

Prevalence of *Trichomonas vaginalis* Isolates with Resistance to Metronidazole and Tinidazole[∇]

Jane R. Schwebke* and Frank J. Barrientes

Department of Medicine, University of Alabama at Birmingham, ZRB 239, 1530 3rd Ave. S., Birmingham, Alabama 35294-0007

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Clinical isolates of *Trichomonas vaginalis* were obtained from women consecutively enrolled in a study of partner notification. Testing of susceptibility to metronidazole and tinidazole was performed. Resistance to metronidazole and tinidazole was present in 17/178 (9.6%) and 1/178 (0.56%) strains, respectively. In vitro resistance was poorly correlated with clinical response to treatment.

Trichomoniasis is a sexually transmitted parasitic infection associated with preterm birth and human immunodeficiency virus acquisition and transmission (2, 4, 10). Most infections are cured by a single dose of metronidazole; however, resistance among *Trichomonas vaginalis* strains has been noted for years (6, 7) and there is anecdotal evidence that resistance may be increasing. Tinidazole, a drug related to metronidazole, may be useful for treating strains of *T. vaginalis* that are resistant to metronidazole (12). There is very limited information on the prevalence of resistance to metronidazole among clinical isolates of *T. vaginalis*, especially temporal trends in resistance. Prevalence values of 2.5% and 5% have been reported in two studies (9, 11). However, no surveillance system exists to detect resistance. We conducted susceptibility testing for metronidazole and tinidazole among clinical isolates of *T. vaginalis* in order to determine baseline resistance rates and to begin examining trends in resistance over time.

Women attending the Jefferson County Department of Health Sexually Transmitted Diseases Clinic in Birmingham, AL, with newly diagnosed *T. vaginalis* infections were invited to participate in a study of partner notification methods for trichomoniasis. All women were initially treated with a 2-g stat dose of metronidazole. As part of this study, cultures for *T. vaginalis* were performed with In-pouch TV culture pouches (BioMed Diagnostics, White City, Oregon). Cultures were transported to the laboratory for further incubation and preparation for susceptibility testing. Isolates were collected between May 2003 and May 2005.

Susceptibility testing was performed according to the protocol used by the Centers for Disease Control and Prevention (CDC) (modified Meingassner method; 3, 7, 8) to determine the minimal lethal concentration (MLC) of drug for each isolate. In brief, trichomonads were grown in Diamond's medium formula 1025 containing Trypticase soy broth, yeast extract, maltose, and calf serum; adjusted to a pH of 5.9; and supplemented with streptomycin at 1 g/liter and penicillin G at 100 U/ml. A hemocytometer was used to calculate the trichomonad population to a concentration of 66,666 cells/ml. Isolates were

tested in microtiter plates under anaerobic and aerobic conditions. MLCs for metronidazole and tinidazole were tested in triplicate. Drugs were serially diluted, with dilutions ranging from 400 µg/ml to 0.2 µg/ml. Plates were then incubated at 37°C for 46 to 50 h and read with a Zeiss Axiovert inverted microscope. The microplate cell in which no motile trichomonads were visualized was reported as the MLC. Control strains included CDC 955 (resistant) and CDC 520 (sensitive), both kindly provided by Evan Secor at the CDC.

Clinical isolates were available from 209 women. Isolates from 31 women lost viability during passage, leaving 178 viable isolates. Rates of resistance to metronidazole and tinidazole are shown in Table 1. Resistance to metronidazole, defined as an aerobic MLC of greater than or equal to 50 µg/ml, was present in 17/178 (9.6%) isolates. For the majority of the resistant isolates (14/17 [82.4%]), the MLCs were 50 to 100 µg/ml. For the rest of the resistant isolates (3/17 [17.6%]), the MLCs were 200 to 400 µg/ml (moderate to high resistance). Tinidazole resistance was present in only one isolate, for which the MLC was 50 µg/ml. This isolate also had high-level resistance to metronidazole (MLC of 400 µg/ml). Anaerobic MLCs of >3.0 µg/ml, which have been associated with resistance (5), were present in 13/178 (7.3%) strains for metronidazole, compared to 2/178 (1.1%) strains for tinidazole ($P = 0.003$). However, there was no apparent correlation between elevated anaerobic MLCs and resistance as defined by aerobic MLCs.

Clinical outcomes for women with resistant strains did not appear to be correlated with the MLCs. A total of 12/209 (5.7%) women remained positive for *T. vaginalis* at the first follow-up visit, all of whom except 1 denied interim unprotected sexual contact. Among the women who had strains of *T. vaginalis* with in vitro resistance, only two failed initial therapy with a 2-g single dose of metronidazole. Both women had isolates for which the MLC was 100 µg/ml. Neither of the two women with isolates demonstrating high-level resistance was a clinical failure. Eight women who failed initial therapy had fully susceptible strains, and two of the women who were clinical failures had strains which lost viability prior to susceptibility testing.

To our knowledge, this study represents only the third published report of surveillance of resistance of *T. vaginalis* to metronidazole and the second report of surveillance of resistance to tinidazole. Müller et al. published results of screening

* Corresponding author. Mailing address: University of Alabama at Birmingham, ZRB 239, 1530 3rd Ave. S., Birmingham, AL 35294-0007. Phone: (205) 975-5665. Fax: (205) 975-7764. E-mail: schwebke@uab.edu.

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TABLE 1. MLCs for clinical isolates of *T. vaginalis*

Aerobic MLC ($\mu\text{g/ml}$)	Interpretation of level of resistance to metronidazole ^a	MLC ($\mu\text{g/ml}$)	
		Metronidazole	Tinidazole
<50	Practically none	161	177
50	Very low	11	1
100	Low	3	0
200	Moderate	2	0
>200	High	1	0

^a Determined in accordance with reference 6.

for resistance to metronidazole among clinical isolates of *T. vaginalis* obtained from women attending a sexually transmitted disease clinic in Columbus, Ohio, between July 1982 and October 1983 (8). They studied women who were diagnosed with trichomoniasis and treated with directly observed therapy with a 2-g stat dose of metronidazole. They reported a 27% clinical failure rate and found that for 44% of the isolates the MLC of metronidazole was $\geq 50 \mu\text{g/ml}$. However, their study population included mainly women who had already failed therapy for trichomoniasis with metronidazole. They reported a correlation between response to therapy and in vitro resistance to metronidazole (8).

A 2001 study from Spain (9) reported on resistance to metronidazole and tinidazole in 91 *T. vaginalis* isolates collected between 1995 and 1999 from female sex workers and patients presenting to a gynecology clinic. Two patient isolates (2.2%) exhibited low-level resistance to metronidazole (50 $\mu\text{g/ml}$), and none were resistant to tinidazole.

A more recent report (1), designed to determine the prevalence of metronidazole-resistant *T. vaginalis* in a general population, studied 42 *T. vaginalis* isolates from adolescent females attending a primary care clinic in Atlanta, GA. Two isolates (4.8%) were metronidazole resistant (MLC of $> 50 \mu\text{g/ml}$), and three (7.1%) were borderline resistant (MLC = 50 $\mu\text{g/ml}$).

The only other published data on metronidazole and tinidazole resistance among isolates of *T. vaginalis* are based on 104 clinical isolates sent to the CDC for testing between 1995 and 2001 because of suspected resistance on the basis of clinical failure. In this study, increased metronidazole resistance was correlated with increased tinidazole resistance; however, MLCs of tinidazole were significantly lower than MLCs of metronidazole (3).

In summary, among women enrolled in a study of partner notification for trichomoniasis, resistance to metronidazole, as defined by aerobic MLCs, was present in 9.6%. However, in vitro resistance to metronidazole was poorly correlated with clinical response to treatment. Rates of resistance to tinidazole were significantly lower than rates of resistance to metronidazole. Ongoing surveillance of this cohort will provide data on temporal trends in resistance to these two drugs.

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