The microbial flora of the vagina contains high concentrations of a composite population of bacteria (11, 21). It is dominated mainly by lactobacilli that maintain an acidic pH by production of lactic acid (24). Alterations in this ecosystem can lead to bacterial vaginosis (BV) and Candida vaginitis, which account for 90% of vaginal infections (10).

BV is a polymicrobial syndrome characterized by alteration of the vaginal flora, where the normally occurring Lactobacillus species are overgrown by endogenous bacteria (24). In particular, high concentrations of Gardnerella vaginalis and Atopobiobium vaginae have been shown to be important microbiological markers (1, 18, 27). The association between the presence of A. vaginae and BV has been highlighted only recently (8), thanks to its detection by molecular techniques. Although its exact role is not yet fully understood, the association between A. vaginae and BV is well established (1, 17, 18, 27), as is its involvement, together with G. vaginalis, in the biofilm present on the vaginal epithelium during BV (25).

The therapies of choice for BV are systemic or topical metronidazole and clindamycin. Results suggest that nifuratel has a better spectrum of activity, being highly active against G. vaginalis and A. vaginae without affecting lactobacilli.

The bacterial strains tested were both clinical isolates and standard strains (5). The ranges of concentrations tested were 0.125 to 256 μg/ml for nifuratel and metronidazole and 0.125 to 64 μg/ml for clindamycin. Inocula were prepared in brucella broth to a 0.5 McFarland standard (1 × 108 to 2 × 108 CFU/ml) by suspending colonies cultivated on Columbia blood agar 5% (vol/vol) sheep blood (Labobasi, Novazzano, Switzerland) for 3 days at 36 ± 1°C under anaerobic conditions (A. vaginae) or in a CO2-enriched atmosphere (G. vaginalis and Lactobacillus spp.).

Plates were prepared according to CLSI standard protocols (3). The ranges of concentrations tested were 0.125 to 256 μg/ml for nifuratel and metronidazole and 0.125 to 64 μg/ml for clindamycin.

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Brucella agar supplemented with 5 μg hemin, 1 μg vitamin K1 per ml, and 5% (vol/vol) sheep blood (Labobasi) and containing the appropriate antibiotic concentration was inoculated with 2 μl of the bacterial suspension and incubated for 3 days at 36 ± 1°C under anaerobic conditions (A. vaginae) or in a CO2-enriched atmosphere (G. vaginalis and Lactobacillus spp.).

Quality controls show that supplemented brucella agar medium and the final DMSO concentration in the medium (1%) were identified by DNA amplification and sequencing (Microsynth, Balgach, Switzerland) (29).

Strains of A. vaginae (n = 10; CCUG 38953T, CCUG 42099, CCUG 43049, CCUG 44061, CCUG 44116, CCUG 44125, CCUG 44156, CCUG 44258, CCUG 48515, and CCUG 55226; Culture Collection Center, University of Göteborg, Göteborg, Sweden), G. vaginalis (n = 22; ATCC 14018 and 21 clinical isolates), and Lactobacillus spp. (n = 20; Lactobacillus crispatus CCUG 27076A and 4 clinical strains; L. iners CCUG 24626 and 2 clinical strains; L. gasseri CCUG 24836; and L. jensenii CCUG 35572T and 11 clinical strains) were tested.

Stock solutions of nifuratel (Polichem, Lugano-Pazzallo, Switzerland) and metronidazole (Sigma-Aldrich, Munich, Germany) were prepared in dimethyl sulfoxide (DMSO; Sigma-Aldrich) to a concentration of 51.2 mg/ml. Clindamycin (Sigma-Aldrich) was dissolved in water to a concentration of 0.64 mg/ml. Stock solutions were immediately used or stored at −60°C. Working solutions were obtained by serial 2-fold dilutions in DMSO (nifuratel and metronidazole) or water (clindamycin).
did not affect the bacterial growth of all tested strains. Moreover, the MICs of the two control strains, G. vaginalis ATCC 14018 and Bacteroides fragilis ATCC 25285, were in the acceptable ranges for metronidazole and clindamycin (data not shown).

Our results (Table 1) show that clindamycin is highly active against both G. vaginalis (MIC for 90% of the strains tested [MIC<sub>90</sub>, 0.25 µg/ml] and A. vaginae (MIC<sub>90</sub>, <0.125 µg/ml), in accordance with previous studies on G. vaginalis (12, 15) and A. vaginae (5). Metronidazole was partially active against G. vaginalis (MICs, <0.125 to 256 µg/ml) and A. vaginae (MICs, 8 to 256 µg/ml). These results are also in accordance with previously published data (5, 12, 15). Nifuratel was more active on G. vaginalis and A. vaginae than metronidazole, with MICs ranging from <0.125 to 4 µg/ml and from <0.125 to 1 µg/ml, respectively.

All tested Lactobacillus strains were highly susceptible to clindamycin (MICs, 0.125 to 1 µg/ml) and resistant to metronidazole (MICs, ≥256 µg/ml). Overall, nifuratel was not effective against lactobacilli (MIC<sub>90</sub>, ≥256 µg/ml). Only L. iners strains (n = 3) appeared to be more sensitive to nifuratel than the other species, with MICs of 8, 16, and 256 µg/ml. It is interesting that previous studies have shown that L. iners is more common than other lactobacilli in samples that have a Nugent score of >4 (6) and after metronidazole treatment (9). Moreover, it seems to predispose to some extent to the occurrence of abnormal vaginal microflora (28). Although these observations deal only with three L. iners strains, they suggest that nifuratel could not only be useful in the eradication of bacteria associated with BV, like G. vaginalis and A. vaginae, but also encourage the development of species of Lactobacillus other than L. iners. Further analysis should be performed to confirm these partial observations.

In conclusion, our results suggest that nifuratel is a good potential candidate for the first-line treatment of BV. Indeed, it is active in vitro against the pool of bacteria recognized to cause BV and, conversely, does not affect the normal flora of lactobacilli. Based on these encouraging results, two pivotal clinical studies on oral and topical treatments are ongoing in order to confirm if this antibiotic offers a real advantage over standard BV treatments.

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REFERENCES


