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Challenges and Persistent Questions in Treatment of Trichomoniasis

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Abstract

Trichomoniasis is a sexually transmitted disease (STD) caused by infection with the protozoan parasite *Trichomonas vaginalis*. It is considered the most prevalent non-viral sexually transmitted disease worldwide. Recently, the infection has been associated with adverse outcomes of pregnancy and increased risks of HIV acquisition and transmission, besides the association with cervical and prostate cancers. The consequences of trichomoniasis are likely much greater than previously recognized, both at the individual and the community level. Since many cases are asymptomatic, and the most common approach used for diagnosis (wet mount) is also one of the least sensitive, millions of *T. vaginalis* infections remain undiagnosed and therefore untreated. The purpose of this review is to address what is known about the treatment of *T. vaginalis* infections and what additional approaches could be pursued. The increasing recognition of the potential public health implications of trichomoniasis has resulted in greater attention to improving effectiveness of the interventions for affected individuals. Currently, treatment relies almost solely on one class of drugs, the 5-nitroimidazoles, which causes concern should widespread drug resistance arise. There are also concerns regarding which 5-nitroimidazole to use as not all of them are active against *T. vaginalis*. Finally, new therapeutic targets and active compounds with treatment potential are considered.

Keywords

Trichomoniasis; treatment; 5-nitroimidazoles; pregnancy; neonates; children; mechanism of action; resistance; prevention; new alternatives

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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INTRODUCTION

Trichomoniasis is a sexually transmitted disease (STD) caused by infection with the protozoan parasite *Trichomonas vaginalis*. It is often considered the most prevalent non-viral sexually transmitted disease with estimates of almost 250 million infections worldwide [1]. Previously, *T. vaginalis* was thought of as simply a nuisance infection with no larger public health implications. However, in recent years, trichomoniasis has been associated with adverse outcomes of pregnancy and increased risks of HIV acquisition and transmission [2–7]. In addition, the association between *T. vaginalis* infection and cervical and prostate cancers has been described [8–10]. In the United States, trichomoniasis accounts for health care costs of $24 million per year [11]. These associations are compounded by the higher risk of *T. vaginalis* infection in persons with lower socioeconomic status who may have reduced access to healthcare [12]. Thus, the consequences of trichomoniasis are likely much greater than previously recognized, both at the individual and the community level.

Because many infections are asymptomatic, the most common approach used for diagnosis (wet mount) is also one of the least sensitive, and the fact that most infections are detected by passive surveillance, millions of *T. vaginalis* infections remain undiagnosed and therefore untreated. The health implications of undiagnosed or asymptomatic infections are not well understood but women can present with symptomatic infections several years after their last reported sexual encounter, suggesting that quiescent *T. vaginalis* infections can remain for long periods of time. Other evidence for persistent asymptomatic infection comes from active surveillance studies using sensitive nucleic acid detection that show older individuals have increased prevalence, the opposite pattern of what is observed with most other STDs [13, 14]. Asymptomatic individuals who have unprotected intercourse do present an infection risk to their sex partners.

The increasing recognition of the potential public health implications of trichomoniasis has resulted in greater attention to improving effectiveness of the interventions for affected individuals. Currently, treatment relies almost solely on one class of drugs, the 5-nitroimidazoles. This approach works for the vast majority of trichomoniasis patients but reliance on a limited armamentarium is concerning should widespread drug resistance arise. There are also adverse events that have been associated with the use of the 5-nitroimidazoles, many of which are valid while others are not supported by the current literature. The purpose of this review is to address what is known about the treatment of *T. vaginalis* infections and what additional approaches could be pursued.

TRICHOMONIASIS TREATMENT

Guidelines for treatment of trichomoniasis were updated in the United Kingdom and United States in 2014 and 2015, respectively [15, 16]. Both sets of guidelines recommend similar treatments with 2 g of metronidazole or tinidazole in a single oral dose or twice daily treatments of 500 mg metronidazole for 7 days. Although tinidazole is more potent than metronidazole *in vitro*, it is not available in a generic form and in the United States is not covered by some insurance plans [16, 17]. Fortunately, in a study of 538 *T. vaginalis*

isolates obtained from women attending STD clinics in 6 US cities, over 95% of isolates were susceptible to metronidazole *in vitro* [18]. However, that still leaves an estimated 160,000 persons in the United States [19], and perhaps more than 10 million worldwide that require an alternative treatment. Unfortunately, there are no other known oral drugs effective for treating trichomoniasis. Furazolidone, paromomycin sulfate, povidone iodine, and boric acid have all shown some efficacy as intravaginal treatments [20–25] but are much less effective than metronidazole or tinidazole [26]. Use of these drugs is limited to patients who have severe 5-nitroimidazole hypersensitivity or whose infections are highly resistant to metronidazole and tinidazole.

METRONIDAZOLE AND TINIDAZOLE SIDE EFFECTS – TRUTH VERSUS MYTH

Since its introduction in 1959, metronidazole has been the standard treatment for *T. vaginalis* infections. The therapy is effective for the vast majority of infected individuals and it is well tolerated with patients suffering few or no serious adverse events when treated with standard regimens. Commonly noted side effects include nausea, vomiting, headache, vertigo, diarrhea, disulfiram-like alcohol intolerance, and a metallic taste in the mouth [27–29]. More serious carcinogenic/mutagenic and teratogenic effects have also been attributed to 5’-nitroimidazole drugs [27], but the evidence for metronidazole and tinidazole causing such serious adverse events are far from conclusive.

Some persons demonstrate an apparent allergy to metronidazole that can be manifested as a hypersensitivity reaction with urticaria, rash, pruritus, bronchospasm, and fever. These reactions can also be observed in patients treated with tinidazole, because both drugs have similar chemical structures [30]. While some of these hypersensitivity events are self-reported and may simply represent a patient’s dislike of the drug’s taste and recommendation for avoiding alcohol, others have been directly observed and can be life threatening [31]. For persons with mild hypersensitivity (hive, rash), a short course desensitization protocol can be used to cure their infections [31] but treatment with 5-nitroimidazoles should be avoided for persons who have severe anaphylactic reactions. Studies on metronidazole hypersensitivity in women infected with *T. vaginalis* are mostly limited to case reports and the frequency and severity of these reactions have not been directly studied [32]. Additional data are needed to better understand the frequency and presentation of hypersensitivity induced by 5’-nitroimidazole drugs.

Another adverse reaction attributed to metronidazole is the disulfiram-like alcohol intolerance. It is very common to warn against the intake of alcohol while taking metronidazole, but the nature of the adverse events associated with alcohol intake while being treated with metronidazole is poorly understood. The disulfiram-like reaction can be produced by the intake of metronidazole and ethanol at the same time. This interaction can result in acetaldehyde accumulation in the blood, inducing hepatic, cardiac, and arrythmogenic toxicity [33]. By contrast, many studies have failed to observe a disulfiram-like reaction induced by ingestion of alcohol with metronidazole. A review of the adverse events associated with metronidazole and ethanol failed to produce conclusive data that the

reaction occurs every time [34]. In addition, Visapaa *et al*. [35] performed a study with 12 healthy male volunteers treated with metronidazole in association with ethanol and did not demonstrate any significant adverse effect. In another study, drugs thought to induce disulfiram-like reaction (chloramphenicol, furazolidone, metronidazole, and quinacrine) were administered to rats and the hepatic activities of alcohol and aldehyde dehydrogenases were evaluated. Metronidazole and quinacrine did not cause disulfiram-like effects, because they did not inhibit hepatic aldehyde dehydrogenase nor increase blood acetaldehyde [36]. Additional *in vitro*, animal models and clinical studies have not demonstrated a disulfiram-like association between metronidazole and ethanol [37]. Thus, there is conflicting data on the association of metronidazole with adverse events when alcohol is consumed.

Similarly, while metronidazole is considered mutagenic in bacteria and carcinogenic in rodents, making teratogenicity a concern, the occurrence of these effects in humans is less clear. This is an important question due to the widespread use of metronidazole; however, long term follow up studies of large numbers of people treated with this drug do not exist. The mechanism of action of metronidazole provides biologic plausibility for mutagenicity, the active nitro group is able to interact with DNA and damage it. But mutagenicity has never been documented in humans [38]. Falagas *et al*. [39] investigated the association of metronidazole treatment with cancer incidence in humans and found that the incidence of cancer among persons who had received metronidazole was nearly identical to that among metronidazole nonusers. Nevertheless, it is important to highlight that these results were obtained for short term exposure to metronidazole in accordance with the recommended trichomoniasis treatment.

Akyol *et al*. [40] used the sister-chromatid exchange technique to measure DNA damage in a study of 20 female patients diagnosed with *T. vaginalis* infection. This technique detects the interchange of DNA replication products through nucleic acid breakage and reunion. In a comparison of 14 patients who received 250 mg metronidazole three times a day with 6 patients who were treated with 400 mg nalidixic acid twice a day for 10 days, the authors observed no relationship between metronidazole treatment and genotoxicity. The genotoxicity of metronidazole was also evaluated using leukocytes from healthy donors. Comet and micronucleus assays were performed and demonstrated no significant cytotoxic or genotoxic effects after metronidazole treatment [41]. Moreover, an association between metronidazole administration during pregnancy and teratogenic or mutagenic effects in newborns and infants has not been demonstrated [42–44]. Together, these studies suggest that while metronidazole and tinidazole side effects are important considerations, the commonly accepted disulfiram-like reaction and carcinogenic or teratogenic effects of metronidazole and tinidazole are not supported by recent studies. Further investigations are needed to inform the use of these drugs.

TREATMENT DURING PREGNANCY

Although often considered a non-complicated STD, trichomoniasis has been associated with severe health consequences in women. The complications caused by *T. vaginalis* infection in the female reproductive tract include infertility [45, 46], pelvic inflammatory disease [47], premature rupture of membrane [48], preterm delivery, low birth weight, and mother-

to-child transmission of *T. vaginalis* [2]. Longer term childhood health consequences have also been associated with maternal *T. vaginalis* infection [49, 50].

The mechanisms by which *T. vaginalis* infection cause pregnancy complications remain unclear. The immunoinflammatory response to trichomonads and the complex host-parasite relationship with the vaginal microbiota appear to play crucial functions in generating pregnancy complications (see review [51]). Recently, Fichorova *et al.* [52] suggested a possible pathogenic role for the innate inflammatory responses elicited by *Trichomonas vaginalis* virus that may be released by the dying parasites during metronidazole treatment. This inflammation may be linked to preterm birth and acquisition of HIV and other STDs.

In the past, metronidazole treatment for trichomoniasis during pregnancy was controversial. Although a few case reports speculated that use of metronidazole during the first 6 to 7 weeks of pregnancy results in midline facial defects in infants, as well as low birth weight, preterm birth rate, or a higher 2-year mortality rate, most retrospective cohort studies do not find an association between metronidazole and teratogenicity [53–55]. Furthermore, a meta-analysis that evaluated 32 studies did not find metronidazole to be teratogenic [56, 57]. Overall, the evidence suggests that metronidazole therapy during pregnancy, including the first trimester, does not lead to congenital malformations. In this context, several studies reinforce the value of trichomoniasis treatment during pregnancy [58–61].

While antibiotic treatment of chlamydia, trichomoniasis, bacterial vaginosis and gonorrheal infection in pregnancy appears to be effective to clear organisms [62–64] some studies were unclear whether treatment of *T. vaginalis* infection has any effect on pregnancy outcomes [54, 62]. A Cochrane review [65] that was performed to evaluate the impact of interventions for chlamydia, trichomoniasis, bacterial vaginosis and gonorrheal infection during pregnancy did find that infection screening and treatment for pregnant women before 20 weeks' gestation reduced preterm birth and preterm low birth weights. Moreover, the infection diagnosis and treatment schedules are associated with cost savings when used for the prevention of preterm birth [65].

The Centers for Disease Control and Prevention (CDC) recommends testing for trichomonas in women presenting with vaginal discharge and treatment with metronidazole in pregnant women diagnosed with this infection [16]. The suggested treatment regimen for symptomatic pregnant women is the same as for other women, 2g oral metronidazole in a single dose, and can be administered at any stage of pregnancy. To reduce neonatal exposure to metronidazole, interruption of breastfeeding is recommended by some clinicians for 12 to 24 hours after a single 2 g dose of metronidazole. Treatment of women with 400 mg metronidazole three times daily for 7 days produced a lower concentration in breast milk and was considered compatible with breastfeeding over an extended duration [66]. Because animal data attribute moderate teratogenic or mutagenic risks to tinidazole and no specific safety studies have been performed, tinidazole is not recommended for pregnant women. In post-partum women, breastfeeding should be postponed for 72 hours following a single 2-g dose of tinidazole [16].

TREATMENT IN NEONATES AND CHILDREN INFECTED WITH *T. VAGINALIS*

Although rare, *T. vaginalis* can be vertically transmitted from mother to newborn during delivery. Trussell *et al.* reported the first case of newborn *T. vaginalis* infection in 1942 [67]. Trichomoniasis can cause urinary tract infections and vaginitis in infants that may persist for up to 9 months after birth [68] and in some cases can be associated with pulmonary complications. Most case reports of neonatal infection have been successfully treated with metronidazole [68–79]. The public health impact of these infections is not defined because the prevalence of newborn *T. vaginalis* infection is still unknown [80].

Trichomoniasis transmission to neonates is proposed to occur through direct vulvovaginal contamination during birth or through ingestion of maternal secretions. If ingested the parasite may contaminate the vagina through deposit in stool [75, 79, 81]. Nosocomial transmission of *T. vaginalis* has not been reported and this route of infection is unlikely to occur because the parasite exists only in the vegetative, trophozoite state and does not develop environmentally resistant cyst forms that are important in transmission of other protozoan parasites [75]. In addition, it is believed that the effect of maternal estrogens on the vaginal epithelium may predispose newborn female infants to infection [79, 82], with resolution once maternal hormone concentrations have dissipated. Fever, irritability, cloudy-white vaginal discharge, urinary tract infection, and respiratory distress can occur with *T. vaginalis* infection in infants [80].

In addition to genitourinary infections, infants can develop respiratory tract infections with *T. vaginalis* and the parasite can be cultured from nasopharyngeal secretions from infants with significant respiratory distress [73, 81, 83–86]. These studies suggest that *T. vaginalis* may be an unrecognized cause of neonatal pneumonia or chronic lung disease. No clearly defined risk factors for development of *T. vaginalis*–associated respiratory tract disease have been documented, although studies have indicated a possible role for the mother's immunologic status. Clinical diagnosis of respiratory distress an ill infant is often difficult, and infection with *T. vaginalis* should be considered when the etiology is not clear [82, 84, 86].

Regardless of pregnancy stage and newborn age, symptomatic pregnant women as well as the infant should be tested for *T. vaginalis* infection and considered for treatment. In addition to potentially preventing the complications of premature delivery, treatment of the woman and her sexual partners during pregnancy can reduce the likelihood that the baby will get infected [75]. Metronidazole therapy has been shown to clear the organisms, and infants with symptomatic infection have shown subsequent improvement [73, 86]. Further work is needed on the incidence of newborn infection with *T. vaginalis*.

When children are found to have *T. vaginalis* infection, sexual abuse or consensual sexual activity should be considered. The identification of an STD in a child, in addition to medical implications, can have serious legal implications. The presence of an STD is often used to support the suspicion of sexual abuse, and the identification of an STD in a child will prompt an investigation of possible abuse [87, 88]. The identification of *T. vaginalis* in these situations is strongly associated with sexual activity [87, 89].

TREATMENT OF RESPIRATORY *T. VAGINALIS* INFECTIONS

Trichomonas vaginalis is almost exclusively found in the urogenital tract of humans. But there are two other trichomonad species that commonly infect humans: *Trichomonas tenax* in the oral cavity and *Pentatrichomonas hominis* in the intestinal tract [90]. *Trichomonas spp*. are generally site-specific; however, Duboucher *et al*. [91] have detected a pulmonary coinfection with *T. vaginalis* and *Pneumocystis* in an AIDS-positive male patient. The diagnosis was achieved by identification of small-subunit rRNA (SSU rRNA) gene sequences that revealed *T. vaginalis* in a bronchoalveolar lavage. Because the patient was treated with trimethoprim-sulfamethoxazole and recovered, the standard therapy with metronidazole was not attempted.

Another case report of trichomonal disease outside the genital tract was described in a healthy 17-year-old male admitted to an intensive care unit following multiple trauma, who developed purulent sinusitis on the 4th day of hospitalization. The diagnosis of *T. vaginalis* infection in his respiratory tract was confirmed by microscopic detection of numerous trophozoites in the sinus aspirate. Further investigation revealed orofacial sexual exposure of the patient to a partner with trichomoniasis. The patient was treated with an antibiotic regimen containing metronidazole and recovered. Thus, although only a few cases have been reported, as mentioned above for infants the colonization of the respiratory tract by *T. vaginalis* and production of clinical symptomatology can also occur in adolescents [92].

MECHANISM OF ACTION OF NITROIMIDAZOLES

Metronidazole and tinidazole enter the parasite by passive diffusion in an inactive form. They are converted to the active form by electron donation from components of the parasite's redox pathway to create a nitro-radical anion. The nitro-radical anion destabilizes the DNA helix and causes strand breakage, leading to inhibition of DNA synthesis and disruption of normal parasite replication and transcription, resulting in cell death within 2 or 3 generations [93, 94]. Only anaerobic organisms have a sufficiently low redox potential to activate the 5-nitroimidazoles, further reducing the concern that these drugs have mutagenic effects in aerobic mammalian cells.

Reduction of 5-nitroimidazole drugs can result from electron donation from a number of enzymes and cofactors. Ferrodoxin, pyruvate-ferrodoxin oxidoreductase (PFOR), and malic enzyme are all present in the hydrogenosome, the organelle by which trichomonads generate ATP as they do not have mitochondria. The flavin enzyme thioredoxin reductase can also reduce 5-nitroimidazole drugs [95]. However, in this pathway, the toxicity of the activated drug is associated with its covalent binding of proteins associated with the thioredoxin redox pathway and disruption of its function.

TREATMENT FAILURES

The first description of metronidazole failing to cure a *T. vaginalis* infection was reported in 1962, just 3 years after metronidazole was introduced for its treatment [96]. This rapid appearance of resistance, coupled with the absence of any major outbreaks of metronidazole resistance and more recent genetic data, argues against treatment-induced resistance and in

favor of a natural drug tolerance among a certain population of *T. vaginalis* isolates [97]. While treatment failures primarily represent drug insensitive parasite infections rather than simply treatment noncompliance, not every isolate from women who have failed repeated treatment is resistant to metronidazole *in vitro* [26]. Anecdotally, pregnant women who fail treatment with 5-nitroimidazoles are often successfully treated with the same drug regimen after delivery, but this observation has not been verified by a well-controlled study. Similarly, women with trichomoniasis and coinfection with HIV-1 can fail standard metronidazole treatment, even when the infecting isolate is sensitive to drug *in vitro* [98]. Other women whose infections do not respond to oral treatment can be cured by intravenous administration of drug, suggesting that poor intestinal absorption of drug may be responsible for some treatment failures [99].

In most cases, drug resistance can be overcome by providing higher doses of metronidazole for longer, or by prescribing tinidazole or combination therapy [100, 101]. However, this is not always successful as isolates with very high resistance to metronidazole, are also cross resistant to tinidazole, indicating a shared mechanism of resistance for both drugs [17, 102]. Often, women will show a temporary improvement in symptoms immediately after treatment, only to have them return 3 weeks later. This is consistent with the interpretation that 5-nitroimidazole resistance is relative and not absolute.

The mechanism of 5-nitroimidazole resistance in *T. vaginalis* is not completely understood. Downregulation of enzymes that reduce metronidazole to its active form, such as PFOR and ferredoxin, as well as reduction on the trichomonads hydrogenosome size were shown to be associated with laboratory-generated resistance in *T. vaginalis* [103–105]. However, resistant clinical isolates do not exhibit reduced transcription of the PFOR, ferredoxin, malic enzyme or hydrogenase genes [106–108]. Even the disruption of the gene encoding ferredoxin did not cause a resistant phenotype [109]. Moreover, no correlation between hydrogenosome number and the drug-resistant status of *T. vaginalis* isolates was observed, suggesting that clinical metronidazole resistance is not associated with smaller hydrogenosomes [110].

The enzyme flavin reductase 1 (FR1) from *T. vaginalis*, formerly known as NADPH oxidase, was isolated, identified, and characterized as a potential metronidazole resistance mechanism in *T. vaginalis* [111]. Flavin reductase reduces oxygen to hydrogen peroxide using flavin mononucleotide as a cofactor [112, 113]. FR1 activity is diminished or even absent in clinical metronidazole-resistant isolates [114, 115]. Anaerobic resistance to metronidazole is hypothesized to result from defective metronidazole-activating pathways, including the PFOR-ferredoxin couple [103, 116] and thioredoxin reductase [95]. Flavin reductase is part of the antioxidative defense in *T. vaginalis* and indirectly reduces molecular oxygen to hydrogen peroxide *via* free flavins. A reduced or absent flavin reductase activity in metronidazole-resistant *T. vaginalis* results in elevated intracellular oxygen levels and ineffective action of metronidazole. Leitsch *et al.* [111] suggest that the inactivation of FR1 acts as a mechanism underlying metronidazole resistance not only in laboratory strains with anaerobic resistance but also in many clinically resistant isolates of *T. vaginalis*. The flavin inhibitor diphenyleneiodonium confers resistance on metronidazole sensitive isolates through decreasing thioredoxin reductase and flavin reductase activities [117].

Moreover, *T. vaginalis* may have a symbiotic relationship with *Mycoplasma hominis*, a pathogenic bacterium associated with urogenital and respiratory infections [118, 119]. The frequency of this association varies widely, from 20 to 92% [107, 120–122]. The consequences of this symbiotic relationship are the increase of the cytopathogenic effect of *T. vaginalis* against epithelial cells [123] and the upregulation of the *in vitro* proinflammatory response of human monocytes [124]. In addition, some studies have demonstrated a relationship between *M. hominis*-*T. vaginalis* coinfection and increased metronidazole tolerance *in vitro* [122, 125]. However, other studies demonstrated no association of mycoplasma infection of *T. vaginalis* with clinical metronidazole resistance [107, 121].

NEW ALTERNATIVES FOR TRICHOMONIASIS TREATMENT – ARE THERE ANY?

Although, the frequency of 5-nitroimidazole-resistant *T. vaginalis* infections is relatively low, the danger of relying on one class of drugs when demonstrated resistance exists, combined with the need to identify treatments for persons allergic to these drugs, has stimulated research into identifying alternative therapies for treating trichomoniasis. Ideally, an alternative therapy could be taken orally, would be well tolerated, and would be effective against trichomonads via a different pathway than the 5-nitroimidazoles. This last criteria is supported by the work of Upcroft *et al.* [126], who tested the efficacy of 12 5'-nitroimidazole derivatives and one lactam-substituted nitroimidazole against *T. vaginalis* isolates. Eleven of the compounds had activity against metronidazole sensitive isolates but none were effective against metronidazole resistant parasites, showing that cross-resistance exists and effective compounds not in the 5'-nitroimidazole class are required.

Alternative classes of drugs with demonstrated activity against *T. vaginalis* include the benzo[*f*]cinnoline *N*-oxides. One of the derivatives bearing a C-6-nitro group was 6.4 times more active than metronidazole [127]. Similarly, the sulfonamides sulphimidazole, sulphamethoxazole, trimoethropim were compared with metronidazole for activity again metronidazole-sensitive and metronidazole-resistant isolates. Sulphimidazole was active against both sensitive and resistant isolates, demonstrating the potential of combining two functional groups, a 5-nitroimidazole and a sulphonamide, onto one compound [128]. Five 3-alkoxy- or 3-hydroxy-1-[ω-(dialkylamino)alkyl]-5-nitroindazole derivatives also demonstrated potent activity against *T. vaginalis* [129].

A new approach in medicinal chemistry has been to modify compounds with known biological activity to create more potent derivatives. For example, Kumar *et al.* [130] modified the structure of metronidazole without altering the nitro group that is responsible for the anti-*T. vaginalis* activity. Addition of dithiocarbamates to metronidazole resulted in compounds that had 3–10 fold greater activity against sensitive isolates and 10–20 greater activity to resistant isolates than did unmodified metronidazole. Synthesized metronidazole-chalcone conjugates also demonstrated up to fourfold greater activity than metronidazole against resistant *T. vaginalis* isolates while performing similarly to sensitive isolates. Thus, these compounds are possible candidates to treat metronidazole resistant *T. vaginalis*

infections [131]. However, whether cross-resistance to these compounds can also occur is an important question that must be evaluated before these derivatives are considered as potential therapy for trichomoniasis.

Along with derivatives that contain dual active groups, derivatives with dual activities may also be important for the future of treating *T. vaginalis* infections. This is particularly true as metronidazole is available as a generic drug, making it unlikely that it would be cost-effective to develop and get approval for an entirely new drug designed to only treat trichomoniasis, even if it showed much greater activity against resistant infections. An example of this approach is the work of Bala *et al.* [132], who tested 17 morpholin/piperidin-1-yl-carbamodithioate spermicidal compounds for their activity against trichomonads. Sixteen of the compounds were active against metronidazole-sensitive isolates, and 15 were toxic for metronidazole-resistant parasites at concentrations comparable to metronidazole. The safety of the compounds was tested using cytotoxic assays on HeLa human cervical cell lines. They were also evaluated for compatibility with vaginal flora. Along with the spermicidal and trichomonicidal activities, these compounds demonstrated antifungal potential. The morpholin/piperidin derivatives bind sulfhydryls, suggesting that their activity results from absorption of free thiol and inhibition of hexokinase activity. Additional studies demonstrated that dithiocarbamate-thiourea hybrid compounds may also inhibit reverse transcriptase, which could also have implications for reducing transmission of HIV-1 [133]. In addition, preliminary *in vivo* pharmacokinetics of the most active compound was performed in rabbits and the new compound was safer than nonoxynol-9. In a similar study, fifteen N-alkyl/aryl-4-(3-substituted-3-phenylpropyl) piperazine-1-carbothioamide derivatives were tested for anti-*T. vaginalis*, spermicidal, antifungal and reverse transcriptase inhibitor activities along with preliminary safety evaluation by HeLa cell cytotoxicity assays and vaginal flora compatibility. The most promising compound, which is an oxo derivative, completely inhibited *T. vaginalis* growth at 46.72 μM and demonstrated a clinical safety profile similar to nonoxynol-9 [134].

By contrast to chemically synthesized derivatives, another strategy is use of natural compounds such as macrolide antibiotics. Pentamycin is a polyene macrolide antibiotic, produced by *Streptomyces penticus*. This compound exhibits a broad spectrum of antimicrobial activity, probably acting on the membrane function of different microorganisms. Pentamycin has been evaluated against *Candida albicans* and approved for the topical treatment of bacterial and fungal vaginitis [135]. Intravaginal pentamycin was also effective for the treatment of trichomoniasis and was well-tolerated and well-accepted by patients, consistent with previous clinical trials and comparative studies [136]. In addition, Kranzler *et al.* [137], tested pentamycin against four isolates of *T. vaginalis* with different metronidazole susceptibilities. The drug was active independent of metronidazole resistance. Pentamycin at 22 μM eradicated 100% of the parasites after 1 h of treatment. Because pentamycin is approved for intravaginal use, it is a promising alternative for treatment of trichomoniasis [137].

An additional group of compounds obtained from the nature are the dermaseptins, which are cationic peptides found on the skin cells of Brazilian frogs from the family Phyllomedusinae. These compounds provide an innate defense against infections and are

selectively lytic for certain bacteria, protozoa and fungi at micromolar concentrations. As part of a study to identify the motifs responsible to their activity against microorganisms, synthetic variants of dermaseptin S1 were evaluated for activity against *T. vaginalis*, *Herpes simplex* virus and Papillomavirus. The results showed that the synthetic peptides inhibited the pathogens tested, indicating a potential against sexually transmitted microorganisms [138].

Antimicrobial peptides (AMPs) are another example of natural antibiotic peptides that can provide some degree of non-specific host protection against a variety of microbial pathogens. One of these AMPs, prophenin 2 adversely affects the integrity and viability of *T. vaginalis*. These peptides are potential alternatives to treat trichomoniasis with both the propeptide and processed peptide demonstrating activity. Furthermore, pro-prophenin 2 and prophenin 2 have less hemolytic activity and are less susceptible to the parasite cysteine proteinases that can degrade and inactivate the peptides than are other known AMPs. Thus, they are potential candidates for alternative treatment of trichomoniasis [139].

Another approach for a new trichomoniasis treatment is to repurpose FDA-approved compounds for a new therapeutic indication. This is an attractive strategy because the approval process for a new indication takes less time and is less costly than for completely new drug applications. The aminoglycoside antibiotics, which are currently used to treat tuberculosis and *Pseudomonas* bacterial infections, are one example as they also have activity against *T. vaginalis* [140]. Another example is miltefosine, an alkylphosphocholine that was first used to treat cutaneous metastasis from mammary carcinoma. Subsequent studies demonstrated that this compound is active against a variety of parasite genera, including *Schistosoma, Leishmania, Trypanosoma, Entamoeba, Acanthamoeba*, and *Giardia*. In Germany, Colombia and India, miltefosine has been used in oral treatment of visceral leishmaniasis. Against *T. vaginalis*, miltefosine is active against both metronidazole-sensitive and -resistant isolates through an antiproliferative effect and ultrastructural alterations that are indicative of apoptosis [141, 142]. Finally, we recently screened 1040 drugs of the US Drug Collection Library for activity against susceptible and resistant *T. vaginalis* isolates [143]. Only 8% of the drugs tested reduced the metronidazole-susceptible *T. vaginalis* isolates growth. The non-5-nitroimidazole drugs disulfiram and nithiamide showed the best activity against trichomonads when tested individually. Albendazole and coenzyme B12 were the most promising compounds in combination with metronidazole or tinidazole for treatment with highly resistant *T vaginalis* infections. The study reinforces the challenges in developing new therapeutic alternatives for the 5-nitroimidazoles.

The changes in the of *T. vaginalis* transcriptome in response to tetracycline were analyzed by next-generation RNA-based sequencing [144]. Tetracycline was active against both metronidazole-sensitive and -resistant *T. vaginalis* isolates, induced apoptotic-like changes, and altered the carbohydrate metabolism and aminoacyl-tRNA synthetase pathways. Finally, tetracycline caused disruption on the hydrogenosomal membrane potential and the antioxidant system. These findings suggested that tetracycline may have therapeutic potential for treating metronidazole-resistant *T. vaginalis* [144]. A new strategy being explored to treat trichomoniasis is the use of proton-pump inhibitor drugs, such as omeprazole, pantoprazole, and rabeprazole that inhibit the enzyme uridine nucleoside

ribohydrolase, a fundamental enzyme in uridine salvage. Because *T. vaginalis* lacks the enzymes for *de novo* synthesis of the purine [145] and pyrimidine [146] rings, nucleosides must be taken up from host cells and/or the extracellular millieu. Thus, enzymes involved in the salvage pathway are potential therapeutic targets. Omeprazole, pantoprazole and rabeprazole were active against *T. vaginalis* at submicromolar concentrations, showing promise as alternatives for *T. vaginalis* infection treatment [147].

Another novel strategy is to target the *T. vaginalis* endobiont viruses that can be sensed by the human host and may serve as critical targets for modifying therapeutic paradigms and prevention of inflammatory sequelae caused by the virus [52]. Interfering with the virus's stimulation of the host innate immune response in the reproductive tract of pregnant and non-pregnant women may decrease the pathology associated with infection.

Despite the efforts made by several research teams, identifying new *T. vaginalis* cellular targets remains an important gap in rational drug development. Beyond understanding the internal biochemical pathways of the parasite, new targets may be revealed through further study of the host-pathogen relationship of this well-adapted extracellular parasite with very complex mechanisms of pathogenicity that uses a multi-faceted machinery to evade hostile environments. Although numerous new compounds with potential against *T. vaginalis* have been described in the literature (Table 1), no new treatments have progressed to clinical trials and as a result, metronidazole and tinidazole remain the only drugs approved by the FDA to treat *T. vaginalis* infection.

PREVENTION

Randomized controlled trials have demonstrated that behavioral interventions and male circumcision protected against viral STDs, although the magnitude of the effect is more limited than that demonstrated with treatment or vaccines. Circumcision may also reduce risk of trichomoniasis among men and their female partners; however, these data were less consistent [148]. In general, treatment interventions for all STDs and vaccines for viral STDs showed the most promising positive effects. Conversely, vaginal microbicides and physical barrier methods demonstrated few or no significant positive effects with respect to preventing STDs [148]. As reported in randomized controlled trials of interventions to prevent STDs, the use of female condoms was particularly low, with only 7% of Kenyan women reporting "consistent" use [149], and Thai sex workers reported using them for just 12% of sex acts [150]. In fact, current STD control is hampered by several behavioral, biological, and implementation challenges, including a large proportion of asymptomatic infections, lack of feasible diagnostic test availability in some developing countries, antimicrobial resistance, repeat infections, and barriers to intervention access, availability, and scale-up [151]. An important component of the prevention and control of STDs is based in behavioral education, early diagnosis and treatment, including asymptomatic infections and in immunization when a vaccine is available [152]. Together, treatment programs and the development of new vaccines afford an opportunity to implement effective control and elimination strategies for the major neglected diseases, including trichomoniasis [153].

The lack of vaccines against most of the major parasitic diseases has made chemotherapy the only option for treatment. However, most non-malaria parasitic infections have only a single, or a single class, of drugs approved for treatment, resulting in an increased risk of resistance emerging. Vaccines for parasitic infections would be another control strategy but there are currently no highly effective vaccines for human parasitic infections. A better understanding of the molecular mechanisms that control the expression of parasitic genes involved in transmission, pathogenicity and immune evasion is needed to develop novel and necessary vaccines. The progress of molecular techniques and the sequencing of their genomes offers the opportunity to undertake comparative genomics of the genes involved in regulation of gene expression and, possibly, in the production of effective vaccines [154–156].

Unfortunately, development of new vaccines has been hampered by numerous issues, such as the large chemical, and hence immunologic, diversity of protective antigens [157]. Other challenges for developing an effective vaccine against trichomoniasis are the lack of long-lasting humoral immunity and the absence of good animal models for *T. vaginalis* infection [158, 159]. Only two candidates for a trichomonas vaccine have been submitted for clinical trials in the last 50 years, with no success [160, 161]. Some reports have tested possible vaccine strategies in animal models. A FDA approved adjuvant, Alhydrogel, formulated with live, whole *T. vaginalis* was tested in the immunized mouse model, with potential applicability [162]. Recently, the same authors demonstrated that *T. vaginalis* infection induces vaginal $CD4^+$ T-cell infiltration, evoking local and systemic immune responses and conferring significantly greater protection against vaginal infection than seen in unvaccinated mice [163]. Another exciting development is the description of an experimental infection model in the pigtailed macaque (*Macaca nemestrina*). This species naturally harbors lactobacilli, has a vaginal pH of 5.5–8.0, sustains *T. vaginalis* infection for up to 2 weeks and responds to metronidazole treatment [164].

Besides the already tested candidates, some biochemical targets for vaccine development have been proposed. Iron is an essential nutrient and virulence factor for *T. vaginalis* since it regulates pathogenicity. *T. vaginalis* expresses lactoferrin-binding proteins and use holo-lactoferrin as an iron source for *in vitro* growth. Sera from patients with trichomoniasis contain antibodies that recognize the purified lactoferrin receptor protein [165]. Hence, the *T. vaginalis* receptor is immunogenic and, therefore, may serve as potential vaccine target [166, 167]. Cywes-Bentley *et al.* [157] are more audacious in proposing a broad-spectrum vaccine eliciting immunity against a wide range of major human and animal pathogens. The target for this vaccine would be poly-N-acetylglucosamine (PNAG), a conserved surface polysaccharide produced by major bacterial, fungal, and protozoal parasites (including *T. vaginalis*), along with malarial sporozoites and blood-stage forms. The authors proposed that all these infections can be targeted for vaccination using this single antigen.

FINAL CONSIDERATIONS

The public health impact of trichomoniasis is becoming increasingly understood, including the direct and indirect costs of *T. vaginalis* infection [12, 19]. For example, because of the role *T. vaginalis* may play in increased HIV transmission, screening and treatment of trichomoniasis is estimated to reduce the medical costs associated with lifetime HIV care

by $167 millon per year [168]. Furthermore, these attributable costs and indeed the overall prevalence of trichomoniasis are based on diagnosis with a relatively insensitive test, the wet mount. As more sensitive nucleic acid detection tests are employed, it is likely that more *T. vaginalis* asymptomatic infections will be detected. While trichomoniasis is not a reportable disease and unlikely to become one [169], there remains a need to determine whether asymptomatic trichomoniasis has health consequences for individuals or their sex partners. Going forward, it is anticipated that reliance on 5-nitroimidazoles alone will become an increasingly untenable public health strategy. Greater effort is needed to identify alternatives to prevent or treat trichomoniasis prior to these anticipated challenges rather than after they have emerged.

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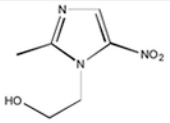
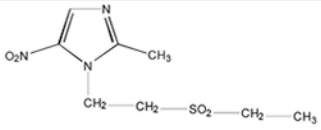
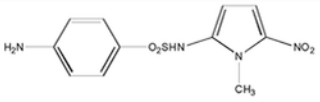
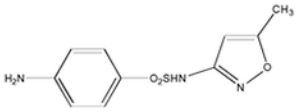
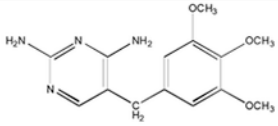
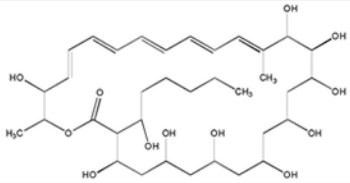
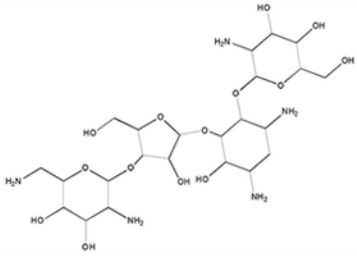
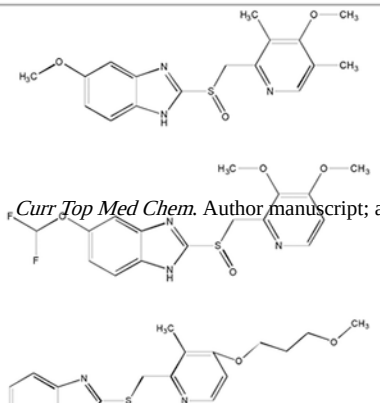
Table 1.

Old drugs versus new promising alternatives for the trichomoniasis treatment.

Compound	Chemical structure	Mode of action	Dose	Reference
Old drugs				
Metronidazole		5-nitro group is reduced within the cell or organelle by an appropriate electron donor such as ferredoxin.	2 g orally in a single dose	[16]
Tinidazole		5-nitro group is reduced within the cell or organelle by an appropriate electron donor such as ferredoxin.	2 g orally in a single dose	[16]
Old drugs with new use				
Sulphimidazole		NE	1.68 (MLC)	[128]
Sulphamethoxazole		Sulfonamides inhibit the enzymatic conversion of pteridine and p-aminobenzoic acid (PABA) to dihydropteroic acid by competing with PABA for binding to dihydrofolate synthetase, an intermediate of tetrahydrofolic acid (THF) synthesis. THF is required for the synthesis of purines and dTMP and inhibition of its synthesis inhibits parasite growth.	>789 (MLC)	[170]
Trimethropim		Inhibition of dihydrofolate reductase, another step in THF synthesis, and therefore synergic action with the sulfonamides.	>689 (MLC)	
Pentamycin		Distortion and malfunction of the membrane caused by polyenes bind to cell membrane sterols.	22 (EC_{100})	[137]
Paromomycin		Inhibition of protein synthesis induced by paromomycin bind to the RNA, causing misreading and premature termination of translation	NI	[140]
Miltefosine		Interaction with the cell membrane resulting in cell lysis	8.0 – 40 (EC_{50})	[142]
Omeprazole, pantoprazole, rabeprazole		Inhibition of inosine nucleoside ribohydrolase	0.3 – 14.5 (IC_{50} of enzyme inhibition)	[147]

NE – not evaluated; NI – not informed; GI – growth inhibition