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

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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).

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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) $\leq 25\,\mu g\,mL^{-1}$ are considered MTZ-sensitive. An MLC of $50\,\mu g\,mL^{-1}$ represents low-level resistance, MLCs of $100–200\,\mu g\,mL^{-1}$ moderate-level resistance and MLCs $\geq 400\,\mu g\,mL^{-1}$ 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.*, 1986*a*, 1986*b*; 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).

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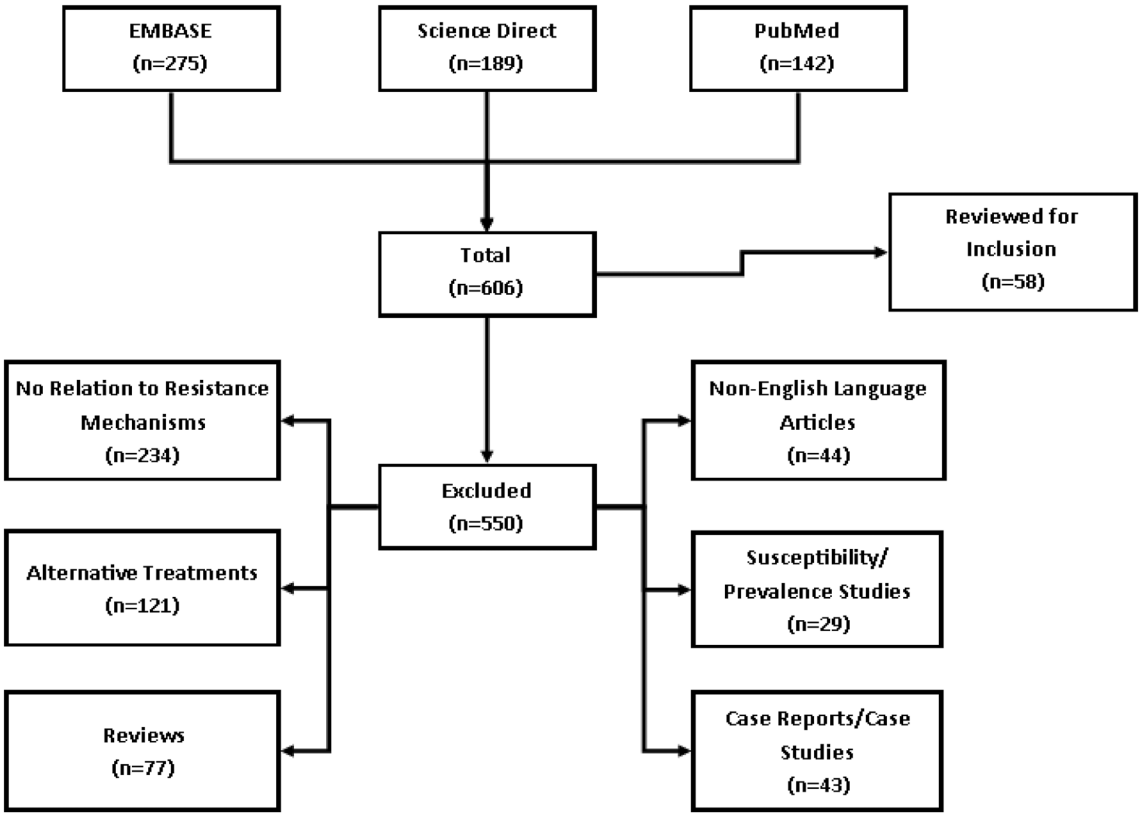

Fig. 1. Flow chart. Flow chart of the databases used to perform the systematic review of the literature. Included are the number of articles reviewed for study inclusion and those that were excluded for various reasons.

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 H_2O_2, 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.*, 1986*a*, 1986*b*; 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 H_2O_2 and increases intracellular O_2 leading to inactivation (oxidation) of MTZ (Leitsch *et al.*, 2012; Leitsch *et al.*, 2014). Although increased O_2 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 CO_2 (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).

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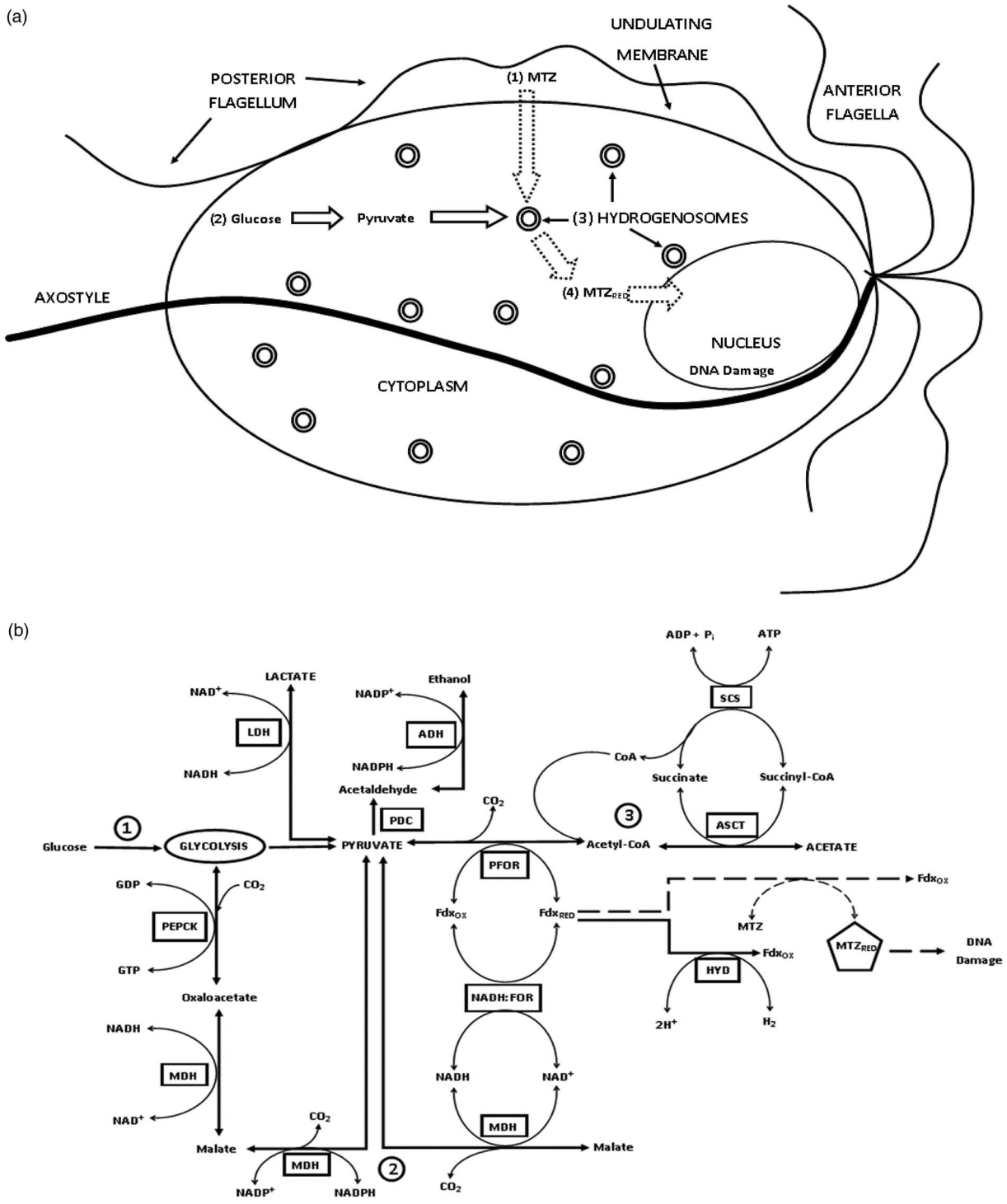

Fig. 2. MTZ activation through the energy production pathway in *T. vaginalis*. (a) Overview of *T. vaginalis* and the MTZ drug activation pathway. (1) MTZ enters the cell by passive diffusion and is subsequently metabolized in the hydrogenosome. (2) Pyruvate is produced from glucose through glycolysis 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 H_2 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; H_2, hydrogen; CO_2, 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: succinate CoA-transferase; SCS, succinyl CoA synthetase; OX, oxidized; RED, reduced.

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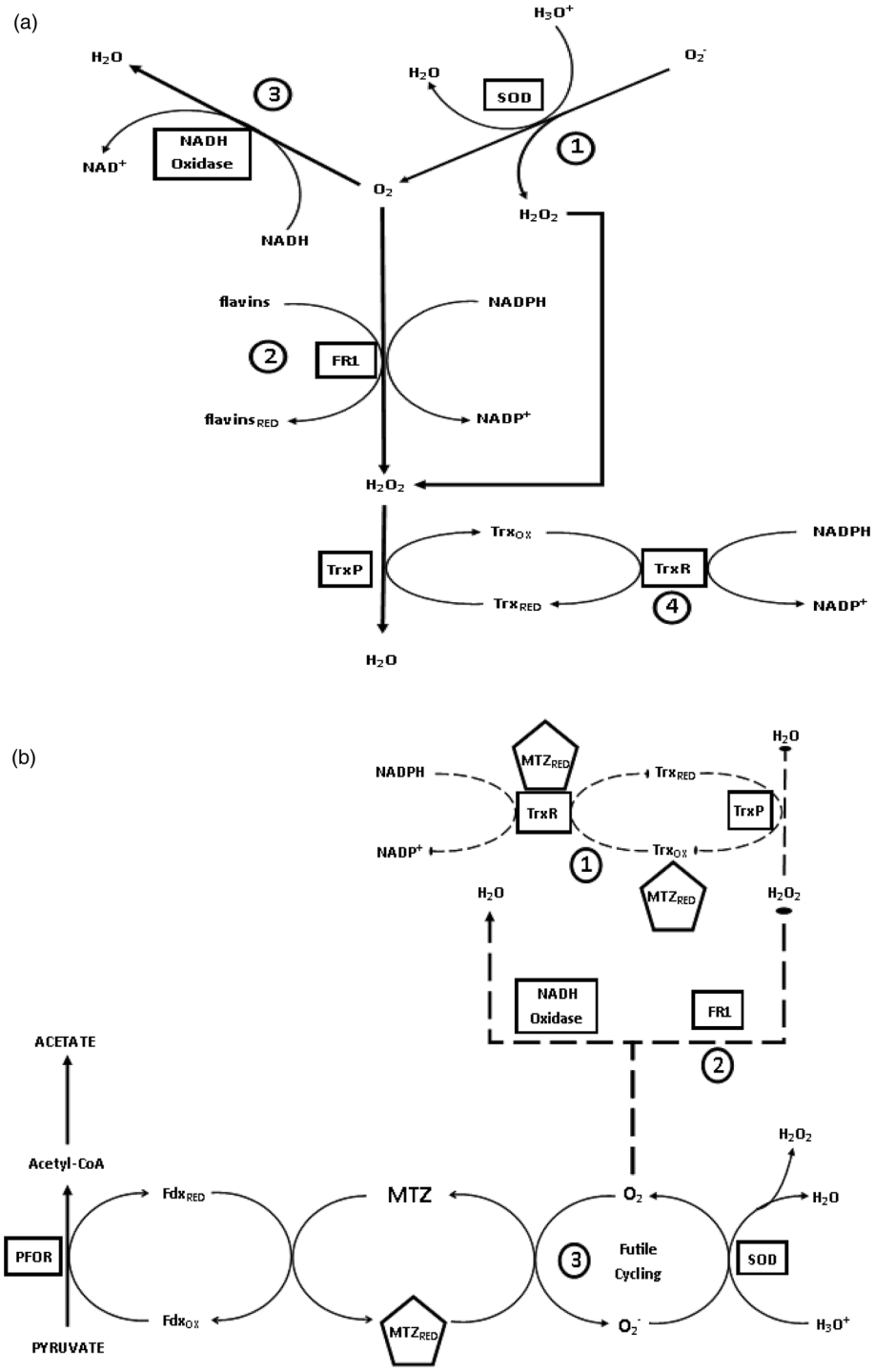

Fig. 3. Aerobic resistance to MTZ arises from deficient O_2 scavenging pathways. (a) Normal *T. vaginalis* antioxidant defense pathways – (1) ROS, such as O_2^-, can be harmful to trichomonads, and multiple pathways can control and 'neutralize' ROS. SOD reduces O_2^- to molecular O_2 while also producing water and H_2O_2. (2) Intracellular O_2 can be reduced to H_2O_2 by FR1 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) FR1 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; FR1, 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

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(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

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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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Ethical standards. Not applicable.

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