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Review

Trichomoniasis: evaluation to execution

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ABSTRACT

Trichomoniasis is the most common sexually transmitted disease, caused by a motile flagellate non-invasive parasitic protozoan, *Trichomonas vaginalis* (*T. vaginalis*). More than 160 million people worldwide are annually infected by this protozoan. *T. vaginalis* occupies an extracellular niche in the complex human genito-urinary environment (vagina, cervix, penis, prostate gland, and urethra) to survive, multiply and evade host defenses. *T. vaginalis* (strain G3) has a ~160 megabase genome with 60,000 genes, the largest number of genes ever identified in protozoans. The *T. vaginalis* genome is a highly conserved gene family that encodes a massive proteome with one of the largest coding (expressing ~4000 genes) capacities in the trophozoite stage, and helps *T. vaginalis* to adapt and survive in diverse environment. Based on recent developments in the field, we review *T. vaginalis* structure, patho-mechanisms, parasitic virulence, and advances in diagnosis and therapeutics.

Contents

Abbreviations: Ad, adhesion molecules; AT, agglutination test; CCC, cation-chloride cotransporter; CBD, cell-binding domain; CDC, Centers for Disease Control and Prevention; CE, cytopathic effects; ePKs, eukaryotic protein kinases; ECM, extracellular matrix; Fdx, ferredoxin; FN, fibronectin; GBD, gelatin-binding domain; GPI, glycosylated phosphatidylinositol; GU, gonococcal urethritis; HIV, human immunodeficiency virus; HPV, human papillomavirus; IL-8, interleukin 8; LRR, leucine-rich repeat; LPG, lipophosphoglycan; LF, lytic factors; Mz, metronidazole; MzR, metronidazole-resistant; MAPK, mitogen-activated protein kinase; NTD, N-terminal domain; NGU, non-gonococcal urethritis; NAAT, nucleic acid amplification test; Pap, Papanicolaou; PE, phospholipids phosphatidylethanolamine; PCR, polymerase chain reaction; KOH, potassium hydroxide; NADPH, reduced nicotinamide adenine dinucleotide phosphate; STI, sexually transmitted infections; snRNA, small nuclear RNAs; TMA, transcription-mediated amplification; tRNAs, transfer RNA; TM, transmembrane; TKL, tyrosine kinase-like; UTR, untranslated region; UTI, urinary tract infection; VECs, vaginal epithelial cells; VSP, variant surface proteins.

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1. Introduction

Trichomoniasis is an extremely common cosmopolitan non-viral genitourinary sexually transmitted infection (STI) in humans caused by the ancient protist *Trichomonas vaginalis*, and described decades before *Chlamydia trachomatis* and human papilloma virus (HPV) infection (Table 1). Worldwide 160–180 million people are affected annually by trichomoniasis. Among this population about 154 million people are in resource-limited settings, 8–10 million are in the United States and 11 million are in Europe [1]. In US the prevalence among women ranges from 2.8% (adolescents) to 51% (black communities) [2], and 51% in black men [2]. Every year a large number of asymptomatic women are attending an obstetrics and gynecology clinic. WHO has estimated that trichomoniasis accounts almost half of all curable STIs [3]. Despite these studies and high prevalence, it is one of the most poorly studied parasites with respect to virulence properties, pathogenesis and immunopathogenesis. With the development of reverse transcriptase polymerase chain reaction (RT-PCR) and nucleic acid amplification tests for *T. vaginalis* DNA, our understanding of the epidemiology of this pathogen has improved and suggests that as many as one-third of infections in women are asymptomatic. At present, the majority of studies are limited to adolescents and young adult women, but the prevalence of the infection has been found to increase with age. There are even fewer studies detailing its incidence and prevalence among men. More recently, acquired data provide evidence that the interactions of *T. vaginalis* with vaginal epithelial cells are exceedingly complex. In this review we summarize the recent series of new findings on *T. vaginalis* ultrastructure, pathogenesis, immunopathogenesis and new developments in diagnostics and therapeutics. Due to space limitation, we emphasize mostly recent review articles, as they include many original findings contributing to the current state of knowledge.

2. Structure

T. vaginalis is a flagellated parasitic protozoan (protist), typically pyriform but occasionally amoeboid in shape, extracellular to genitourinary tract epithelium with a primarily anaerobic lifestyle (Fig. 1, Table 2) [4,5]. *T. vaginalis* is a member of the parabasilid lineage of microaerophilic eukaryotes that have unique energy-producing double membrane organelles known as hydrogenosomes [6]. Hydrogenosomes lack cytochromes, mitochondrial respiratory chain (RC) enzymes and DNA, but catalyze carbohydrate (fermentation) and produce molecular hydrogen by a diverse group of iron-only [Fe]-hydrogenases that possess a conserved H-cluster with four different sets of functional domains. *T. vaginalis* hydrogenosomes contain the complete machinery required for mitochondria-like organelles with FeS cluster and putative cytosolic auxiliary proteins for biogenesis of cytosolic FeS protein [7].

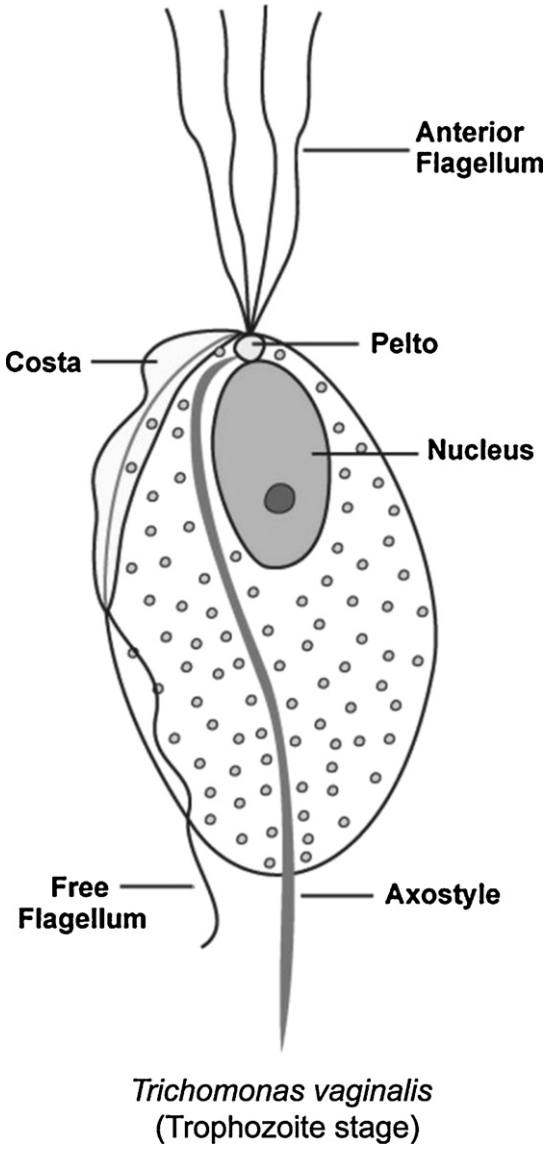

Fig. 1. Morphology of *T. vaginalis*.

3. Genome and transcription machinery

T. vaginalis has a large genome (strain G3, 176,441,227 bp) with ~60,000 protein coding genes, organized into six chromosomes [7–9] (Table 2). The *T. vaginalis* genome contains a large number of histone genes, and most of them organize as gene pairs in a

Table 1
Trichomonas vaginalis (*T. vaginalis*) – Time line (For detail see Refs. [1,4,5,8]).

1836	• Alfred Donne identified *T. vaginalis* as motile microorganisms in the purulent, frothy leukorrhea of women presenting with vaginal discharge and genital irritation.
1916	• O. Hohne used the term "trichomoniasis" to describe the clinical condition of trichomoniasis, wherein *T. vaginalis* colonizes the vaginal mucosa.
1934–1939	• L. Procaccini identified and classified *T. vaginalis* as a venereal disease in a group of Italian soldiers serving in the Eastern Italian Army in Ethiopia.
1940	• R.E. Trussell found that *T. vaginalis* produced vaginitis in 9 of 20 women by culturing *T. vaginalis*.
1959	• D.H. Clark and E. Solomos developed routine culture examination for *Trichomonas vaginalis*
1960s–1970s	• The research focused on biochemical tests and microscopic examination to understand the growth characteristics and behavior of *T. vaginalis*.
1960s	• 5-Nitroimidazoles is used for the management of trichomoniasis.
1980s–2000s	• The immunologic methods and molecular biology techniques are used to study the pathogenesis and immunology of *T. vaginalis*.
2007	• Joint project on the *T. vaginalis* genome sequencing from the Institute for Genomic Research (TIGR), the Center for the Advancement of Genomics (TCAG), the J. Craig Venter Science Foundation, the Joint Technology Center and the Institute for Biology Energy Alternatives (IBEA) used whole-genome shotgun methodology (contains 1.4 million shotgun reads assembled into 17,290 scaffolds at ~7.2× coverage) to identify the genome for *T. vaginalis* (strain G3, ~160 megabase genome, Mbp).

Table 2
Trichomonas vaginalis's (*T. vaginalis*) – Key characteristics related to morphology, genetics, pathogenicity, metabolism and life cycle (For detail see Refs. [4–32]).

Shape	• Amoeboid or pyriform (pear shaped)
Size	• ~9–23 × 7 μm (average 13 μm)
Flagella	• Anterior four [9(2)+2 arrangement]
	• Recurrent one (originate from blepharoplast)
Internal organalles	• Nucleus, Axostyle, Costa, Pelta
	• Cytoskeleton (made up of actin, tubulin, kinesin and dynenin)
	• Hydrogenosome without cytochrome, electron transport chain (ETC) enzymes and DNA
Genome	• 176,441,227 bp (strain G3), Six chromosomes
	• ~65% Repetitive genome (includes virus-like, transposan-like, retrotransposan-like and unclassified repeats)
	• 32.7% Guanine and cytosine (G+T) rich regions
	• 65 Genes with short introns and conserved 12-nt sequence (5′-ACTAACACACAG-3′) at the 3′splice-site
	• ~60,000 Protein coding genes
	• 74 Functional core histones including 11 H2A/H2B gene pairs, 6 solitary H2A, 3 solitary H2B, 19 H3/H4 gene pairs, 2 solitary H3 and 3 solitary H4
	• 5-Spliceosomal small nuclear RNA (snRNA-U1, U2, U4, U5 and U6) with conserved sequences and motifs similar to eukaryotes, lack 59- trimethylguanosine cap that typical of snRNAs
	• ~250 Ribosomal DNA (rDNA)
	• Transfer RNA (tRNAs) for all 20 amino acids
	• ~927 Protein kinase coding genes (ePKs)
Surface proteins	• ~3000, Grouped into three major categories (BspA-like proteins, GP63-like proteins, adhesins and other proteins)
Energy sources	• Carbohydrate via fermentative metabolism, and amino acids through arginine dihydrolase metabolism; enzyme amino-transferases and glutamate dehydrogenase synthesizes glutamate, aspartate, alanine, glutamine and glycine; enzyme cysteine synthase synthesizes cysteine; synthesize proline from arginine; synthesize phospholipids; metabolize threonine.
Adhesion proteins	• Adhesion with host vaginal epithelial cells (VECs) through adhesion molecules (AP65, AP51, AP33, AP23), fibronectin binding protein, laminin binding protein and glycolipids (Lipophosphoglycan, LPG)
Life cycle	• Only trophozoite stage, no cystic stage known

head-to-head manner. *T. vaginalis* has more metazoan eukaryotic transcription machinery than protistan. Introns are present in 65 genes with conserved regulatory motifs and characteristics are similar to *Giardia lamblia*, but not to other metazoan introns. *T. vaginalis* has five spliceosomal small nuclear RNAs (snRNA-U1, U2, U4, U5, and U6) without cap structure [10]. The protein coding genes in *T. vaginalis* are transcribed by RNA polymerase II [7]. U1, U2, U4, and U5 genes are transcribed by RNA polymerase II, whereas the U6 gene is transcribed by RNA polymerase III [9]. A metazoan-like TATA element is absent in trichomonad promoter, but an initiator (Inr) sequence or Inr promoter elements are present in ~75% of 5′ untranslated region (UTR) sequences of the protein-coding genes. The Inr is the only element in metazoan protein encoding genes known to be a functional analog of the TATA box for accurate transcription initiation [7]. The Inr element is located within 20 nucleotides upstream of the start codon and may be as close as 6 nucleotides. This motif with the consensus sequence TCA + 1Py(T/A), surrounds the transcription start site of all genes studied so far. *T. vaginalis* has an Inr-binding protein, a 39 kDa polypeptide (IBP39). IBD39 has two domains, connected by a proteolytically sensitive linker, whereas the N-terminal domain binds Inr (IBD) and the C-terminal domain (CTD) binds the RNA pol II large subunit [11]. IBD domain is a lineage-specific DNA binding domain that is utilized by specific transcription factors in *T. vaginalis*.

The *T. vaginalis* genome encodes membrane trafficking machinery, pathogenic proteins for endocytosis of host proteins and phagocytosis of bacteria and host cells that help active endocytosis and support the phagocytic life-style [7,8]. *T. vaginalis* has a massively amplified gene family for encoding distinct eukaryotic protein kinases (ePKs, ~880), atypical protein kinases (~40), cytosolic tyrosine kinase-like (TKL, ~124) genes without receptor serine/threonine ePKs of the TKL family, heterotrimeric guanine nucleotide–binding proteins, and components of the mitogen-activated protein kinase (MAPK) pathways, suggesting yeast-like signal transduction mechanisms. Inactive kinases are also found that make up to 17% of the *T. vaginalis* kinome and may act as substrates and scaffolds for assembly of signaling complexes [7,8]. ePK accessory domains are important for regulating signaling pathways. There are nine accessory domains in 8% of the *T. vaginalis* ePKs. The regulation of protein kinase functions and cell signaling in *T. vaginalis* are, however, less complex than that in higher eukaryotes [5,8].

4. Surface proteins

T. vaginalis has over 300 candidate surface proteins which belong to ten different protein families with at least one inferred transmembrane (TM) domain and share one or more features with other pathogen surface proteins. Three surface protein families are BspA-like proteins (TpLRR-containing proteins), GP63-like proteins and adhesins or others [12,13] (Table 2). The BspA-like proteins are the largest gene family encoding potential surface proteins and share a specific type of leucine-rich repeat (LRR), the TpLRR. The GP63-like proteins are the second largest gene family of candidate surface proteins encoding 77 paralogues, and 53 possess potential TM domains. GP63 are metalloproteinases (MMP) belonging to the metzincin class (EC 3.4.24.36), characterized by the motif HExxHxxGxxH (x represents any amino acid residues) forming an extended zinc-binding motif and a catalytic site. Additional candidates of this family are 28 subtilisin-like serine proteases, nine different serine proteases and five calpain-like cysteine proteases. The calpain-like cysteine proteases possess 22–23 identifiable TM domains and function as surface proteases that transport important protein fragments or small peptides for energy generation from amino acids and redox balancing. The third surface protein family of *T. vaginalis* shares domains with other surface proteins related to mucosal pathogens. The members of this family are identified at different stages of infection during mucosal contact and help *T. vaginalis* to escape from the host adaptive immune response.

5. Energy sources

T. vaginalis is a highly predatory obligate parasite that phagocytoses bacteria, vaginal epithelial cells (VECs) and erythrocytes, and is itself ingested by macrophages [5,8]. *T. vaginalis* uses carbohydrates as its main energy source via fermentative metabolism under aerobic and anaerobic conditions. *T. vaginalis* lacks the ability to synthesize many macromolecules *de novo*, particularly purine, pyrimidine, and many lipids, including cholesterol. These nutrients are acquired from the vaginal secretions or through phagocytosis of host and bacterial cells. *T. vaginalis* has a broad range of transport capabilities that facilitate transport of complex carbohydrates and amino acids through the members of the cation-chloride co-transporter (CCC) family proteins, and help survival by reflecting osmotic changes in a mucosal environment. *T. vaginalis* has an unusual biosynthetic pathway for synthesis of non-protein lipid anchors (inositol-

phosphoceramide) of surface lipophosphoglycans (LPG) [14]. With a microaerophilic (aerobic) lifestyle, *T. vaginalis* uses redox and antioxidant systems to counter the detrimental effects of oxygen and express a wide range of genes encoding for defense molecules including superoxide dismutases, thioredoxin reductases, peroxiredoxins, and rubrerythrins.

6. Pathogenesis

Trophozoites of *T. vaginalis* are transmitted from person to person through sexual intercourse [5,16–18]. Non-sexual transmission of *T. vaginalis* is rare. Cystic stages are unknown for *T. vaginalis*. The trophozoite attaches to mucosal surfaces of the lower urogenital tract and divides by longitudinal binary fission. *In vitro* studies suggest that trophozoites have a 4–28 day incubation period. *T. vaginalis* survives long-term in the varying and adverse acidic environment of the vagina through various successful host parasitisms. VEC surface or extracellular matrix (ECM) proteins play different roles in binding diverse target molecules from the host or other microbes from the mucosal surfaces that are mediated by *T. vaginalis* surface proteins including LPG, cytokines, cytoskeletal protein α-actinin.

6.1. Adhesion

T. vaginalis possesses multiple mechanisms for colonization in the vaginal tract due to a dynamic hormonal influence from the menstrual cycle on the exfoliation of VECs and a constantly changing environment. After cytoadherence, *T. vaginalis* transforms to an amoeboid structure with increasing cell-to-cell surface contact, forming cytoplasmic projections that interdigitate with target cells. The interactions of *T. vaginalis* with mucin, VECs and ECM molecules persist in a non-self-limiting fashion. *T. vaginalis* releases cysteine proteinases into the vaginal milieu resulting in desquamation of the vaginal and cervical epithelia [19–21]. This facilitates efficient cytotoxicity toward host cells with complex interactions similar to the situation for other cell–cell contacts.[71]LPG is a major adherence factor in *T. vaginalis*, although studies of the molecular basis of adhesion of *T. vaginalis* to human cells have revealed that several other genes [including adhesion proteins (AP), fibronectin (FN)-binding protein, laminin-binding protein, α-actinin, enolase, phosphoglucomutase, and conserved GTP-binding protein (GTP-BP)] expressions are up-regulated [7,22–24]. At protein level four major adhesion proteins (AP65, AP51, AP33 and AP23), GAPDH and several hypothetical proteins are up-regulated in a specific receptor-ligand fashion that depends on time, temperature and pH, prompting *T. vaginalis* to become flat and laminate itself to the host cell [7,22–24]. Much of the evidence for the role of AP in pathogenesis has come from co-culture experiments in which antibodies to AP are shown to reduce parasite adhesion and subsequent cytopathic effects (CE) on host cells. Additionally, laminin- and FN-binding proteins are also up-regulated in *T. vaginalis* cytoadherence to the vaginal epithelium. *T. vaginalis* AP molecules bind laminin of the host target cells. FN-binding protein binds ECM components such as collagen, fibrin and heparan sulfate proteoglycans (e.g. syndecans). *T. vaginalis* binds to multiple FN domains including the N-terminal domain (NTD), the cell-binding domain (CBD) and the gelatin-binding domain (GBD). During these processes, iron, calcium and phosphatase are essential nutrients for differential gene expression in *T. vaginalis* to survive, grow, and colonize in the vaginal hostile environment.

The ap65-1 gene encodes a 65 kDa malic enzyme involved in cytoadherence. The transcription of this gene is critically regulated by the coordination of two similar but opposite oriented DNA regulatory regions, MRE-1/MRE-2r and MRE-2f; both of the regulatory proteins bind for multiple Myb-like proteins and binding varies with iron concentrations [7,25,26]. Myb1 proteins bind ap65-1 promoter at a proximal site in higher iron levels, whereas Myb2 protein binds in iron-depleted conditions [7,26]. Another iron-inducible nuclear protein (Myb3) binds only to the MRF-1 element. Moreover, Myb2 and Myb3 co-activate basal and iron-inducible ap65-1 transcription against Myb1 through conditional and competitive promoter entries. The cysteine proteinases are necessary for efficient AP-mediated adhesion of *T. vaginalis* to target cells. Since *T. vaginalis* is unable to synthesize lipids, erythrocytes may be the prime source of the fatty acids as well as iron, as an important nutrient for the trophozoids.

6.2. Hydrolases and cytotoxic molecules

A wide range of hydrolases (20–110 kDa) has been identified in *T. vaginalis* as cytoplasmic cysteine proteinases [5,7,8]. These hydolases have trypsin-like activity and function as cell-detaching factors by degrading proteins (such as laminin, fibronectin, and other components) of the ECM and aid in the release of host cells from tissue and mucosal desquamation. *T. vaginalis* proteinases of 25, 27 and 34 kDa are specifically hydrolyzed synthetic substrates with arginine–arginine residues, whereas other proteinases have activity over a wide substrate range. Four different cysteine proteinase genes of *T. vaginalis* have ~45% homology to cysteine proteinase genes of *Dictyostelium discoideum*, and are L-cathepsin and H-papain type proteinases [27]. These proteinases allow *T. vaginalis* to traverse the protective mucus barrier of host epithelium.

T. vaginalis produces several cytotoxic molecules and mediate cytotoxicity by damaging the target cell plasma membrane. Some of these molecules have perforin-like activity and create pores in erythrocyte membranes [5,7,8]. *T. vaginalis* also secretes different lytic factors (LF) with phospholipase A2 activities to destroy nucleated cells, erythrocytes and specifically degrade phosphatidylcholine, underlying its unique pathogenesis.

6.3. Host response and innate immunity mechanism

T. vaginalis evades the immune system through complement-mediated destruction, molecular mimicry and coating itself with host plasma proteins [28–31]. Natural infection of *T. vaginalis* to humans seems to produce immunity that is only partially protective. *T. vaginalis* has unique abundant cell surface lipophosphoglycan (LPG) [28], a carbolipid molecule ($2–3 \times 10^6$ copies/parasite) that similar to prokaryotic glycoconjugates. LPG plays a major pathogenic and immunoregulatory role to help adherence with VECs; triggers leukocytes to secretes interleukin-8 (IL-8), parasite-specific immunoglobulin G (IgG), IgA, Th1 cytokines, leukotrienes, reactive nitrogen intermediates and macrophage inflammatory protein-3α; induce nitric oxide synthase (iNOS); priming of helper T cells and promote transmigration of neutrophils across the endothelium [28–31]. *T. vaginalis* produces immuno-suppressive cytokines (IL-10, TGFβ) and causes caspase-mediated apoptosis in T-cells, macrophages and dendritic cells.

Moreover, recent comprehensive compositional and structural analysis of *T. vaginalis* revealed that LPG has specific LPG domains with proinflammatory properties, and its outer branch saccharide and ceramide phospho-inositol glycan core (CPI-GC) activates NFκB, ERK1/2 and MEK1/2 (28). Furthermore, *T. vaginalis* induces COX-2 expression, and up-regulates and activates toll-like receptors (TLR2, 4, and 9) via the p38 mitogen-activated protein kinase (MAPK) pathway. LPG CPI-GC contains terminal poly-N-acetylactosamine repeats that represent the ligand for the animal lectins called galectins [28]. Cervical and VECs release galectin-1

Table 3
Trichomonas vaginalis (*T. vaginalis*) – Transmission, clinical signs and symptoms (For detail see Refs. [5,16,17,18]).

Transmission	• Through sexual intercourse • Affects genitourinary tract
Incubation period	• 4–28 days
Symptoms	• In females 1. Vaginal discharge, green to brown color (42%), frothy (~10%) 2. Foul odor of vaginal discharge (50%) 3. Edema or Erythema (22–37%) 4. Pruritus 5. Colpitis mascularis (Strawberry cervis) by punctate hemorrhages (50%) 6. Vaginal itching 7. Urinary tract irritation 8. Soreness 9. Dysuria 10. Lower abdominal pain 11. Elevated pH greater than 4.5, normal pH of the vagina is 3.8–4.4) 12. Presence of amines, vaginal leucocytosis, vulvar erythema, purulent with white blood cells (WBCs) • In males 1. Symptoms of inflammation 2. Urethral discharge 3. Urethritis or gonococcal urethritis 4. Irritation 5. Non-gonococcal urethritis (NGU) or prostatitis 6. Epididymitis 7. Reduced sperm function 8. Infertility

and galectin-3 upon *T. vaginalis* infection and modulate the inflammatory responses in opposite fashion (galectin-1 suppressing and galectin-3 enhancing leucocyte response to inflammatory response) [30,31]. Also galectin-1 promotes viral attachment, whereas HIV-1 infected cells enhance viral replication, gelatin-3 and cytokine expression in VECs [31,32]. Thus, natural infection with *T. vaginalis* results in priming of acquired immune responses. Moreover, studies from the patients infected with *T. vaginalis* and HIV indicated that innate immunity involves chemotaxis and subsequent influx of neutrophils [1,2,28–31].

7. Clinical signs and symptoms

Humans are the only natural host of *T. vaginalis*. There are two additional species of trichomonas (*T. tenax*, *T. hominis*) that infect humans but do not cause any diseases. The evidence for sexual transmission of trichomoniasis is unequivocal. Prevalence is highest among women with multiple sexual partners and with other STIs [33–35]. In women, *T. vaginalis* adheres to and damages VECs and causes vaginitis. Women with symptomatic trichomoniasis have a wide range of symptoms ranging from a relatively asymptomatic state to severe inflammation (Table 3). Nearly half of all women with *T. vaginalis* are asymptomatic. Trichomonads also affect the bladder, urethra and paraurethral glands as urinary tract infection. Moreover, women with trichomoniasis have several complications associated with adverse pregnancy outcome, preterm birth or premature labor, low birth weight, premature rupture of membranes, greater risk of tubal infertility,

Table 4
Trichomonas vaginalis (*T. vaginalis*) – Diagnostic tests. In tables different *T. vaginalis* diagnostic tests are given for female vaginal swab and urinary samples. For males, diagnostic tests are restricted. However, for males, urinary sediments, urethral swab culture, wet mount and PCR are used. These diagnostic tests for males are ~60% sensitive and highly specific except PCR test, which is >90% sensitive and >90% specific (For detail see Refs. [34,36–43]).

	Characteristics	Sensitivity	Specificity
4A. Old diagnostic tests (Microscopic evaluation)			
1. Papanicolaou (Pap) smear	• Direct visualization of motile trichomonads in saline preparation • Perform within 10–20 min of sample collections	50%	90%
2. Staining techniques	• Can detect 3-trichomonads/ml Most common dyes are acridine orange, leishman stain, periodic acid-schiff stain, Fontana dye	30–60%	Less specific
3. Wet mount	• A physiological saline preparation of vaginal secretions • Trichomonads can be identified by size (similar to WBC), shape and quivering/twitching motility • Require ~104 trichomonads/ml vaginal fluid	50-60%	>90%
4. The agar plate technique	• A timesaving culture technique with microscopic examination of the whole clinical material obtained, require a turnaround time of 2–6 days • Eliminates the need for slide preparations. • Favorable for screening a low-risk population	>90%	>90%
4B. New diagnostic tests			
1. Broth culture (Gold standard)	• Require ~300–500 trichomonads/ml to culture at 37 °C for 2–7 days • Culture media types (Diamond's media for culture in glass tube; InPouch TV media, a double pouched soft transparent plastic container, Biomed Diagnostics, USA; Trichosel media)	~85–95%	>95–100%
2. Odor test	• Whiff test (amine odor test) • Perform by mixing vaginal secretions with 10% potassium hydroxide (KOH), gives strong fishy odor	–	Poor specificity due to similar results with bacterial vaginosis
3. XenoStrip-Tv technology	• Antigen specific color immunochromatographic "dipstick" test (mouse antibody bound to a nitrocellulose membrane) • Results can be obtained within 10 min	~66%	100%
4C. Newest diagnostic test			
1. Affirm VPIII test	• Unamplified RNA test (Becton Dickinson, Sparks, Maryland, USA) • Rapid expensive test, require a complete laboratory, 30–60 min to perform	<90% (false +ve from dead organisms)	99%
2. Rapid antigen test	• Point-of-care lateral flow test strip device (OSOM TV, Genzyme Diagnostic, Cambridge, MA, USA) • Detect *T. vaginalis* membrane proteins within 10 min	83–90%	99–100%
3. Nucleic acid amplification test (NAAT)	• Use of analyte-specific reagents to analyze transcription-mediated amplification (TMA)	96–98% (vaginal swab), 88% (urine specimen)	>98%
4. TV Polymerase chain reaction (PCR)-based test	• Provides a good alternative to culture for vaginal specimens • Limited availability	64–89%	97–100%

Table 5
Management of trichomoniasis (For detail see Refs. [5,44–46]).

Recommended regimens	Dose	Cure rate	Avoid medication
1. Metronidazole	2 g orally in a single dose OR 500 mg orally twice for 7 days	~90–95%.	First trimester of pregnancy and during lactation
2. Tinidazole	2 g orally in a single dose	>90%	During pregnancy, lactation or breastfeeding (should be with held ~12–24 h or 3 days after the last treatment), or in patients with blood dyscrasia or active neurological disorders

atypical pelvic inflammatory disease, amplified HIV transmission/acquisition, and increased risk of cervical cancer [5].

The prevalence and spectrum of trichomoniasis in males are less well characterized but the infection appears to be asymptomatic in 70% of men [5] (Table 3). In sexually active men, however, *T. vaginalis* causes urethritis, balanoposthitis, prostatitis, cystitis, and epididymo-orchitis and increases the risk of HIV transmission. *T. vaginalis* serves as a vector for the transmission of various infections. The oxidative nature of male genital fluid is, however, hypothesized to be inhibitory to certain pathogenic factors for the protozoan [5]. The presence of zinc in prostatic fluid acts as cytotoxic factor for the trichomonad. In contrast, the vagina has a reducing environment, which may contribute to the activation of some pathogenic mechanisms of *T. vaginalis*. Furthermore, the levels of secretory leukocyte protease inhibitor are significantly lower in *T. vaginalis* infected patients [5].

8. Diagnostic tests

A wide range of classic symptoms are associated with infection by *T. vaginalis* (Table 3), but these symptoms are similar to other STIs and cannot be used as a diagnostic markers for *T. vaginalis*. Thus, accurate, reliable, convenient, and inexpensive laboratory diagnostic tests play a key role in the diagnosis of *T. vaginalis*. These diagnostic tests are classified as old (Papanicolaou, Pap and wet mount), newer (culture and RNA), and newest (rapid antigen and nucleic acid amplification) techniques [34,36–42] (Table 4). Detailed guidelines regarding the diagnosis of trichomoniasis are available in the 2006 Sexually Transmitted Disease Treatment Guidelines published by the Centers for Disease Control and Prevention (http://www.cdc.gov/) [43]. Most commonly urine specimens are used for the diagnostic tests in male. For women, however, endocervical and vaginal swab specimens are more reliable.

9. Nitroimidazoles

Since the 1960s, systemic treatment with 5-nitroimidazoles is the cornerstone of management of trichomoniasis. Currently metronidazole (Mz) and the other 5-nitroimidazoles (tinidazole, ornidazole, and secnidazole) have become the standard therapies for treating *T. vaginalis* [5,44] (Table 5). Mz is a 5-nitroimidazole, a heterocyclic compound with a nitro group on the fifth position at imidazole ring, derived from the *Streptomyces* antibiotic azomycin. As an inactive molecule, Mz enters *T. vaginalis* through passive diffusion and forms a cytotoxic nitro radical anion by anaerobic reduction through hydrogenosomes by pyruvate-ferredoxin oxidoreductase (PFOR). In this process, Mz acts as an electron sink by capturing the electrons from reduced ferredoxin, which would normally be donated to hydrogen ions to form hydrogen gas in the hydrogenase reaction. This process is induced by Mz in a concentration gradient manner in *T. vaginalis*. Finally, nitro radicals bind transiently to DNA, disrupt the DNA and cause cell death. Mz gel is effective in less than half the cases of trichomoniasis. Tinidazole is a second-generation nitroimidazole with a plasma elimination half-life twice that of Mz (12–14 h vs. 6–7 h). Moreover, tinidazole penetrates better into male reproductive tissues than Mz. At present, Mz and tinidazole are the only approved drugs for treatment of trichomoniasis in the USA.

An emerging problem with Mz is rising Mz-resistance (MzR) against *T. vaginalis* (~2.5–5% of reported cases), failure to cure the infection with two consecutive courses, and development of allergic reactions [45,46]. Resistance affects both aerobic and anaerobic mechanisms of metabolism. Resistance by aerobic mechanisms possibly arises by reduced transcription of the ferredoxin gene with decreased trafficking and activation of Mz in the cell, affecting the oxygen-scavenging pathways [5]. In anaerobic resistance, the activities of POFR and hydrogenase are decreased with concomitant reduction in hydrogen production by the impaired oxygen-scavenging mechanisms in the hydrogenosome. Since oxygen is a highly efficient electron receptor, increased levels of cellular (hydrogenosomal) oxygen result in impaired reduction and activation of Mz. If Mz is not reduced, the concentration of the Mz is same in the intra- and extra-cellular environments, and no additional Mz enters into the cells [5]. Additionally, in the presence of oxygen, reduced Mz changes into free radical and is oxidized back to the original drug, which ultimately reduces to become superoxide anion. This process is known as futile cycling and causes only limited cell damage by the superoxide anion rather than nitro free radicals. To overcome resistance to Mz, clinicians can treat the patients with tinidazole or Mz at 2 gm orally for 10–14 days, since the ferredoxin levels and Mz resistance are inversely related.

10. Conclusions

Trichomoniasis is an extremely common infection, but it has remained ignored as a public health issue. A significant development has taken place in diagnosis and therapeutics to resolve trichomoniasis. Current treatment with nitroimidazoles is reliable and inexpensive, but the number of resistant strains is increasing. Moreover, nitroimidazoles treatments are not recommended for pregnant and lactating women due to incomplete establishment of safety issues. Therefore, more studies are needed to determine the most sensitive diagnostic kits with high specificity associated with rapid read-outs as well as to expand therapeutics in both men and women.

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References

[1] McClelland RS. *Trichomonas vaginalis* infection: can we afford to do nothing? J Infect Dis 2008;197:487–9.
[2] Shafir SC, Sorvillo FJ, Smith L. Current issues and considerations regarding trichomoniasis and human immunodeficiency virus in African–Americans. Clin Microbiol Rev 2009;22:37–45.

[3] World Health Organization (WHO). Global prevalence and incidence of selected curable sexually transmitted infections. 2001. WHO/HIV-AIDS/2001.02/CDC/CSR/EDC/2001.10.
[4] Benchimol M. Trichomonads under microscopy. Microsc Microanal 2004;10(5):528–50.
[5] Sood S, Kapil A. An update on *Trichomonas vaginalis*. Indian J Sex Transm Dis 2008;29:7–14.
[6] Sutak R, Dolezal P, Fiumera HL, et al. Mitochondrial-type assembly of FeS centers in the hydrogenosomes of the amitochondriate eukaryote *Trichomonas vaginalis*. Proc Natl Acad Sci USA 2004;101(28):10368–73.
[7] Gomez C, Esther Ramirez M, Calixto-Galvez M, Medel O, Rodríguez MA. Regulation of gene expression in protozoa parasites. J Biomed Biotechnol 2010;2010:726045.
[8] Carlton JM, Hirt RP, Silva JC, et al. Draft genome sequence of the sexually transmitted pathogen *Trichomonas vaginalis*. Science 2007;315:207–12.
[9] Simoes-Barbosa A, Meloni D, Wohlschlegel JA, et al. Spliceosomal snRNAs in the unicellular eukaryote *Trichomonas vaginalis* are structurally conserved but lack a 5′-cap structure. RNA 2008;14(8):1617–31.
[10] Zubácová Z, Cimburek Z, Tachezy J. Comparative analysis of trichomonad genome sizes and karyotypes. Mol Biochem Parasitol 2008;161:49–54.
[11] Lau AOT, Smith AJ, Brown MT, Johnson PJ. *Trichomonas vaginalis* initiator binding protein (IBP39) and RNA polymerase II large subunit carboxy terminal domain interaction. Mol Biochem Parasitol 2006;150(1):56–62.
[12] Hirt RP, Noel CJ, Sicheritz-Ponten T, et al. *Trichomonas vaginalis* surface proteins: a view from the genome. Trends Parasitol 2007;23(11):540–7.
[13] Vogel C, Chothia C. Protein family expansions and biological complexity. PLoS Comput Biol 2006;2(5):e48.
[14] Fichorova RN, Trifonova RT, Gilbert RO, et al. *Trichomonas vaginalis* lipophosphoglycan triggers a selective upregulation of cytokines by human female reproductive tract epithelial cells. Infect Immun 2006;74(10):5773–9.
[15] Coombs GH, Westrop GD, Suchan P, et al. The amitochondriate eukaryote *Trichomonas vaginalis* contains a divergent thioredoxin-linked peroxiredoxin antioxidant system. J Biol Chem 2004;279:5249–56.
[16] Hawkes S, Santhya KG. Diverse realities: sexually transmitted infections and HIV in India. Sex Transm Infect 2002;78(Suppl 1):i31–9. Erratum in: Sex Transm Infect 2005;81(3):282.
[17] Schwebke JR, Burgess D. Trichomoniasis. Clin Microbiol Rev 2004;17(4):794–803.
[18] Singh BN, Lucas JJ, Fichorova RN. Trichomonas vaginalis: pathobiology and pathogenesis. In: Khan NA, et al., editors. Emerging Protozoan Pathogens. London, UK: Taylor & Francis Group; 2007. p. 411–55.
[19] Westrop GD, Goodall G, Mottram JC, et al. Cysteine biosynthesis in *Trichomonas vaginalis* involves cysteine synthase utilizing O-phosphoserine. J Biol Chem 2006;281(35):25062–75.
[20] Sommer U, Costello CE, Hayes GR, et al. Identification of *Trichomonas vaginalis* cysteine proteases that induce apoptosis in human vaginal epithelial cells. J Biol Chem 2005;280(25):23853–60.
[21] Yadav M, Dubey ML, Gupta I, Malla N. Cysteine proteinase 30 (CP30) and antibody response to CP30 in serum and vaginal washes of symptomatic and asymptomatic *Trichomonas vaginalis* infected women. Parasite Immunol 2007;29:359–65.
[22] Alderete JF, Benchimol M, Lehker MW, et al. The complex fibronectin–*Trichomonas vaginalis* interactions and Trichomonosis. Parasitol Int 2002;51(3):285–92.
[23] Crouch ML, Benchimol M, Alderete JF. Binding of fibronectin by *Trichomonas vaginalis* is influenced by iron and calcium. Microb Pathog 2001;31(3):131–44.
[24] Lubick KJ, Burgess DE. Purification and analysis of a phospholipase A2-like lytic factor of *Trichomonas vaginalis*. Infect Immun 2004;72(3):1284–90.
[25] Kucknoor AS, Mundodi V, Alderete JF. The proteins secreted by *Trichomonas vaginalis* and vaginal epithelial cell response to secreted and episomally expressed AP65. Cell Microbiol 2007;9(11):2586–97.
[26] Hsu H-M, Ong S-J, Lee M-C, Tai J-H. Transcriptional regulation of an iron-inducible gene by differential and alternate promoter entries of multiple Myb proteins in the protozoan parasite *Trichomonas vaginalis*. Eukaryot Cell 2009;8(3):362–72.
[27] León-Sicairos CR, León-Félix J, Arroyo R. tvcp12: a novel *Trichomonas vaginalis* cathepsin L-like cysteine proteinase-encoding gene. Microbiology 2004;150:1131–8.
[28] Singh BN, Hayes GR, Lucas JJ, et al. Structural details and composition of *Trichomonas vaginalis* lipophosphoglycan in relevance to the epithelial immune function. Glycoconj J 2009;26:3–17.
[29] Yadav M, Gupta I, Malla N. Kinetics of immunoglobulin G, M, A and IgG subclass responses in experimental intravaginal trichomoniasis: prominence of IgG1 response. Parasite Immunol 2005;27:461–7.
[30] Fichorova RN. Impact of *T. vaginalis* infection on innate immune responses and reproductive outcome. J Reprod Immunol 2009;83:185–9.
[31] Than NG, Kim SS, Abbas A, et al. Chorioamnionitis and increased galectin-1 expression in PPROM—an anti-inflammatory response in the fetal membranes? Am J Reprod Immunol 2008;60:298–311.
[32] Than NG, Erez O, Wildman DE, et al. Severe preeclampsia is characterized by increased placental expression of galectin-1. J Matern Fetal Neonatal Med 2008;21:429–42.
[33] Schwebke JR, Hook 3rd EW. High rates of *Trichomonas vaginalis* among men attending a sexually transmitted diseases clinic: implications for screening and urethritis management. J Infect Dis 2003;188(3):465–8.
[34] Soper D. Trichomoniasis: under control or undercontrolled? Am J Obstet Gynecol 2004;190(1):281–90.
[35] Weinstock H, Berman S, Cates Jr W. Sexually transmitted diseases among American youth: incidence and prevalence estimates, 2000. Perspect Sex Reprod Health 2004;36:6–10.
[36] Huppert JS, Mortensen JE, Reed JL, et al. Rapid antigen testing compares favorably with transcription-mediated amplification assay for the detection of *Trichomonas vaginalis* in young women. Clin Infect Dis 2007;45(2):194–8.
[37] Lowe NK, Neal JL, Ryan-Wenger NA. Accuracy of the clinical diagnosis of vaginitis compared with a DNA probe laboratory standard. Obstet Gynecol 2009;113(1):89–95.
[38] Aslan DL, Gulbahce HE, Stelow EB, et al. The diagnosis of *Trichomonas vaginalis* in liquid-based Pap tests: correlation with PCR. Diagn Cytopathol 2005;32(6):341–4.
[39] Huppert JS, Batteiger BE, Braslins P, et al. Use of an immunochromatographic assay for rapid detection of *Trichomonas vaginalis* in vaginal specimens. J Clin Microbiol 2005;43(2):684–7.
[40] Hardick A, Hardick J, Wood BJ, et al. Comparison between the Gen-Probe transcription-mediated amplification *Trichomonas vaginalis* research assay and real-time PCR for *Trichomonas vaginalis* detection using a Roche LightCycler instrument with female self-obtained vaginal swab samples and male urine samples. J Clin Microbiol 2006;44(11):4197–9.
[41] Van Der Pol B, Kraft CS, Williams JA. Use of an adaptation of a commercially available PCR assay aimed at diagnosis of chlamydia and gonorrhea to detect *Trichomonas vaginalis* in urogenital specimens. J Clin Microbiol 2006;44(2):366–73.
[42] Sena AC, Miller WC, Hobbs MM, et al. *Trichomonas vaginalis* infection in male sexual partners: implications for diagnosis, treatment, and prevention. Clin Infect Dis 2007;44:13–22.
[43] Centers for Disease Control and Prevention, Workowski KA, Berman SM. Sexually transmitted diseases treatment guidelines. MMWR Recomm Rep 2006;55(August (RR-11)):1–94. Erratum in: MMWR Recomm Rep 2006;55(September (36)):997.
[44] Löfmark S, Edlund C, Nord CE. Metronidazole is still the drug of choice for treatment of anaerobic infections. Clin Infect Dis 2010;50(Suppl 1):S16–23.
[45] Cudmore SL, Delgaty KL, Hayward-McClelland SF, et al. Treatment of infections caused by metronidazole-resistance *Trichomonas vaginalis*. Clin Microbiol Rev 2004;17(4):783–93.
[46] Das S, Huengsberg M, Shahmanesh M. Treatment failure of vaginal trichomoniasis in clinical practice. Int J STD AIDS 2005;16(4):284–6.