients in the metronidazole group were completely free from abnormal vaginal discharge, and 75.00% (n = 9) had a normal lactobacillus flora, and the pH (4.61 ± 0.09 to 4.15 ± 0.05, P = 0.001) and Nugent scores (7.49 ± 1.34 to 2.08 ± 0.60, P < 0.001; Figure 1) significantly decreased from baseline to follow-up. In the clindamycin group, 50.00% (n = 6) of the study participants were free from abnormal vaginal discharge, but only 41.67% (n = 5) had a normal lactobacillus flora. The pH value (4.88 ± 0.11 to 4.65 ± 1.44, P = 0.279) and Nugent scores (7.45 ± 1.34 to 3.42 ± 0.65, P < 0.001; Figure 1) in the clindamycin group also decreased from baseline to follow-up. There was no statistically significant differences in the two groups in terms of vaginal discharge, lactobacillus flora grade, and Nugent scores recorded at the follow-up visit (Table 4), while the pH value at the follow-up visit significantly differed between the two groups (P=0.003), suggesting better vaginal acidification in the metronidazole group.

A significant decrease in colonization by *G. vaginalis* (P < 0.001) was observed among the baseline and follow-up visits in both treatment groups, although the difference between the two groups was not statistically significant (c^2 = 3.01, P = 0.083; Figure 2). A significant decrease in colonization by *A. vaginae* (47.0% to 0.0%) and *Prevotella species* (23.8% to 0.0%) was observed between the baseline and follow-up visit in the metronidazole group. A significant decrease in colonization by *A. vaginae* (44.6% to 8.3%) while the population of *Prevotella* spp. remained nearly unchanged (26.2% to 25%) from the baseline to follow-up visits in women treated with clindamycin, with significant differences between the two groups (Fisher's Exact Test, P = 0.006; c^2 = 17.65, P < 0.001; Figure 2). The presence of H_2O_2-producing lactobacilli is considered as an indicator of optimal vaginal ecology, and clindamycin

treatment (19.6% to 0.0%) resulted in a significantly larger decrease (c^2 = 16.971, P < 0.001; Figure 2) in colonization by g-producing *Lactobacillus* species than metronidazole treatment (22% to 25%).

Antimicrobial susceptibilities of lactobacilli

As the effect of clindamycin and metronidazole on lactobacilli may potentially affect clinical efficacy, we determined the MIC of these antibiotics against clinical *Lactobacillus* isolates using the microdilution method. As seen in Table 5, all ten *Lactobacillus* isolates (100.00%) displayed high-level resistance to metronidazole with MICs of ≥1024; while eight of the ten were susceptible (MIC ≤ 2 μg/mL, 80.00%) and two were resistant (MIC ≥ 8 μg/mL, 20.00%) to clindamycin. The clinical *Lactobacillus* isolates were significantly more resistant to metronidazole than to clindamycin (Figure 3 & Table 3; P = 0.001). In general, most *Lactobacillus* isolates were inhibited by a maximum of 0.5–1 μg/mL of clindamycin. None of the examined isolates were susceptible to metronidazole. The MICs of the two antimicrobial agents against *G. vaginalis* were lower than those against the *Lactobacillus* isolates.

Discussion

Principal Findings Of The Study And Clinical Implications

G. vaginalis, which is the only species in the genus *Gardnerella*, is one of the primary causes of BV, the most common vaginal infection identified in women of childbearing age worldwide [20]. We used 16S rRNA sequencing to demonstrate the importance of *G. vaginalis* for BV, which can be massively underestimated, and reported an isolation rate of 40.31%. This rate varies across different studies due to the broad diversity in patient recruitment, methods, and diagnostic criteria [21].

Metronidazole and clindamycin are the current gold standards to treat BV, and come recommended by the CDC and ACOG [3, 8]. Two antimicrobial therapies have been shown to have equivalent clinical efficacy allowing recurrence rates of 50% after 6 months [22]. Metronidazole possesses excellent activity against obligate anaerobes but is ineffective against aerobes and facultatives [23], while clindamycin has broader spectrum activity against gram-positive aerobes and anaerobes [24]. Our results of antimicrobial susceptibility testing confirmed *G. vaginalis* was significantly more resistant to metronidazole than clindamycin *in vitro*. However, metronidazole is active *in vivo* with a relative higher cure rate and better vaginal acidification than clindamycin, which may due to two possible mechanisms: i) BV-associated bacteria *in vivo* may be sensitive to the hydroxymetabolite of metronidazole, requiring enzymatic reduction within the cell [25]; or more likely, ii) metronidazole acts indirectly through synergism, killing anaerobes that provide a substrate to BV-associated bacteria [26].

Our results also demonstrated that BV is not a single entity but a combination of different microbial communities that may be dominated by anaerobes, a combination of *G. vaginalis*, *Prevotella*, and *A. vaginae*, or other abnormal subtypes of mixed organisms, consistent with other studies [26]. Both *G. vaginalis* and *A. vaginae* decreased significantly following treatment with either regimen. *Prevotella*

species (23.8–0.0%) colonization significantly decreased in the metronidazole group than in the clindamycin group (26.5–25.0%), which is similar to those in other studies. This finding may be partly due to high-level clindamycin-resistant *Prevotella* strains, although *Prevotella* spp. showed no resistance to metronidazole, which is a cause of concern [27, 28]. Among all *Prevotella* species, *Prevotella bivia* may account for most of the multidrug-resistant isolates [29]. This data emphasizes the need for identification of BV-associated bacteria and periodic monitoring of their susceptibility to guide empirical treatment. As the antibiotic resistance of these microorganisms to antibiotics are variable or even increasing [30], none of the antibiotics will be able to cover the bacterial spectrum with sufficient efficacy to accomplish complete cure. Hence, we might need to individualize the treatment to recurrent BV according to the subtype of BV-associated bacteria, so that the optimal therapeutic scheme can be selected.

It is noteworthy that metronidazole therapy may have shown a statistically significant improvement in the normal flora index (a composite of *Lactobacillus* spp. flora and vaginal pH) of BV patients than clindamycin therapy, which is consistent with the findings reported in literature [31]. To further verify the hypothesis, we tested the susceptibility of *Lactobacillus* isolates to clindamycin and metronidazole. Overall, all *Lactobacillus* isolates displayed high-level resistance to metronidazole (MIC $\geq$ 1024) but 80.00% were susceptible and 20.00% were resistant to clindamycin. Accordingly, the clinical case is often made in favor of metronidazole because metronidazole spares vaginal lactobacilli, whereas clindamycin eradicates them. These may partly because *Lactobacillus* species are a facultatively anaerobic, gram-positive, non-spore-forming bacteria that are the dominant bacterial species in the vaginal microbiome, and metronidazole exerts antibacterial effects only in an anaerobic environment, and possesses no clinically relevant activity either against facultative anaerobes or obligate aerobes [23]. Clindamycin most likely not only eliminates pathogenic microorganisms but also lowers the number of hydrogen peroxide- and lactic acid-producing lactobacilli, which are in good agreement with data from other studies [9, 32], thus leading to a lower cure rate than that of metronidazole, contrary to the results of the in vitro MIC assays. Currently used antimicrobial agent(s) may lead to antimicrobial resistance and inhibition of the normal microbiota, striking differences were observed. The inhibition of healthy microorganisms lengthens the overall restoration time of the normal vaginal ecosystem [33]. Although our study did not specifically examine idiographic strains, it is possible that a decrease or absence of most *Lactobacillus* species after therapy, which could be a marker for long-term treatment failure in independently maintaining the healthy vaginal niche [34, 35]. It is considered that *Lactobacillus* probiotics may support restoration of the normal dominant state of lactobacilli and hold promise for the cure and prevention of BV [36]. We recommend the administration of *Lactobacillus* probiotics for 5–7 days after the last dose of clindamycin therapy.

In the present study, lactobacilli were not identified to the species level. A greater understanding of these mechanisms should clarify whether clindamycin and metronidazole have different effects on various *Lactobacillus* strains, which would be more informative when monitoring the treatment outcome. Future studies should focus on the longer-term effects of various antibiotic regimens on the recurrence rates of BV and the susceptibility of *Prevotella* spp. to clindamycin.

In conclusion, we found that the CDC-recommended regimens of 500 mg oral metronidazole twice a day for 7 days or intravaginal 5 g clindamycin cream at bedtime for 7 days have roughly equivalent clinical efficacy for managing BV. Both metronidazole and clindamycin remain the most commonly used antimicrobial agents to treat BV. Metronidazole is more active *in vivo* and better vaginal acidification than clindamycin, despite its relatively limited *in vitro* activity against *G. vaginalis* when compared with clindamycin. Treatment of recurrent BV needs individualization according to the subtype of BV-associated bacteria; for instance, when antibiotic susceptibility assay indicates *Prevotella* species, metronidazole is the better choice. As *Lactobacillus* spp. are the dominant healthy bacterial species in vaginal microbiota maintaining healthy microbial homeostasis, metronidazole were found to have no *in vitro* activity against lactobacilli recovered from the vagina, whereas clindamycin, which has a broader antibacterial spectrum, may inhibit them. In this respect, we recommend the administration of *Lactobacillus* probiotics 5–7 days after the last dose when choosing clindamycin therapy.

Abbreviations

BV: Bacterial vaginosis; CDC, Centers for Disease Control; ACOG: American College of Obstetricians and Gynecologists; PCR: polymerase chain reaction; MRS: de Man, Rogosa and Sharpe; CLSI: Clinical and Laboratory Standards Institute; MIC: minimum inhibitory concentration; VMES: Vaginal Microecology Evaluation System; DGGE: denaturing gradient gel electrophoresis; SD: standard deviation.

Declarations

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Not applicabl

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Availability of data and materials

All the data supporting our findings is contained within the manuscript.

Authors' contributions

The main experimental conception and design: ZL; Performed the experiments: TL, FW, and ZZ; Analyzed the data and contributed reagents: XZ and HB; Writing the manuscript: TL. All of the authors approved the final version.

Ethics approval

This study was approved by the Ethics Committee of Beijing Obstetrics and Gynecology Hospital (2019-KY-030-01), and was conducted in accordance with the Declaration of Helsinki principles.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 The Nugent score system of vaginal discharge

Scores	*Lactobacillus*	*Gardnerella* and *Bacteroides*	*Mobiluncus*
0	≥30/OML	0	0
1	6-30/OML	≤1/OML	1-5/OML or ≤1/OML
2	1-5/OML	1-5/OML	≥30/OML or 6-30/OML
3	≤1/OML	6-30/OML	
4	0	≥30/OML	

OML, oil immersion lens, HPL, high-power lens.

Table 2 *Lactobacillary* grade based on Gram staining under oil microscope

Grade	microscopic diagnosis of *Lactobacillus*
I	Numerous pleiomorph *lactobacilli*, no other bacteria
IIa	Mixed flora, but predominantly *lactobacilli*
IIb	Mixed flora, but proportion of *lactobacilli* severely decreased due to increased number of other bacteria
III	*Lactobacilli* severely depressed or absent because of overgrowth of other bacteria

I and IIa are defined as normal *lactobacillary* flora, whereas IIb and III are defined as abnormal *lactobacillary* flora.

Table 3 In vitro antimicrobial susceptibilities of 28 *Gardnerella vaginalis* isolates and 10 *Lactobacillus* isolates (Fisher's Exact Test)

Isolates	Antimicrobial agent	N	Susceptible	Intermediary	Resistant	*P* value
Gardnerella vaginalis	Metronidazole	28	9	1	18	0.002
	Clindamycin	25[a]	20	0	5	
Lactobacillus	Metronidazole	10	0	0	10	0.001
	Clindamycin	10	8	0	2	

[a] Three of the 28 isolates did not grow well on clindamycin susceptibility test media.

Table 4 The clinical and laboratory characteristics of pre- (baseline) and post-treatment for metronidazole and clindamycin

	Metronidazole		Clindamycin		*P* value[a]
	Pre-treatment (Baseline)	Post-treatment	Pre-treatment (Baseline)	Post-treatment	
PH value	4.61±0.09	4.15±0.05*	4.88±0.11	4.65±1.44	0.003
Nugent scores	7.49±1.34	2.08±0.596*	7.45±1.34	3.42±0.65*	0.143
Abnormal vaginal discharge	12(100%)	5(41.67%)	12(100%)	6(50.0%)	0.682
Abnormal *Lactobacillus*[b]	12(100%)	3(25.00%)	12(100%)	7(58.33%)	0.214

[a] compared between two groups at follow-up visit(post-treatment); [b] I and IIa are defined as normal *lactobacillary* flora, whereas IIb and III are defined as abnormal *lactobacillary* flora; * indicates that *P*□0.05 performed by *t* test for independent samples between Pre-treatment and Post-treatment.

Table 5 Minimum inhibitory concentration (MIC) of Metronidazole and Clindamycin for clinical *lactobacillus* isolates (μg/mL)

Isolate No.	Metronidazole	Clindamycin
1	≥1024	≤0.25
2	≥1024	16
3	≥1024	0.5
4	≥1024	0.5
5	≥1024	128
6	≥1024	≤0.25
7	≥1024	0.5
8	≥1024	1
9	≥1024	1
10	≥1024	≤0.25

All data are the average of two separate experiments in duplicates. All assays conducted resulted in identical results for all substances (no standard deviation).

Figures

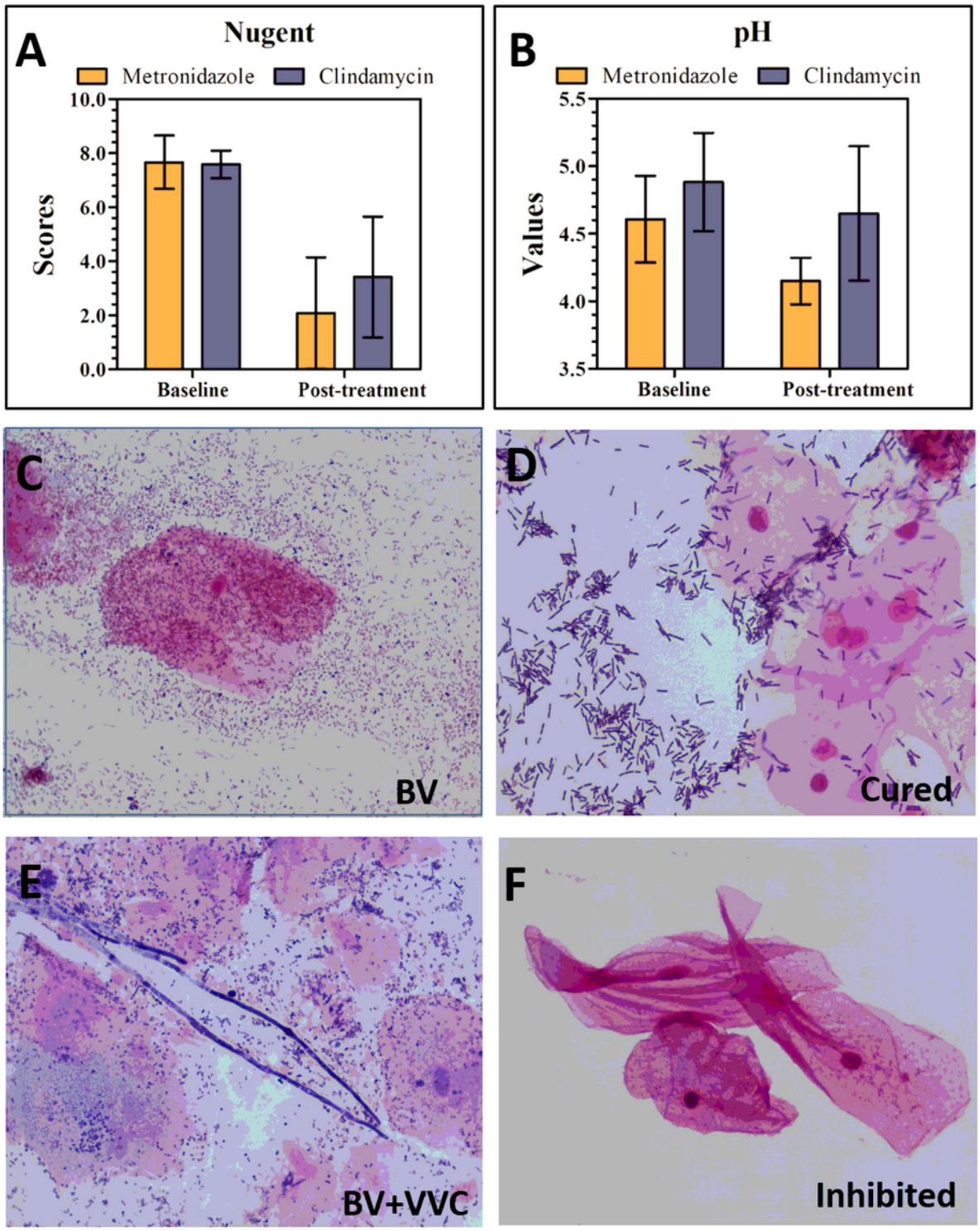
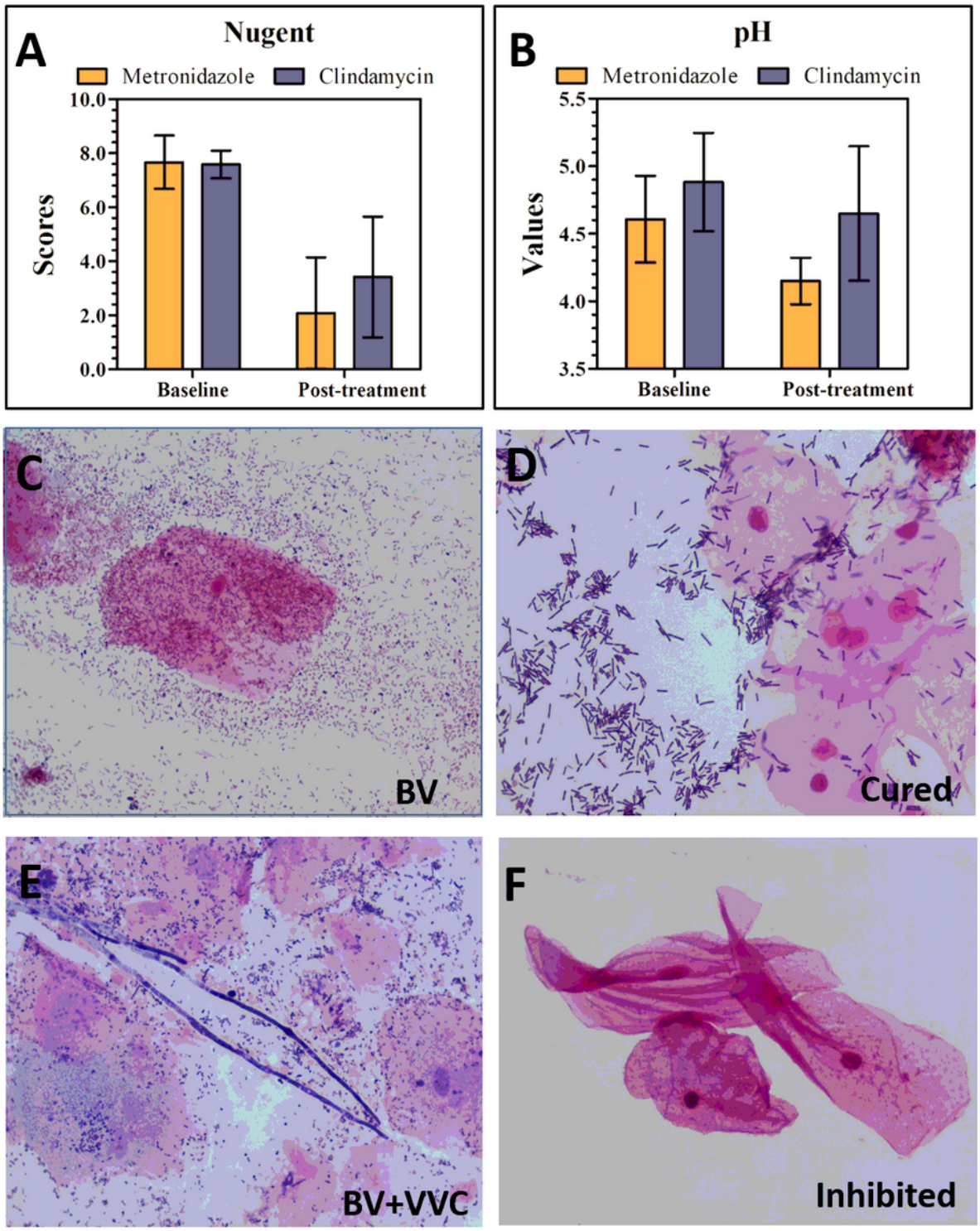


Figure 1

Changes in laboratory characteristics from baseline among women with bacterial vaginosis (BV) after therapy with metronidazole or clindamycin, evaluated by the Vaginal Microecology Evaluation System. Quantitative analysis of Nugent scores (A); quantitative analysis of pH values (B); untreated BV (baseline, C); cured BV (post-treatment, D); BV+VVC (post-treatment, E); and inhibited vaginal flora (post-treatment, F). VVC: Vulvovaginal candidiasis. Data represent the mean ± standard deviation (n = 12).



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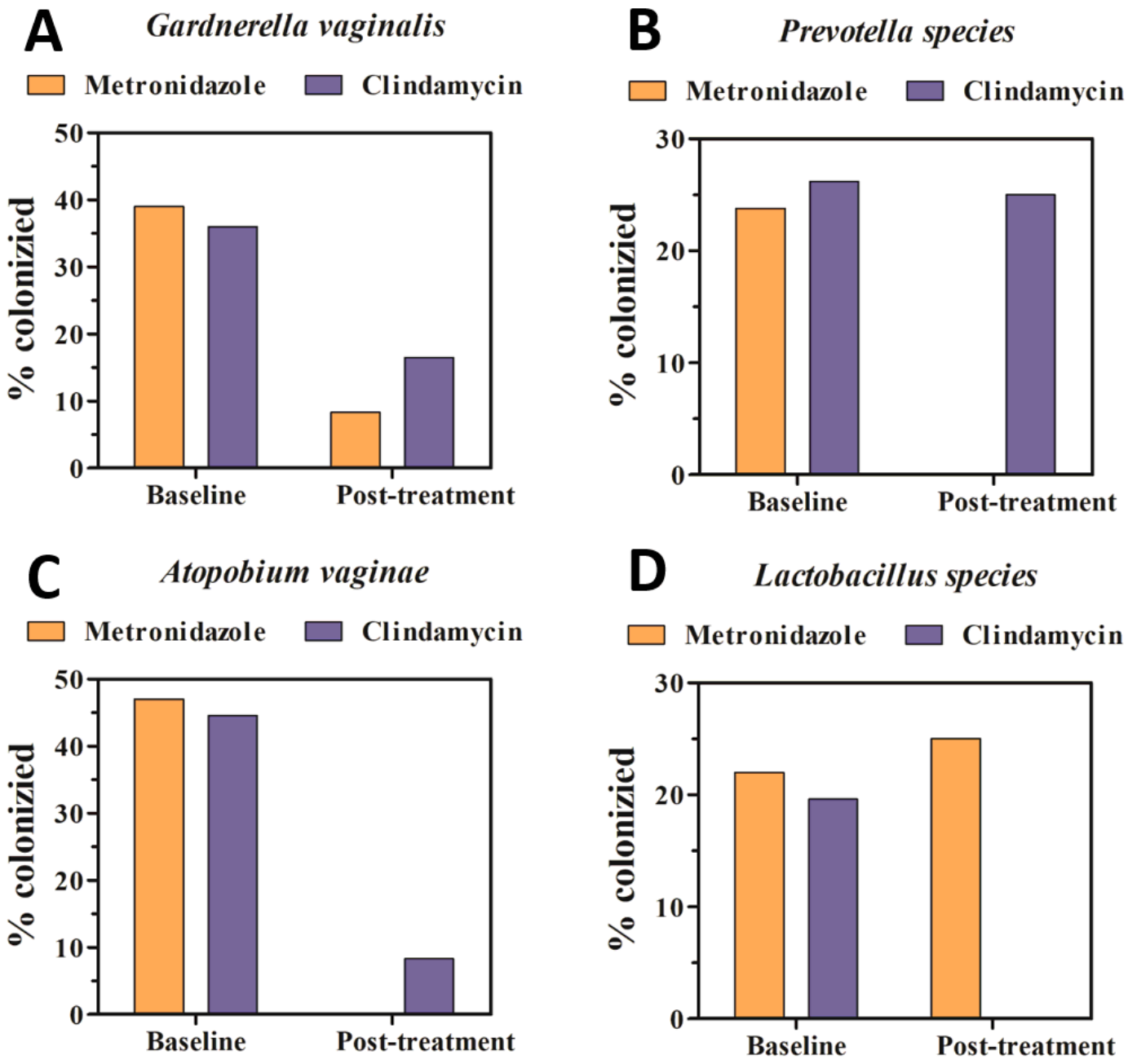
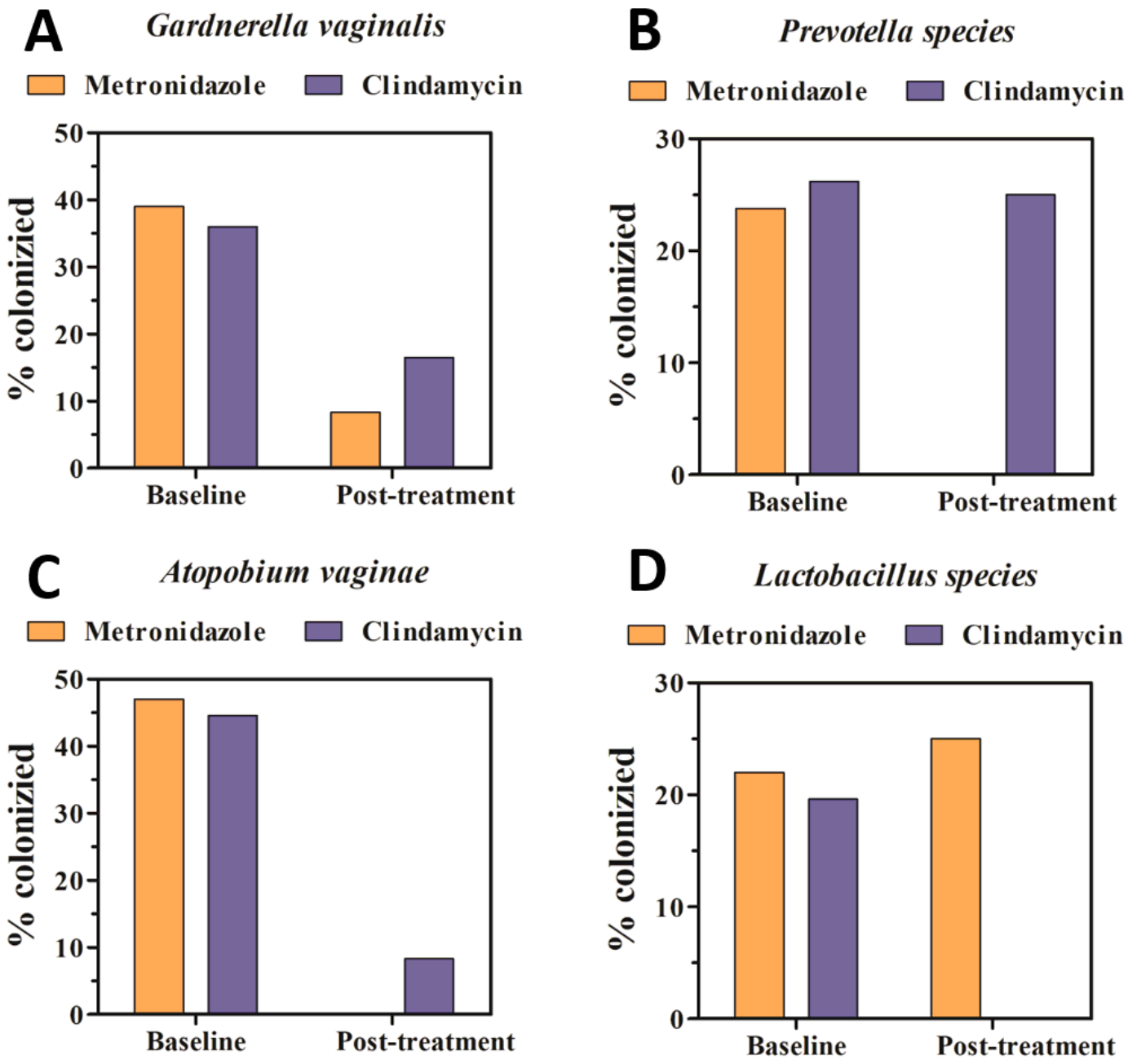


Figure 2

Changes in the vaginal microbiota from baseline among women with BV after therapy with metronidazole or clindamycin.



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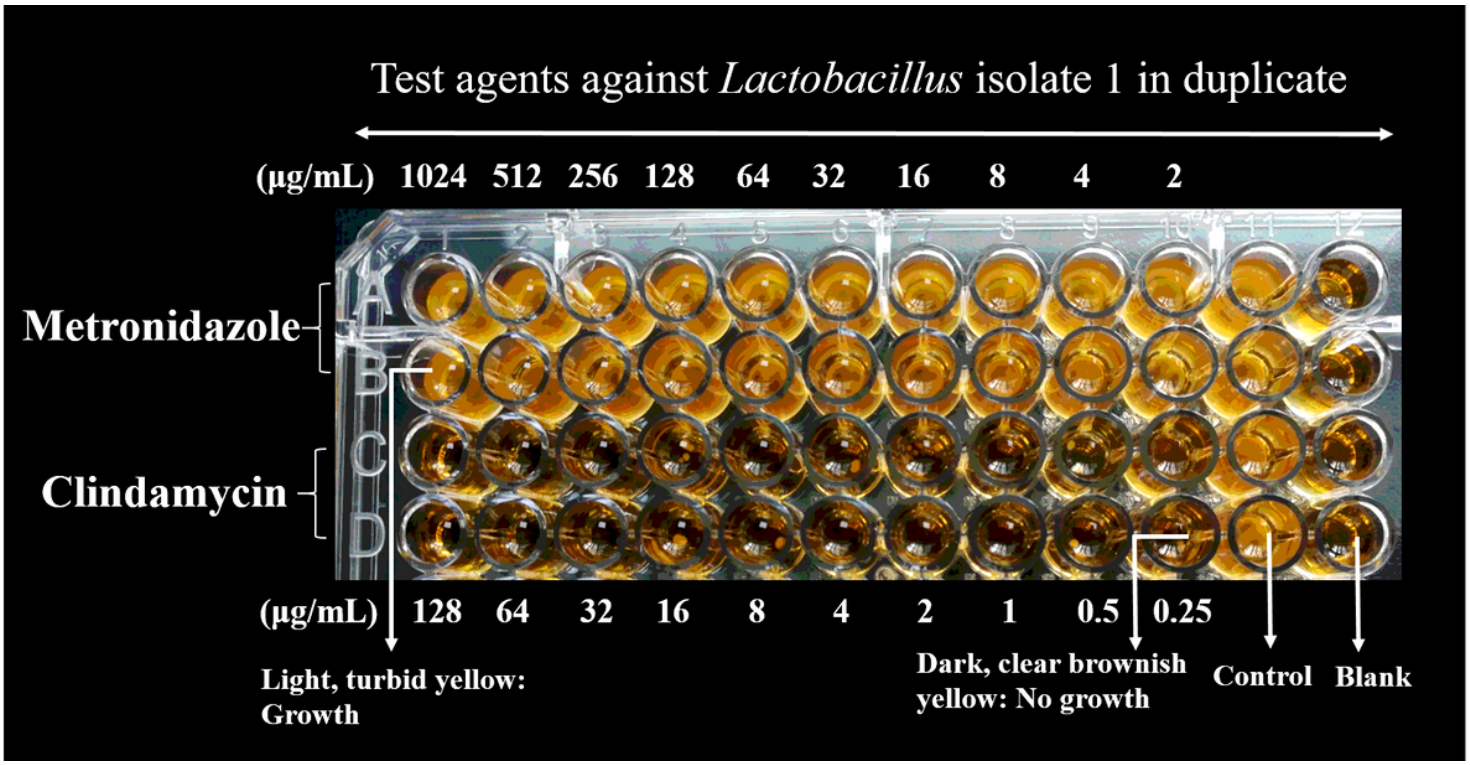
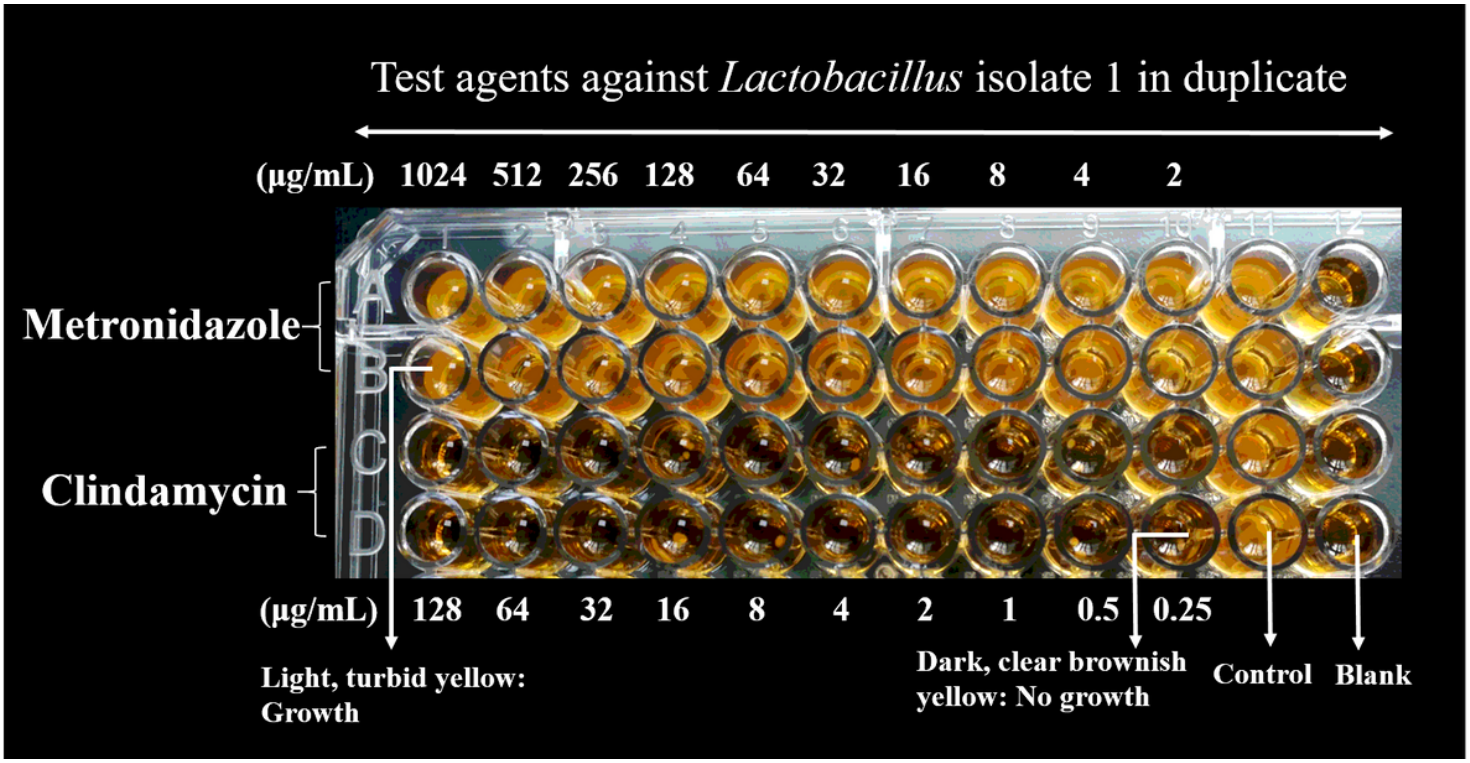


Figure 3

Antimicrobial susceptibility testing of metronidazole and clindamycin against clinical Lactobacillus isolates in a microtiter plate broth format. The minimal inhibitory concentration (MIC) endpoint was defined as the lowest concentration of metronidazole or clindamycin that inhibited visible growth of the test isolate. Brownish, dark yellow wells were considered as wells without growth and light turbid yellow wells were considered as wells with growth. Wells without antimicrobial agents were used as the control, and wells without bacteria and antimicrobial agents were considered as the blank.



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