

In Vitro Activity of Nifuratel on Vaginal Bacteria: Could It Be a Good Candidate for the Treatment of Bacterial Vaginosis?[▽]

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Bacterial vaginosis is characterized by a shift of the physiological flora to a diverse spectrum of bacteria, where *Gardnerella vaginalis* and *Atopobium vaginae* are the most important markers. In this study, the antimicrobial activity of nifuratel against *G. vaginalis*, *A. vaginae*, and lactobacilli was compared with that of the two currently used antibiotics 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 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 H₂O₂ and lactic acid production (14). 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 *Atopobium 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. Previous studies reported cure rates of 70 to 96% for both antibiotics, with recurrence rates of 49 to 66%, following 7 days of therapy (2, 13, 16).

Inadequate diagnosis (23), pharmacologic resistance (20), and persistence of an adherent bacterial biofilm after treatment (26) seem to be the main reasons for failures of BV treatment and eradication, as well as the presence of a complex microbial population with putative resistance to antimicrobials.

Nifuratel is a nitrofurantoin derivative with strong activity against *Trichomonas vaginalis* (4, 7) and a broad spectrum of antibacterial action (7, 19, 22). The purpose of this study was to investigate the potential of nifuratel in the treatment of BV and compare it with metronidazole and clindamycin against *G. vaginalis*, *A. vaginae*, and lactobacilli.

The bacterial strains tested were both clinical isolates and reference strains. Clinical isolates from vaginal swabs (pro-

vided by Maditest, Vevey, Switzerland) were grown and identified following standard protocols. In addition, *Lactobacillus* strains 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).

Plates were prepared by following CLSI standard protocols (3). The ranges of concentrations tested were 0.125 to 256 $\mu\text{g/ml}$ for nifuratel and metronidazole and 0.125 to 64 $\mu\text{g/ml}$ for clindamycin.

Inocula were prepared in brucella broth to a 0.5 McFarland standard (1×10^8 to 2×10^8 CFU/ml) by suspending colonies cultured on Columbia blood agar 5% (vol/vol) sheep blood (Labobasi, Novazzano, Switzerland) for 3 days at $36 \pm 1^{\circ}\text{C}$ under anaerobic conditions (*A. vaginae*) or in a CO₂-enriched atmosphere (*G. vaginalis* and *Lactobacillus* spp.).

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 $36 \pm 1^{\circ}\text{C}$ under anaerobic conditions (*A. vaginae*) or in a CO₂-enriched atmosphere (*G. vaginalis* and *Lactobacillus* spp.) (3).

Quality controls show that supplemented brucella agar medium and the final DMSO concentration in the medium (1%)

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TABLE 1. Frequency distribution of MICs, MIC₅₀, MIC₉₀, and MIC ranges for *G. vaginalis*, *A. vaginae*, and *Lactobacillus* spp.

Organism (total no. of isolates) and antibiotic	No. of isolates with MIC (µg/ml) of:													MIC range	MIC ₅₀	MIC ₉₀		
	<0.125	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256				>256	
<i>A. vaginae</i> (n = 10)																		
NIF ^a		1	2	5	2											0.125–1	0.5	1
MTZ ^b								1	2	3	2	1	1			8–256	32	128
CLI ^c	10															<0.125	<0.125	<0.125
<i>G. vaginalis</i> (n = 22)																		
NIF	3		1	4	3	7	4									<0.125–4	2	4
MTZ	4						1	2	6	5	1	1	1	1		<0.125–>256	16	128
CLI	20			2												<0.125–0.5	<0.125	<0.125
<i>Lactobacillus</i> spp. (n = 20)																		
NIF								2	1		1		2	14		8–>256	>256	>256
MTZ													20			>256	>256	>256
CLI	19				1											<0.125–1	<0.125	<0.125

^a NIF, nifuratel.
^b MTZ, metronidazole.
^c CLI, 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 [MIC₉₀], 0.25 µg/ml) and *A. vaginae* (MIC₉₀, <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 (MIC₅₀, >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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