Single-dose versus 7-day-dose metronidazole for the treatment of trichomoniasis in women: an open-label, randomised controlled trial

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Summary

Background Among women, trichomoniasis is the most common non-viral sexually transmitted infection worldwide, and is associated with serious reproductive morbidity, poor birth outcomes, and amplified HIV transmission. Single-dose metronidazole is the first-line treatment for trichomoniasis. However, bacterial vaginosis can alter treatment efficacy in HIV-infected women, and single-dose metronidazole treatment might not always clear infection. We compared single-dose metronidazole with a 7-day dose for the treatment of trichomoniasis among HIV-uninfected, non-pregnant women and tested whether efficacy was modified by bacterial vaginosis.

Methods In this multicentre, open-label, randomised controlled trial, participants were recruited at three sexual health clinics in the USA. We included women positive for *Trichomonas vaginalis* infection according to clinical screening. Participants were randomly assigned (1:1) to receive either a single dose of 2 g of metronidazole (single-dose group) or 500 mg of metronidazole twice daily for 7 days (7-day-dose group). The randomisation was done by blocks of four or six for each site. Patients and investigators were aware of treatment assignment. The primary outcome was *T vaginalis* infection by intention to treat, at test-of-cure 4 weeks after completion of treatment. The analysis of the primary outcome per nucleic acid amplification test or culture was also stratified by bacterial vaginosis status. This trial is registered with ClinicalTrials.gov, number NCT01018095, and with the US Food and Drug Administration, number IND118276, and is closed to accrual.

Findings Participants were recruited from Oct 6, 2014, to April 26, 2017. Of the 1028 patients assessed for eligibility, 623 women were randomly assigned to treatment groups (311 women in the single-dose group and 312 women in the 7-day-dose group; intention-to-treat population). Although planned enrolment had been 1664 women, the study was stopped early because of funding limitations. Patients in the 7-day-dose group were less likely to be *T vaginalis* positive at test-of-cure than those in the single-dose group (34 [11%] of 312 *vs* 58 [19%] of 311, relative risk 0·55, 95% CI 0·34–0·70; p<0·0001). Bacterial vaginosis status had no significant effect on relative risk (p=0·17). Self-reported adherence was 96% in the 7-day-dose group and 99% in the single-dose group. Side-effects were similar by group; the most common side-effect was nausea (124 [23%]), followed by headache (38 [7%]) and vomiting (19 [4%]).

Interpretation The 7-day-dose metronidazole should be the preferred treatment for trichomoniasis among women.

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Introduction

Among women, trichomoniasis is estimated to be the most common non-viral sexually transmitted infection (STI) worldwide, and is as prevalent as chlamydia, gonorrhoea, and syphilis combined.[1] Trichomoniasis is not a reportable disease, but an estimated 143 million new cases occur globally each year in women aged 15–29 years.[1] The prevalence of trichomoniasis has been estimated to be around 5% among women worldwide[1] and around 1·8% among women in the USA, but nearly five times higher among African American women.[2]

Trichomoniasis has been associated with serious reproductive morbidity (eg, vaginitis, cervicitis, urethritis, and endometritis)[3] and poor birth outcomes (eg, premature rupture of membranes, low birthweight, and preterm delivery) in women.[4] Several studies have found that trichomoniasis co-occurs with other STIs[5] and amplifies HIV acquisition.[6]

Both WHO and the US Centers for Disease Control and Prevention (CDC) recommend a single 2 g dose of oral metronidazole or tinidazole as first-line treatment and a 7-day dose of oral metronidazole (400 mg or 500 mg twice daily for 7 days) as second-line treatment for *Trichomonas vaginalis* infections.[7,8] In most settings, metronidazole is used more often than tinidazole, because purchase costs are lower.[9]

Evidence has shown that the single-dose treatment of metronidazole might be insufficient to treat trichomoniasis. A meta-analysis[10] of six published studies found that women who received the 7-day-dose metronidazole had 46% fewer

Research in context

Evidence before this study

Among women, trichomoniasis is the most common non-viral sexually transmitted infection worldwide. It has been associated with increased reproductive and perinatal morbidity, and amplified HIV acquisition. A single 2 g dose of oral metronidazole is the recommended first-line treatment of trichomoniasis, with a 7-day dose (400 or 500 mg twice daily for 7 days) as second-line treatment. A meta-analysis of six published studies found that multiple doses of metronidazole resulted in fewer treatment failures than single-dose treatment. However, five of the studies were done more than 30 years ago. In our 2010 study with HIV-infected women, we found that women receiving the 7-day-dose metronidazole treatment were half as likely to be *Trichomonas vaginalis*-positive at test-of-cure compared with those receiving the single-dose treatment, but this difference was mostly found among women with co-occurring bacterial vaginosis. The purpose of this study was to re-evaluate the efficacy of single-dose metronidazole compared with 7-day-dose metronidazole among HIV-uninfected women, and to examine treatment differences by bacterial vaginosis status.

Added value of this study

Our findings add to the evidence that 7-day-dose metronidazole is superior to single-dose metronidazole for the treatment of trichomoniasis in women, irrespective of bacterial vaginosis status.

Implications of all the available evidence

The 7-day dose should be the preferred first-line treatment for *T vaginalis* infection. Treatment guidelines for trichomoniasis in HIV-uninfected women should be refined accordingly.

treatment failures than did women who received the single dose, and the proportion of patients with positive test-of-cure (TOC) after single-dose metronidazole ranged from 6·2% to 18%. Five of the six studies concluded that single-dose treatment was similar to the 7-day treatment.[11–15] However, all but one of these studies were done over 30 years ago, when clinical trial protocols were not as rigorous as today.[16] Most of the studies were not adequately powered to detect meaningful differences, and all were done before the availability of nucleic acid amplification tests (NAATs), which are three-to-five times more sensitive than microscopy for the diagnosis of trichomoniasis.[17–21] The most recent study that was included in the meta-analysis was our 2010 multicentre study of trichomoniasis treatment.[22] We used newer clinical trial methods but only included HIV-infected women with laboratory-diagnosed *T vaginalis*. We found that women receiving the 7-day-dose metronidazole treatment were half as likely to be *T vaginalis*-positive at TOC compared with those receiving the single-dose treatment.[22] Subsequent analysis, however, found superiority in the 7-day-dose group only among the patients who had bacterial vaginosis according to the Nugent score, suggesting that altered vaginal flora could have interfered with the single-dose treatment.[23]

The purpose of this study was to re-evaluate the efficacy of single-dose metronidazole compared with 7-day-dose metronidazole among HIV-uninfected women, using a sample size with sufficient power and advanced *T vaginalis* diagnostics. A secondary aim was to examine treatment differences by bacterial vaginosis status.

Methods

Study design and participants

This was a multicentre, open-label, randomised controlled trial comparing the efficacy of 7-day-dose metronidazole treatment with the standard single-dose metronidazole treatment of trichomoniasis. An open-label design was used to simulate real-world conditions and accommodate for the possibility of lack of adherence and sexual re-exposure among women who received treatment over multiple days.

Women who attended one STI clinic in each of three US cities (Birmingham, AL; Jackson, MS; and New Orleans, LA) for clinical screenings and who had a *T vaginalis* infection were referred to study staff by their clinical providers.

Inclusion criteria for participants were female sex, English speaking, reached the age of majority (between 18 and 19 years, depending on site requirements), positive for *T vaginalis* infection according to clinical screening (microscopy or NAAT) confirmed by at least one study test (NAAT or culture), willingness to refrain from all alcohol use during treatment and for 24 h after treatment, willingness to be treated with metronidazole, and willingness to be randomly assigned to either of the study groups.

The exclusion criteria were HIV infection, pregnancy or breastfeeding, previous enrolment in this study, incarceration, any medical contraindications to metronidazole (eg, currently taking disulfiram, phenytoin, or warfarin), a history of alcoholism or known liver damage, treatment with any medication used to treat trichomoniasis or bacterial vaginosis (including metronidazole, tinidazole, secnidazole, acetarsol, boric acid, furazolidone, and paromomycin) within the previous 14 days, inability or unwillingness to provide informed consent, unwillingness to be randomised, and inability or unwillingness to return for a follow-up visit 4 weeks after completion of treatment.

The study received ethics approval from the institutional review boards of Tulane University, Louisiana State University Health Sciences Center (LSUHSC), University of Alabama at Birmingham, University of Mississippi Medical Center, Jefferson County Department of Health, and the Mississippi State Department of Health. An independent Data Safety and Monitoring Board monitored the data every 6 months, with a priori stopping rules. The

study was stopped early because of funding limitations and never reached half of the anticipated sample size, so an interim analysis could not be done. Because of the sensitive nature of the surveys both at enrolment and TOC, a Certificate of Confidentiality was also obtained from the US Department of Health and Human Services. Women who discovered they were pregnant while taking the medication or who had any serious adverse events were followed up according to the institutional review board's protocol for each site.

Study enrolment was open from Oct 6, 2014, to April 26, 2017. The study was completed on June 5, 2017. After eligibility screening and written informed consent, the participants were asked to take a survey, provide urine for pregnancy testing, and self-collect vaginal swabs for *T vaginalis* culture, NAAT, and Gram stains.

Randomisation and masking

Participants were randomly assigned (1:1) to receive either 500 mg of metronidazole twice daily for 7 days (7-day-dose group) or the standard single dose of 2 g of metronidazole (single-dose group). Group assignment was done with sealed, sequentially numbered envelopes that contained the randomly chosen treatment group. These envelopes were prepared before the start of the study by a non-investigator who used SAS software to develop a randomisation scheme of blocks of four or six for each site. A list containing the envelope number and allocation group was kept in an electronic file that was not accessed until the end of the study. Envelopes were kept at each site, and study staff pulled envelopes sequentially and documented the treatment group, envelope number, and lot number of the treatment received. All patients, clinicians, and study staff were aware of the allocation, but the treatment group was masked from all laboratory technicians.

Procedures

The surveys at baseline and at the TOC appointment (4 weeks after completion of treatment) were done with an audio computer-assisted self-administered interview (ACASI) software to collect demographics, substance use, vaginal hygiene practices, contraception use, and sexual behaviour data for each participant. Symptoms commonly reported with trichomoniasis (unusual vaginal discharge, vaginal odour, vaginal itching or irritation, painful urination, pelvic pain)[24] were systematically elicited by the ACASI. The survey at the TOC appointment was similar to the survey at baseline, but it had additional questions about sexual exposure, treatment adherence, partner treatment, symptoms during follow-up, and side-effects of the medication (including nausea, vomiting, and headaches). The survey was modelled after surveys from our previous studies.[22,25]

For *T vaginalis* NAAT, self-collected vaginal swabs were tested for *T vaginalis* with the Aptima *Trichomonas vaginalis* Assay (Hologic, Bedford, MA, USA), which uses transcription-mediated amplification and hybridisation protection assay technologies. At the time of the study, this assay was allowed for investigational use only, and was performed by use of the direct tube sampling system. The assay is now available for clinical use.[26] All tests were done at the LSUHSC laboratory (New Orleans, LA, USA), according to the manufacturer's instructions.

For *T vaginalis* culture, self-collected vaginal swabs were placed into the *T vaginalis* culture InPouch medium (Biomed Diagnostics, White City, OR, USA), incubated at 37°C and read following the manufacturer's protocol by trained personnel. Three readings were done over 7 days. The cultures were considered *T vaginalis*-positive if any live trichomonads were detected, and *T vaginalis*-negative after three negative pouch readings.[27]

Gram stains for Nugent score determination were read by an experienced technician at the LSUHSC laboratory. Samples were periodically evaluated for quality assurance following Clinical Laboratory Improvement Amendments by the US Food and Drug Administration (FDA).[28] Women were considered to have bacterial vaginosis if they had a Nugent score of 7 or higher.[29]

Participants in the single-dose group were treated with 2 g of oral metronidazole (four pills of 500 mg each) in a single dose. Participants in the 7-day-dose group were treated with 500 mg oral metronidazole (one pill) taken twice daily for 7 days. Participants in both groups were asked to take the first dose at the clinic under direct observation by study staff and were offered crackers or cookies to prevent nausea. For the participants in the treatment group, the remaining pills were dispensed in a container with a child-proof cap.

Participants in both groups were asked to tell all their sexual partners of their exposure to *T vaginalis* and to encourage them to seek treatment. Because of legal or institutional restrictions, none of the participating clinics routinely provided expedited partner treatment (presumptive treatment for trichomoniasis given to partners without a clinic visit) but some were given expedited partner treatment at the clinic's discretion.

All participants received the standardised advice by study personnel to refrain from unprotected sexual intercourse until symptoms had resolved, and until they and their partners had completed the treatment regimen. Participants were also advised to refrain from alcohol consumption while taking the medication and for 24 h after completion. Participants were informed of the possibility of adverse events related to treatment with metronidazole, and of the possibility of a change in taste sensation and discolouration of urine. Participants in the treatment group were also advised on the importance of taking all doses of the medication.

A TOC appointment was scheduled 4 weeks after completion of treatment, with an appointment window of 3–12 weeks. Women were not screened before the 3-week TOC, because of the potential for false-positive NAAT results from remnant *T vaginalis* DNA.[30,31] Participants

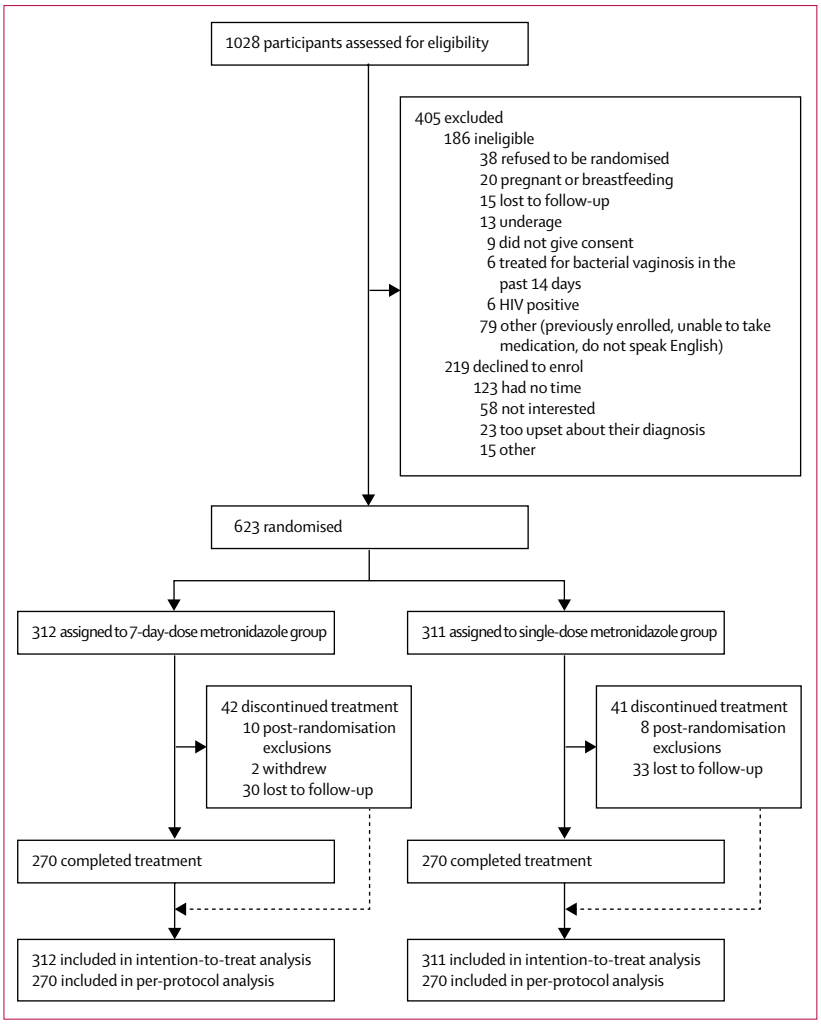

Figure: **Trial profile**

provided their contact information and were given an appointment card and compensation worth US$20. Participants were reminded of their TOC visit by use of their preferred method of contact. At the TOC appointment, data collection included a survey and self-collected vaginal swabs for NAAT, culture, and Gram stain; participants who completed the TOC visit received compensation worth $50.

Outcomes

The primary outcome was a *T vaginalis*-positive result at the TOC appointment (ie, 4 weeks after completion of treatment), with NAAT or culture. NAATs were centrally assessed, but the cultures were done at the laboratory of each study site. Allocation was masked from technicians at all sites. The trial hypothesis was that women receiving the 7-day-dose metronidazole would be less likely to have a *T vaginalis*-positive TOC than women receiving single-dose metronidazole, and that this effect would be observed only in women with bacterial vaginosis. Stratification by bacterial vaginosis status was the secondary analysis of the primary outcome.

The secondary outcomes were to find the probable cause of a *T vaginalis*-positive result at TOC using the genotyping results and sexual histories, and to find out if most of the infections are due to treatment failure, re-exposure, sexual exposure to a new partner, or reduced susceptibility of *T vaginalis* to metronidazole. Results for the secondary outcomes will be presented in a separate report.

Statistical analysis

We calculated that a sample size of 1664 participants would provide statistical power of 80%. Our calculations were based on inputs from our unpublished pilot study with HIV-uninfected women, and from our previous study of trichomoniasis treatment in HIV-infected women.[22] We assumed a *T vaginalis*-positive TOC rate of 16·8% for single-dose treatment and 10·7% for 7-day-dose treatment, inflated the sample size by 15% for the potential of interclass correlation between the three sites, and inflated the sample size by a further 20% to account for the potential loss to follow-up.

Study data were collected and managed with REDCap electronic data capture tools (version 7.4) hosted at Tulane University (New Orleans, LA, USA).[1] REDCap is a secure, web-based application designed to support data capture for research studies. It provides an intuitive interface for validated data entry, audit trails for tracking of data manipulation and export procedures, automated export procedures for seamless data downloads to common statistical packages, and procedures to import data from external sources.

We measured the baseline characteristics associated with trichomonas infection.[2,32] The primary outcome analysis of *T vaginalis* infection at TOC was by intention to treat. Missing outcome data were imputed 20 times for participants who were lost to follow-up, using the fully conditional method in SAS (version 9.4) PROC MI. Because the sites did not enrol the same number of participants, we had to account for differences between sites.[33] Generalised estimating equation (GEE) regression methods were used, including an exchangeable correlation matrix and clustering by site. Rate differences and 95% CIs, as well as GEE-derived relative risks and 95% CIs, were calculated. Intention-to-treat analyses for all randomised participants were stratified by baseline bacterial vaginosis status.

Four sensitivity analyses of the primary outcome were also done. We redid all analyses by reclassifying all missing results as negative at TOC in the intention-to-treat population, by reclassifying all missing results as positive at TOC in the intention-to-treat population, by using the *T vaginalis* culture results as the outcome (to remove the possibility of false positives by *T vaginalis* NAAT) in the per-protocol population and by using

NAAT and *T vaginalis* culture results as the outcome in the per-protocol population.

Adverse events and serious adverse events were compared by treatment group. Statistical analyses were done either with the χ^2 test or Fisher's exact test. All analyses were done using SAS version 9.4, under the supervision of the team biostatistician.

The trial is registered with ClinicalTrials.gov, number NCT01018095, and with the FDA, number IND118276.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Participants were recruited from Oct 6, 2014, to April 26, 2017. The study was completed on June 5, 2017. Of the 1028 women with *T vaginalis* infections that were approached, 623 enrolled in the study (figure). The study was stopped early because of funding limitations, and we enrolled 623 (37%) of the 1664 patients that were planned for our intended sample size. Of the 623 women enrolled in the study, 312 women were randomly assigned to the 7-day-dose group and 311 to the single-dose group. All women received the dose allocated to them and were included in the intention-to-treat population.

Baseline characteristics were well balanced between groups (table 1). Most women who enrolled were African American (591 [96%] of 619) and the median age was 27 years (range 18–64). The study site in Birmingham, AL, recruited 400 (64%) of 623 participants. At baseline, 294 (48%) of 609 women had bacterial vaginosis per Nugent criteria, and 231 (37%) of 618 did vaginal douching at least once per month. Participants had the option of not answering any question; this accounts for variations in the denominators for some of the baseline variables.

Participants were screened for *T vaginalis* via NAAT and culture to confirm their clinical screening. In the 7-day-dose group, 239 (77%) of 312 were tested by NAAT and culture, 16 (5%) were tested by NAAT only, and 57 (18%) were tested by culture only. In the single-dose group, 237 (76%) of 311 were tested by NAAT and culture, 14 (5%) were tested by NAAT only, and 60 (19%) were tested by culture only.

Of the 623 patients included in the study, 540 (87%) returned for their follow-up visit. The mean time from completion of treatment to TOC visit was 4·9 weeks (SD 1·6) for the study sample, and did not differ between groups (p=0·60). The proportion of patients who did not return for follow-up did not differ significantly between treatment groups (42 [14%] of 312 patients in the 7-day-dose group *vs* 41 [13%] of 311 patients in the single-dose group, p=0·92) or between study sites (19 [12%] of 155 in Jackson, MS; 13 [19%] of 68 in New Orleans, LA; 51 [13%] of 400 in Birmingham, AL; p=0·33). Sexual exposure (either to baseline or a new partner) during follow-up did not differ significantly between treatment groups (99 [37%] of 270 in the 7-day-dose group, 89 [33%] of 270 in the single-dose group, p=0·37).

	7-day-dose metronidazole group	Single-dose metronidazole group
Demographics		
Age ≥30 years	116/310 (37%)	132/309 (43%)
African American	298/310 (96%)	293/309 (95%)
Study site		
Jackson, MS	77/312 (25%)	78/311 (25%)
New Orleans, LA	34/312 (11%)	34/311 (11%)
Birmingham, AL	201/312 (64%)	199/311 (64%)
Substance use behaviour		
Binge drinking in past week	57/310 (18%)	56/309 (18%)
Smoking	145/310 (47%)	142/308 (46%)
Sexual behaviours		
More than one male sexual partner	119/310 (38%)	122/309 (40%)
Any number of female partners	27/310 (9%)	30/309 (10%)
Vaginal douching at least once a month	117/309 (38%)	114/309 (37%)
Use of hormonal contraception	60/310 (19%)	58/309 (19%)
Taking the 7-day dose will be difficult or somewhat difficult	91/310 (29%)	96/309 (31%)
Clinical factors		
Bacterial vaginosis per Nugent score	146/306 (48%)	148/303 (49%)
Trichomoniasis in past year per self-report	53/309 (17%)	54/307 (18%)
Bacterial vaginosis in past year per self-report	48/310 (15%)	65/308 (21%)
Yeast infection in past year per self-report	87/301 (29%)	76/297 (26%)
Symptoms of trichomoniasis	248/311 (80%)	248/311 (80%)
Expedited partner treatment	21/307 (7%)	27/309 (9%)

Table 1: **Baseline characteristics of the intention-to-treat population**

Adherence to treatment was lower in the 7-day-dose group than in the single-dose group (253 [96%] of 265 *vs* 264 [99%] of 266, p=0·006). Of the 603 women who reported partners at baseline, 43 (7%) received expedited partner treatment (2 g single-dose metronidazole) for their sexual partners, with no substantial difference in provision of treatment by group (table 1).

Overall, 80 (15%) of 540 patients were positive at TOC. One patient was assessed by culture only, 17 by NAAT only, and 62 by both NAAT and culture. In the intention-to-treat analysis (with data imputed for participants who were lost to follow-up), the proportion of women who were positive at TOC was lower in the 7-day-dose group than in the single-dose group (34 [11%] of 312 women *vs* 58 [19%] of 311 women, p=0·0008, relative risk [RR] 0·55, 95% CI 0·34–0·70). When bacterial vaginosis was present, women in the 7-day-dose group were less likely to have a positive result at TOC than women in the single-dose group (p=0·0002, RR 0·59, 0·43–0·80). In the absence of bacterial vaginosis, women receiving the 7-day dose were also less likely to have a positive result at TOC compared

	7-day-dose metronidazole group	Single-dose metronidazole group	7-day-dose *vs* single-dose difference (95% CI)	Relative risk (95% CI)	p value*
Primary outcome analyses by intention to treat†					
Trichomonas vaginalis infection at test-of-cure	34/312 (11%)	58/311 (19%)	−7·8 (−2·2 to −13·3)	0·55 (0·34 to −0·70)	<0·0001
Among patients with bacterial vaginosis at baseline	16/125 (13%)	26/125 (21%)	−8·0 (−12·8 to −20·8)	0·59 (0·43 to 0·80)	0·0002
Among patients without bacterial vaginosis at baseline	13/139 (9%)	24/140 (17%)	−7·8 (−0·2 to −15·8)	0·57 (0·45 to 0·71)	<0·0001
Sensitivity analyses of primary outcome					
All missing TOC results reclassified as negative	29/312 (9%)	51/311 (16%)	−7·1 (−1·9 to −12·4)	0·57 (0·45 to 0·71)	<0·0001
All missing TOC results reclassified as positive	71/312 (23%)	92/311 (30%)	−6·8 (−0·1 to −13·7)	0·77 (0·70 to 0·85)	<0·0001
T vaginalis culture results as outcome‡	22/269 (8%)	41/270 (15%)	−7·0 (-1·3 to −12·7)	0·54 (0·39 to 0·75)	0·0002
NAAT and *T vaginalis* culture results as outcome‡	29/270 (11%)	51/270 (19%)	−8·2 (−2·2 to −14·1)	0·57 (0·45 to 0·71)	0·008

TOC=test-of-cure. NAAT=nucleic acid amplification test. *Relative risks and p values were derived from generalised estimating equation (GEE) analysis. †Missing data imputed using the fully conditional method in SAS. ‡In the per-protocol population.

Table 2: **Primary outcome and sensitivity analyses**

	7-day-dose metronidazole group (n=270)	Single-dose metronidazole group (n=270)	p value
Any adverse event during follow-up	89 (33%)	90 (33%)	0·90
Adverse events			
Nausea	63 (23%)	61 (23%)	0·84
Vomiting	13 (5%)	6 (2%)	0·10
Headache	23 (9%)	15 (6%)	0·18
Dizziness	6 (2%)	2 (1%)	0·15
Bad or metallic taste in mouth	4 (2%)	3 (1%)	0·70
Vaginal itching	3 (1%)	1 (<1%)	0·32*
Fatigue	2 (1%)	0	0·32*
Rash	2 (1%)	0	0·32*
Other side-effects†	9 (4%)	9 (3%)	1·00
Serious adverse events			
Spontaneous abortion‡	0	2 (1%)	0·32*

*Adverse events were compared with Fisher's exact test. †Yeast infection, abdominal pain, numbness, dysuria, loss of appetite, altered mental status, myalgia, generalised itching, lightheadedness, restlessness, verbal intimidation by partner, and stiff neck. ‡Two women tested negative at baseline and discovered they were pregnant during follow-up.

Table 3: **Adverse events and serious adverse events**

with women receiving the single dose (RR 0·57, 0·45–0·71, p<0·0001). Bacterial vaginosis status had no significant effect on relative risk ($p_{interaction}$=0·17).

When all missing results were classified as negative, the women in the 7-day-dose group had fewer positives at TOC than did those in the single-dose group (table 2). Findings were similar when all missing results were classified as positive. When *T vaginalis* culture was used as the outcome, women receiving the 7-day dose were less likely to be positive at TOC than women receiving the single dose. Per-protocol analysis of complete cases showed that women in the 7-day-dose group had fewer positive *T vaginalis* results than those receiving the single dose.

Of those patients who returned for the TOC, 179 (33%) reported a treatment-related side-effect (table 3). The most common was nausea (23%), followed by headache (7%) and vomiting (4%). The proportion of patients with these events did not differ significantly by study group. Two spontaneous abortions were reported among women who tested negative for pregnancy at baseline and discovered they were pregnant during follow-up. Both were in the 7-day-dose group.

Discussion

Trichomoniasis is highly prevalent worldwide and has been associated with increased reproductive and perinatal morbidity,[3,4] and amplified HIV acquisition.[1,6] In this randomised trial, treatment of trichomoniasis with 7-day-dose oral metronidazole (500 mg twice daily for 7 days) resulted in 45% fewer positive results after 4 weeks than single-dose oral metronidazole (2 g single dose) among HIV-uninfected women. These findings are consistent with our 2010 multicentre study[22] of trichomoniasis treatment with HIV-infected women, and a previous meta-analysis[10] of six published studies of trichomoniasis treatment.

We did not find treatment differences according to bacterial vaginosis status, by contrast with our previous study with HIV-infected women.[23] The reason for this result is not entirely clear. Although the validity of a Nugent score can be affected by the technician's expertise,[29] the same technician read the Gram stains in both studies, so this variability is not a likely factor. One possible explanation of this result is that host factors affected treatment efficacy; HIV status seems to affect the vaginal microbiota with and without presence of bacterial vaginosis.[35] Future studies should examine how the vaginal microbiota affects the treatment of trichomoniasis.

Concomitant bacterial vaginosis is common among women with trichomoniasis.[36] 48% of the women in this study had bacterial vaginosis per Nugent score, yet

bacterial vaginosis had not been diagnosed by the referring clinical provider. Amsel criteria[37] had been used to test for bacterial vaginosis at the clinics. Symptoms for bacterial vaginosis and trichomoniasis are often similar,[7] and Amsel has low predictability for bacterial vaginosis.[38] 7-day-dose metronidazole is the first-line treatment for bacterial vaginosis[7] and is more effective than single-dose metronidazole for trichomoniasis, providing further rationale for recommending 7-day-dose metronidazole as first-line treatment for trichomoniasis.

The high rates of positive *T vaginalis* results at TOC in both treatment groups are of concern; 11% of patients receiving the 7-day dose tested positive at TOC, and 19% of patients receiving the single dose tested positive at TOC. These findings suggest that the CDC re-commendation to rescreen women treated for trichomoniasis should be upheld even for those women that receive the 7-day dose.

Although NAAT has greater sensitivity to detect *T vaginalis* than culture, it is not clear how long remnant *T vaginalis* DNA stays in vaginal fluids when it is no longer a live parasite. We decided to request TOC visits 4 weeks after completion of treatment because three previous studies found 0–15% false positives if the TOC visits were 3 weeks after completion of treatment.[30,31,39] When we tested for *T vaginalis* with culture, we found a reduced presence of *T vaginalis* at TOC in both treatment groups, but the relative risk was similar (table 2). This result suggests that false positives from NAAT did not have a major effect on our results.

We chose to use an open-label study design to factor in real use conditions, although masking participants to treatment is often preferred. Because of the open-label study design, treatment duration and follow-up times were different for each treatment group, which could have resulted in differences in sexual exposure. However, we did not find any significant differences in sexual exposure between groups after treatment. Although self-reported sexual behaviour might not be accurate, we used computer-assisted interviews, which have been shown to reduce social desirability bias.[40,41] Furthermore, reporting bias should be the same across groups, as all participants received similar counselling.

An important limitation of the study was that enrolment was much lower than planned; maybe our sample size calculation was too conservative. Fewer participants could result in reduced power for the primary outcomes and less certainty about the effect measure, particularly for the analysis stratified by bacterial vaginosis. Our calculations were based on inputs from our unpublished pilot study with HIV-uninfected women, and from our previous study of trichomoniasis treatment in HIV-infected women.[22] It is possible we did not have enough power to detect a difference by bacterial vaginosis status, but the relative risks by bacterial vaginosis status did not indicate a trend, so it is unlikely that a larger sample size would have led to a different conclusion.

Single-dose treatment for trichomoniasis has long been favoured over 7-day-dose treatment, because adherence to treatment is not an issue, especially if the patient is under direct observation by a clinician. Despite their willingness to be randomised, 30% of the women thought it would be difficult or somewhat difficult to take the medicine for 7 days. Indeed, in this study self-reported adherence was higher among patients receiving the single dose compared with patients receiving the 7-day dose, but adherence in both arms was very high. Providers who have concerns about patient adherence will need to consider the benefits of the 7-day dose and the convenience of the single dose.

The study population consisted of non-pregnant women with trichomoniasis living mainly in urban areas, with a high rate of co-occurring bacterial vaginosis. The majority of women who enrolled were African American. Although our results might not be generalisable to all women with trichomoniasis, they are probably generalisable to most, since the study population represents those at high risk for trichomoniasis.[7,32] Our study population was mostly symptomatic, but most women with trichomoniasis are asymptomatic,[42] and the effect of asymptomatic trichomoniasis on reproductive morbidity is not well known.[7] More studies of women with asymptomatic trichomoniasis are needed.

About a third of the cohort had side-effects that were minimal and did not appear to differ by treatment group. There were, however, two reported spontaneous abortions among women receiving the 7-day dose. The difference between groups might be due to chance, and reviews[43,44] have found that multiple doses of metronidazole are safe in pregnancy. Strengths of the study were that randomisation appeared to work well, as baseline characteristics were similar by group (table 1), loss to follow-up was minimal (13%), and the treatment group was masked from the laboratory technicians when outcome measures were recorded. Moreover, the intention-to-treat, modified intention-to-treat, and other sensitivity analyses were consistent with each other. Combined with our previous work,[45,46] this study provides strong evidence that 7-day-dose metronidazole is a better treatment option for women with trichomoniasis than single-dose metronidazole, and recommendations should be adapted accordingly.

Contributors

PK, NS, and LM contributed to the data analysis. PK, CAM, LAM, RAL, JRS, LB, SNT, NS, and DHM wrote the Article. CAM, LAM, RAL, JRS, LB, and SNT contributed to the data collection. DHM, PA, WES, MB, and JMC did the laboratory work and wrote the corresponding sections of the Article.

Declaration of interests

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