**In vitro activity of secnidazole against Atopobium vaginae, an anaerobic pathogen involved in bacterial vaginosis**

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**Abstract**

Bacterial vaginosis is a polymicrobial syndrome. The most important marker for bacterial vaginosis is the presence of Gardnerella vaginialis and Atopobium vaginae. In this study, the in vitro susceptibilities to metronidazole and secnidazole of 16 strains of *A. vaginae* were tested with the agar dilution method. We observed an MIC range for metronidazole of 4–64 mg/L (MIC₅₀ 8 mg/L; MIC₉₀ 32 mg/L) and an MIC range for secnidazole of 4–128 mg/L (MIC₅₀ 16 mg/L; MIC₉₀ 64 mg/L). According to these findings, we can conclude that the activity of secnidazole is similar to that of metronidazole.

**Keywords:** *Atopobium vaginae*, bacterial vaginosis, metronidazole, resistance, secnidazole

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Bacterial vaginosis (BV) is a polymicrobial syndrome in which the lactobacilli of the normal microflora are overgrown by Gram-positive and Gram-negative bacteria and for which high concentrations of *Gardnerella vaginalis* and, recently, *Atopobium vaginae* have been recognized as important microbiological markers [1,2], whereby a high concentration of *A. vaginae* is a better marker than a high concentration of *G. vaginalis* [2,3]. Both species are frequently found together in the biofilm formed in BV [4]. Metronidazole and clindamycin are the preferred treatment regimens. In this study, the in vitro activities of secnidazole against members of the *Atopobium* group were tested.

Secnidazole is a member of the family of 5-nitro-imidazoles, as is metronidazole. Secnidazole could be a valuable alternative for metronidazole, owing to a longer half-life and fewer side effects [5]. The longer half-life makes single-dose applications possible, which could avoid treatment failures caused by patient compliance. Previously, the susceptibility to secnidazole was determined by Dubreuil et al. [6] for several strictly anaerobic bacteria, including bacteria involved in BV, although *A. vaginae* was not included. High MIC values of *A. vaginae* for metronidazole have been reported [7,8].

For this study, 16 strains of *A. vaginae*, two strains of *Atopobium parvulum* and one strain each of *Atopobium rimae* and *Atopobium minutum* were tested. Six strains of *A. vaginae* (CCUG 44258, CCUG 42099, CCUG 44125, CCUG 44061 and CCUG 38953) and one strain each of *A. minutum* (CCUG 31167), *A. rimae* (CCUG 31168) and *A. parvulum* (CCUG 32760) were obtained from the Culture Collection of the University of Göteborg. All other strains were clinical vaginal isolates obtained during studies at the Laboratory Bacteriology Research from 2003 until 2006. In addition, two quality control strains were included, i.e. *Bacteroides thetaiotaomicron* CCUG 34778 and *Bacteroides fragilis* CCUG 4856.

For both metronidazole (Dr Ehrenstorfer, Augsburg, Germany) and secnidazole (Ethypharm, Houdan, France), a stock solution of 5120 mg/L was prepared. Secnidazole was dissolved in dimethylsulphoxide (DMSO), and the DMSO concentration was afterwards adjusted to 10% with sterile distilled water. Two-fold dilution series in distilled water were made for both antibiotics, as previously described [9].

One millilitre of each dilution was incorporated in 19 mL of Mueller–Hinton agar, supplemented with 5% sterile defibrinated sheep blood (E&O Laboratories, Bonnybridge, UK) at 50°C. Plates contained Mueller–Hinton agar with serial two-fold dilutions of antimicrobial agents between 256 and 0.5 mg/L, and two negative control plates to which 1 mL of distilled water or 1 mL of a 10% DMSO solution was added. The plates were dried with open lids for 1 h and stored at 4°C. Plates were pre-incubated for 5 h in an anaerobic workstation (BugBoxPlus; LED techno, Heusden-Zolder, Belgium).
Strains were cultured on Mueller–Hinton agar + 5% sheep blood (Becton Dickinson, Erembodegem, Belgium) for 72 h in an anaerobic workstation (BugBoxPlus; LED Techno) at 37°C. Strains were harvested and suspended in sterile saline (0.9% NaCl) at a density equal to a McFarland standard (Bio-Mérieux, Boxtel, The Netherlands) of 0.5. Immediately after inoculation of the strains with a Steers replicator (Mast Systems, London, UK), plates were incubated in the anaerobic workstation for 72 h at 37°C.

The MIC values for metronidazole and secnidazole were, respectively, 1 and 2 mg/L for both control strains, B. fragilis CCUG 4856 and B. thetaiotaomicron CCUG 34778; these values are within the expected range. Both negative control plates showed dense growth for all strains, and no difference was seen between the negative control plates containing 0.5% DMSO and distilled water. For the 16 strains of A. vaginae, we observed an MIC range for metronidazole of 4–64 mg/L (MIC50, 8 mg/L; MIC90, 32 mg/L) and an MIC range for secnidazole of 4–128 mg/L (MIC50, 16 mg/L; MIC90, 64 mg/L). The results for all the strains are listed in Table 1.

This study was carried out to compare the in vitro MIC values of Atopobium strains for secnidazole in comparison with metronidazole. It is difficult to link these values to susceptibility breakpoints, for several reasons. Different authorities (CLSI, EUCAST) have determined breakpoints for resistance for metronidazole, but not yet for secnidazole. CLSI breakpoints are general for all nitro-imidazole molecules, but EUCAST criteria are only specified for metronidazole. In addition, not much is known about the pharmacokinetics of these compounds in the vagina.

With use of a topical treatment, high concentrations of metronidazole can be achieved, although the most common administration route is the oral one. Davis et al. [10] reported that a maximum concentration of 26 mg/L could be reached in the vaginal fluid 6 h after oral administration of 2 g of metronidazole, at which time it equals the serum concentration. These data correspond to those of Matilla et al. [11], showing that the maximal serum concentration of 9.4 mg/L is reached 1.9 h after oral administration of 500 mg of metronidazole. The half-life of metronidazole ranges from 7 to 9.7 h, in contrast to the half-life of secnidazole, which ranges from 17 to 28.8 h with a Cmax of 35.7–46.3 mg/L for a 2-g dose [12]. Recent studies confirm these previous results (B. Dickey, 2009, to be published). Therefore, it seems appropriate that EUCAST and CLSI should propose new criteria for secnidazole in accordance with these findings.

Bradshaw et al. [2] reported that, despite a significant reduction of A. vaginae numbers after treatment with oral metronidazole (400 mg twice daily for 7 days), BV cases with both A. vaginae and G. vaginalis had much higher rates of recurrent BV (83%) than those with G. vaginalis only (38%). This could possibly be explained by acquired resistance, e.g. through the presence and activation of nim genes [13] or by the biofilm that is present in BV [4]. According to a recent meta-analysis of published results [14], treatment of both partners may be important for the eradication of BV.

### TABLE 1. Susceptibilities of Atopobium strains to metronidazole and secnidazole

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Metronidazole MIC (mg/L)</th>
<th>Secnidazole MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopobium minutum</td>
<td>CCUG 31167</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Atopobium panulum</td>
<td>CCUG 32760</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Atopobium panulum</td>
<td>TiG29</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Atopobium rimae</td>
<td>CCUG 31168</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Atopobium vaginae</td>
<td>CCUG 38937</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Atopobium vaginae</td>
<td>CCUG 42099</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Atopobium vaginae</td>
<td>CCUG 44061</td>
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<td>16</td>
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<td>Atopobium vaginae</td>
<td>CCUG 44116</td>
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<td>16</td>
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<td>8</td>
<td>8</td>
</tr>
<tr>
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<td>CCUG 44258</td>
<td>4</td>
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<tr>
<td>Atopobium vaginae</td>
<td>FB010-06</td>
<td>64</td>
<td>128</td>
</tr>
<tr>
<td>Atopobium vaginae</td>
<td>FB101-3C</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Atopobium vaginae</td>
<td>FB106B</td>
<td>8</td>
<td>16</td>
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<tr>
<td>Atopobium vaginae</td>
<td>FB130-CNAB-2aD</td>
<td>16</td>
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<tr>
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<td>FB145-SA-14A</td>
<td>8</td>
<td>16</td>
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<tr>
<td>Atopobium vaginae</td>
<td>FB158-CNAB-2C</td>
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<td>Atopobium vaginae</td>
<td>FB160-CNAB-7</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
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<td>PB200309-T1-4</td>
<td>16</td>
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<td>Atopobium vaginae</td>
<td>PB200309-T1-8</td>
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<td>PB2003189-T1-4</td>
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<td>8</td>
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<tr>
<td>Bacteroides fragilis</td>
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<td>1</td>
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<tr>
<td>Bacteroides thetaiotaomicron</td>
<td>CCUG 34778</td>
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</tr>
</tbody>
</table>

T, type strain.

**Transparency Declaration**

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### References


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Prevalence of acquired AmpC β-lactamases in Enterobacteriaceae lacking inducible chromosomal ampC genes at a Spanish hospital from 1999 to 2007

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Abstract

In 2007, a significant increase in acquired ampC genes in Enterobacteriaceae from 0.06% in 1999 to 1.3% was observed. Proteus mirabilis showed the highest prevalence (0.95%) and CMY-2 was the most prevalent AmpC enzyme (66.7%). Other enzymes such as CMY-4, DHA-1, ACC-1, and three new enzymes called CMY-25, CMY-27 and CMY-40 were detected. Seven out of the 117 isolates (6%) also produced an extended-spectrum β-lactamase. As acquired AmpC enzymes are likely to become a serious public health issue worldwide, close surveillance is necessary to curb their spread.

Keywords: AmpC β-lactamases, antimicrobial resistance mechanism, epidemiology of resistance

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Acquired AmpCs appeared in the late 1980s and have been detected mainly in isolates of Klebsiella spp., Escherichia coli, Proteus mirabilis and Salmonella spp. although they have also been identified in other species including natural AmpC producers [1]. These enzymes confer resistance to most β-lactams – including cefamycins – with the exception of cefepime and carbapenems [2].

Most acquired ampCs derive from chromosomal ampC genes of the family Enterobacteriaceae (Citrobacter freundii, Enterobacter spp., Morganella morganii and Hafnia alvei) whereas the origin of others remains unknown. Isolates harbouring acquired ampCs are usually multi-resistant [3–6], limiting the therapeutic options even further. In this context, we aimed to determine the prevalence of acquired AmpCs in Enterobacteriaceae isolates lacking inducible chromosomal ampC genes at a Spanish hospital from January 1999 to December 2007.

Isolates were obtained from routine cultures at the Hospital de la Santa Creu i Sant Pau (Barcelona, Spain). When there were multiple isolates from a patient within a 30-day period, only one was considered for analysis. Isolates were identified using standard methods [7]. The disc diffusion susceptibility test was performed according to Clinical Laboratory Standards Institute (CLSI) guidelines [8], using commercially available discs (Bio-Rad, Marnes La Coquette, France). The production of Extended-spectrum beta-lactamase (ESBL) was studied using the double-disc synergy test.