Bacterial Vaginosis, *Atopobium vaginae* and Nifuratel

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**Abstract:** As bacterial vaginosis (BV) is a potential cause of obstetric complications and gynecological disorders, there is substantial interest in establishing the most effective treatment. Standard treatment - metronidazole or clindamycin, by either vaginal or oral route – is followed by relapses in about 30% of cases, within a month from treatment completion. This inability to prevent recurrences reflects our lack of knowledge on the origins of BV. *Atopobium vaginae* has been recently reported to be associated with BV in around 80% of the cases and might be involved in the therapeutic failures. This review looks at the potential benefits of nifuratel against *A. vaginae* compared to the standard treatments with metronidazole and clindamycin. In *vitro*, nifuratel is able to inhibit the growth of *A. vaginae*, with a MIC range of 0.125-1 µg/mL; it is active against *G. vaginalis* and does not affect lactobacilli. Metronidazole is active against *A. vaginae* only at very high concentrations (8-256 µg/mL); it is partially active against *G. vaginalis* and also has no effect on lactobacilli. Clindamycin acts against *A. vaginae* with an MIC lower than 0.125 µg/mL and is active on *G. vaginalis* but it also affects lactobacilli, altering the vaginal environment. These observations suggest that nifuratel is probably the most valid therapeutic agent for BV treatment.

**Keywords:** Antibiotic resistance, *Atopobium vaginae*, bacterial vaginosis, nifuratel, review.

**BACTERIAL VAGINOSIS**

**Epidemiology and Pathogenesis**

Bacterial vaginosis (BV) is one of the most frequent female lower genital tract infections, not only in pregnancy but throughout the reproductive life. Studies from Europe and the USA have found prevalence between 4.9% and 36.0% [1]. The first signs of BV are radical changes in the vaginal ecosystem. H2O2-producing lactobacilli, and 36.0% [1]. The first signs of BV are radical changes and the USA have found prevalence between 4.9%

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**Complications of BV**

BV has aroused interest in the last few years being considered as a predisposing factor for HIV, Type II *Herpes simplex* virus, *Chlamydia trachomatis* infections, as well as for trichomoniasis and gonorrhrea [13, 14]; BV can be also a cause for complications like late abortion [15], premature rupture of the amniotic membrane [16], chorio-amnionitis [17], *post-partum* endometritis [18, 19, 20], and failure of in *vitro* fertilization and embryo transfer [13, 14]. Particular attention has been recently paid to *Atopobium vaginae*, a newly identified bacterium, belonging to the *Coriobacteriaceae* family, which is believed to be at least a partial cause of the above mentioned complications [13]. The genus *Atopobium*, described for the first time in 1992, includes bacteria previously classified as lactobacilli. Rodriguez first identified *A. vaginae* in a study on vaginal lactobacilli [21]. *A. vaginae* 16s rRNA gene differs from the other species belonging to *Atopobium* genus by approximately 3-8% [22, 23]; this enabled Rodriguez to identify it as a new species. The isolate can be distinguished from *A. minutum*, *A. parvulum*,

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and A. rimae by biochemical tests and protein electrophoresis of the whole cell (Table 1). Gram stain shows A. vaginae as a small coccus, rounded or oval, or rods, visible as single cells, in pairs or in short chains (Fig. 1).

![Fig. (1). A) Grey-white colonies of A. vaginae after 48h culture in anaerobic conditions. B) Gram staining shows Gram-positive bacteria, with A. vaginae visible as single cells, in pairs or short chains. Geissdorfer et al. 2003 [41].](image)

| Enzyme                   | A. vaginae | A. minutum | A. parvulum | A. rimae |
|--------------------------|------------|------------|-------------|----------|
| Acid phosphatase         | +          | -          | +           | +        |
| Alanine arylaminidase    | -          | -          | +           | -        |
| Arginine dihydrolase     | +          | +          | -           | -        |
| Arginine arylaminidase   | +          | +          | +           | -        |
| Histidine arylaminidase  | +          | -          | -           | -        |
| B-Galactosidase          | -          | -          | +           | -        |
| Leucine arylaminidase    | +          | +          | -           | -        |
| Proline arylaminidase    | +          | +          | -           | -        |
| Pyroglutamic acid arylaminidase | - | v | + | + |
| Glycine arylaminidase    | +          | -          | +           | -        |
| Serine arylaminidase     | +          | -          | -           | -        |
| Thyroxine arylaminidase  | -          | -          | +           | -        |

+, the enzyme is expressed constitutively; -, the enzyme is absent and cannot be induced; v, expression of the enzyme is variable Modified, from Rodriguez et al. 1999 [21].

This aerobic facultative, gram-positive bacterium cannot be easily isolated by classical microbiological methods [14, 24]. It is hardly detected in healthy women vaginal fluid but is commonly found in the vagina of patients with BV: 50% according to Burton [25, 26], 70% according to Ferris [27], and more than 95% according to Verhelst et al. [24] and Verstraeten et al. [28]. In symptomatic BV it has been detected together with Gardnerella vaginalis in the biofilm adherent to the vaginal mucosa [24]. This was confirmed by Swidsinski et al. [7] who, by examining the composition and structural organization of the biofilm, found that Gardnerella vaginalis accounted for 60-95% of the film mass. In addition, in 70% of biopic samples, Atopobium vaginae accounted for the 1-40% of the film mass. Lactobacillus concentrations were lower than 10^6 CFU/mL, making up only 5% of the biofilm (Fig. 2).

**Therapy**

Concerning the pharmacological therapy, CDC recommends either oral or topical (vaginal gel) metronidazole once a day for 5 days as first choice for BV. Efficacy is comparable to topical clindamycin [29]. Cure rates, following intravaginal treatment with metronidazole or clindamycin, account for 80-90% at the end of treatment and one month after the end of therapy [13, 14, 30]. However, three months after the end of therapy the rate of relapses can overcome 30%. Persistence of an adherent bacterial biofilm, containing mostly G. vaginalis and A. vaginae, seems to be the main reason for failure of BV treatment [30]. Suppressive treatment with metronidazole gel and physiological approaches (use of probiotics or acidifying) have been investigated with variable results [31]. Moreover, long-term treatment with metronidazole is not recommended because of the high incidence of gastrointestinal adverse reactions, the risk of peripheral neuropathy, and Candida super infection [32].
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Antibiotic Sensitivity

Failures with metronidazole in patients with recurrent or persistent BV [33, 34] might conceivably reflect the newly found mechanism of formation of a biofilm containing G. vaginalis together with A. vaginae [7, 9, 13, 28] (Fig. 3). The fact that A. vaginae is resistant to metronidazole, and that the bacterium creates a biofilm in which it is associated with G. vaginalis, complicates the response to the antibiotic [9, 13, 28]. Though clindamycin is more active than metronidazole against both G. vaginalis and A. vaginae, its negative effects on lactobacilli leave the way open to microbial disorders that can cause frequent super infections and recurrences. Moreover, an increasing resistance to antibiotics that act like clindamycin, by blocking protein synthesis has been reported. A randomized prospective trial compared 119 women assigned to two therapeutic regimens for BV: either metronidazole vaginal gel for five days, or clindamycin vaginal tablets for three days. The clinical efficacy was comparable in the two arms: after 7-12 days about 80% of the patients were cured, but this percentage fell down to about 50% after 35-45 days. Following clindamycin treatment – but not metronidazole - there was a steep rise in the percentage of women with at least one clindamycin resistant strain isolated. Moreover, 70-90 days after the end of treatment, about 80% of the women who received clindamycin presented in their vaginal swabs anaerobic bacteria resistant to that drug [35].

Togni et al. [36] compared the in vitro susceptibility of A. vaginae to nifuratel, metronidazole and clindamycin. Susceptibility to metronidazole was variable, with MIC ranging from 8 to 256 µg/mL. Nifuratel and clindamycin inhibited the growth of all the tested strains, with MIC from 0.125 to 1 µg/mL and below 0.125 µg/mL, respectively (Table 2). The findings related to metronidazole and clindamycin are in line with previously published studies [37].

Table 2. MIC Ranges (µg/mL) and MIC₅₀ (µg/mL) of Metronidazole, Clindamycin and Nifuratel against Atopobium vaginae

| Antimicrobial Agent | MIC Range (µg/ml) | MIC₅₀ (µg/ml) |
|--------------------|------------------|--------------|
| Metronidazole      | 8 - 256          | 32           |
| Clindamycin        | < 0.125          | < 0.125      |
| Nifuratel          | 0.125 - 1        | 0.5          |

Togni et al. 2011 [30].

In the same study, the activity of these antibiotics was assayed on lactobacilli and G. vaginalis. Either nifuratel and metronidazole did not affect the normal lactobacterial flora, while clindamycin inhibited all tested strains of lactobacilli. Nifuratel and metronidazole were both highly active against G. vaginalis (Fig. 4). The susceptibility of Atopobium vaginae to metronidazole and clindamycin, and the action on lactobacilli and G. vaginalis were in line with previous reports [37-39]. To summarise, nifuratel was active against A. vaginae and G. vaginalis strains without affecting lactobacilli; metronidazole was active against A. vaginae, but only at very high concentrations, partially active against G. vaginalis, and did not affect lactobacilli; clindamycin was extremely effective against A. vaginae and G. vaginalis, but it also affected the lactobacilli, altering the vaginal ecosystem.

CONCLUSIONS

The discovery of the presence of Atopobium vaginae in the vaginal ecosystem improves the basic understanding of
the pathogenesis of BV [28]. This bacterium is presumably the main reason for failures or recurrences after BV treatment with metronidazole, since it is found in 80-90% of cases of relapse [40]. Prospective studies are now needed to show whether metronidazole-resistant microorganisms, such as *Atopobium vaginae*, are involved in recurrences. Information to date suggests that nifuratel is probably the most valid therapeutic agent for BV, as it is highly active against *Gardnerella vaginalis* and *Atopobium vaginae*, without affecting lactobacilli which are fundamental for the system health and balance [30].

**CONFLICT OF INTEREST**

Declared none.

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