In Vitro Susceptibility of Trichomonas vaginalis to 50 Antimicrobial Agents

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We determined the susceptibilities of five strains of Trichomonas vaginalis, one of which was metronidazole resistant, to 50 antimicrobial agents. For the metronidazole-susceptible strains, the most active agents were metronidazole, tinidazole, mebendazole, furazolidone, and anisomycin. Against the resistant strain mebendazole, furazolidone, and anisomycin were the most active. Antifungal agents, beta-lactams, macrolides, aminoglycosides, and folic acid antagonists were ineffective against all strains.

Trichomoniasis, the illness caused by the flagellated protozoan Trichomonas vaginalis, is a common sexually transmitted disease estimated to affect at least two million to three million women annually (16). Although symptoms are usually mild to moderate, significant morbidity can occur. Treatment has been revolutionized with the introduction of the 5-nitrimidazoles, principally metronidazole and tinidazole (3, 5, 7). Most trichomonads are susceptible to metronidazole (MIC, ≤1 μg/ml), but metronidazole has been found to be mutagenic for bacteria and carcinogenic for laboratory animals and is contraindicated in pregnancy (3, 5). Moreover, recent T. vaginalis strains for which MICs are high (as high as 100 μg/ml) have been isolated from patients who failed metronidazole therapy (8, 14, 15). Few other antimicrobial agents have been found to be active against trichomonads in vitro, and with the exception of furazolidone and clotrimazole, few have been used to treat infections caused by resistant isolates (10, 16, 19). Clearly, there is a need for alternative antitrichomonal agents. Therefore, we determined the susceptibilities of five T. vaginalis strains, one of which was metronidazole resistant, to 50 antimicrobial agents.

Five T. vaginalis strains (one patient isolate, three metronidazole-resistant laboratory strains, and one metronidazole-resistant laboratory strain) were tested. The strains were maintained in Diamonds medium (1). Before the beginning of any experiment, all cultures were examined for motility at mid logarithmic growth to guarantee viability. The MIC method of Forsgren and Wallin was used (5). Briefly, trichomonads were added to serial twofold dilutions of the antimicrobial agents in screw-top vials. Since resistance is more readily detected under aerobic than anaerobic conditions, all experiments were run in duplicate with positive and negative controls under both anaerobic conditions (brewer’s jars activated with GasPaks [BBL Microbiology Systems, Cockeysville, Md.] or medium supplemented with ascorbic acid in screw-top vials filled to the top) and aerobic conditions (37°C in room air in loosely capped vials) as previously described (13). After incubation at 37°C for 72 h, samples were examined microscopically for motility. The lowest concentration of drug in which there were no motile organisms was defined as the MIC. From each tube, 0.1 ml was transferred to 4 ml of fresh medium. After 5 days, samples were examined for the presence of viable trichomonads. The highest dilution (lowest drug concentration) in which no growth was observed was defined as the minimum lethal concentration (MLC).

Most of the antimicrobial agents tested had little activity against these strains of T. vaginalis. We tested 50 drugs, including folic acid antagonists (pyrimethamine-sulfadoxine, sulfadiazine, sulfisoxazole), beta-lactams (ampicillin, ceftazolin, cefoxitin, moxalactam, piperacillin), tetracyclines (chlortetracycline, doxycycline, minocycline), other antibiotics (bicozamycin, chloramphenicol, clindamycin, erythromycin, nalidixic acid, neomycin, rifampin, vancomycin), antiparasitics (chloroquine, diloxanide furoate, emetine, niclosamide, niridazole, pentamidin, praziquantel, primaquine, quinine sulfate, spiramycin, thiabendazole), antifungal agents (amphotericin B, clotrimazole, ketoconazole, miconazole, nystatin), and miscellaneous agents (acyclovir, allopurinol, dimethyl sulfoxide, indomethacin, verapamil), and very few had any appreciable antitrichomonal activity (MIC, >100 μg/ml). Only 10 drugs had MICs of <100 μg/ml against these strains. Five of these agents had significant trichomonacidal activity, as shown in Table 1. Five other drugs—furaltadone, quinacrine, cycloheximide, puromycin, and oxamniquine—had moderate activity, with MICs between 25 and 100 μg/ml. The MIC was determined by examining samples of culture medium at 72 h for motility of the trichomonads. We found that observable motility (MIC) as an endpoint correlated imperfectly with viability (MLC), as determined by regrowth of trichomonads in subculture. For the active drugs, the MIC was usually one to three dilutions less than the MLC, except for mebendazole, which consistently had an MLC lower than the MIC.

We tested all strains under both aerobic and anaerobic conditions to maximize our ability to study the resistant strain. Metronidazole resistance was more apparent when trichomonads were grown aerobically, but, except for metronidazole and tinidazole, assay conditions made little difference. Under either aerobic or anaerobic conditions, anisomycin, mebendazole, and furazolidone were active against the metronidazole-resistant strain. We used two different methods to maintain an anaerobic environment; the first was with anaerobic jars (GasPak), and the second utilized ascorbic acid added to medium in filled screw-top jars (13). Both methods gave similar and reproducible results.

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TABLE 1. In vitro susceptibilities of five strains of T. vaginalis to the five most active antimicrobial agents

<table>
<thead>
<tr>
<th>Strain and growth conditions</th>
<th>Metronidazole</th>
<th>Tinidazole</th>
<th>Furazolidone</th>
<th>Mebendazole</th>
<th>Anisomycin</th>
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<tr>
<td></td>
<td>MIC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>MLC&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>MLC</td>
<td>MIC</td>
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<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>12.5</td>
<td>6.2</td>
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<tr>
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<td>50</td>
<td>3.1</td>
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<tr>
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<td>1.6</td>
<td>0.4</td>
<td>1.6</td>
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<tr>
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<td>0.8</td>
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<tr>
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</table>

<sup>a</sup> The MIC was defined as the concentration of drug (in micrograms per milliliter) at which no trophinal motility was observed.

<sup>b</sup> The MLC was defined as the concentration of drug (in micrograms per milliliter) at which no viable organisms were detected by subculture.

<sup>c</sup> Strain A was a laboratory isolate known to be metronidazole resistant.

<sup>d</sup> An anaerobic environment was maintained by adding ascorbic acid to the medium in tightly capped screw-top jars.

The most effective drug for the treatment of T. vaginalis vaginitis is metronidazole, but because of concern with the carcinogenic and teratogenic potential, there has been a search for alternative therapies (3, 7, 16). T. vaginalis strains that are somewhat resistant to metronidazole were also recently isolated, adding to the obvious need for new antitrichomonal agents (4, 8, 12, 14, 15). The standard regimen for susceptible trichomonads is 250 mg of metronidazole three times daily for 7 days or a single 2-g dose, but for resistant strains no uniform therapeutic strategy has been established (11, 15). Increased dosage, intravenous therapy, and prolonged oral therapy with metronidazole have been variably successful in treating resistant strains (2, 15). This is not surprising, since the peak level of metronidazole in serum after oral administration of a 500-mg dose is 10 μg/ml and it is approximately 40 μg/ml after a 2-g dose; the MICs for resistant trichomonads have been reported to vary between 50 and 250 μg/ml (4, 9, 20). Other therapies that have been used include applications to the vagina of furazolidone and clotrimazole, as well as vinegar douches.

Of the 50 antimicrobial agents that we tested, only five—metronidazole, tinidazole, anisomycin, furazolidone, and mebendazole—had significant trichomonal activity. When we examined the metronidazole-resistant strain, anisomycin, furazolidone, and mebendazole were the most active agents. It is unlikely that these agents will be clinically useful. Anisomycin, a heterocyclic antibiotic first studied in the 1950s, was found in animals to cause irritation when given systemically (6). Mebendazole, a microtubular inhibitor used to treat helminths, is teratogenic; and the safety of furazolidone, a nitrofuran used to treat giardia, is not established (17, 18). Five other agents—furalaltadone (an antitrypanosomal drug), oxamniquine (an antischistosomal drug), pyrvinium (an antiprotozoal drug), quinacrine (an anti-giardia drug), and cycloheximide (an antifungal drug)—had moderate activity. Whether these would be clinically useful is unknown, but it is unlikely since systemic administration of all of these agents is not associated with significant levels in serum (17).

Clotrimazole has been used for the therapy of trichomoniasis in pregnancy (10). In previous studies, 100 mg of clotrimazole intravaginally for 6 days relieved symptoms in 48 to 89% of women with trichomoniasis, but long-term cure was not documented (19). Recently, Krieger et al. (9) tested several T. vaginalis strains in a time-kill assay against clotrimazole and found MICs greater than 100 μg/ml for 90% of the isolates, although several strains were susceptible. All five of the strains we tested were resistant to clotrimazole, and MICs were >100 μg/ml. Since we do not know the intravaginal concentrations of clotrimazole, it is difficult to draw conclusions about its antiparasitic effect in vivo. However, on the basis of our data it is difficult to advocate the use of this agent.

Trichomoniasis is predominantly a local mucosal disease, but metronidazole, the most effective therapy, is administered systemically. Clinical cure is due in part to the excellent tissue penetration of metronidazole, as well as the usually exquisite susceptibility of T. vaginalis (3, 5, 11). When metronidazole MICs for T. vaginalis strains are high, clinically resistant vaginitis may result, since vaginal levels of metronidazole will most likely be lower than the MIC. We have found the licensed agents furazolidone and mebendazole and the experimental agent anisomycin to be active against metronidazole-resistant strains and one metronidazole-resistant laboratory strain of T. vaginalis. All of these agents when given systemically have potential toxicity, but their efficacies and toxicities when applied locally have not been adequately studied. Possibly, optimum therapy of metronidazole-resistant trichomoniasis may need to include both systemic and intravaginal dosing. Whether this will be a useful and successful approach to therapy for metronidazole-resistant trichomonads needs further study.

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**LITERATURE CITED**