Trichomonas vaginalis: Common, curable and in the diagnostic spotlight

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LEARNING OBJECTIVES

1. Discuss the historical and epidemiological spectra of *Trichomonas vaginalis.*
2. Define the clinical characteristics of trichomoniasis in men and women.
3. Explain the methods for clinical diagnosis of *T. vaginalis* infection.
4. Compare and contrast the treatment strategies for trichomoniasis in women.

ABBREVIATIONS: CDC - Centers for Disease Control and Prevention, FDA - Food and Drug Administration, MDx - molecular diagnostics, NAAT - nucleic acid amplification test, NGU - non-gonococcal urethritis, NHANES - National Health and Nutrition Examination Surveys, PID - pelvic inflammatory disease: POC - point of care, PPV - positive predictive value, STD - sexually transmitted disease, STI - sexually transmitted infection

INDEX TERMS: Molecular diagnostics, sexually transmitted disease, sexually transmitted infections, *Trichomonas vaginalis*, trichomoniasis

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Trichomonas vaginalis is the most common curable sexually transmitted infection (STI) worldwide.[1] The 2001-2004 National Health and Nutrition Examination Surveys (NHANES) report that American women between the ages of 14 and 49 are infected with *T. vaginalis* with a prevalence of approximately 3% - greater than that of *C. trachomatis* and *N. gonorrhoeae*.[2,3] Additional studies have highlighted that, unlike *C. trachomatis* and *N. gonorrhoeae*, *T. vaginalis* affects all age groups and the prevalence is highest in women over 40 years of age.[4-7] Annually, it is estimated that more than half of the approximately 248 million new *T. vaginalis* infections occur in males worldwide.[1] However, the majority of prevalent *T. vaginalis* infections are detected in women, which is consistent with the chronic nature of these infections in the female urogenital tract. Most infections in women are asymptomatic, however *T. vaginalis* has been linked to lower and upper reproductive tract disease syndromes including vaginitis, cervicitis and pelvic inflammatory disease (PID). Pregnancy-related complications include pre-term birth and infertility. In men, *T. vaginalis* is most commonly a cause of non-gonococcal urethritis (NGU) but infections tend to be transient and frequently asymptomatic. In this article, we concisely discuss the current knowledge on *T. vaginalis* epidemiology and the developing trends in diagnosis of this incredibly prevalent STI.

Disease spectrum of T. vaginalis infections

Currently, trichomoniasis is not a notifiable disease in the USA and so therefore, compared to other STIs, relatively little is known about the epidemiology and pathogenesis of *T. vaginalis* infections. The spectrum of disease in women is characterized by symptoms of vaginitis including discharge, and localized vulvar pruritus. Classical disease signs include purulent vaginal discharge, and vaginal and vulvar erythema. The urethra

is commonly infected with accompanying symptoms of dysuria in women with vaginal infection. In addition, colpitis macularis, or "strawberry cervix" is a highly specific sign of infection but is only accurately diagnosed during colposcopy. The dogmatic notion that *T. vaginalis* infections are overtly symptomatic in women has been unequivocally overturned. In fact, up to 80% of *T. vaginalis* infections are asymptomatic in women depending on the clinical setting.[3] Despite the asymptomatic nature of infection, *T. vaginalis* has been linked to several inflammatory syndromes in women thus highlighting the importance for screening initiatives in subjects at risk for STIs. *T. vaginalis* infection in women is also associated with increased susceptibility to HIV acquisition and HIV shedding in co-infected individuals. In males, *T. vaginalis* is most commonly a cause of NGU but infection is frequently asymptomatic and the majority of cases are reported to clear spontaneously within 10 days.[8-11] Together, the importance of asymptomatic males as transmitters to women is enormous since *T. vaginalis* infections in women, with or without symptoms, can persist for at least several months.[11] Like the bacterial STIs, complicated *T. vaginalis* infections in men can lead to more severe inflammatory outcomes including prostatitis, urethral stricture, epididymitis and infertility.[12]

Diagnosis of Trichomoniasis

The history of *T. vaginalis* diagnostics dates back to the early part of the 19th century when motile organisms were visible on wet mount preparations of vaginal swab specimens. Strikingly, direct microscopic observation remained the standard of diagnostic care until *in vitro* culture methods were introduced in 1949.[11] Several studies have reported low sensitivities of *T. vaginalis* detection using wet mount microscopy that range from 44-68%.[13] In addition, sensitivity can be negatively impacted by even short time intervals (10-30 minutes) between swab collection and microscopic examination.[14] Despite low sensitivity and the need for rapid processing, microscopy remains the most commonly used technique for diagnosis of trichomoniasis due to ease and cost-effectiveness. Culture of trichomonads from female urogenital specimens using Diamond's broth medium is substantially more sensitive compared to wet mount microscopy (96% vs. 36-82%, respectively)[11] but requires additional supplies and equipment including an incubator for growing cultures. Although both techniques rely upon a trained microscopist, wet mount microscopy and culture methods are highly specific for *T. vaginalis* detection (>99%)[11,13] primarily due to the characteristic tumbling motility of viable organisms.

Microscopy-based techniques were followed by the first generation of nucleic acid amplification tests (NAATs). Collectively, NAATs have dramatically enhanced our understanding of *T. vaginalis* epidemiology, pathogenesis, and helped to identify a previously underappreciated population of asymptomatic infections in men and women. Contemporary NAATs are both highly specific and sensitive, and several have been adapted to high-throughput instrumentation. Unlike microscopy, NAATs require no subjective interpretation of the test results and have faster turn-around times compared to culture. Enhanced sensitivity is the clear advantage of NAATs compared to culture and wet mount microscopy. The most widely used culture methods utilize the InPouch TV (BioMed Diagnostics, White City, OR) device or inoculation of specimen material into Diamond's medium. These tests are moderately sensitive ranging from 44-75%[13] and require three to seven days before results are available.

It was not until the late 1990's when the first NAAT was FDA-approved for diagnostic testing in the USA. Currently, development and implementation of *T. vaginalis* testing platforms into clinical laboratories are top industry priorities in the realm of STI diagnostics. As of this 2014 publication, only two NAATs are FDA-approved and marketed for *T. vaginalis* detection in the USA: 1) the APTIMA TV test (GenProbe, Inc., San Diego, CA) for use on the TIGRIS and PANTHER platforms; and 2) the ProbeTec *Trichomonas vaginalis* Q^x Amplified DNA Assay for use on the BD Viper platform (Beckton Dickinson, Franklin Lakes, NJ). Targeting a conserved region of 16s rRNA molecules, the APTIMA TV test utilizes the combined technologies of transcription medicated amplification (TMA) and a hybridization protection assay (HPA). By targeting a high-copy transcript in the *T. vaginalis* cell, the FDA-approved APTIMA TV test is characterized by very high sensitivity values ranging from 95-100% depending on the specimen type and gold standard comparator.[13] According to the manufacturer's package insert, sensitivity values range from 92-100% for the ProbeTec *Trichomonas vaginalis* Q^x Amplified DNA

Assay among both symptomatic and asymptomatic women when compared to a composite comparator of wet mount and culture. The ProbeTec assay utilizes strand displacement assay (SDA) technology as described previously.[15] Taken together, modern NAATs substantially improve sensitivity of *T. vaginalis* detection from several specimen types, and serve both as diagnostic and screening tools in symptomatic and asymptomatic women. Unfortunately, no NAAT has been approved for *T. vaginalis* detection in men. However, as clinical research clarifies the value of screening in asymptomatic men and women, combined with the high frequency of vaginitis, it is predicted that the volume of NAATs for *T. vaginalis* will exceed those for *C. trachomatis* and *N. gonorrhoeae* in the coming years.

In contrast to high-throughput NAATs typically run at hospital or reference laboratories, point of care (POC) tests for STIs facilitate the initiation of treatment during the initial clinic visit. This approach ensures compliance with the treatment regimen since most first line therapies are single STAT doses of antibiotics. Ideally, POC tests allow differentiation of etiologic agents to substantially reduce the number of patients that are managed syndromically. In the accompanying *Mycoplasma genitalium* article in this series, we describe how POC testing could substantially reduce syndromic management of NGU in men and non-gonococcal cervicitis in women where treatment guidelines fall short to effectively treat all causative agents. Over the last decade, POC tests for *T. vaginalis* have been available that utilize either immunochromatographic test strips (OSOM Trichomonas Rapid Test; Sekisui Diagnostics, California, USA) or non-amplification nucleic acid detection technology (BD Affirm VPIII; Beckton Dickinson, Maryland, USA). The sensitivities of the rapid diagnostic tests parallel those of culture-based tests and are consistently higher than wet mount microscopy (40-95%).[13] Despite lower sensitivity, the positive predictive value (PPV; the ability to detect truly positive subjects) for the available POC tests can be increased to levels of contemporary NAATs by using them in populations where prevalence is high (e.g. women with signs of disease). As for NAATs, no FDA-approved POC tests are available for *T. vaginalis* diagnosis in men. Unfortunately, wet mount microscopy is insensitive for detection of trichomonads from male urogenital specimens and, in general, testing paradigms for men are lacking considering the substantial number of incident infections.

Treatment of T. vaginalis infections

Effective therapies for *T. vaginalis* infections are available for men and women diagnosed with trichomoniasis. The current CDC-recommended first-line therapy for *T. vaginalis* infection is a single 2-gram dose of metronidazole or tinidazole – both of which are FDA-approved for trichomoniasis therapy in men and women. Current recommendations for patients who fail stat metronidazole are to treat with a single dose of tinidazole or an extended regimen of metronidazole (500 mg twice daily for seven days). Fortunately, treatment failures are infrequent following single dose metronidazole therapy in patients for whom re-infection can be excluded. In short, non-susceptibility rates to the currently available therapies are low,[9] and parasitological cure rates are high (86-100%) in women.[16] Failure of the recommended second line regimens should be followed by treatment with oral metronidazole or tinidazole at 2 grams daily for five days. In patients with persistent *T. vaginalis* infection after extended metronidazole/tinidazole regimens, a combination of oral and topically applied agents have been anecdotally effective and may be indicated in cases of chronic treatment failure.[16]

Subjects with *T. vaginalis* infection are up to twice as likely to acquire HIV infection[17] thus underscoring the global importance for continued investigation of this prevalent STI. Interestingly, a recent randomized clinical trial showed that women with HIV responded better to extended metronidazole (500 mg twice daily for seven days) compared to a single 2-gram dose and had lower rates of re-infection upon follow up.[18] It is possible that special considerations should be applied to subjects with HIV, AIDS or who are immunocompromised. However, data are lacking regarding *T. vaginalis* disease and therapies in HIV-positive individuals despite high rates of co-infection.

Conclusions

As the most common parasitic protozoan infection in developed countries, *T. vaginalis* infections are widespread and disproportionally affect women in both prevalence and disease sequelae. Considering the substantial proportion of asymptomatic infections, the true impact of *T. vaginalis* testing will be realized fully

when these assays are used as screening tests in both men and women. In lieu of relatively low sensitivities, POC tests for *T. vaginalis* including wet mount microscopy remain valuable tools to initiate stat therapy in clinical settings where follow up is unlikely. Interestingly, because *T. vaginalis* infections are more common in women over 40 years of age, the apparent risk for pathological sequelae without symptoms is high and efforts to screen should be guided to all age groups rather than specifically to young adults. We still have much to learn regarding the epidemiology and pathogenesis of *T. vaginalis* infections. With FDA-approved NAATs entering clinical laboratories in the USA and abroad, we are on the cusp of a deeper understanding of clinical disease and optimized therapeutic approaches in both men and women.

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