

ORIGINAL ARTICLE

# The incidence of *Trichomonas vaginalis* infection in women attending nine sexually transmitted diseases clinics in the USA

Maria L Alcaide,<sup>1</sup> Daniel J Feaster,<sup>2</sup> Rui Duan,<sup>2</sup> Stephanie Cohen,<sup>3</sup> Chanelle Diaz,<sup>4</sup> Jose G Castro,<sup>1</sup> Matthew R Golden,<sup>5</sup> Sarah Henn,<sup>6</sup> Grant N Colfax,<sup>3</sup> Lisa R Metsch<sup>7</sup>

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<sup>1</sup>Division of Infectious Diseases, University of Miami Miller School of Medicine, Miami, Florida, USA

<sup>2</sup>Department of Public Health Sciences, University of Miami Miller School of Medicine, Miami, Florida, USA

<sup>3</sup>San Francisco Department of Public Health, San Francisco, California, USA

<sup>4</sup>University of Miami Miller School of Medicine, Miami, Florida, USA

<sup>5</sup>University of Washington, Seattle, Washington, USA

<sup>6</sup>Whitman-Walker Health, Washington DC, USA

<sup>7</sup>Department of Sociomedical Sciences, Mailman School of Public Health, Columbia University, New York, New York, USA

## Correspondence to

Dr Maria L Alcaide, Division of Infectious Diseases, University of Miami Miller School of Medicine, 1120 NW 14th St, suite 849 (D-90), Miami, FL 33136; USA; [malcaide@med.miami.edu](mailto:malcaide@med.miami.edu)

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## ABSTRACT

**Objectives** Trichomoniasis (TV) is associated with an increased risk of acquisition of sexually transmitted diseases (STDs) and HIV. The purpose of this study is to evaluate factors associated with incidence TV among female STD clinic attendees in the USA.

**Methods** Data were collected from women participating in a randomised controlled trial evaluating brief risk reduction counselling at the time of HIV testing to reduce sexually transmitted infections (STIs) incidence in STD clinics. Participants recruited from STD clinics underwent STI testing at baseline and 6-month follow-up. TV testing was performed using Nucleic Acid Amplification Test.

**Results** 1704 participants completed study assessments. Prevalence of TV was 14.6%, chlamydia 8.6%, gonorrhoea 3.0%, herpes simplex virus 2 44.7% and HIV 0.4%. Cumulative 6-month incidence of TV was 7.5%. Almost 50% of the incident TV cases had TV at baseline and had received treatment. Factors associated with incidence of TV were having chlamydia, TV and HIV at baseline: TV relative risk (RR)=3.37 (95% CI 2.35 to 4.83,  $p<0.001$ ); chlamydia RR=1.92 (95% CI 1.23 to 2.99,  $p=0.04$ ); and HIV=1.59 (95% CI 1.01 to 2.50,  $p=0.047$ ).

**Conclusions** Prevalent and incident TV is common among STD clinic attendees; and baseline TV is the main risk factor for incident TV, suggesting high rates of reinfection or treatment failures. This supports the importance of rescreening women after treatment for TV, evaluating current treatment regimens and programmes to ensure treatment of sexual partners.

**Clinical trial number** NCT01154296.

## INTRODUCTION

Trichomoniasis is caused by the protozoan parasite *Trichomonas vaginalis* (TV) and is the most common non-viral sexually transmitted infection (STI) worldwide.<sup>1</sup> TV can affect both men and women, but the health consequences of TV occur primarily in women. TV is associated with severe obstetric and gynaecological complications such as pelvic inflammatory disease, preterm delivery and spontaneous abortions.<sup>2</sup> TV also increases the reproductive mucosa vulnerability to STIs and HIV, and thus women with TV have an increased risk of acquiring and transmitting STIs and HIV.<sup>3, 4</sup>

Despite the deleterious health effects of TV, trichomoniasis is a neglected STI. TV is not a reportable disease, and partner notification

programmes are not available in most clinic settings.<sup>5</sup> In addition, due to lack of evidence to support screening asymptomatic women, screening for TV is not routinely done and most settings use wet mount as a diagnostic tool only in the presence of symptoms. Wet mount has poor sensitivity, and Nucleic Acid Amplification Test (NAAT) or TV culture are superior for accurate diagnosis but are costly and not widely available.<sup>6, 7</sup>

In the USA, it is estimated that 3.1% of women of reproductive age are infected with TV, and prevalence rates up to 50% have been reported in certain populations.<sup>8–11</sup> Risk factors associated with prevalent TV infection in the USA are African-American race, older age, drug use (crack and marijuana), multiple sexual partners and infection with gonorrhoea,<sup>12–14</sup> but factors associated with TV incidence in high-risk women attending a sexually transmitted diseases (STD) clinic have seldom been studied. The purpose of this study is to evaluate the factors associated with incidence of TV among female STD clinic attendees.

## METHODS

### Study procedures

This study presents data collected from women participating in Project AWARE, a randomised controlled trial evaluating brief risk reduction counselling at the time of HIV testing as a tool to reduce STI incidence in individuals seeking care in STD clinics in the USA.<sup>15</sup> Project AWARE was conducted in nine STD clinics in the USA in 2010. Participating STD clinics were located in three geographical regions: (1) west: Los Angeles and San Francisco (California), Portland (Oregon) and Seattle (Washington); (2) south: Columbia (South Carolina), Jacksonville and Miami (Florida); and (3) northeast: Pittsburgh (Pennsylvania) and Washington DC.

Enrolment criteria for Project AWARE included being (1) a recipient of care at one of the participating STD clinics; (2) 18 years of age or older; (3) negative or unknown HIV status; (4) able to communicate in English; (5) willing to be tested for STIs and HIV; (6) willing to sign a medical record release to permit abstraction of STI tests, results and treatment; and (7) a resident in the vicinity of the clinic. Because Project AWARE only tested women for TV, the study population for this analysis was restricted to the trial's female participants.

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Project AWARE compared the cumulative 6-month incidence of STIs (chlamydia, gonorrhoea, TV, syphilis, herpes simplex virus 2 (HSV-2) and HIV) in individuals receiving rapid HIV testing with brief patient-centred risk reduction counselling versus rapid HIV testing with information only. Participants received STI and HIV testing at baseline and at 6 months along with a risk-behaviour assessment using audio computer-assisted self-interview (ACASI). Women underwent testing for trichomoniasis at baseline and 6 months. A detailed description and the primary outcome findings of Project AWARE have been previously published.<sup>15</sup>

### Assessments

Sociodemographic characteristics, illegal substance use and sexual risk behaviours were assessed using ACASI and included age, race/ethnicity, employment, education, income, unprotected receptive vaginal intercourse with primary or non-primary partner(s), drug, alcohol and tobacco use. Race/ethnicity was categorised as four mutually exclusive categories: Hispanic, if the participant reported being Hispanic; black, non-Hispanic, if the participant reported being black but not Hispanic; white, non-Hispanic, if the participant reported being white only; and other for all other participants. Employment was categorised as full-time, occasional (day labourer), part-time and unemployed. Yearly income was categorised in US\$ as <\$20 000, \$20 000–\$40 000 and >\$40 000. US regions were categorised as west, south and northeast. Problematic alcohol drinking at baseline was defined as five or more drinks on a typical day when the participant was drinking in the past six months or six or more drinks on one occasion in the past six months. Use of drugs included use of club drugs, marijuana, cocaine, heroin, hallucinogens, phencyclidine, amphetamine, tranquilisers or barbiturates, or narcotic medications. Risk factors were assessed at baseline and at 6 months. Risk factors at baseline included risks in the 6 months prior to enrolment and were self-reported.

### Risk reduction counselling intervention and Project AWARE outcomes

Project AWARE participants were randomised to receive individual patient-centred risk reduction counselling based on an evidence-based model or information alone. The elements of the intervention were focused on the patient's specific risk behaviours. Project AWARE approached 14 948 patients for participation, 6239 consented to be screened and assessed for eligibility, and of those, 1227 were excluded. The number of participants randomised was 5.012 (2505 received risk reduction counselling and 2507 received information alone). In total, 2039 participants in the intervention group (81.4%) and 2032 (81.1%) in the control group had complete follow-up STI data. Cumulative 6-month STI incidence by study group was not significantly different at the 6-month follow-up. Details of the results of Project AWARE have previously been published.<sup>15</sup>

### Laboratory assessments

Participants of Project AWARE underwent testing for STIs at baseline and 6-month follow-up for chlamydia, gonorrhoea, TV (women only), syphilis, HSV-2 and HIV. Chlamydia and gonorrhoea testing was done using the Aptima Combo-2 (gen-Probe Diagnostics) in vaginal swabs.<sup>16</sup> TV testing was done using NAAT Gen-Probe Diagnostics TV Analyte Specific Reagent in vaginal swabs.<sup>17</sup> Sensitivity and specificity of these tests are

above 95%. The use of NAAT for TV diagnostics was not standard of care in all the clinics and was done as a study procedure. When women presented with vaginal symptoms, wet mount testing was performed by qualified STD clinic laboratory personnel, and TV was diagnosed by direct visualisation of TV. Syphilis and HSV-2 testing and interpretation of results have been previously described.<sup>18</sup>

All positive infections at baseline received appropriate treatment according to the Centers for Disease Control and Prevention (CDC) STD guidelines.<sup>19</sup> Treatment for TV was metronidazole 2 g orally one time. Women diagnosed with trichomoniasis were advised to notify their sexual partners and given a note regarding recommended treatment. Expedited partner therapy was available in clinics located in the west. Treatment of sexual partners was not assessed.

### Trichomoniasis incidence

An incident case of trichomoniasis was defined as a participant who had a positive test for TV at 6-month follow-up if (1) her baseline TV test was negative or (2) her baseline test was positive and she received adequate treatment. Adherence to treatment was self-reported.

### Statistical analysis

Analyses were conducted using SAS V9.3 (SAS Institute) and Mplus V7.3. The primary outcome was cumulative 6-month trichomoniasis incidence. The frequency and means of baseline sociodemographic characteristics, illegal substance use, sexual risk behaviours and STIs were calculated. The relative risk (RR) of trichomoniasis incidence in the univariate and multivariate models was estimated by Poisson regression with robust error variance.<sup>20</sup> Univariate models are performed on available data for each predictor variable. Multivariate models used the EM-algorithm as implemented in Mplus due to non-overlapping missing data among some predictors. All tests were two-sided at the nominal  $\alpha$  level of 0.05.

## RESULTS

### Characteristics of study participants

The study population was 1704 women out of the 5012 participants of Project AWARE. In total, 859 women were assigned to risk reduction counselling and most received the intervention (842/850, 99.1%). And 854 women were assigned to rapid testing with information only and all of them received only a rapid HIV test. A total of 1459 (85.6%) women completed the follow-up assessments and follow-up laboratories for trichomoniasis. Table 1 describes the demographic characteristics, substance use and risk behaviours of study participants. The average age was 30.2 years. More than half of the women were African-American, had completed high school education and were unemployed. Income was low (<US\$20 000) in 84.2% of the women. Regional distribution was as follows: 36.7% women were recruited in the west, 37.7% were recruited in the south and 25.6% were recruited in the northeast.

Approximately 12% of participants had problematic alcohol drinking and almost 40% reported smoking. The number of unprotected sex acts with primary and non-primary partners in the six months prior to enrolment were high, and the mean number of partners was 2.1. Over one-third of women had engaged in unprotected sex while high on drugs or alcohol during the prior six months. Rates of drug use were high as >50% of participants had used any type of illicit drugs in the six months prior to enrolment (879, 51.6%) (results not shown in the table).

**Table 1** Univariate and multivariate analysis of demographic characteristics, illegal substance use, risk behaviours and STIs associated with cumulative 6-month trichomoniasis incidence

	N (total= 1704)		Incident TV		Univariate			Multivariate (N=1459)		
	N (mean)	% (SD)	N	%	RR	95% CI	p Value	RR	95% CI	p Value
<i>Demographics</i>										
Age (years)*	30.2	10.4			<b>1.26</b>	<b>1.11 to 1.42</b>	<b>&lt;0.001</b>	1.01	0.92 to 1.24	0.345
<i>Race/ethnicity</i>										
Black	958	56.2	90	9.4	<b>4.05</b>	<b>2.13 to 7.71</b>	<b>&lt;0.001</b>	1.92	0.94 to 3.91	0.072
Hispanic	190	11.2	7	3.7	1.38	0.51 to 3.73	0.525	1.67	0.65 to 4.31	0.287
Other	98	5.6	2	2.0	0.90	0.20 to 4.02	0.888	1.13	0.24 to 5.28	0.876
White	458	26.9	10	2.2	1					
<i>Employment</i>										
Full-time	324	19.1	9	2.8	<b>0.30</b>	<b>0.15 to 0.61</b>	<b>&lt;0.001</b>	0.49	0.24 to 1.02	0.058
Occasional	55	3.2	2	3.6	0.41	0.10 to 1.62	0.205	0.50	0.08 to 3.12	0.458
Part-time	289	17.0	10	3.5	<b>0.41</b>	<b>0.22 to 0.78</b>	<b>0.006</b>	0.55	0.30 to 1.00	0.051
Unemployed	1033	60.7	88	8.5	1.00			1.00		
<i>Education</i>										
Greater than high school	993	58.3	37	3.7	<b>0.38</b>	<b>0.22 to 0.63</b>	<b>&lt;0.001</b>	0.81	0.49 to 1.37	0.435
High school	467	27.4	51	10.9	1.11	0.68 to 1.80	0.672	1.32	0.83 to 2.09	0.236
Less than high school	244	14.3	21	19.3	1.00			1.00		
<i>Income US\$/year</i>										
>40 000	74	4.5	2	2.7	0.39	0.10 to 1.56	0.184	1.54	0.48 to 4.94	0.465
Between 20 000 and 40 000	188	11.3	8	4.3	0.64	0.32 to 1.29	0.208	1.50	0.70 to 3.19	0.293
<20 000	1397	84.2	96	6.9	1.00			1.00		
<i>US region</i>										
West	625	36.7	12	1.9	<b>0.20</b>	<b>0.10 to 0.41</b>	<b>&lt;0.001</b>	<b>0.50</b>	<b>0.25 to 0.99</b>	<b>0.047</b>
South	643	37.7	59	9.2	1.17	0.79 to 1.74	0.438	0.97	0.66 to 1.43	0.874
Northeast	436	25.6	38	8.7	1.00			1.00		
<i>Substance abuse and sexual risk factors</i>										
Smoking at baseline	680	39.9	62	9.1	<b>2.16</b>	<b>1.48 to 3.15</b>	<b>&lt;0.001</b>	1.36	0.92 to 2.03	0.124
Problematic drinking at baseline	202	11.9	14	6.9	1.11	0.64 to 1.94	0.709	0.87	0.49 to 1.53	0.625
Number of unprotected sex acts with primary partnert	17.4	50.1			1.00	0.98 to 1.02	0.955	0.98	0.94 to 1.02	0.223
Number of unprotected sex acts with non-primary partnert	5.6	24.2			<b>1.05</b>	<b>1.01 to 1.08</b>	<b>0.017</b>	<b>1.11</b>	<b>1.03 to 1.18</b>	<b>0.003</b>
Number of partners (unprotected sex)†	2.1	7.8			1.37	0.79 to 1.99	0.217	1.01	0.74 to 1.38	0.950
Number of unprotected sex acts while high†	7.4	32.1			1.02	0.98 to 1.07	0.244	1.03	0.95 to 1.11	0.525
<i>Baseline STI</i>										
Trichomoniasis	245	14.6	54	22.0	<b>5.89</b>	<b>4.14 to 8.40</b>	<b>&lt;0.001</b>	<b>3.37</b>	<b>2.35 to 4.83</b>	<b>&lt;0.001</b>
Gonorrhoea	51	3.0	8	15.7	<b>2.75</b>	<b>1.44 to 5.27</b>	<b>0.002</b>	1.33	0.73 to 2.43	0.355
Chlamydia	145	8.6	18	12.4	<b>2.31</b>	<b>1.45 to 3.71</b>	<b>0.001</b>	<b>1.92</b>	<b>1.23 to 2.99</b>	<b>0.004</b>
Herpes simplex virus 2	757	44.7	77	10.2	<b>2.85</b>	<b>1.90 to 4.29</b>	<b>&lt;0.001</b>	1.40	0.92 to 2.14	0.114
HIV	6	0.4	2	33.3	4.40	0.88 to 22.01	0.072	<b>1.59</b>	<b>1.01 to 2.50</b>	<b>0.047</b>
Syphilis	8	0.5	1	12.5	3.29	0.60 to 18.17	0.171	1.05	0.28 to 3.97	0.942
Vaginal symptoms	918	62.9	89	9.7	<b>2.62</b>	<b>1.63 to 4.21</b>	<b>&lt;0.001</b>	1.58	0.97 to 2.55	0.064

Statistically significant values are given in bold (p<0.05).

\*Relative risk for every increase in age in 10 years.

†Relative risk for every increase of 10 acts or partners.

RR, relative risk; STI, sexually transmitted infection; TV, *Trichomonas vaginalis*.

### Baseline rates of STIs

Baseline prevalence of chlamydia was 8.6% and of gonorrhoea 3.0%. Eight women were diagnosed with syphilis (0.5%). Seven were diagnosed with late latent syphilis and one with secondary syphilis. Almost half of the participants were seropositive for HSV-2. HIV prevalence was low (0.4%).

### Prevalence and incidence of trichomoniasis

Prevalence of trichomoniasis by NAAT testing at baseline was 14.6% (245 women participants). The rates of coinfections with trichomoniasis and chlamydia or gonorrhoea were low at baseline: 8 women had TV and gonorrhoea (0.5%), 24 TV and chlamydia (1.4%) and 7 had the TV, chlamydia and gonorrhoea

(0.4%). All participants diagnosed with TV at baseline received treatment and reported adherence with the prescribed regimen.

In total, 918 women (62.9%) presented with vaginal symptoms. Out of the 245 women with positive NAAT for TV, 191 (77.9%) were symptomatic and had wet mount performed. And 147 of the symptomatic women (77.0%) had diagnosis of TV by wet mount examination.

Out of 1459 women who completed the study, 109 were infected with TV by the 6-month follow-up visit. The incidence of TV infection was 7.5% per 6 months. Out of the 109 incident cases, 55 (50.5%) were negative at baseline and 54 (49.5%) were positive at baseline and had been treated. At follow-up, out of the 109 women with positive NAAT for TV,

37 were symptomatic (33.9%) and had a wet mount performed. Twenty-four (64.9%) had diagnosis of TV by wet mount examination. The rates of incident coinfections at 6 months with TV and chlamydia or gonorrhoea were low: two women had TV and gonorrhoea (0.1%), eight TV and chlamydia (0.6%) and one woman had three TV, chlamydia and gonorrhoea (0.1%).

#### Factors associated with incident trichomoniasis

Univariate and multivariate analysis of factors associated with cumulative 6-month trichomoniasis incidence are illustrated in [table 1](#). In univariate analysis, demographic, illegal substance use and risk factors associated with incident TV were age, black race, unemployment, education lower than high school, study regions other than the west, cigarette smoking and unprotected sex with non-primary partners. Presenting with vaginal symptoms and having other STIs at baseline (trichomoniasis, chlamydia, gonorrhoea, and HSV-2) was also associated with incident TV. In multivariate analysis, the only behavioural risk factor associated with TV acquisition was unprotected sex with non-primary partner(s) (RR=1.10, 95% CI 1.03 to 1.17,  $p=0.005$ ). The western study region had a lower RR for incident TV (RR=0.5, 95% CI 0.25 to 0.99,  $p=0.047$ ). Having CT, TV or HIV at baseline were the main risk factors for acquiring TV, and baseline TV had the highest RR: RR for baseline TV=3.37 (95% CI 2.35 to 4.83,  $p<0.001$ ); RR for baseline CT=1.92 (95% CI 1.23 to 2.99,  $p=0.004$ ); RR for HIV=1.59 (95% CI 1.01 to 2.50,  $p=0.047$ ).

#### DISCUSSION

This is the first multicentre study evaluating incident TV in female STD clinic attendees in the USA. TV infection has a high prevalence and incidence rate in this setting. We found baseline prevalence of TV to be 14.6%, and cumulative 6-month incident TV to be 7.5%. This is consistent with what has previously been reported in the literature in other high-risk settings.<sup>13 14 21</sup> In symptomatic women, wet mount missed >20% of TV cases diagnosed by using NAAT. Factors associated with the acquisition of TV in our sample were unprotected sex with non-primary partner(s) and having TV, chlamydia or HIV infections at baseline.

Estimates for incident TV in the USA have recently been published using the National Diseases and Therapeutic Index and outpatient clinic data from 2008. It was estimated that 680 000 incident TV infections occurred in the USA, but the authors concluded that the quality and reliability of the data to estimate TV incidence was poor.<sup>22</sup> Incident TV by world region has been recently published by the WHO using data from years 1990 to 2005. These data suggest that incident TV is high worldwide with the highest rates in sub-Saharan Africa and intermediate rates in Europe and the USA.<sup>23</sup> These studies only present estimates and highlight the need for more accurate data on TV incidence.

Several small studies have also evaluated incident TV in the USA and other settings. A recent study of African-American female adolescents participating in an HIV/STI prevention trial in Atlanta ( $n=701$ ) found that TV prevalence was 20% and incident infections were associated with cigarette smoking, alcohol use, chlamydia and gonorrhoea acquisition and TV infection at baseline.<sup>21</sup> Another study with female adolescents in Indiana ( $n=332$ ) found that incident TV was associated with older age, new sexual partner and baseline TV.<sup>24</sup> Miller *et al*<sup>14</sup> found that TV was very common among women drug users ( $n=135$ ) in New York City (38%), and acquisition of TV was associated with having more than one sex partner in the prior 30 days.

Incident TV in sub-Saharan Africa has recently been evaluated in women participating in an HIV prevention trial in South Africa. The rate of incident TV was 8.6% per year and incident TV was associated with baseline TV, number of sexual partner and having other STIs at baseline.<sup>25</sup> The only behavioural risk factor associated with incident TV in our sample was having unprotected sex with non-primary partner(s).

In our study, incident TV infection was also associated with baseline CT and HIV. The primary risk factor for incident trichomoniasis in our study was having trichomoniasis at baseline, and we believe the most likely reason for incident cases is reinfection. Partner notification programmes and expedited partner therapy were not available in all the study clinics, and the clinics that had expedited partner therapy located in the west had significantly lower incident cases. Although patient-delivered partner treatment may be a cost-effective method, its use does not result in decreasing reinfection rates when compared with standard referral.<sup>26 27</sup> Other possibility of incident TV in women with baseline TV is failure of treatment or relapses. Both treatment failures and relapses have been reported in the literature, particularly in individuals with HIV infection and when using a single-dose regimen.<sup>28 29</sup> However, whereas we saw elevated TV incidence associated with HIV, HIV rates in our sample were low. Another possible reason is low treatment adherence since adherence to treatment was self-reported and participants may not have adhered to treatment recommendations. Lastly, resistance to metronidazole could explain incident TV in women with baseline TV, but resistance is low in STD clinic settings.<sup>30</sup>

In regards to TV screening, we used NAAT testing in all participants regardless of the presence of symptoms. In most STD clinic settings, TV testing for the diagnosis of trichomoniasis is performed only in symptomatic women by using wet mount testing. Poor sensitivity of wet mount compared with NAAT has previously been reported,<sup>31</sup> and in our study we confirmed that many cases of TV would be missed by using wet mount alone. Although the benefit of treating asymptomatic women for TV has not been established and the use of NAAT may detect non-viable organisms, we believe that the incident cases at a relatively long follow-up (6 months) are related to sexual behaviours and not to the use of different tests. This study emphasises the need to improve TV diagnostic tools in STD clinics.

This study has several limitations: (1) the demographics, risk behaviours and treatment of sexual partners data collected were self-reported, although the use of ACASI limited desirability bias; (2) this study did not distinguish among new infections, reinfections and treatment failures and our incidence analysis considered all follow-up cases as incident cases; (3) participants were enrolled in an STI prevention trial that may have influenced the behaviours and TV rates 6 months after enrolment, although rates of TV and other STI were not affected by the intervention;<sup>15</sup> (4) almost 15% of the participants were lost to follow-up; and (5) the nine research sites may not be representative of all STD clinic in the US or other settings.

Our study provides recent quality and reliable data on TV incidence in high-risk women attending STD clinics in the USA and enrolled in a large STI prevention trial. Our findings highlight the high rates of reinfection or treatment failures and emphasise the need to evaluate rescreening women after treatment for trichomoniasis by using highly sensitive and specific NAAT testing and ensuring appropriate time to avoid detection of non-viable organisms. In addition, these results call for the need to evaluate different treatment regimens (single dose vs a 1-week course of metronidazole) and programmes that will ensure treatment of sexual partners.



## Key messages

- ▶ Prevalent and incident *Trichomonas vaginalis* infection is common among sexually transmitted diseases clinic attendees in the USA.
- ▶ The main factors associated with incident *T. vaginalis* infection are baseline trichomoniasis, chlamydia and HIV infection.
- ▶ High rates of reinfection or treatment failures emphasise the need to rescreen women for trichomoniasis, to evaluate different regimens and to ensure treatment of sexual partners.

**Handling editor** Jackie A Cassell

**Contributors** MLA, DJF and RD collaborated in the drafting of the manuscript. MLA, DJF, RD, SC, CD, JGC, MRG, SH, GNC and LRM provided critical revision of the manuscript. LRM, DJF, GNC and MRG were involved in the design of the study. MLA, SC, CD, JGC, SH and MRG were involved in participant activities and collection of data. DJF and RD performed the statistical analysis. LRM, DJF, MRG, GNC, SH and JGC provided study supervision, administrative, technical and material support.

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**Competing interests** None declared.

**Patient consent** Obtained.

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