Antibiotic susceptibility of *Atopobium vaginae*
Ellen De Backer*1, Rita Verhelst1, Hans Verstraelen2, Geert Claeys1, Gerda Verschraegen1, Marleen Temmerman2 and Mario Vaneechoutte1

Address: 1Department of Clinical Chemistry, Microbiology & Immunology, Ghent University Hospital, UGent, Ghent, Belgium and 2Department of Obstetrics & Gynaecology, Ghent University Hospital, UGent, Ghent, Belgium

Email: Ellen De Backer* - ellekendb@yahoo.com; Rita Verhelst - Rita.Verhelst@UGent.be; Hans Verstraelen - Hans.Verstraelen@UGent.be; Geert Claeys - Geert.Claeys@UGent.be; Gerda Verschraegen - Gerda.Verschraegen@UGent.be; Marleen Temmerman - Marleen.Temmerman@UGent.be; Mario Vaneechoutte - Mario.Vaneechoutte@UGent.be
* Corresponding author

Abstract

**Background:** Previous studies have indicated that a recently described anaerobic bacterium, *Atopobium vaginae* is associated with bacterial vaginosis (BV). Thus far the four isolates of this fastidious micro-organism were found to be highly resistant to metronidazole and susceptible for clindamycin, two antibiotics preferred for the treatment of BV.

**Methods:** Nine strains of *Atopobium vaginae*, four strains of *Gardnerella vaginalis*, two strains of *Lactobacillus iners* and one strain each of *Bifidobacterium breve*, *B. longum*, *L. crispatus*, *L. gasseri* and *L. jensenii* were tested against 15 antimicrobial agents using the Etest.

**Results:** All nine strains of *A. vaginae* were highly resistant to nalidixic acid and colistin while being inhibited by low concentrations of clindamycin (range: < 0.016 µg/ml), rifampicin (< 0.002 µg/ml), azithromycin (< 0.016 – 0.32 µg/ml), penicillin (0.008 – 0.25 µg/ml), ampicillin (< 0.016 – 0.94 µg/ml), ciprofloxacin (0.023 – 0.25 µg/ml) and linezolid (0.016 – 0.125 µg/ml). We found a variable susceptibility for metronidazole, ranging from 2 to more than 256 µg/ml. The four *G. vaginalis* strains were also susceptible for clindamycin (< 0.016 – 0.047 µg/ml) and three strains were susceptible to less than 1 µg/ml of metronidazole. All lactobacilli were resistant to metronidazole (> 256 µg/ml) but susceptible to clindamycin (0.023 – 0.125 µg/ml).

**Conclusion:** Clindamycin has higher activity against *G. vaginalis* and *A. vaginae* than metronidazole, but not all *A. vaginae* isolates are metronidazole resistant, as seemed to be a straightforward conclusion from previous studies on a more limited number of strains.

**Background**

Bacterial vaginosis is considered a common vaginal disorder in women of reproductive age. Whereas normal vaginal microflora consists of lactobacilli, especially *L. crispatus* [1-4], the disturbed vaginal microflora is characterized by the overgrowth of *Gardnerella vaginalis* and anaerobic bacteria such as *Mobiluncus* spp., *Mycoplasma hominis* and *Prevotella* spp. Recently several research groups showed – by means of cloning of the 16S rRNA-gene [3,5], by Terminal Restriction Fragment Length Polymorphism analysis of the 16S rRNA gene (T-RFLP) [6,7], by specific PCR [5,8,9], by Denaturing Gradient Gel Electrophoresis of the 16S rRNA gene (DGGE) [10] and by FISH [3,11] – that a previously unrecognized organism,
Atopobium vaginae, was strongly associated with bacterial vaginosis and with Gardnerella vaginalis.

During the last decade, the interest for bacterial vaginosis increased because of reports of adverse sequelae of this disorder, such as preterm birth [12-14], pelvic inflammatory disease [15,16] and postpartum endometritis [17]. In addition, several publications showed that an altered vaginal microflora is linked to an increased susceptibility to the acquisition of HIV [18,19] and other sexually transmitted infectious agents such as Neisseria gonorrhoeae and Chlamydia trachomatis [18,20].

The severity of the consequences of such sequelae asks for an adequate treatment of bacterial vaginosis. Currently the preferred antibiotic treatment regimen consists of clindamycin or metronidazole (oral or intravaginal). Recurrence rates of up to 30% within 3 months after treatment have been reported [21,22]. This recurrence might be due to the survival of metronidazole or clindamycin resistant bacteria in the vagina, although Beigi et al. [23] showed recently that less than one percent of the cultivable vaginal anaerobic bacteria is resistant to metronidazole.

Another possible explanation might be the presence of some fastidious to unculturable, metronidazole resistant organism, a role for which A. vaginae is a likely candidate, since Geißdörfer et al. [24] and Ferris et al. [10] reported an MIC of > 32 µg/ml for metronidazole for the four isolates that were tested.

Here, we studied the susceptibility of nine other A. vaginae isolates for 15 antimicrobial agents by using the Etest and for comparison we included a limited number of other important vaginal bacteria such as lactobacilli, G. vaginalis and bifidobacteria and we compared our results with those of previously published articles.

Methods

Nine strains of Atopobium vaginae, four strains of Gardnerella vaginalis, two strains of Lactobacillus iners and one strain each of Bifidobacterium breve, B. longum, Lactobacillus crispatus, L. gasseri and L. jenseni were tested against 15 antibiotics (ampicillin, azithromycin, bacitracin, cefuroxime, ciprofloxacin, clindamycin, colistin, doxycycline, kanamycin, linezolid, metronidazole, nalidixic acid, penicillin, rifampicin and vancomycin) [see Additional file 1]. Six strains of A. vaginae (CCUG 42099, CCUG 44116, CCUG 44258, CCUG 38953T, CCUG 44125, CCUG 44061) were kindly provided by the Culture Collection of Göteborg (Sweden). The other strains were obtained from studies by cultivating vaginal samples (with informed consent) and identified by tDNA-PCR and 16S rRNA gene sequencing [4].

Strains were cultivated anaerobically using the GasPak anaerobic envelope system (Becton Dickinson, Erembodegem, Belgium) at 37 °C on Trypticase Soy Agar (TSA) + 5% sheep blood (Becton Dickinson). Epsilometer tests (Etest AB Biodisk, Solna, Sweden) were used to determine the minimal inhibitory concentrations (MIC). An inoculum taken from TSA + 5% sheep blood was suspended in 0.5 ml of physiological water and adjusted to the turbidity of a 1 McFarland standard. Before inoculating the plates and testing for metronidazole, plates were anaerobically incubated during 24 h. After application of the epsilometer, test plates were anaerobically incubated at 37°C. The inhibitory concentration was defined as the value on the test strip scale at which the inhibition zone intersected the strip edge, according to the manufacturers’ instructions. MIC-values were read after 48 h for G. vaginalis, Lactobacillus spp. and Bifidobacterium spp. and after 72 h for A. vaginae.

Results and discussion

Value and reproducibility of Etest for MIC-determination of anaerobes

In this study we used the Etest for MIC-determination of vaginal bacteria towards 15 antibiotics. Croco et al. [25] concluded, from a study whereby the Etest was compared with the reference agar dilution method, that the Etest was a quantitatively accurate and reproducible method for routinely testing the antimicrobial susceptibilities of anaerobes, in particular of organisms associated with BV. Discordances were reported only for clindamycin susceptibility testing of lactobacilli and chloramphenicol susceptibility testing of the Bacteroides fragilis group. Schieven et al. [26] reported an agreement between the Etest and the reference agar dilution method within 1 log, dilution of 87.4% for clindamycin and 85.1% for metronidazole for a total of 150 anaerobic isolates tested.

Antimicrobial susceptibility testing of Atopobium vaginae

The antibiotic susceptibility for ampicillin, azithromycin, bacitracin, cefuroxime, ciprofloxacin, clindamycin, colistin, doxycycline, kanamycin, linezolid, metronidazole, nalidixic acid, penicillin, rifampicin and vancomycin obtained for nine Atopobium vaginae isolates in this study is listed in additional file 1 [see Additional file 1].

We focused our research on the antibiotic susceptibility profile of Atopobium vaginae. In contrast to the results of Ferris et al. [10] and Geißdörfer et al. [24], who reported MIC-values of more than 32 µg/ml for all four A. vaginae isolates tested (Table 1), we found a great variability in susceptibility for metronidazole, ranging from 2 to more than 256 µg/ml. When taking into account 16 µg/ml as the breakpoint between sensitive and resistant (NCCLS guidelines), only four out of 9 strains in our study could be accounted as intermediately susceptible or resistant. All
nine A. vaginae isolates in our study showed MIC-values of more than 256 µg/ml for nalidixic acid and colistin while being inhibited by low concentrations of clindamycin (range: < 0.016 – 0.047 µg/ml), azithromycin (< 0.016 – 0.25 µg/ml), penicillin (0.008 – 0.047 µg/ml), ampicillin (< 0.016 – 0.94 µg/ml), ciprofloxacin (0.023 – 0.25 µg/ml) and linezolid (0.016 – 0.125 µg/ml) (Table 1).

We previously described the existence of a second genotype within A. vaginae. To this genotype belongs isolate PB2003/189-T1-4, of which the 16S rRNA gene sequence differs at 23 positions compared to the type strain, but is identical to that of an isolate no longer in our collection of which the sequence was submitted [Genbank: AJ585206] [5]. This genotype 2 isolate showed a marked difference only for azithromycin (i.e. MIC of 0.32 µg/ml) with the other isolates (MIC of < 0.016 µg/ml).

### Antimicrobial susceptibility testing of Gardnerella vaginalis

We tested 4 strains of Gardnerella vaginalis. By using the Etest method in an anaerobic environment we found in general slightly lower values for ampicillin (range: < 0.016 – 0.047 µg/ml), penicillin (0.004 – 0.047 µg/ml), cefuroxime (< 0.016 – 0.125 µg/ml) and rifampicin (0.5 – 0.75 µg/ml) (Table 1), compared to other publications [25,27,28] [see Additional file 2]. The range of the MIC-values of Gardnerella vaginalis for clindamycin (range: < 0.016 – 0.047 µg/ml), doxycycline (0.38 – 0.75 µg/ml), kanamycin (12 – 16 µg/ml), metronidazole (0.25 – 32 µg/ml), nalidixic acid (> 256 µg/ml) and vancomycin (0.125 – 0.38 µg/ml) is comparable with other studies [25,27-31] [see Additional file 2].

G. vaginalis is, according to NCCLS standards for anaerobic bacteria, susceptible to ampicillin (range: < 0.016 – 0.047 µg/ml), penicillin (0.004 – 0.047 µg/ml) and azithromycin (< 0.016 – 0.047 µg/ml). In 1993, Goldstein et al. [29] reported that 20% of G. vaginalis strains were resistant to metronidazole (MIC ≥ 16 µg/ml). In 2002, the same group reported a resistance of 29% to metronidazole for...
Agents tested in this study.

There were no literature data to compare the susceptibility of L. iners and L. jensenii for the antimicrobial agents tested in this study.

**Antimicrobial susceptibility testing of Bifidobacterium spp**

Both of the tested Bifidobacterium strains are sensitive to a low amount of ampicillin (B. longum: 0.25 µg/ml, B. breve: 0.38 µg/ml), azithromycin (B. longum: 0.064 µg/ml, B. breve: 0.25 µg/ml), clindamycin (B. breve and B. longum: < 0.016 µg/ml), linezolid (B. longum: 0.25 µg/ml, B. breve: 0.38 µg/ml), penicillin (B. longum: 0.19 µg/ml, B. breve: 0.5 µg/ml), rifampicin (B. longum: 0.25 µg/ml, B. breve: 0.19 µg/ml) and vancomycin (B. longum: 0.38 µg/ml, B. breve: 1 µg/ml) [see Additional file 2]. The strains tested showed comparable results with previous publications except for linezolid and kanamycin [see Additional file 2], where we obtained a lower value than described [33-35].

**Conclusion**

Bacterial vaginosis is a polymicrobial disease and the organisms involved are likely to be in symbiotic relationship to each other for various metabolic requirements. Antimicrobial treatment may affect susceptible members of the consortia which may negatively alter the microenvironment for resistant organisms, such as A. vaginae and G. vaginalis.

By knowing the antibiotic susceptibility of the vaginal species it might be possible to develop new regimens for the treatment of recurrent bacterial vaginosis.

For example, this study showed that metronidazole resistance of A. vaginae is not an intrinsic feature. Further research needs to make clear whether this metronidazole resistance might be acquired by the presence and activation of nim-genes [36]. Metronidazole resistance up to 29% [30] has been described for G. vaginalis but mechanisms are not yet clarified. Possibly this could be due to the lack of nitroreductases necessary to produce the hydroxymetabolite of metronidazole, which has stronger antibiotic activity than the parent compound.

Lactobacilli are resistant to metronidazole and it has been demonstrated that recolonization of the vagina by H₂O₂-producing lactobacilli after metronidazole treatment occurs more frequently compared to clindamycin treatment [37].

Clindamycin, which is frequently used as a treatment for bacterial vaginosis, has indeed higher activity against G. vaginalis and A. vaginae than metronidazole, but L. crispatus is more susceptible to clindamycin than L. gasseri with as a consequence that a regimen of clindamycin can remove also the H₂O₂-producing lactobacilli from the vaginal microflora (Table 2). Hydrogen peroxide production is generally believed to be an important factor in the preservation of a normal vaginal microflora [1], i.e. in the build up of vaginal colonisation resistance. There-
fore, it is possible that clindamycin treatment may diminish the vaginal colonisation resistance. Moreover, resistance to clindamycin seems to develop more readily than resistance to metronidazole, as becomes apparent from clinical studies comparing both antibiotics [23,37].

Competing interests
The author(s) declare that they have no competing interests.

Authors' contributions
EDB, RV, GC, GV and MV participated in the development of the study design, the analysis of the study samples, analysis and interpretation of the data, and in the writing of the report. HV and MT participated in the development of the study design, analysis and interpretation of the data, and in the writing of the report. All authors read and approved the final manuscript.

Additional File 1
Antibiotic susceptibility testing of vaginal bacteria for 15 antibiotics. The table lists the antibiotic susceptibility for ampicillin, azithromycin, bacitracin, cefazoline, ciprofloxacin, clindamycin, colistin, doxycycline, linezolid, metronidazole, nalidixic acid, penicillin, rifampicin and vancomycin obtained for nine strains of Atopobium vaginae, four strains of Gardnerella vaginalis, two strains of Lactobacillus iners and one strain each of Bifidobacterium breve, B. longum, Lactobacillus crispatus, L. gasseri and L. jensenii.

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Additional File 2
Comparison between the results from our study and those from other publications. The table lists the results from our study for the antibiotic susceptibility of Bifidobacterium breve, B. longum, Gardnerella vaginalis, Lactobacillus crispatus and L. gasseri compared to those from other publications.

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