



Antimicrobial susceptibility of anaerobic bacteria

Susceptibility of bacterial vaginosis (BV)-associated bacteria to secnidazole compared to metronidazole, tinidazole and clindamycin

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ABSTRACT

Secnidazole, a 5-nitroimidazole with a longer half-life, is structurally related to metronidazole and tinidazole. For treatment of bacterial vaginosis (BV), secnidazole is a suitable single-dose oral drug having a longer serum half-life than metronidazole. The objective of this study was to evaluate the antimicrobial susceptibility of vaginal isolates of facultative and anaerobic bacteria to secnidazole, metronidazole, tinidazole and clindamycin.

A total of 605 unique BV-related bacteria and 108 isolates of lactobacilli recovered from the human vagina of US women during the years 2009–2015 were tested for antimicrobial susceptibility by the agar dilution CLSI reference method to determine the minimal inhibitory concentration (MIC).

The MIC₉₀ (μg/mL) for secnidazole was similar to metronidazole and tinidazole for *Anaerococcus tetradius* (secnidazole: MIC₉₀ 2; metronidazole: MIC₉₀ 2; tinidazole: MIC₉₀ 4), *Atopobium vaginae* (32; >128; 128), *Bacteroides* species (2; 2; 2), *Fingoldia magna* (2; 2; 4), *Gardnerella vaginalis* (128; 64; 32), *Mageibacillus indolicus* (2; 2; 2), *Megasphaera*-like bacteria (0.5; 0.25; 0.5), *Mobiluncus curtisii* (128; >128; >128) and *Mobiluncus mulieris* (>128; >128; >128), *Peptoniphilus lacrimalis* (4; 4; 4) and *Peptoniphilus harei* (2; 2; 4), *Porphyromonas* species (0.25; 0.5; 0.25), *Prevotella bivia* (8; 8; 8), *Prevotella amnii* (2; 1; 2) and *Prevotella timonensis* (2; 2; 2). In this evaluation, 14 (40%) of 35 *P. bivia*, 5 (14%) of 35 *P. amnii* and 21 (58%) of 36 *P. timonensis* isolates were resistant to clindamycin with MIC values of >128 μg/mL. Secnidazole, like metronidazole, was superior to clindamycin for *Prevotella* spp., *Bacteroides* spp., *Peptoniphilus* spp., *Anaerococcus tetradius* and *Fingoldia magna*. Clindamycin had greater activity against *Atopobium vaginae*, *Gardnerella vaginalis* and *Mobiluncus* spp. compared to the nitroimidazoles. All 27 *Lactobacillus crispatus*, 26 (96%) of 27 *L. jensenii*, 5 (19%) of 27 *L. gasseri* and 18 (67%) of 27 *L. iners* isolates were susceptible to clindamycin (MIC ≤2) while the MIC₉₀ for all lactobacilli tested was >128 μg/mL for secnidazole, metronidazole and tinidazole.

Secnidazole has similar in vitro activity against the range of microorganisms associated with BV compared to metronidazole or tinidazole. Further, secnidazole spares lactobacilli, a characteristic which is desirable in drugs used to treat bacterial vaginosis.

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1. Introduction

Bacterial vaginosis (BV) is a common vaginal syndrome affecting 29% of reproductive age women in the United States [1]. BV is characterized by a shift in vaginal microbiota from *Lactobacillus*

dominance to a high diversity microbiome with increased *Gardnerella vaginalis*, *Atopobium vaginae* and other anaerobic microorganisms, such as *Megasphaera*, *Sneathia*, and *Leptotrichia* species [2,3]. BV has been found to be associated with an increased risk of sexually transmitted infections, including *Chlamydia trachomatis* [4], *Neisseria gonorrhoeae* [4], herpes simplex virus 1 and 2 [5,6], human immunodeficiency virus (HIV) [7,8], and *Trichomonas vaginalis* [4]. In addition, BV is a risk factor for reproductive health sequelae including pelvic inflammatory disease (PID) [9] and pre-term birth [10].

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The 2015 Centers for Disease Control and Prevention (CDC) recommended treatments for BV include oral metronidazole taken twice a day for seven days, five days of an intravaginal metronidazole gel, or seven days of an intravaginal clindamycin cream [11]. Other FDA approved treatments for BV include tinidazole taken orally for multiple days [12], a single dose metronidazole gel [13] and a single dose clindamycin cream treatment [14]. Even with a variety of antimicrobial agents available for the treatment of BV, recurrence occurs after 12 months for almost 60% of women [15]. Therefore, new therapeutic strategies are needed to more effectively treat this common condition.

Secnidazole, a second-generation 5-nitroimidazole, has a longer half-life than both metronidazole and tinidazole (~20 vs ~8 vs ~14 h) [16] and therefore has the potential of becoming a suitable single oral dose to treat women with BV. Outside of the United States, secnidazole is used not only to treat BV but also a variety of other infections including trichomoniasis, giardiasis and amoebiasis [17,18]. A single dose of secnidazole was shown in a European study to provide a therapeutic response similar to that of oral metronidazole twice-a-day for 7 days for treatment of BV [19]. Secnidazole, like other nitroimidazoles, also has limited activity against beneficial microbes such as *Lactobacillus* species, a preferred characteristic for an antibiotic used to treat vaginal infections. The objective of this study was to evaluate the antimicrobial susceptibility of vaginal isolates of facultative and anaerobic bacteria to secnidazole compared to metronidazole, clindamycin and tinidazole.

2. Materials and methods

2.1. Bacterial isolates

A total of 605 BV-associated bacteria isolates and 108 isolates of lactobacilli were recovered from the human vagina of US women during the years 2009–2015 in human subject protocols approved by the University of Pittsburgh IRB. Vaginal cultures were performed as previously described [20]. The *G. vaginalis* isolates were identified by their characteristic colony morphology, beta hemolysis on human bilayer agar with Tween (Becton Dickinson, Rockville, MD), Gram stain showing gram-variable pleomorphic rods, and negative catalase reaction. DNA from the anaerobic bacteria was extracted using PrepMan™ Ultra Sample Preparation Reagent (Applied Biosystems, Foster City, CA). 16S rDNA for restriction fragment length polymorphism (RFLP) analysis was completed using HaeIII (Promega, Madison, WI) restriction enzyme for identification of *A. vaginae*, *Bacteroides* species, *Mageeibacillus indolicus*, *Mobiluncus* species, and *Prevotella* species. HinfI (Promega, Madison, WI) restriction enzyme was used to identify *A. tetradius*, *F. magna* and *Peptoniphilus* species. Both HaeIII and HinfI restriction enzymes were used to identify *Megasphaera*-like bacteria, novel bacteria which have not yet been placed into a taxonomic group. *Porphyromonas* species were identified using both HaeIII and TaqI (Promega, Madison, WI) restriction enzymes. *Lactobacillus* species were identified using repetitive sequence polymerase chain reaction fingerprinting [21] and if needed, 16S rDNA for RFLP analysis using HpyCH4V (New England Biolab, Ipswich, MA) restriction enzymes. The RFLP patterns for each species were confirmed by 16S rDNA sequences compared to the GenBank data library using the nucleotide BLAST program.

The following organisms were included in this susceptibility study: *Anaerococcus tetradius* (n = 30); *Atopobium vaginae* (n = 25); *Bacteroides* species (n = 27); *Finegoldia magna* (n = 30); *Gardnerella vaginalis* (n = 110); *Mageeibacillus indolicus* (n = 11); *Megasphaera*-like type 1 (n = 76) and type 2 (n = 47); *Mobiluncus curtisii* (n = 51) and *M. mulieris* (n = 12); *Peptoniphilus harei* (n = 30) and

P. lacrimalis (n = 30); *Porphyromonas* species (n = 20); *Prevotella amnii* (n = 35), *P. bivia* (n = 35) and *P. timonensis* (n = 36); and *Lactobacillus crispatus* (n = 27), *L. gasseri* (n = 27), *L. iners* (n = 27) and *L. jensenii* (n = 27).

2.2. Agar dilution susceptibility testing

The vaginal isolates were evaluated for susceptibility to secnidazole (Symbiomix, Newark, NJ), metronidazole, tinidazole and clindamycin (all from Sigma-Aldridge, St. Louis, MO) using the anaerobic agar dilution method described by the Clinical and Laboratory Standards Institute [22,23].

The concentrations of antimicrobial agents used ranged from 0.03 to 128 µg/mL. Prior to testing, *Lactobacillus* species were cultivated on Columbia agar with 5% sheep blood (Becton Dickinson, Rockville, MD) and incubated in anaerobic jars, *G. vaginalis* isolates were cultivated anaerobically on human bilayer agar with Tween (Becton Dickinson, Rockville, MD), and all other isolates were grown anaerobically on Brucella agar (Hardy Diagnostics, Santa Maria, CA). The isolates were isolated to purity and suspended in Brucella broth (Becton Dickinson, Rockville, MD) at a 0.5 McFarland suspension. Using a Steer's replicator, Brucella agar (Remel, Lenexa, KS) plates with 5% Laked Sheep Blood (Hardy Diagnostic, Santa Maria, CA) and varying concentrations of test agent alongside a no drug growth control were inoculated and incubated in anaerobic jars for 48 h at 37 °C. The lowest antibiotic concentration yielding marked reduction to no growth was read as the Minimum Inhibitory Concentration (MIC). Three control strains, *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, and *Clostridium difficile* ATCC 700057 (American Type Culture Collection, Rockville, MD) were used to ensure quality of testing. *G. vaginalis* ATCC 14018 was also used as a supplemental control only when testing *G. vaginalis* isolates. The microbiological susceptibility and resistant breakpoints for clindamycin (≤ 2 µg/mL and ≥ 8 µg/mL) and metronidazole (≤ 8 µg/mL and ≥ 32 µg/mL) as defined by CLSI [22,23] were used for interpretation of MIC results. CLSI does not have a defined susceptibility or resistant breakpoint for either secnidazole or tinidazole.

3. Results

The 713 female genital tract isolates were evaluated for susceptibility to secnidazole, clindamycin, metronidazole and tinidazole. As summarized in Tables 1–4, MIC₅₀ and MIC₉₀ values were similar for secnidazole, metronidazole and tinidazole for *Anaerococcus tetradius*, *A. vaginae*, *Bacteroides* species, *Finegoldia magna*, *G. vaginalis*, *Mageeibacillus indolicus*, *Megasphaera*-like bacteria, *Mobiluncus curtisii* and *M. mulieris*, *Peptoniphilus harei* and *P. lacrimalis*, *Porphyromonas* species, *Prevotella bivia*, *P. amnii* and *P. timonensis*, and all four *Lactobacillus* species.

BV-associated bacteria had susceptibility patterns for clindamycin and the three nitroimidazoles that distributed into three distinct groups. The first group of BV-associated bacteria (Table 1) consisted of anaerobic gram-negative rods and anaerobic gram-positive cocci which were susceptible to metronidazole based on a CLSI susceptibility breakpoint of ≤ 8 µg/mL. For secnidazole, only two *P. bivia* isolates had a MIC value of 16 µg/mL. One *F. magna* isolate also had a MIC of 16 µg/mL for tinidazole. All of the other isolates had MIC values ≤ 8 µg/mL for secnidazole and tinidazole. Clindamycin resistance, on the other hand, was observed in 38% of *Prevotella* species, 30% of *Bacteroides* species, 38% of *Peptoniphilus* species, 20% of *Anaerococcus tetradius* isolates, and 33% of *Finegoldia magna* isolates tested. All of the *Prevotella* and *Bacteroides* species resistant to clindamycin had MIC values of > 128 µg/mL.

A second group of BV-associated bacteria (Table 2) consisted of

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