

A systematic review of the literature on mechanisms of 5-nitroimidazole resistance in *Trichomonas vaginalis*

Review

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Abstract

Background. *Trichomonas vaginalis* is the most common non-viral sexually transmitted infection. 5-Nitroimidazoles [metronidazole (MTZ) and tinidazole (TDZ)] are FDA-approved treatments. To better understand treatment failure, we conducted a systematic review on mechanisms of 5-nitroimidazole resistance.

Methods. PubMed, ScienceDirect and EMBASE databases were searched using keywords *Trichomonas vaginalis*, trichomoniasis, 5-nitroimidazole, metronidazole, tinidazole and drug resistance. Non-English language articles and articles on other treatments were excluded.

Results. The search yielded 606 articles, of which 550 were excluded, leaving 58 articles. *Trichomonas vaginalis* resistance varies and is higher with MTZ (2.2–9.6%) than TDZ (0–2%). Resistance can be aerobic or anaerobic and is relative rather than absolute. Differential expression of enzymes involved in trichomonad energy production and antioxidant defenses affects 5-nitroimidazole drug activation; reduced expression of pyruvate:ferredoxin oxidoreductase, ferredoxin, nitroreductase, hydrogenase, thioredoxin reductase and flavin reductase are implicated in drug resistance. *Trichomonas vaginalis* infection with *Mycoplasma hominis* or *T. vaginalis* virus has also been associated with resistance. *Trichomonas vaginalis* has two genotypes, with greater resistance seen in type 2 (vs type 1) populations.

Discussion. 5-Nitroimidazole resistance results from differential expression of enzymes involved in energy production or antioxidant defenses, along with genetic mutations in the *T. vaginalis* genome. Alternative treatments outside of the 5-nitroimidazole class are needed.

Introduction

Trichomonas vaginalis is the parasitic protozoan causing the sexually transmitted infection (STI) trichomoniasis. Trichomoniasis is the most common non-viral STI affecting an estimated 3.7 million U.S. women and men and 143 million people worldwide (Rowley *et al.*, 2012; Newman *et al.*, 2015). It is more common in women, particularly older women (Sutton *et al.*, 2007; Patel *et al.*, 2018). In addition, there is a pronounced racial disparity with African Americans having a higher prevalence than other racial groups (Patel *et al.*, 2018). Trichomoniasis is frequently asymptomatic in women; in a nationally representative sample of U.S. women aged 14–49 with *T. vaginalis* infection, only 9.5% reported vaginal discharge, 7.3% reported vaginal itch and 7.0% reported vaginal odour (Sutton *et al.*, 2007). Among women who are symptomatic, signs and symptoms may include a thin, frothy vaginal discharge, odour, genital inflammation, pruritus, dysuria and/or dyspareunia (Wolner-Hanssen *et al.*, 1989; Swygard *et al.*, 2004). *Trichomonas vaginalis* is usually asymptomatic in men (Sena *et al.*, 2007), although it can cause penile discharge and/or dysuria in the setting of non-gonococcal urethritis (Schwebke *et al.*, 2011). It may also interfere with male and female fertility, although few studies have been conducted to directly investigate this (Meites *et al.*, 2015). Infected women and men with *T. vaginalis* also have an increased risk of acquisition and transmission of HIV (Kissinger and Adamski, 2013) whereas pregnant women have an increased risk of preterm delivery and other adverse birth outcomes (Silver *et al.*, 2014).

Currently approved treatments for trichomoniasis include metronidazole (MTZ) and tinidazole (TDZ); 5-nitroimidazoles (WHO, 2017). Other drugs in this class include secnidazole (SEC), nimorazole, ornidazole and azanidazole. SEC is used outside the USA to treat *T. vaginalis* (Rossignol *et al.*, 1984), as well as other infections including *Helicobacter pylori* and *Giardia lamblia* (Ahuja *et al.*, 1998; Escobedo *et al.*, 2003). SEC is currently FDA-approved for the treatment of bacterial vaginosis in the USA but not for trichomoniasis (Schwebke *et al.*, 2017). A phase 3 clinical trial to evaluate the safety and effectiveness of a single dose of SEC for the treatment of trichomoniasis in women is ongoing (NCT03935217).

MTZ is the 5-nitroimidazole most commonly used in clinical practice for *Trichomonas* treatment, as it is generic and inexpensive. MTZ was first introduced in 1959 (Cosar and Julou, 1959; Durel *et al.*, 1960); *in vitro* resistance in *T. vaginalis* was observed by 1962 (Robinson, 1962). Trichomonads with minimal lethal concentrations (MLCs) $\leq 25 \mu\text{g mL}^{-1}$ are considered MTZ-sensitive. An MLC of $50 \mu\text{g mL}^{-1}$ represents low-level resistance, MLCs of $100\text{--}200 \mu\text{g mL}^{-1}$ moderate-level resistance and MLCs $\geq 400 \mu\text{g mL}^{-1}$ high-level resistance. The prevalence of resistance to MTZ and TDZ ranges between 2.2 and 9.6% (Schmid *et al.*, 2001; Schwebke and Barrientes, 2006; Krashin *et al.*, 2010; Kirkcaldy *et al.*, 2012) and 0 and 2% (Perez *et al.*, 2001; Schwebke and Barrientes, 2006), respectively. Resistance to MTZ is associated with cross-resistance to other 5-nitroimidazoles. 5-Nitroimidazole-resistant trichomoniasis is concerning, as few alternative treatments exist.

To better understand treatment failure due to 5-nitroimidazole drug resistance, we performed a systematic literature review on mechanisms of 5-nitroimidazole resistance in *T. vaginalis*. An improved understanding of this resistance may inform development of novel therapeutic options outside of the 5-nitroimidazole class.

Methods

A systematic review of the literature focusing on original research on mechanisms of 5-nitroimidazole resistance in *T. vaginalis* was performed between January and August 2019 by authors KJG and PJK. The review included articles ranging from the introduction of MTZ as a treatment for trichomoniasis in 1959 to August 2019. The databases used included PubMed, ScienceDirect and EMBASE. Keywords were 5-nitroimidazole, metronidazole, tinidazole, secnidazole, drug resistance, *Trichomonas vaginalis* and trichomoniasis. Reference lists from selected publications identified by these keywords were also reviewed to identify any additional articles that may be relevant.

Evaluated articles had to contain original research on mechanisms of 5-nitroimidazole resistance in *T. vaginalis*. This included how resistance can arise, descriptions of proteins and enzymes involved in metabolic pathways affecting drug activation, enzymes altered in drug-resistant *T. vaginalis* isolates and whether or not clinical resistance was relative or absolute. Research aimed at describing genetic components of resistance, i.e. point mutations leading to inactivation or dysfunction of an enzyme involved in drug activation and other metabolic processes affecting resistance were also considered. Non-English language articles, case reports, case series or reviews were excluded. Research articles involving treatment of other microorganisms besides *T. vaginalis*, articles describing non-5-nitroimidazole treatments for *T. vaginalis* and articles focusing solely on susceptibility testing and prevalence of resistance were also excluded.

If it was unclear whether an article was appropriate for inclusion, author CAM assisted in an additional assessment. An Excel spreadsheet was created during the database search that contained all the papers that met the inclusion criteria. The spreadsheet was subsequently made available to all authors for analysis of the relevant literature.

Results

The search yielded 606 articles: 275 from EMBASE, 142 from PubMed and 189 from ScienceDirect (Fig. 1). Of these 606 articles, 550 were excluded for various reasons listed in Fig. 1. Thus, 58 articles relevant to 5-nitroimidazole resistance mechanisms in *T. vaginalis* were included in this review.

Metabolic pathways of *T. vaginalis* and their effects on 5-nitroimidazole resistance

Energy production pathways in *T. vaginalis*

Trichomonas vaginalis obtains most of its energy through carbohydrate metabolism, which involves breaking down glucose to produce lactate, acetate, molecular hydrogen and adenosine triphosphate (ATP). Malate can also serve as an energy source. Figures 2a and b depict the structure (Honigberg and King, 1964; Honigberg and Brugerolle, 1990; Bouchemal *et al.*, 2017) and energy production pathways of *T. vaginalis*. One pathway begins in the cytoplasm where glucose undergoes glycolysis to form pyruvate, which is then passively transported into hydrogenosomes (Fig. 2a). Inside the hydrogenosome (Fig. 2b), the enzyme pyruvate:ferredoxin oxidoreductase (PFOR) facilitates oxidation (loss of electrons) of pyruvate by ferredoxin (Fdx) to produce acetyl-CoA. The reduced Fdx is oxidized by the enzyme hydrogenase (HYD), which uses the electrons gained from Fdx to produce molecular hydrogen (H_2). Figure 2b depicts a second energy production pathway in the hydrogenosomes that uses malate (Dolezal *et al.*, 2004; Hrdy *et al.*, 2005). This pathway utilizes malate dehydrogenase (MDH) and nicotinamide adenine dinucleotide:ferredoxin oxidoreductase (NADH:FOR). Malate is decarboxylated by MDH to yield pyruvate and CO_2 , transferring electrons to NAD^+ whereas pyruvate enters the first pathway to produce acetyl-CoA and ATP. Similar to PFOR, NADH:FOR facilitates the transfer of electrons to Fdx, allowing the reaction to proceed to H_2 production. ATP and acetate production in the hydrogenosomes occurs through a substrate-level phosphorylation catalysed by two enzymes: acetate:succinate CoA-transferase (ASCT) and succinyl-CoA synthetase (SCS), called the ASCT/SCS cycle (Fig. 2b; pathway 3).

Antioxidant defense pathway in *T. vaginalis*

The antioxidant defense pathway takes place in the cytosol of *T. vaginalis*. Figure 3a illustrates this pathway under normal conditions, i.e. in the absence of a 5-nitroimidazole. The reactive oxygen species (ROS), superoxide (O_2^-), is reduced to molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) by the enzyme superoxide dismutase (SOD). The O_2 is then reduced by either nicotinamide adenine dinucleotide (NADH) oxidase to H_2O or by flavin reductase 1 (FR1) to H_2O_2 . The H_2O_2 produced by these processes must be further reduced to avoid its cytotoxic side-effects. This is accomplished indirectly by the activity of the flavin enzyme thioredoxin reductase (TrxR) and its accompanying protein and enzyme thioredoxin (Trx) and thioredoxin peroxidase (TrxP), respectively. TrxR facilitates the reduction of Trx by nicotinamide adenine dinucleotide phosphate (NADPH). Reduced Trx subsequently activates enzymes involved in other metabolic pathways. Importantly, Trx activates TrxP, which then reduces H_2O_2 to H_2O .

Drug activation of 5-nitroimidazoles in *T. vaginalis*

MTZ or other 5-nitroimidazoles used for *Trichomonas* treatment enter the parasites in an inactive form through passive diffusion (Muller, 1986). 5-Nitroimidazoles need to be reduced in order to actively interfere with *T. vaginalis* processes (Fig. 2a). MTZ is activated in the hydrogenosomes by the energy-production pathway through competition with HYD for the electrons of Fdx (Fig. 2b) (Lloyd and Kristensen, 1985; Lloyd *et al.*, 1986). Both Fdx and nitroreductase (NTR) can reduce MTZ to its active nitro radical anion form (Yarlett *et al.*, 1985; Yarlett *et al.*, 1986a, 1986b; Pal *et al.*, 2009). In this active form, MTZ interferes with, and disrupts, DNA synthesis and repair, leading to cell death (Declerck and De Ranter, 1986).

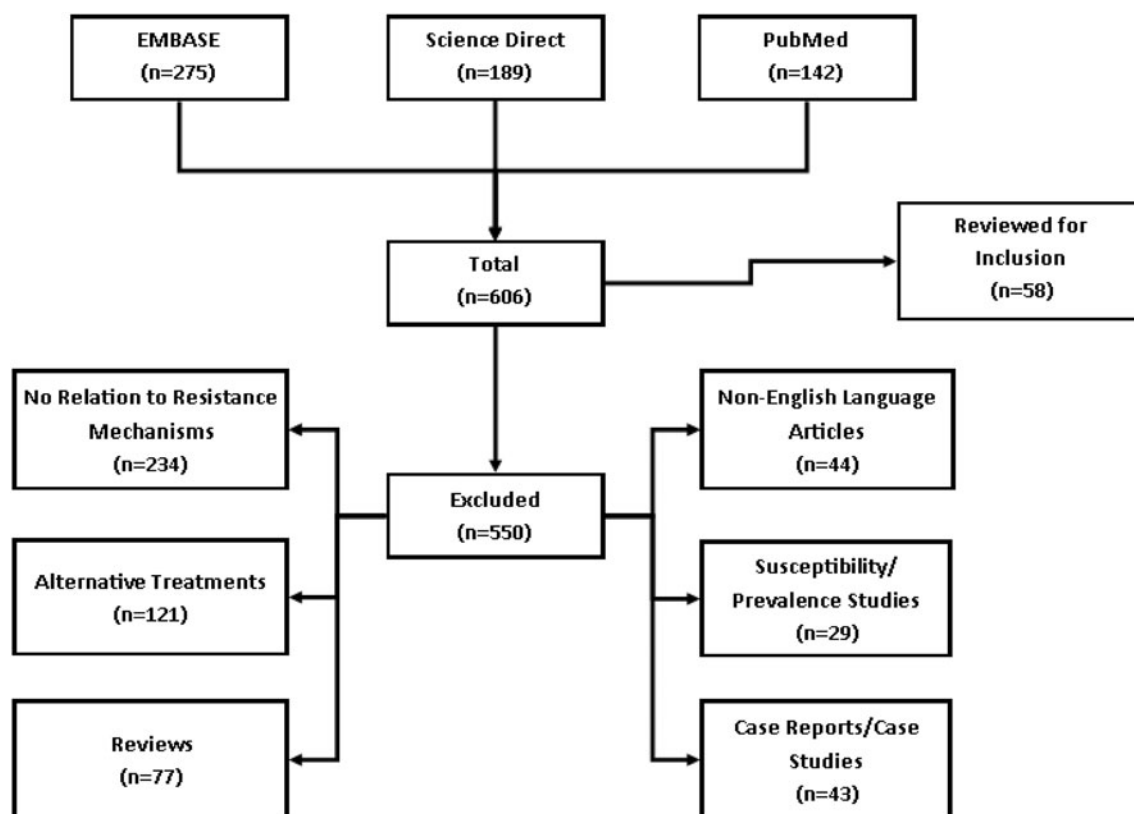


Fig. 1. Flow chart. Flow chart of the databases used to perform the systematic review of the literature. Included are the number of articles reviewed for study inclusion and those that were excluded for various reasons.

TrxR, such as Fdx and NTR, can activate MTZ (Leitsch *et al.*, 2009). This provides an alternative theory to the mechanism of action and target metabolic mechanisms that 5-nitroimidazoles affect. Upon activation, MTZ can form a 'covalent adduct' with TrxR and other enzymes and proteins involved in the antioxidant defense pathway. The inactivation of TrxR prevents Trx activation which in turn blocks TrxP from reducing H₂O₂, which is cytotoxic. Left unchecked, this will lead to death of the organism. Thus, antioxidant defenses (i.e. oxygen scavenging mechanisms) could be the main mechanism of action of MTZ instead of DNA disruption.

Aerobic vs anaerobic resistance to 5-nitroimidazoles in *T. vaginalis*

5-Nitroimidazole resistance in *T. vaginalis* can be either aerobic (*in vivo* clinical resistance) or anaerobic (laboratory-induced, *in vitro* resistance) (Kulda *et al.*, 1993; Tachezy *et al.*, 1993). Aerobic resistance is the most commonly observed form of resistance and may be a potential first step in the development of anaerobic resistance (Kulda, 1999). Proposed mechanisms for aerobic and anaerobic resistance to 5-nitroimidazoles in *T. vaginalis* include multiple enzymes and proteins involved in energy production as well as oxygen-scavenging mechanisms (Coombs, 1978; Land *et al.*, 2001; Rasoloson *et al.*, 2001; Leitsch *et al.*, 2010; Paulish-Miller *et al.*, 2014).

Aerobic resistance

Aerobic resistance arises due to deficiencies associated with oxygen-scavenging mechanisms of the antioxidant-defense pathway (Lloyd and Pedersen, 1985; Yarlett *et al.*, 1986a, 1986b; Ellis *et al.*, 1994). Decreased or absent FR1, TrxR and possibly

NADH oxidase and SOD activity leads to increased intracellular oxygen levels (Fig. 3b) (Muller and Gorrell, 1983; Rasoloson *et al.*, 2001; Leitsch *et al.*, 2010). Downregulated expression of FR1 in response to toxic TrxR-MTZ adducts limits the production of H₂O₂ and increases intracellular O₂ leading to inactivation (oxidation) of MTZ (Leitsch *et al.*, 2012; Leitsch *et al.*, 2014). Although increased O₂ levels and MTZ inactivate cytosolic metabolic pathways, the hydrogenosomal metabolic pathways are still functional in aerobically resistant *T. vaginalis* strains. This is characterized by increased rates of glucose consumption and increased production of acetate and CO₂ (Ellis *et al.*, 1992).

Anaerobic resistance

Trichomonas vaginalis isolates that exhibit anaerobic resistance tend to have much higher *in vitro* MLC values compared to aerobically-resistant isolates. Anaerobic resistance has mainly been observed *in vitro* and induced under laboratory conditions rather than arising clinically. It is characterized by the disruption of enzymes that participate in the energy production pathway (Land *et al.*, 2001; Land *et al.*, 2002). Decreased levels of hydrogenosomal enzymes have been associated with smaller hydrogenosomes in laboratory-induced resistant strains compared to MTZ-sensitive strains (Wright *et al.*, 2010). However, loss of PFOR activity alone does not lead to resistance as Fdx functions in the malate-dependent pathway through NADH:FOR and MDH (Fig. 2b) (Rasoloson *et al.*, 2002). Thus, full resistance only occurs when both PFOR and NADH:FOR activities are lost. Another alternative energy pathway proposed in resistant *T. vaginalis* strains bypasses PFOR and the need to pass on electrons to Fdx. It involves the enzyme keto acid oxidoreductase (KOR), which, like PFOR, can reduce pyruvate and other metabolites to meet the energy demands of *T. vaginalis* (Brown *et al.*, 1999).

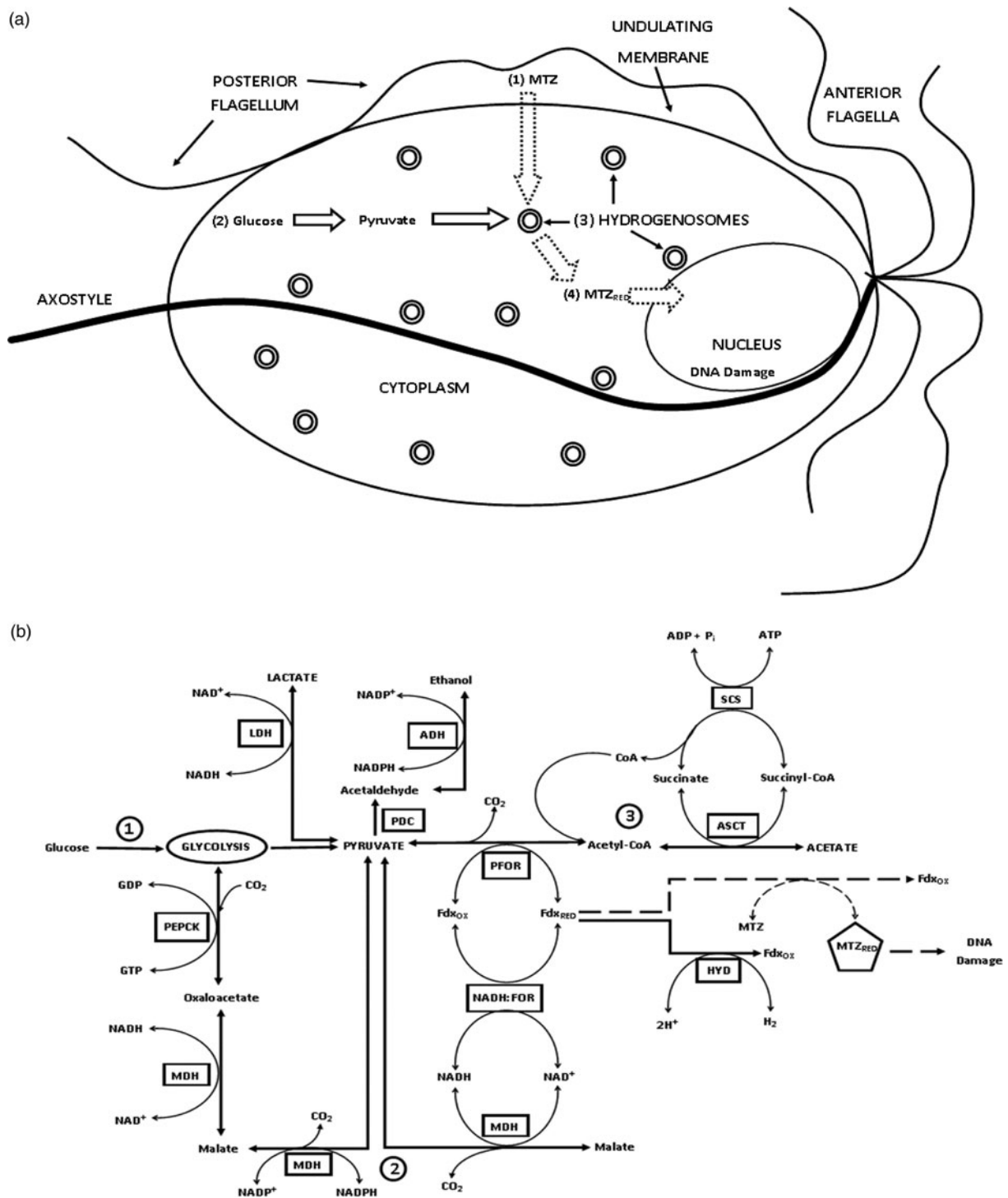


Fig. 2. MTZ activation through the energy production pathway in *T. vaginalis*. (a) Overview of *T. vaginalis* and the MTZ drug activation pathway. (1) MTZ enters the cell by passive diffusion and is subsequently metabolized in the hydrogenosome. (2) Pyruvate is produced from glucose through glycolysis in the cytosol. (3) Inside the hydrogenosome, PFOR facilitates oxidative decarboxylation of pyruvate by reducing and transferring electrons to Fdx. Fdx then reduces the nitro group of MTZ, which creates a cytotoxic nitro radical anion. (4) Activated MTZ then interacts with *T. vaginalis* DNA causing damage and death of the organism. (b) Detailed energy production and MTZ drug activation pathway in the hydrogenosome. Solid line: Major energy production pathway: Pyruvate is the major intermediate product produced from glucose through the glycolytic pathway (pathway 1) as well as through an alternative malate-dependent pathway (pathway 2). PFOR then transfers electrons from pyruvate to the electron acceptor Fdx, producing acetyl-CoA, which is through ASCT/SCS cycle reduced to acetate, producing ATP (pathway 3). The reduced Fdx is used to produce H₂ by the HYD enzyme. Dashed line: Drug activation pathway – Once MTZ enters the hydrogenosome, it competes with HYD for the electron carrying Fdx. MTZ is reduced to a nitro radical anion that interacts with *T. vaginalis* DNA causing damage and death of the organism. PFOR, pyruvate: ferredoxin oxidoreductase; Fdx, ferredoxin; HYD, hydrogenase; H₂, hydrogen; CO₂, carbon dioxide; MTZ, metronidazole; MDH, malate dehydrogenase; NADPH, nicotinamide adenine dinucleotide phosphate; NADH:FOR, nicotinamide adenine dinucleotide:ferredoxin oxidoreductase; PEPCK, phosphoenolpyruvate carboxykinase; GTP/GDP, guanosine triphosphate/guanosine diphosphate; PDC, pyruvate decarboxylase; LDH, lactate dehydrogenase; ADH, alcohol dehydrogenase; ASCT, acetate: succinyl CoA-transferase; SCS, succinyl CoA synthetase; OX, oxidized; RED, reduced.

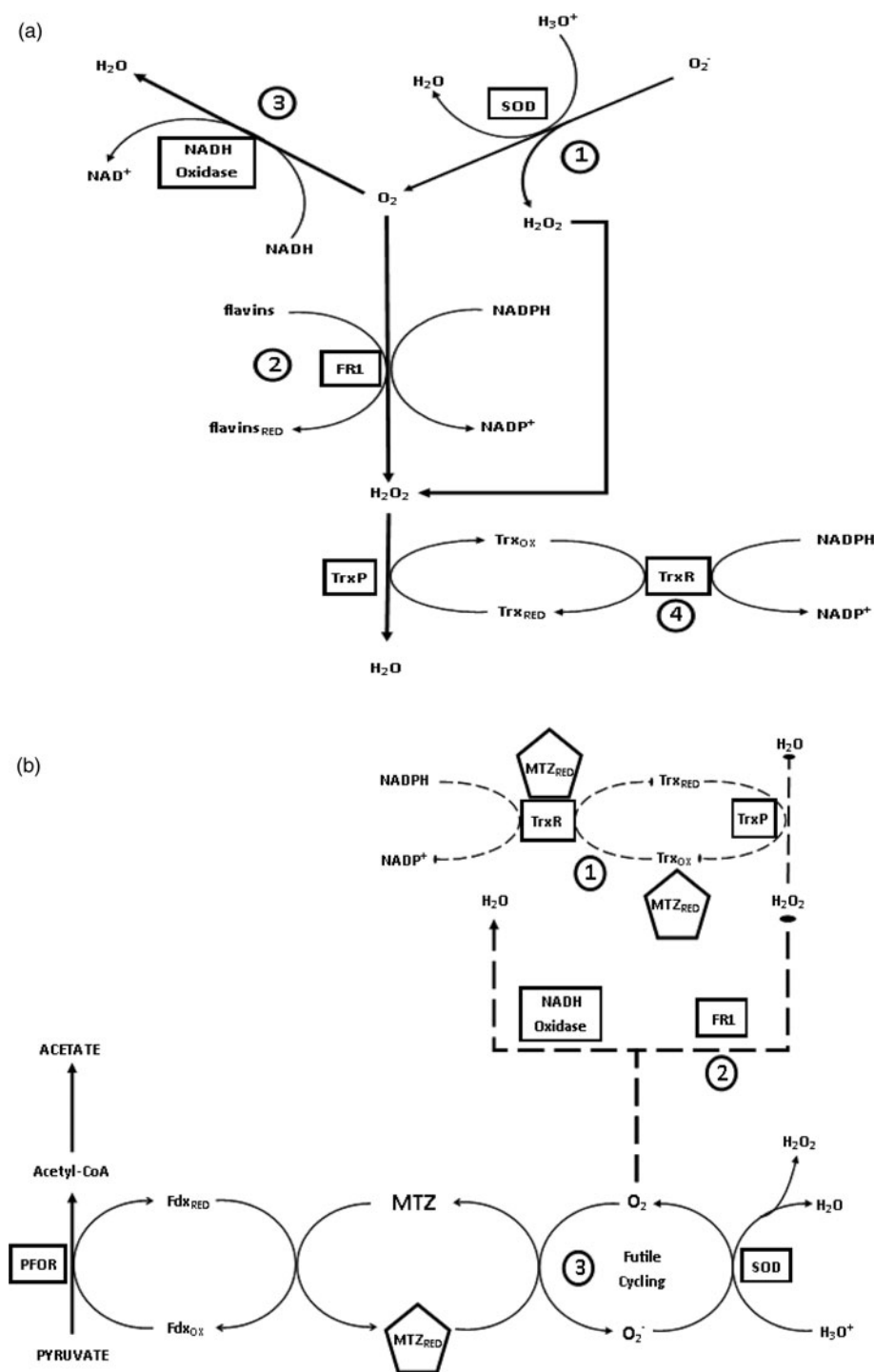


Fig. 3. Aerobic resistance to MTZ arises from deficient O_2 scavenging pathways. (a) Normal *T. vaginalis* anti-oxidant defense pathways – (1) ROS, such as O_2^- , can be harmful to trichomonads, and multiple pathways can control and ‘neutralize’ ROS. SOD reduces O_2^- to molecular O_2 while also producing water and H_2O_2 . (2) Intracellular O_2 can be reduced to H_2O_2 by FRI or by (3) NADH oxidase into H_2O . The cytotoxic H_2O_2 produced by these pathways further metabolized by the trichomonad. (4) H_2O_2 can be indirectly reduced to H_2O , through the activation of the flavin-containing enzyme TrxR by NADPH. Electrons from NADPH are transferred from TrxR to Trx, which activates thioredoxin-dependent peroxidases such as TrxP to reduce H_2O_2 to H_2O . (b) Aerobic resistance pathway – (1) TrxR, like Fdx and NTR, can reduce MTZ. Once reduced, MTZ can form covalent adducts with TrxR and Trx. These adducts inhibit the activities of the enzymes, leading to increased levels of cytotoxic H_2O_2 . (2) FRI and NADH oxidase activities are significantly decreased or absent in MTZ resistant strains. (3) Intracellular O_2 levels increase in the absence of normally functioning O_2 -scavenging pathways, resulting in the inactivation of MTZ from its nitro radical anion in a process known as futile cycling. ROS, reactive oxygen species; O_2^- , superoxide; SOD, superoxide dismutase; O_2 , oxygen; H_2O_2 , hydrogen peroxide; FRI, flavin reductase 1; NADH, nicotinamide adenine dinucleotide; TrxR, thioredoxin reductase; NTR, nitroreductase; NADPH, nicotinamide adenine dinucleotide phosphate; Trx, thioredoxin; TrxP, thioredoxin peroxidase; MTZ, metronidazole; OX, oxidized; RED, reduced.

Genetics of 5-nitroimidazole resistance

The role of genetic diversity and differential gene expression in 5-nitroimidazole resistance

Many techniques have been used to characterize and analyse the genotypic, phenotypic and genetic relatedness of *T. vaginalis* strains from different geographical regions. These techniques involve analysing random-amplified polymorphic DNA patterns, restriction fragment length polymorphisms, multi-locus sequence typing and internal transcribed spacer (ITS) region sequences (Vanacova *et al.*, 1997; Snipes *et al.*, 2000; Stiles *et al.*, 2000; Hampl *et al.*, 2001; Abdel-Magied *et al.*, 2017). Genotypic and phenotypic analyses demonstrate the genetic relatedness of MTZ-resistant *T. vaginalis* strains and suggest *T. vaginalis* can be divided into two distinct populations (Cornelius *et al.*, 2010;

Rabiee *et al.*, 2012). Type 1 populations are the more ancestral lineage whereas type 2 populations appear to have diverged more recently (Conrad *et al.*, 2012), with type 2 having higher mean MLCs for MTZ (Bradic *et al.*, 2012; Conrad *et al.*, 2012). Some genes involved in 5-nitroimidazole resistance possess mutations and are differentially expressed in resistant strains (Mead *et al.*, 2006; Bradic *et al.*, 2017).

Fdx gene mutations

Some MTZ-resistant *T. vaginalis* isolates have decreased *fdx* gene transcription (Quon *et al.*, 1992). Two different point mutations have been identified at nucleotide positions –178 and –239 upstream of the transcription start site. The –239 mutation is more relevant and is characterized by an A→T transversion

(adenine to thymidine). One study observed a 28-bp region surrounding the –239 mutation that binds a 23-kDa DNA-binding protein thought important for transcription of the *fdx* gene (Quon *et al.*, 1992). MTZ-resistant isolates with this mutation have a lower binding affinity for DNA-binding protein that could lead to reduced transcription. This –239 A→T mutation has been observed more recently (Heidari *et al.*, 2013). An additional study analysed the amino-acid sequence of Fdx and determined regions coding for amino-acids 43–48 are more susceptible to mutation (Wiwanitkit, 2008).

NTR 4/6 gene mutation

NTR is one of the enzymes responsible for the reduction of 5-nitroimidazoles (Pal *et al.*, 2009). A recent study found single-nucleotide polymorphisms (SNPs) in two NTR genes (*ntr4* and *ntr6*) (Paulish-Miller *et al.*, 2014). These SNPs were nonsense mutations (introduction of a stop codon) associated with MTZ resistance. Isolates with either mutation or both were more likely to belong to the type 2 population, which is associated with greater MTZ resistance (Paulish-Miller *et al.*, 2014).

ITS1 point mutation

The ITS regions that flank the 5.8S subunit of the ribosomal DNA gene of *T. vaginalis* are not as highly conserved as the ribosomal coding sequences they flank, leading to a greater frequency of genetic polymorphisms between organisms of the same species. A point mutation at the 66th nucleotide of the ITS region consisting of a cysteine being replaced with a thymidine (C66T) is associated with higher levels of MTZ resistance (Snipes *et al.*, 2000; Xiao *et al.*, 2006; Kazemi *et al.*, 2010).

Iron metabolism affects gene expression in *T. vaginalis*

Iron plays an important role in the metabolic processes (as a cofactor in many enzymes) and transcriptional regulation of genes for key enzymes in *T. vaginalis* (Argaez-Correa *et al.*, 2019). The restriction of iron in *T. vaginalis* leads to the down-regulation of genes important for energy metabolism and drug activation like PFOR and Fdx (Horvathova *et al.*, 2012). Decreased expression leads to development of 5-nitroimidazole resistance. Iron is a key structural and metabolic component of PFOR and Fdx; decreasing iron stores available to *T. vaginalis* isolates decrease their ability to synthesize these critically important enzymes. Supplemental addition of iron with MTZ treatment decreases MLCs under both aerobic and anaerobic conditions in MTZ-sensitive strains (Elwakil *et al.*, 2017).

Expression of surface carbohydrate genes

Trichomonas vaginalis membranes contain glycoproteins and glycolipids that act as cell receptors and are involved in host–parasite interactions. The plasma membranes of MTZ-resistant and MTZ-sensitive *T. vaginalis* strains differ in their surface carbohydrate structures (Dias Filho *et al.*, 1992). *Trichomonas vaginalis* also has a P-glycoprotein gene (*Tvpgp1*) that codes for an ATP binding cassette (ABC) transmembrane protein (Johnson *et al.*, 1994). ABC proteins are involved in the transport of drugs out of cells. *Tvpgp1* is expressed 2–20-fold higher in MTZ-resistant *T. vaginalis* strains compared to sensitive strains (Johnson *et al.*, 1994).

Extracellular ATP and ADP level modulation by *T. vaginalis*

Extracellular ATP is involved in host–parasite interactions such as signal transduction, cytoadherence, immune response and inflammation. The enzymes that *T. vaginalis* employs for the hydrolysis of extracellular ATP, adenosine diphosphate (ADP) and adenosine monophosphate, are NTPDase1 and ecto-5'-nucleotidase; these enzymes can be incorporated into the plasma membrane

of *T. vaginalis* and are involved in signal transduction and cytoadhesion. Prolonged exposure/treatment with MTZ or TDZ results in the inhibition of these enzymes in MTZ-sensitive strains (Tasca *et al.*, 2003). Thus, modulation of extracellular ATP and ADP levels may be a defense strategy of *T. vaginalis* in response to unfavourable environmental conditions (i.e. drug pressure) (Tasca *et al.*, 2003).

Relative vs absolute resistance in *T. vaginalis*

Compared to resistance mechanisms in many bacteria, resistance to 5-nitroimidazoles in *Trichomonas* appears to be relative rather than absolute. Based upon how quickly resistance to MTZ appeared after its introduction (Robinson, 1962), the mechanism(s) for drug resistance may have already existed and clinical treatment failure may be more of a function of drug tolerance rather than developed drug resistance. For example, *T. vaginalis* infections unresponsive to currently recommended doses of MTZ (i.e. 2 g single oral dose or 500 mg orally twice daily for 7 days) can often be treated by increasing dosages (i.e. 2–4 g of MTZ orally daily for 3–14 days) (Lossick *et al.*, 1986). This may be because the enzymes that activate MTZ are important in other critical cellular functions and complete loss of these enzymes would result in parasite death. In addition, some *T. vaginalis* isolates have been found to be clinically more resistant than others with similar MLC values to MTZ (Lossick *et al.*, 1986). This suggests that complex interactions between drug levels in the vaginal mucosa, the intra-vaginal redox potential (which may regulate the amount of drug taken up by the parasites), and the composition of the vaginal microbiota (which may modify the amount of available drug) may contribute to the level of drug resistance (Lossick *et al.*, 1986). The major limiting factor of high dose treatment in such cases is the amount of drug that the patient can safely tolerate. Significant nausea, metallic taste, sensorium changes and peripheral neuropathy have occurred in patients receiving 4 g of MTZ for 14 days (Lossick *et al.*, 1986). Thus, the risks and benefits of treating patients with MTZ-resistant trichomoniasis with daily doses of MTZ exceeding 3 g should be carefully considered; alternative treatments outside of 5-nitroimidazoles may need to be considered in these cases (Workowski *et al.*, 2015).

Other factors supporting the assertion that *Trichomonas* resistance is relative rather than absolute are the similar prevalence of the two genotypes (type 1 and type 2) in different regions of the world (Conrad *et al.*, 2012) and the apparent lack of 'outbreaks' of drug resistance that might be expected if resistance developed in response to drug pressure.

The role of *T. vaginalis* co-infections and 5-nitroimidazole resistance

Early treatment failures to MTZ were not readily attributed to resistance but instead to the presence of a diverse population of micro-organisms that may metabolize MTZ before it can reach *T. vaginalis* for uptake in sufficient concentrations to kill the parasite (Edwards *et al.*, 1979; Ingham *et al.*, 1982). Some data suggest that 5-nitroimidazole resistance may also be associated with co-infection of *T. vaginalis* by *Mycoplasma hominis* or *T. vaginalis* virus (TVV) (Wang, 1984; Fichorova *et al.*, 2017).

Trichomonas vaginalis virus (TVV)

TVV is a 4.5 kbp dsRNA virus that infects some *T. vaginalis* isolates (Wang and Wang, 1985). Four strains of TVV have been described since its discovery in the mid-1980s with TVV1 being the most common strain, followed by TVV2 and TVV3, with TVV4 being least common (Graves *et al.*, 2019). TVV2 and

TVV3 are associated with expression of the surface immunogen P270. Some studies found no clear associations of TVV presence on MTZ resistance (Wang and Wang, 1985; Flegr *et al.*, 1987), whereas another study found increased MTZ susceptibility in TVV-infected *T. vaginalis* (Malla *et al.*, 2011); additional research is needed to clarify the effect of TVV infection on 5-nitroimidazole resistance in *T. vaginalis*.

Mycoplasma hominis

Mycoplasma hominis is a bacterial pathogen of the lower genital tract that naturally infects *T. vaginalis* (van Belkum *et al.*, 2001). Some studies have found that *M. hominis* infection of *T. vaginalis* was associated with increased MTZ resistance (Xiao *et al.*, 2006; Furnkranz *et al.*, 2018). However, other studies indicate *M. hominis* infection has no clinical significance (Butler *et al.*, 2010; Fraga *et al.*, 2012; da Luz Becker *et al.*, 2015). *Mycoplasma hominis* can downregulate expression of Fdx, FR1 and PFOR genes involved in MTZ resistance mechanisms (Furnkranz *et al.*, 2018); however, similar to TVV, how *M. hominis* affects *T. vaginalis* susceptibility to MTZ needs further investigation.

Discussion

We found that resistance is facilitated by altered expression and activation of enzymes and proteins involved in *T. vaginalis* energy production and oxygen scavenging. Increased intracellular oxygen concentrations, due to deficient oxygen scavenging, enables development of aerobic resistance, the major form of resistance encountered in clinical isolates. Anaerobic resistance is rarely observed clinically but has been induced under laboratory conditions and is characterized by altered energy metabolism due to reduced expression of enzymes and proteins involved in the main and alternative energy-production pathways. There is no history of 'outbreaks' of drug-resistant trichomoniasis and the relative frequency of the two genotypes of *T. vaginalis* is consistent throughout the world, suggesting that insensitivity to 5-nitroimidazoles may naturally occur rather than being treatment-induced.

Currently recommended treatments in the setting of 5-nitroimidazole resistance are 2 g of oral MTZ or TDZ daily for 7 days (Workowski *et al.*, 2015). Even higher doses of these medications for longer periods of time have been previously used to overcome drug resistance (Lossick *et al.*, 1986). However, patients may be at increased risk of adverse drug effects, as previously described (Lossick *et al.*, 1986). For patients failing to respond to higher doses or longer courses of oral MTZ or TDZ, two additional treatments are available, although not rigorously studied. The first is high dose oral TDZ 2 g daily plus intra-vaginal TDZ 500 mg twice daily for 14 days (Sobel *et al.*, 2001). If this regimen fails, a second option is high dose oral TNZ 1 g three times daily plus intra-vaginal paromomycin (4 g of 6.25% intra-vaginal paromomycin cream nightly) for 14 days (Nyirjesy *et al.*, 2011). Paromomycin is an aminoglycoside with a different mechanism of action (destruction of ribosomal RNA) than 5-nitroimidazoles (inhibition of nucleic acid synthesis by DNA disruption and/or inhibition of enzymes involved in antioxidant defense) (Leitsch *et al.*, 2009; Van Gerwen and Muzny, 2019); combination use of these medications may provide a synergistic effect. It is important to note that all intra-vaginal formulations need to be made at a compounding pharmacy and may be costly (Muzny *et al.*, 2020). In addition, intra-vaginal paromomycin can cause vaginal ulceration and should be discontinued if this occurs, at which time ulcerations may spontaneously regress (Van Gerwen and Muzny, 2019).

The primary limitation of this study is that non-English language articles were excluded. Because of this we may have missed some pertinent information that should have been included. Future reviews on this topic should include addition of non-English language

articles. Despite this limitation, we were able to find a moderately sized body of English-language literature to include in this review. In summary, the data reviewed suggest that alternative methods of treatment using medications outside of the 5-nitroimidazole class are urgently needed as well as a more complete understanding of the mechanisms of 5-nitroimidazole resistance in *T. vaginalis*.

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References

- Abdel-Magied AA, El-Kholya EI, Abou El-Khair SM, Abdelmegeed ES, Hamoudaa MM, Mohamed SA and El-Tantawy NL (2017) The genetic diversity of metronidazole susceptibility in *Trichomonas vaginalis* clinical isolates in an Egyptian population. *Parasitology Research* **116**, 3125–3130.
- Ahuja V, Dhar A, Bal C and Sharma MP (1998) Lansoprazole and secnidazole with clarithromycin, amoxicillin or pefloxacin in the eradication of *Helicobacter pylori* in a developing country. *Alimentary Pharmacology and Therapeutics* **12**, 551–555.
- Argaez-Correa W, Alvarez-Sanchez ME, Arana-Argaez VE, Ramirez-Camacho MA, Novelo-Castilla JS, Coral-Martinez TI and Torres-Romero JC (2019) The role of iron status in the early progression of metronidazole resistance in *Trichomonas vaginalis* under microaerophilic conditions. *Journal of Eukaryotic Microbiology* **66**, 309–315.
- Bouchenal K, Bories C and Loiseau PM (2017) Strategies for prevention and treatment of *Trichomonas vaginalis* infections. *Clinical Microbiology Reviews* **30**, 811–825.
- Bradic M, Secor WE, Conrad M and Carlton JM (2012) Distribution of drug resistance and its genetic basis in global isolates of *Trichomonas vaginalis*. *American Journal of Tropical Medicine and Hygiene* **87**, 313.
- Bradic M, Warring SD, Tooley GE, Scheid P, Secor WE, Land KM, Huang PJ, Chen TW, Lee CC, Tang P, Sullivan SA and Carlton JM (2017) Genetic indicators of drug resistance in the highly repetitive genome of *Trichomonas vaginalis*. *Genome Biology and Evolution* **9**, 1658–1672.
- Brown DM, Upcroft JA, Dodd HN, Chen N and Upcroft P (1999) Alternative 2-keto acid oxidoreductase activities in *Trichomonas vaginalis*. *Molecular and Biochemical Parasitology* **98**, 203–214.
- Butler SE, Augostini P and Secor WE (2010) *Mycoplasma hominis* infection of *Trichomonas vaginalis* is not associated with metronidazole-resistant trichomoniasis in clinical isolates from the United States. *Parasitology Research* **107**, 1023–1027.
- Conrad MD, Gorman AW, Schillinger JA, Fiori PL, Arroyo R, Malla N, Dubey ML, Gonzalez J, Blank S, Secor WE and Carlton JM (2012) Extensive genetic diversity, unique population structure and evidence of genetic exchange in the sexually transmitted parasite *Trichomonas vaginalis*. *PLoS Neglected Tropical Diseases* **6**, e1573.
- Coombs GH (1978) A mechanism of resistance to metronidazole in trichomonads. *British Society for Parasitology: Proceedings* **77**, xxiii.
- Cornelius DC, Mena L, Lushbaugh WB and Meade JC (2010) Genetic relatedness of *Trichomonas vaginalis* reference and clinical isolates. *American Journal of Tropical Medicine and Hygiene* **83**, 1283–1286.
- Cosar C and Julou L (1959) The activity of 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (R. P. 8823) against experimental *Trichomonas vaginalis* infections. *Annales de l'Institut Pasteur* **96**, 238–241.

- da Luz Becker D, dos Santos O, Frasson AP, de Vargas Rigo G, Macedo AJ and Tasca T (2015) High rates of double-stranded RNA viruses and *Mycoplasma hominis* in *Trichomonas vaginalis* clinical isolates in South Brazil. *Infection, Genetics and Evolution* **34**, 181–187.
- Declerck PJ and De Ranter CJ (1986) In vitro reductive activation of nitroimidazoles. *Biochemical Pharmacology* **35**, 59–61.
- Dias Filho BP, Andrade AF, de Souza W, Esteves MJ and Angluster J (1992) Cell surface saccharide differences in drug-susceptible and drug-resistant strains of *Trichomonas vaginalis*. *Microbios* **71**, 55–64.
- Dolezal P, Vanacova S, Tachezy J and Hrdy I (2004) Malic enzymes of *Trichomonas vaginalis*: two enzyme families, two distinct origins. *Gene* **329**, 81–92.
- Durel P, Couture J, Collart P and Girot C (1960) Flagyl (metronidazole). *British Journal of Venereal Diseases* **36**, 154–162.
- Edwards DI, Thompson EJ, Tomusange J and Shanson D (1979) Inactivation of metronidazole by aerobic organisms. *Journal of Antimicrobial Chemotherapy* **5**, 315–316.
- Ellis JE, Cole D and Lloyd D (1992) Influence of oxygen on the fermentative metabolism of metronidazole-sensitive and resistant strains of *Trichomonas vaginalis*. *Molecular and Biochemical Parasitology* **56**, 79–88.
- Ellis JE, Yarlett N, Cole D, Humphreys MJ and Lloyd D (1994) Antioxidant defences in the microaerophilic protozoan *Trichomonas vaginalis*: comparison of metronidazole-resistant and sensitive strains. *Microbiology (Reading, England)* **140**, 2489–2494.
- Elwakil HS, Tawfik RA, Alam-Eldin YH and Nassar DA (2017) The effect of iron on metronidazole activity against *Trichomonas vaginalis* in vitro. *Experimental Parasitology* **182**, 34–36.
- Escobedo AA, Canete R, Gonzalez ME, Pareja A, Cimerman S and Almirall P (2003) A randomized trial comparing mebendazole and secnidazole for the treatment of giardiasis. *Annals of Tropical Medicine and Parasitology* **97**, 499–504.
- Fichorova R, Fraga J, Rappelli P and Fiori PL (2017) *Trichomonas vaginalis* infection in symbiosis with *Trichomonas* virus and *Mycoplasma*. *Research in Microbiology* **168**, 882–891.
- Flegr J, Cerkasov J, Kulda J, Tachezy J and Stokrov J (1987) The dsRNA of *Trichomonas vaginalis* is associated with virus like particles and does not correlate with metronidazole resistance. *Folia Microbiologica* **32**, 345–348.
- Fraga J, Rodriguez N, Fernandez C, Mondeja B, Sariego I, Fernandez-Calienes A and Rojas L (2012) *Mycoplasma hominis* in Cuban *Trichomonas vaginalis* isolates: association with parasite genetic polymorphism. *Experimental Parasitology* **131**, 393–398.
- Furnkranz U, Henrich B and Walochnik J (2018) *Mycoplasma hominis* impacts gene expression in *Trichomonas vaginalis*. *Parasitology Research* **117**, 841–847.
- Graves KJ, Ghosh AP, Kissinger PJ and Muzny CA (2019). *Trichomonas vaginalis* virus: a review of the literature. *International Journal of STD and AIDS* **30**, 496–504.
- Hampf V, Vanacova S, Kulda J and Flegr J (2001) Concordance between genetic relatedness and phenotypic similarities of *Trichomonas vaginalis* strains. *BMC Evolutionary Biology* **1**, 11.
- Heidari S, Bandehpour M, Seyyed-Tabaei SJ, Valadkhani Z, Haghghi A, Abadi A and Kazemi B (2013) Ferredoxin gene mutation in Iranian *Trichomonas vaginalis* isolates. *Iranian Journal of Parasitology* **8**, 402–407.
- Honigberg BM and Brugerolle G (1990). Structure. In Honigberg BM (ed.), *Trichomonads Parasitic in Humans*. New York, NY: Springer, pp. 5–35.
- Honigberg BM and King VM (1964) Structure of *Trichomonas vaginalis* Donn'e. *Journal of Parasitology* **50**, 345–364.
- Horvathova L, Safarikova L, Basler M, Hrdy I, Campo NB, Shin JW, Huang KY, Huang PJ, Lin R, Tang P and Tachezy J (2012) Transcriptomic identification of iron-regulated and iron-independent gene copies within the heavily duplicated *Trichomonas vaginalis* genome. *Genome Biology and Evolution* **4**, 1017–1029.
- Hrdy I, Cammack R, Stopka P, Kulda J and Tachezy J (2005) Alternative pathway of metronidazole activation in *Trichomonas vaginalis* hydrogenosomes. *Antimicrobial Agents and Chemotherapy* **49**, 5033–5036.
- Ingham HR, Sisson PR and Selkon JB (1982) Interactions between microorganisms and metronidazole. *Journal of Antimicrobial Chemotherapy* **10**, 84–87.
- Johnson PJ, Schuck BL and Delgado MG (1994) Analysis of a single-domain P-glycoprotein-like gene in the early-diverging protist *Trichomonas vaginalis*. *Molecular and Biochemical Parasitology* **66**, 127–137.
- Kazemi F, Hooshyar H, Zareikar B, Bandehpour M, Arbabi M, Talari S, Alizadeh R and Kazemi B (2010) Study on ITS1 gene of Iranian *Trichomonas vaginalis* by molecular methods. *Iranian Journal of Parasitology* **5**, 9–14.
- Kirkcaldy RD, Augustini P, Asbel LE, Bernstein KT, Kerani RP, Mettenbrink CJ, Pathela P, Schwebke JR, Secor WE, Workowski KA, Davis D, Braxton J and Weinstock HS (2012) *Trichomonas vaginalis* antimicrobial drug resistance in 6 US cities, STD surveillance network, 2009–2010. *Emerging Infectious Diseases* **18**, 939–943.
- Kissinger P and Adamski A (2013) Trichomoniasis and HIV interactions: a review. *Sexually Transmitted Infections* **89**, 426–433.
- Krashin JW, Koumans EH, Bradshaw-Sydnor AC, Braxton JR, Evan Secor W, Sawyer MK and Markowitz LE (2010) *Trichomonas vaginalis* prevalence, incidence, risk factors and antibiotic-resistance in an adolescent population. *Sexually Transmitted Diseases* **37**, 440–444.
- Kulda J (1999) Trichomonads, hydrogenosomes and drug resistance. *International Journal for Parasitology* **29**, 199–212.
- Kulda J, Tachezy J and Cerkasovova A (1993) In vitro induced anaerobic resistance to metronidazole in *Trichomonas vaginalis*. *Journal of Eukaryotic Microbiology* **40**, 262–269.
- Land KM, Clemens DL and Johnson PJ (2001) Loss of multiple hydrogenosomal proteins associated with organelle metabolism and high-level drug resistance in trichomonads. *Experimental Parasitology* **97**, 102–110.
- Land KM, Delgado MG and Johnson PJ (2002) In vivo expression of ferredoxin in a drug resistant trichomonad increases metronidazole susceptibility. *Molecular and Biochemical Parasitology* **121**, 153–157.
- Leitsch D, Kolarich D, Binder M, Stadlmann J, Altmann F and Duchene M (2009) *Trichomonas vaginalis*: metronidazole and other nitroimidazole drugs are reduced by the flavin enzyme thioredoxin reductase and disrupt the cellular redox system. Implications for nitroimidazole toxicity and resistance. *Molecular Microbiology* **72**, 518–536.
- Leitsch D, Kolarich D and Duchene M (2010) The flavin inhibitor diphenyleneiodonium renders *Trichomonas vaginalis* resistant to metronidazole, inhibits thioredoxin reductase and flavin reductase, and shuts off hydrogenosomal enzymatic pathways. *Molecular and Biochemical Parasitology* **171**, 17–24.
- Leitsch D, Drinic M, Kolarich D and Duchene M (2012) Down-regulation of flavin reductase and alcohol dehydrogenase-1 (ADH1) in metronidazole-resistant isolates of *Trichomonas vaginalis*. *Molecular and Biochemical Parasitology* **183**, 177–183.
- Leitsch D, Janssen BD, Kolarich D, Johnson PJ and Duchene M (2014) *Trichomonas vaginalis* flavin reductase 1 and its role in metronidazole resistance. *Molecular Microbiology* **91**, 198–208.
- Lloyd D and Kristensen B (1985) Metronidazole inhibition of hydrogen production in vivo in drug-sensitive and resistant strains of *Trichomonas vaginalis*. *Journal of General Microbiology* **131**, 849–853.
- Lloyd D and Pedersen JZ (1985) Metronidazole radical anion generation in vivo in *Trichomonas vaginalis*: oxygen quenching is enhanced in a drug-resistant strain. *Journal of General Microbiology* **131**, 87–92.
- Lloyd D, Yarlett N and Yarlett NC (1986) Inhibition of hydrogen production in drug-resistant and susceptible *Trichomonas vaginalis* strains by a range of nitroimidazole derivatives. *Biochemical Pharmacology* **35**, 61–64.
- Lossick JG, Muller M and Gorrell TE (1986) In vitro drug susceptibility and doses of metronidazole required for cure in cases of refractory vaginal trichomoniasis. *Journal of Infectious Diseases* **153**, 948–955.
- Malla N, Kaul P, Sehgal R and Gupta I (2011) The presence of dsRNA virus in *Trichomonas vaginalis* Isolates from symptomatic and asymptomatic Indian women and its correlation with in vitro metronidazole sensitivity. *Indian Journal of Medical Microbiology* **29**, 152–157.
- Mead JR, Fernandez M, Romagnoli PA and Secor WE (2006) Use of *Trichomonas vaginalis* clinical isolates to evaluate correlation of gene expression and metronidazole resistance. *Journal of Parasitology* **92**, 196–199.
- Meites E, Gaydos CA, Hobbs MM, Kissinger P, Nyirjesy P, Schwebke JR, Secor WE, Sobel JD and Workowski KA (2015) A review of evidence-based care of symptomatic trichomoniasis and asymptomatic *Trichomonas vaginalis* infections. *Clinical Infectious Diseases* **61**, S837–S848.
- Muller M (1986) Reductive activation of nitroimidazoles in anaerobic microorganisms. *Biochemical Pharmacology* **35**, 37–41.
- Muller M and Gorrell TE (1983) Metabolism and metronidazole uptake in *Trichomonas vaginalis* isolates with different metronidazole susceptibilities. *Antimicrobial Agents and Chemotherapy* **24**, 667–673.

- Muzny CA, Van Gerwen OT and Kissinger P (2020) Updates in *Trichomonas* treatment including persistent infection and 5-nitroimidazole hypersensitivity. *Current Opinion in Infectious Diseases* **33**, 73–77.
- Newman L, Rowley J, Vander Hoorn S, Wijesooriya NS, Unemo M, Low N, Stevens G, Gottlieb S, Kiarie J and Temmerman M (2015) Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting. *PLoS ONE* **10**, e0143304.
- Nyirjesy P, Gilbert J and Mulcahy LJ (2011) Resistant trichomoniasis: successful treatment with combination therapy. *Sexually Transmitted Diseases* **38**, 962–963.
- Pal D, Banerjee S, Cui J, Schwartz A, Ghosh SK and Samuelson J (2009) *Giardia*, *Entamoeba*, and *Trichomonas* enzymes activate metronidazole (nitroreductases) and inactivate metronidazole (nitroimidazole reductases). *Antimicrobial Agents and Chemotherapy* **53**, 458–464.
- Patel EU, Gaydos CA, Packman ZR, Quinn TC and Tobian AAR (2018) Prevalence and correlates of *Trichomonas vaginalis* infection among men and women in the United States. *Clinical Infectious Diseases* **67**, 211–217.
- Paulish-Miller TE, Augostini P, Schuyler JA, Smith WL, Mordechai E, Adelson ME, Gyax SE, Secor WE and Hilbert DW (2014) *Trichomonas vaginalis* metronidazole resistance is associated with single nucleotide polymorphisms in the nitroreductase genes ntr4Tv and ntr6Tv. *Antimicrobial Agents and Chemotherapy* **58**, 2938–2943.
- Perez S, Fernandez-Verdugo A, Perez F and Vazquez F (2001) Prevalence of 5-nitroimidazole-resistant *Trichomonas vaginalis* in Oviedo, Spain. *Sexually Transmitted Diseases* **28**, 115–116.
- Quon DV, d'Oliveira CE and Johnson PJ (1992) Reduced transcription of the ferredoxin gene in metronidazole-resistant *Trichomonas vaginalis*. *Proceedings of the National Academy of Sciences of the United States of America* **89**, 4402–4406.
- Rabiee S, Bazmani A, Matini M and Fallah M (2012) Comparison of resistant and susceptible strains of *Trichomonas vaginalis* to metronidazole using PCR method. *Iranian Journal of Parasitology* **7**, 24–30.
- Rasoloson D, Tomkova E, Cammack R, Kulda J and Tachezy J (2001) Metronidazole-resistant strains of *Trichomonas vaginalis* display increased susceptibility to oxygen. *Parasitology* **123**, 45–56.
- Rasoloson D, Vanacova S, Tomkova E, Razga J, Hrdy I, Tachezy J and Kulda J (2002) Mechanisms of in vitro development of resistance to metronidazole in *Trichomonas vaginalis*. *Microbiology* **148**, 2467–2477.
- Robinson SC (1962) Trichomonal vaginitis resistant to metronidazole. *Canadian Medical Association Journal* **86**, 665.
- Rossignol JF, Maisonneuve H and Cho YW (1984) Nitroimidazoles in the treatment of trichomoniasis, giardiasis, and amebiasis. *International Journal of Clinical Pharmacology, Therapy, and Toxicology* **22**, 63–72.
- Rowley J, Toskin I and Ndowa F (2012) Global incidence and prevalence of selected curable sexually transmitted infections – 2008. *World Health Organization, Geneva*.
- Schmid G, Narcisi E, Mosure D, Secor WE, Higgins J and Moreno H (2001) Prevalence of metronidazole-resistant *Trichomonas vaginalis* in a gynecology clinic. *Journal of Reproductive Medicine* **46**, 545–549.
- Schwebke JR and Barrientes FJ (2006) Prevalence of *Trichomonas vaginalis* isolates with resistance to metronidazole and tinidazole. *Antimicrobial Agents and Chemotherapy* **50**, 4209–4210.
- Schwebke JR, Rompalo A, Taylor S, Sena AC, Martin DH, Lopez LM, Lensing S and Lee JY (2011) Re-evaluating the treatment of nongonococcal urethritis: emphasizing emerging pathogens – a randomized clinical trial. *Clinical Infectious Diseases* **52**, 163–170.
- Schwebke JR, Morgan FG Jr, Koltun W and Nyirjesy P (2017) A phase-3, double-blind, placebo-controlled study of the effectiveness and safety of single oral doses of secnidazole 2 g for the treatment of women with bacterial vaginosis. *American Journal of Obstetrics and Gynecology* **217**, 678.e1–678.e9.
- Sena AC, Miller WC, Hobbs MM, Schwebke JR, Leone PA, Swygard H, Atashili J and Cohen MS (2007) *Trichomonas vaginalis* infection in male sexual partners: implications for diagnosis, treatment, and prevention. *Clinical Infectious Diseases* **44**, 13–22.
- Silver BJ, Guy RJ, Kaldor JM, Jamil MS and Rumbold AR (2014) *Trichomonas vaginalis* as a cause of perinatal morbidity: a systematic review and meta-analysis. *Sexually Transmitted Diseases* **41**, 369–376.
- Snipes LJ, Gamard PM, Narcisi EM, Beard CB, Lehmann T and Secor WE (2000) Molecular epidemiology of metronidazole resistance in a population of *Trichomonas vaginalis* clinical isolates. *Journal of Clinical Microbiology* **38**, 3004–3009.
- Sobel JD, Nyirjesy P and Brown W (2001) Tinidazole therapy for metronidazole-resistant vaginal trichomoniasis. *Clinical Infectious Diseases* **33**, 1341–1346.
- Stiles JK, Shah PH, Xue L, Meade JC, Lushbaugh WB, Cleary JD and Finley RW (2000) Molecular typing of *Trichomonas vaginalis* isolates by HSP70 restriction fragment length polymorphism. *American Journal of Tropical Medicine and Hygiene* **62**, 441–445.
- Sutton M, Sternberg M, Koumans EH, McQuillan G, Berman S and Markowitz L (2007) The prevalence of *Trichomonas vaginalis* infection among reproductive-age women in the United States, 2001–2004. *Clinical Infectious Diseases* **45**, 1319–1326.
- Swygard H, Sena AC, Hobbs MM and Cohen MS (2004) Trichomoniasis: clinical manifestations, diagnosis and management. *Sexually Transmitted Infections* **80**, 91–95.
- Tachezy J, Kulda J and Tomkova E (1993) Aerobic resistance of *Trichomonas vaginalis* to metronidazole induced in vitro. *Parasitology* **106**, 31–37.
- Tasca T, Borges FP, Bonan CD, De Carli GA, Battastini AM and Sarkis JJ (2003) Effects of metronidazole and tinidazole on NTPDase1 and ecto-5'-nucleotidase from intact cells of *Trichomonas vaginalis*. *FEMS Microbiology Letters* **226**, 379–384.
- Vanacova S, Tachezy J, Kulda J and Flegr J (1997) Characterization of trichomonad species and strains by PCR fingerprinting. *Journal of Eukaryotic Microbiology* **44**, 545–552.
- van Belkum A, van der Schee C, van der Meijden WI, Verbrugh HA and Sluiter HJ (2001) A clinical study on the association of *Trichomonas vaginalis* and *Mycoplasma hominis* infections in women attending a sexually transmitted disease (STD) outpatient clinic. *FEMS Immunology and Medical Microbiology* **32**, 27–32.
- Van Gerwen OT and Muzny CA (2019) Recent advances in the epidemiology, diagnosis, and management of *Trichomonas vaginalis* infection. *F1000Research* **8**, 1–9.
- Wang A (1984) A linear double-stranded RNA in *Trichomonas vaginalis* and its relationship with resistance to metronidazole. *Federation Proceedings* **43**, 1284.
- Wang AL and Wang CC (1985) A linear double-stranded RNA in *Trichomonas vaginalis*. *Journal of Biological Chemistry* **260**, 3697–3702.
- WHO (2017) Essential medicines and health products information portal. 2019, Available at <http://apps.who.int/medicinedocs/en/d/Jh2942e/4.9.html#Jh2942e.4.9>.
- Wiwanitkit V (2008) Identification of weak points prone for mutation in ferredoxin of *Trichomonas vaginalis*. *Indian Journal of Medical Microbiology* **26**, 158–159.
- Wolner-Hanssen P, Krieger JN, Stevens CE, Kiviat NB, Koutsky L, Critchlow C, DeRouen T, Hillier S and Holmes KK (1989) Clinical manifestations of vaginal trichomoniasis. *JAMA* **261**, 571–576.
- Workowski KA, Bolan GA and Centers for Disease and Prevention C (2015) Sexually transmitted diseases treatment guidelines, 2015. *MMWR: Recommendations and Reports* **64**, 1–137.
- Wright JM, Webb RI, O'Donoghue P, Upcroft P and Upcroft JA (2010) Hydrogenosomes of laboratory-induced metronidazole-resistant *Trichomonas vaginalis* lines are downsized while those from clinically metronidazole-resistant isolates are not. *Journal of Eukaryotic Microbiology* **57**, 171–176.
- Xiao JC, Xie LF, Fang SL, Gao MY, Zhu Y, Song LY, Zhong HM and Lun ZR (2006) Symbiosis of *Mycoplasma hominis* in *Trichomonas vaginalis* may link metronidazole resistance in vitro. *Parasitology Research* **100**, 123–130.
- Yarlett N, Gorrell TE, Marczak R and Muller M (1985) Reduction of nitroimidazole derivatives by hydrogenosomal extracts of *Trichomonas vaginalis*. *Molecular and Biochemical Parasitology* **14**, 29–40.
- Yarlett N, Yarlett NC and Lloyd D (1986a) Ferredoxin-dependent reduction of nitroimidazole derivatives in drug-resistant and susceptible strains of *Trichomonas vaginalis*. *Biochemical Pharmacology* **35**, 1703–1708.
- Yarlett N, Yarlett NC and Lloyd D (1986b) Metronidazole-resistant clinical isolates of *Trichomonas vaginalis* have lowered oxygen affinities. *Molecular and Biochemical Parasitology* **19**, 111–116.