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REVIEW
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An Overview of Mucosa-Associated Protozoa: Challenges in Chemotherapy and Future Perspectives

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Parasitic infections caused by protozoans that infect the mucosal surfaces are widely neglected worldwide. Collectively, *Entamoeba histolytica, Giardia lamblia, Cryptosporidium* spp. and *Trichomonas vaginalis* infect more than a billion people in the world, being a public health problem mainly in developing countries. However, the exact incidence and prevalence data depend on the population examined. These parasites ultimately cause pathologies that culminate in liver abscesses, malabsorption syndrome, vaginitis, and urethritis, respectively. Despite this, the antimicrobial agents currently used to treat these diseases are limited and often associated with adverse side effects and refractory cases due to the development of resistant parasites. The paucity of drug treatments, absence of vaccines and increasing problems of drug resistance are major concerns for their control and eradication. Herein, potential candidates are reviewed with the overall aim of determining the knowledge gaps and suggest future perspectives for research. This review focuses on this public health problem and focuses on the progress of drug repositioning as a potential strategy for the treatment of mucosal parasites.

Keywords: Entamoeba histolytica, Giardia lamblia, Cryptosporidium spp., Trichomonas vaginalis, reinfection, treatment-refractory, repurposed drug

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1 INTRODUCTION

Pathogenic protozoa associated with mucosal surfaces represent a significant threat to global health and development. Their control and elimination rely on prevention, diagnosis, and effective treatment. Altogether, *Entamoeba histolytica, Cryptosporidium* spp., *Giardia intestinalis* (syn. *Giardia lamblia, Giardia duodenalis*) contribute significantly to the global burden of gastroenteritis and fit well into the One Health concept (Collaborators, 2017; Shrivastav et al., 2020; Li et al., 2021). In turn, *Trichomonas vaginalis* accounts for one of the most prevalent non-viral sexually transmitted infections (STIs) (Vivancos et al., 2018). However, to a lesser extent, sexual activity that promotes faecal-oral contact can also lead to the transmission of *E. histolytica*, *Cryptosporidium* spp., and *G. intestinalis*. These intestinal infections of interest within the STI scope

pose an important public health challenge and seem to be significantly underestimated (Hung et al., 2012; Escobedo et al., 2014; Billet et al., 2019).

Globally, these mucosa-associated protozoa are one of the leading causes of morbidity and mortality, mainly in areas of intense poverty, in marginal communities of urban centres and rural areas in developing countries (Iyer et al., 2017). The clinical spectrum of diseases produced by these protozoa ranges from asymptomatic (up to 50%) to severe disease, which includes liver abscesses, malabsorption syndrome, vaginitis, and urethritis (Collaborators, 2017; Leung et al., 2019; Li et al., 2021). Moreover, despite a low frequency, trichomoniasis has been implicated in leading to serious adverse effects, such as infertility and cervical cancer (Yang et al., 2018). In pregnancy, *T. vaginalis* is frequently linked with complications, such as premature birth and low birth weight babies (Silver et al., 2014; Leitsch, 2016). While in men, symptomatic infections are rarer (urethritis and prostatitis), but in the long term, trichomoniasis may lead to impaired sperm quality (Mielczarek and Blaszkowska, 2016) and an increased risk of acquisition and transmission of human immunodeficiency virus (HIV) and other STIs (Kissinger and Adamski, 2013; Lazenby et al., 2019) in both sexes.

Remarkably, despite the good and high coverage of sanitary quality, these parasites are still a matter of concern to developed countries (e.g., in European countries and the North American states) (Nisha et al., 2013; Gharpure et al., 2019). The outbreaks seem to be concentrated in the USA, Canada, Australia and the UK (Karanis et al., 2007; Painter et al., 2016). Large outbreaks are associated with treated (disinfected) recreational water venues in the USA. In the last decade, *Cryptosporidium* has emerged as one of the major causes of outbreaks associated with treated aquatic venues. Each year, in the USA, 748,000 cases of cryptosporidiosis (Scallan et al., 2011) and more than 1.2 million cases of *G. intestinalis* are diagnosed (Collier et al., 2021). These parasites are zoonotic agents that are often identified during outbreaks caused by contaminated public water supplies (Nisha et al., 2013; Painter et al., 2015). It is noteworthy that outbreaks of cryptosporidiosis have been related to public drinking water (failures at water treatment facilities), as in the massive Milwaukee cryptosporidiosis outbreak that affected nearly 400,000 people (Mac Kenzie et al., 1994; Efstratiou et al., 2017). However, in some outbreaks, recreational, drinking and fountain waters have been identified as important sources of community infections worldwide (Karanis et al., 2007). As a result, children are more at risk for infection, and cryptosporidiosis is more prevalent in children and immunosuppressed patients. Similarly, children are more likely to have giardiasis and amebiasis than adults (Iyer et al., 2017).

In regard to *T. vaginalis*, in the USA it is one of the most common causes of protozoal infections (Satterwhite et al., 2013; Secor et al., 2014). There were an estimated 2.6 million infections in 2018, and it is also a common cause of symptomatic vaginitis in women (Newman et al., 2015; Hlavsa et al., 2017; Conners et al., 2021). Globally, trichomoniasis mostly affects women between 35 and 40 years of age (Hung et al., 2012; Escobedo et al., 2014).

2 TREATMENT AND SIDE EFFECTS

For many years, some pathogenic protozoa parasites associated with mucosal surfaces were considered "neglected infections of poverty", had low visibility and their studies were unable to attract funding, leading to insufficient development of their diagnosis and treatment options. Currently, they are underdiagnosed, and due to the lack of an effective vaccine, chemotherapy is the only option against these infections.

There are only a small number of drugs available that are effective against these microorganisms. Noteworthy, with different availability across countries, basically two classes of drugs are used in treatment-the nitroimidazole derivatives (MTZ, tinidazole, secnidazole and ornidazole) and benzimidazole derivatives (albendazole and mebendazole), Nitazoxanide, furazolidone, quinacrine, chloroquine and paromomycin. Consequently, rational use of available antiparasitic agents is essential to maintain their usefulness. Although, the cure rate is great, treatment failure can be owing to several factors including noncompliance, resistance and reinfection.

A non-exhaustive list of treatments selected from the literature is depicted in **Table 1**.

Some of the current pharmacological treatments of amebiasis, giardiasis, cryptosporidiosis, and trichomoniasis are discussed in more detail. The most effective and widely used compound is MTZ (a nitroimidazole derivative) that has been the mainstay of protozoan parasite treatment for decades (Leitsch, 2019).

This compound has the ability to reduce and produce nitro radicals in anaerobic microorganisms that inhibit pathogen DNA synthesis (Leitsch, 2019). Events associated with MTZ therapy, such as drug adverse effects or the need for continued dosing past the resolution of disease symptoms, are common (Hernandez Ceruelos et al., 2019). In addition, there is emerging evidence for an increased frequency of therapeutic failure (Leitsch, 2015). In spite of this, the most used medication against these protozoa, except for *Cryptosporidium* spp., is still MTZ. Although its mechanism of action is not fully elucidated, MTZ is activated by thioredoxin reductase and possibly by ferredoxin to generate a nitro-radical anion and a nitroimidazole compound on subsequent reduction. These metabolites compete with the energy metabolism pathway of anaerobic microorganisms, causing toxic effects on trophozoites by inhibiting nucleic acid synthesis, thereby inhibiting protein synthesis and parasite growth (Shrivastav et al., 2020; Weir and Le, 2021). MTZ has been prescribed for over 60 years for luminal and tissue action on *G. intestinalis* and *T. vaginalis*, respectively. Moreover, this drug is currently the standard therapy for treating adults and children with invasive amebiasis. However, treatment for amebiasis is reliant on a single class of agents, the nitroimidazole compounds (Haque et al., 2003). Advantages of MTZ are that it is effective at killing trophozoites, cheap, and can be orally dosed. However, it is unable to kill the infective cyst stage of *E. histolytica* from the colonic lumen, necessitating a multi-drug treatment regimen. Consequently, around 40% of patients treated with MTZ will continue to have parasites in the

TABLE 1 | Current treatment options for amoebiasis, giardiasis, cryptosporidiosis and trichomoniasis.

Diseases	Parasite	Drug	References
Amoebiasis	*E. histolytica*	Nitroimidazoles (Metronidazole, tinidazole, ornidazole, secnidazole), Emetine, Paromomycin Nitazoxanide	(Shirley et al., 2018; Gonzales et al., 2019; Sharma and Ahuja, 2021)
Giardiasis	*G. intestinalis*	Nitroimidazoles (Mebendazole and Tinidazole) Benzimidazole (Albendazole and Mebendazole) Furazolidone, Paromomycin nitazoxanide	(Morgan et al., 1993; Rossignol, 2010; Kappagoda et al., 2011; Vivancos et al., 2018)
Trichomoniasis	*T. vaginalis*	Nitroimidazoles (Metronidazole and Tinidazole)	(Gulmezoglu and Garner, 1998; Meites et al., 2015; Sherrard et al., 2018; Workowski et al., 2021)
Cryptosporidiosis	*Cryptosporidium* spp.	Nitazoxanide	(Rossignol, 2010; Innes et al., 2020)

colonic lumen (Haque et al., 2003) and a second agent (paromomycin or iodoquinol) should be administrated to completely clear the remaining trophozoites and cysts from the colonic lumen. Other unwanted side effects include alcohol intolerance and problems with use during pregnancy and lactation (Roe, 1977).

Unfortunately, resistance to MTZ and tinidazole have been reported in *G. intestinalis* and *T. vaginalis* for several decades (Schwebke and Barrientes, 2006; Kirkcaldy et al., 2012; Paulish-Miller et al., 2014; Leitsch, 2015). Several investigations have identified MTZ-resistant *T. vaginalis* strains associated with treatment-refractory vaginal trichomoniasis (Coelho, 1997; Meri et al., 2000; Schmid et al., 2001; Lin et al., 2020). However, nitroimidazole resistance is relative not absolute, ranging from slight to strong. Similar findings were reported for refractory giardiasis due to MTZ resistance in the parasite in both healthy and immunocompromised individuals (Morch et al., 2008; Nabarro et al., 2015; Carter et al., 2018; Lalle and Hanevik, 2018). So far, there is slight evidence for MTZ resistance in *E. histolytica* (Victoria-Hernandez et al., 2020), but a decrease in 5-nitroimidazole susceptibility can be induced experimentally (Wassmann et al., 1999; Iyer et al., 2017). Notoriously, increased drug resistance leads to higher doses used in the treatment and, therefore, more severe side effects (Hernandez Ceruelos (Hernandez Ceruelos et al., 2019). MTZ is generally well tolerated, but unpleasant side effects are often reported. The most common adverse effects are dose dependent, mild and reversible. Among them, common secondary effects of the nitroimidazoles are gastrointestinal tract symptoms, such as nausea, anorexia, vomiting and metallic or bitter taste, dizziness, ataxia and headache (Hernandez Ceruelos et al., 2019; Diptyanusa and Sari, 2021).

It is also important to note that the risk of treatment failure is linked to reinfection, suboptimal drug dose and drug resistance. In particular, *G. intestinalis* could be sequestrated in the gallbladder or the pancreatic duct, which promotes treatment failure (Escobedo et al., 2016; Lalle and Hanevik, 2018). In turn, except for *T. vaginalis*, it is hard to make this distinction in an endemic area, because reinfection is frequent related to high environmental contamination by infective cysts/oocysts, in association with precarious sanitation conditions. However, treatment failure must be excluded in the case of a recurrence of symptoms after appropriate therapy.

Nitazoxanide (NTZ) is a nitrothiazole pro-drug that also needs to be reduced at its nitro group to be active. NTZ has been shown to be a non-competitive inhibitor of the pyruvate: ferredoxin/flavodoxin oxidoreductases (PFORs) of *T. vaginalis*, *E. histolytica* and *G. intestinalis* (Adagu et al., 2002). Protein disulphide isomerases (PDI) have also been identified as potential targets of NTZ activity. In *G. intestinalis*, which expresses PDI variants, PDI2 and PDI4 expression was shown to be significantly downregulated during *in vitro* treatment with NTZ, indicating that the drug is targeting this enzyme (Muller et al., 2007).

In general, the effects of NTZ on the trophozoite ultrastructure of *T. vaginalis*, *E. histolytica* and *G. intestinalis* include cell swelling and distorted cell shape, a redistribution of vacuoles, plasma membrane damage and the formation of extensive empty areas in the cytoplasm of the protozoa (Cedillo-Rivera et al., 2002). Conversely, NTZ is currently the single approved medicine for the treatment of cryptosporidiosis in individuals who have healthy immune systems. It exhibits moderate clinical efficacy in children and immunocompromised individuals, although the treatment of immunosuppressed patients is remarkably difficult, even with higher doses (Abubakar et al., 2007; Amadi et al., 2009). Common side effects may include: nausea, diarrhoea, dry mouth, skin rash, stomach pain, fatigue, headache, and scleral and urine discoloration (Andersson, 1981; Diptyanusa and Sari, 2021).

3 PROSPECTS ABOUT OF MUCOSA-ASSOCIATED PROTOZOA INFECTIONS PERSISTENCE: A CHALLENGE AHEAD

Mucosa-associated protozoa infections have distinct characteristics that, when considered together, set them apart from other diseases. Of note, the persistence of infection is linked to several causes, including suboptimal drug concentrations, incomplete treatment regimens, reinfection, an immunocompromised state and drug resistance. Understanding of these factors is, thus, relevant and has been demonstrated. With mass drug administration (MDA), continued campaigns have substantially reduced soil-transmitted helminth (STH) infections in many countries. Albendazole is frequently used in MDA campaigns as a principal control tool for intestinal helminth infections. The collateral benefits of preventive chemotherapy might also affect conditions beyond its originally intended targets, such as enteric protozoa like *G. lamblia*, *E. histolytica*

and *Cryptosporidium* spp. However, the therapeutic regimen of treatment with albendazole is suboptimal for these protozoa and may lead to drug resistance (Quihui-Cota and Morales-Figueroa, 2012; Oliveira et al., 2020), although no surveillance system exists to detect resistance. In Pemba Island, Tanzania, persistent parasitic infection was reported following single-dose albendazole, NTZ and albendazole-nitazoxanide (Speich et al., 2013). Conversely, the rates of *Giardia*, *Cryptosporidium* and *E. histolytica* are high in communities where basic sanitary conditions are unsatisfactory or non-existent; consequently, the frequent episodes of reinfection and protozoan persistence are remarkable in these communities, even after treatment (Fantinatti et al., 2020). In turn, cases of recurrent trichomoniasis are likely to occur due to a lack of adherence to the medication or reinfection from an untreated sexual partner (Sena et al., 2014). However, decreased sensitivity to nitroimidazoles has been identified in 2% to 10% of vaginal trichomoniasis cases (Perez et al., 2001; Schwebke and Barrientes, 2006; Kirkcaldy et al., 2012).

Notably, there are important challenges in the context of these infections. The first challenge is the lack of reliable epidemiological data, which precludes one from estimating the actual burden of pathogenic protozoa associated with mucosal surfaces. However, the heart of this challenge is mainly associated with two (some) factors: a high percentage of asymptomatic individuals and limited tests for diagnosis, which are biased and likely contribute to the epidemiological data underestimating the actual global burden of these diseases (Soares et al., 2020).

The second challenge are factors intrinsic to the host and/or parasite. The complex parasite-host relationship involves multiple mechanisms that have been characterized as one of the most important challenges of these times. Despite being complex and poorly investigated, the existence of one organism within another is not uncommon between protists and bacteria and/or viruses. There is an increasing recognition that endosymbionts in protozoa could influence the outcome of a disease as an eco-evolutionary process, such as drug resistance (Barrow et al., 2020). Parasites infected by viruses modify this relationship, adding more complexity to the system that now becomes tripartite. However, these issues remain largely unknown and evidence is conflicting.

Globally, some strains of *T. vaginalis, G. intestinalis, E. histolytica*, and *Cryptosporidium* spp. carry endosymbiotic double-stranded RNA (dsRNA) viruses (Banik et al., 2014; Jenkins et al., 2015), which share common characteristics, with uncharted implications to both the human host and protozoa (Fichorova et al., 2012). However, increasing evidence is accumulating that these protozoa harbour different classes of viruses that are absent from humans, but their role in pathogenicity is still poorly understood. Indeed, much of our understanding of viral endosymbionts of mucosa-associated protozoa is based on the *Trichomonasvirus* (TVV), which has been relatively more studied in some details than other protozoa. The TVV is a dsRNA virus that belongs to the Totiviridae family, which was described and characterized in the 1980s and has at least two core proteins, a capsid protein (CP) and an RNA-dependent RNA polymerase (RdRp). Currently, the TVV has been divided into four distinct viral strains (TVV1, TVV2, TVV3, and TVV4), ranging in size from 4.5 to 5 kbp, based on phylogenetic analyses and comparisons of genomic sequences (Su and Tai, 1996; Liu et al., 1998). The viral genome is never found free in the protozoan cell, and the positive strand viral transcripts synthesized within the viral particle by the CP/RdRp are translocated to the cell cytoplasm to be translated (Barrow et al., 2020). Despite their non-lytic life mode, dsRNA viruses may induce various phenotypic changes that may interfere with the virulence of *T. vaginalis* (Provenzano et al., 1997; Fraga et al., 2012). In the last few years, some studies have reported experimental findings that point toward a positive association between infection and the exacerbation of trichomoniasis symptoms (El-Gayar et al., 2016) while other authors have shown the absence of any correlation (El-Gayar et al., 2016; Graves et al., 2019). Of note, more than one TVV species can coexist in the same *T. vaginalis* cell (Benchimol et al., 2002), and a high percentage of Trichomonasvirus in different protozoan isolates have been reported worldwide, ranging from 30% to 100% (Fichorova et al., 2017), which may lead to the discrepancy of the findings. The implications of these co-infections so far are unclear, but evidence has shown that the TTV released from infected *T. vaginalis* cells induced inflammation upon treatment with metronidazole (MTZ) and may suppress host immune activation (Fichorova et al., 2012; Govender et al., 2020). Each viral strain affects different aspects of the parasite. TVV2 and TVV3 infections are strongly enfolded in the upregulation of the protozoan cysteine proteases (Provenzano et al., 1997), which are involved in modulating *T. vaginalis* cytoadherence to human host cells and in the degradation of the basement membrane, human cellular molecules, and secretory IgAs. While, TVV1 and TVV2 are related to the severity of clinical symptoms of trichomoniasis in humans (Fraga et al., 2012). Conversely, one study reported increased MTZ susceptibility in TVV-infected *T. vaginalis* (Malla et al., 2011), while another report observed drug resistance (Snipes et al., 2000). However, additional research is needed to clarify the effect of TVV infection on 5-nitroimidazole resistance in *T. vaginalis*.

Similar to TVV, *Giardiavirus* was first isolated from assemblage A of *G. intestinalis* (Wang and Wang, 1986) as a dsRNA virus and is a member of the family Totiviridae, which specifically infects trophozoites of the parasite *G. intestinalis*. The virus makes capsids that are released from the host to infect other cells, and when a high viral titre is observed, the growth of the parasite is suspended (Marucci et al., 2021). However, unlike *Leishmaniavirus* and *Trichomonasvirus*, the *Giardiavirus* does not seem to be linked with the virulence of the parasite (Gomez-Arreaza et al., 2017). Another *Giardiavirus* of the family *Totiviridae* is *G. canis* virus (GCV) (Cao et al., 2009), which was isolated from the *G. canis* strain. However, it is important to note that knowledge on *Giardiavirus* has unfortunately made little progress in recent years. Similarly, there are few reports on *Cryptosporidium* and *E. histolytica* virus. *Cryspovirus* is a new genus of protozoan viruses in the family Partitivirida, which infect different species of *Cryptosporidium* oocysts (Nibert et al., 2009).

Although viruses can have complex effects on *Cryptosporidium*, there is so far no well-established information on whether the pathogenicity of parasites is either positively or negatively modulated by *Cryspovirus* infection (Banik et al., 2014; Sharma et al., 2016). Likewise, the presence of virus-like particles in an *E. histolytica* trophozoite has been reported. This virus was described in the 1960s based on electron microscopy studies, which revealed characteristics closely related to Rhabdoviridae (a family of negative-strand RNA viruses) (Ludvik and Shipstone, 1970). Despite being discovered a number of years ago, little was investigated about the viruses involved and their impact on amoebiasis, and no investigation based on clinical perspective has been done (Bird et al., 1974; Banik et al., 2014).

4 PARASITE DRUG-RESISTANCE MECHANISMS

The nitroimidazole metronidazole is clearly one of the best-studied drugs affecting intermediary metabolism in *Entamoeba histolytica*, *G. lamblia* and *Trichomonas vaginalis* (Lofmark et al., 2010), but resistance mechanisms against NTZ and albendazole were also investigated. Notoriously, Metronidazole's mechanism of action describes that of the other nitroimidazoles. However, not all mechanisms that reduce susceptibility to the nitroimidazoles have been described. Resistance to metronidazole is complex but appears to be owing to activation of the prodrug to the active nitroso free radical. Laboratory-induced resistant isolates (Upcroft and Upcroft, 1993) is associated with an downregulation of pyruvate:flavodoxin/ferredoxin oxidoreductase (PFOR) activity, a protein absenting in higher eukaryotic cells (Horner et al., 1999). Metronidazole is a prodrug that present an ability to intracellularly reduce to intermediates nitro radical (Sisson et al., 2000) by electrons coming from PFOR (Upcroft and Upcroft, 2001). This radical causes irreversible damage on intracellular structures of parasites. Some enzymatic pathways of *G. lamblia* and *Trichomonas vaginalis*, were identified that are probable to play a function in 5-nitroimidazole reduction, inclusive of the central metabolic enzyme pyruvate:ferredoxin oxidoreductase (PFOR) together with ferredoxin (Townson et al., 1996; Rasoloson et al., 2002; Leitsch et al., 2011) and thioredoxin reductase (TrxR).

In Giardia, pyruvate:ferredoxin oxidoreductases (PFORs), Giardia lamblia nitroreductase 1 (GlNR1), and thioredoxin reductase (TrxR) are capable to induce the partial reduction reaction that to convert MTZ into toxic metabolites (Nillius et al., 2011; Lalle and Hanevik, 2018).

It is worth to mention that large predictor studies for therapeutic failure are unavailable so far. The resistance slowly induced in laboratory lines could be of a different nature than the rapidly increasing clinical resistance occurred the past decades. Laboratory-induced resistance were obtained from patients several decades ago, most of them belong to the zoonotic assemblage AI, and may not currently represent the main circulating strains (Lalle and Hanevik, 2018). The results reported from laboratory-induced resistant lines showed a variation in the infectivity and molecular phenotypes, suggesting that multiple molecular resistance phenotypes are possible. However, stability of laboratory-induced resistance is generally lost or reduced after removing the drug. MTZ resistances in clinical isolates from patients were demonstrated in a study reported that some isolates were also less MTZ susceptible when tested in a neonatal mouse model (Lemee et al., 2000). Studies with metronidazole-resistant strains have reported, however, that resistance is not always correlated with reduced POR activity (Leitsch, 2015). A study on resistance of clinical isolates of *T. vaginalis* reported that flavin reductase activity was downregulated (Leitsch et al., 2012), or even absent, in metronidazole-resistant strains. It is important to considerate that flavin reductase can reduce oxygen to hydrogen peroxide (Leitsch et al., 2014), so its downregulation might impair oxygen scavenging, and with activation of nitroimidazoles by either inhibiting drug-activating pathways or by reoxidizing a critical, toxic, nitroradical anion intermediate, consequently reduce metronidazole uptake. Likewise, resistance to metronidazole in *E. histolytica* has been associated with oxidative stress mechanisms, including superoxide dismutase and peroxiredoxin, without a significant decrease of the PFOR activity (Wassmann et al., 1999).

NTZ resistance is thought to be due to altered expression of stress response proteins. An expression study on the NTZ laboratory-induced resistance showed several protein chaperones (Hsp70, Hsp90, and Cpn60), (Leitsch, 2015) and surface antigens to be upregulated in expression (Muller et al., 2008). Levels of PFOR were practically unaltered in nitazoxanide-resistant cell lines (Muller et al., 2007), whereas nitroreductase 1 was found downregulated (Nillius et al., 2011). It is important to point out that resistance to NTZ has been reported not only on experimentally induced resistant strains, but also in clinical isolates (Lemee et al., 2000; Reyes-Vivas et al., 2014).

Benzimidazole affect the microtubules assembly, and induction of oxidative stress may also play a role in the antiparasitic mechanism. Albendazole resistance in *Giardia* is correlated with cytoskeletal changes (Upcroft et al., 1996), in the particular emphasis on the median body. Moreover, resistance has been attributable mutation in the beta-giardin gene, leading in amino acid changes have also been associated with reduced susceptibility to albendazole (Jimenez-Cardoso et al., 2009). However, ttreatment failure has also been reported for ABZ either administered alone or in combination with MTZ. The higher levels of efficacy with albendazole require multiple doses.

In fact, given their significant impact on human health and infections of mucosa-associated protozoa being difficult to eradicate, it is necessary to focus on finding preventive and curative therapeutics to control their spread. Thereby, there is an urgent need to develop new therapeutic options that will help fight resistance and increase effectiveness. However, a great barrier is the high costs associated with developing a new drug and low economic returns (Dimasi et al., 2003). In recent years, one alternative approach for lowering the costs of drug discovery

and development for these diseases is to repurpose drugs developed for other indications (Ashburn and Thor, 2004; Jourdan et al., 2020). It is an alternative viable to the identification of a new indication for a known drug or compound with previously established preclinical studies or clinical data can significantly decrease the overall cost and reduce the time required to bring a drug to market (Muller and Hemphill, 2013).

Recent strategies for the development of drugs in the context of these mucosa-associated parasitic protozoa will be discussed in the next section.

5 RECENT APPROACHES TO DRUG DEVELOPMENT

Over the past six decades, the lack of financial incentives has strongly reduced efforts to develop effective treatments. Because of this, new drugs are urgently required for parasitic protozoan infections, and repurposing drugs is a promising approach for the drug development process, a rapid mode and reduces cost. The discovery of new uses for approved drugs has the potential to identify and reveal new targets that aim to maximize the pre-existing preclinical and clinical knowledge accumulated on registered drugs for a new indication outside the scope of its original indication. Thus, it opens a promising avenue for identifying targets for treatment.

Nevertheless, the treatment of parasitic diseases is often complex, owing to the multiple life stages of parasites with differing sensitivities to chemical agents. High-throughput screens of repurposed drug libraries have been used to the screening of drugs for *E. histolytica, G. intestinalis, Cryptosporidium* spp. and *T. vaginalis*, and some of these compounds are shown below. However, one of the available practical approaches to find novel potential candidates with antiprotozoal activity are FDA-approved drugs, which is an attractive way to identify new drugs.

5.1 Advances in Research for Novel Anti-*Giardia* Activity Agents and FDA-Approved Drugs

5.1.1 Fumagilin

This is an antibiotic compound isolated from *Aspergillus fumigatus*, and its efficacy has been demonstrated in the treatment of diarrheal disease caused by *Microsporodia* spp. (Goodgame, 2003; Agholi et al., 2013). Similarly, fumagillin also showed activity against *Giardia* assemblages A and B at submicromolar concentrations in the mouse giardiasis model. The antigiardiasic properties of fumagilin have exhibited effectiveness, even against *in vitro* MTZ resistance, and the *in vivo* animal model was superior to MTZ, being a promising drug candidate for the treatment of giardiasis (Kulakova et al., 2014).

5.1.2 Proton Pump Inhibitors

PPIs are a class of medications widely used for the treatment of gastroesophageal reflux disease and other acid-related disorders. Repurposing of PPIs, such as omeprazole, is effective *in vitro* against *G. lamblia*, and the toxic activity is associated with the inhibition of triosephosphate isomerase (*Gl*TIM), which is a key enzyme in glucose and glycogen metabolism of the parasite (Reyes-Vivas et al., 2014; Lopez-Velazquez et al., 2019). Hernández-Ochoa and colleagues (2017) described two novel PPI derivatives, named BHO2 and BHO3, that showed better antigiardiasic activity than omeprazole in micromolar concentrations, without a cytotoxic effect on mammal cell cultures (Hernandez-Ochoa et al., 2017; Hernandez-Ochoa et al., 2020).

5.1.3 Tetrahydrolipstatin (orlistat)

This is a specific lipase inhibitor derived from lipstatin, which is a lipid produced by *Streptomyces toxytricini* used in the treatment of obesity. Hahn and colleagues (2013) showed experimentally that orlistat inhibited *in vitro* growth of *G. duodenalis* at low micromolar concentrations compared to that of the standard drug MTZ (Hahn et al., 2013).

5.1.4 Spiro Compounds of Isatin

Spiro compounds represent an important class of a small and versatile organic molecule that are known to exhibit versatile biological properties. *In vitro* test of 1*H*-1,2,3-triazole and β-amino-alcohol tethered isatin-β-lactam conjugates displayed anti–*T. vaginalis* activity (Nisha et al., 2013). Also, 1*H*-1,2,3-triazole-tethered isatin-MTZ conjugates also exhibited activity *in vitro* against *T. vaginalis, E. histolytica*, and *G. lamblia* in submicromolar concentrations (Kumar et al., 2018).

5.1.5 Auranofin

The gold(I) complex auranofin was approved by the FDA in 1985 as an oral anti-arthritic agent (used in the treatment of rheumatoid arthritis) was shown to be effective against MTZ-resistant *Giardia*, and auranofin has now progressed into clinical trials (Capparelli et al., 2017).

5.1.6 Disulfiram

This drug blocks an enzyme that is involved in metabolizing alcohol. It is an inhibitor of acetaldehyde dehydrogenase, which is used to support the treatment of alcohol use disorder. It also acts as a cysteine modifying agent. The antigiardiasic activity of disulfiram has shown to be effective against *G. lamblia* trophozoites *in vitro* and in a murine model of giardiasis. Interestingly, the drug has been demonstrated to be more than 2–4 times active against MTZ-resistant than MTZ-sensitive (WB and GS/B) parasites (Kulakova et al., 2014). This drug may act as a novel inactivator of *G. lamblia* triosephosphate isomerase (the endogenous activity and carbamate kinase *in vitro*), which demonstrates that its giardicidal effect may involve the inactivation of more than a single enzyme, increasing its potential as an antigiardial drug (Castillo-Villanueva et al., 2017).

5.2 Advances in Research for Novel Anti-*Entamoeba histolytica* Activity Agents and FDA-Approved Drugs

5.2.1 Antineoplastic Kinase Inhibitors

Recently, antineoplastic kinase inhibitors have emerged as potent amebicidal drugs for amebiasis. The drugs tested

were able to kill *E. histolytica* trophozoites as quickly as MTZ. Moreover, one of these drugs (ibrutinib) also exhibited both amebicidal and cysticidal properties, in contrast to all the drugs currently used in the therapeutic strategy (Sauvey et al., 2021).

5.2.2 Rabeprazole

The thioredoxin system catalyses several redox reactions essential in maintaining a variety of important biological functions in the *Entamoeba*. It is considered a fundamental enzyme system (reducing the redox system and detoxifying the intracellular oxygen) for *E. histolytica* survival under both aerobic *in vitro* and *in vivo* conditions. Consequently, the TrxR/Trx system is an excellent target for antiamebic drug. Rabeprazole (RB), a drug widely used to treat heartburn, is able to inhibit the EhTrxR recombinant enzyme, amebic proliferation and several functions required for parasite virulence, such as cytotoxicity, oxygen reduction to hydrogen peroxide, erythrophagocytosis, proteolysis, and oxygen and complement resistances (Martinez-Perez et al., 2020).

5.2.3 Pencolide

It is a fungal secondary metabolite that shows cysteine deprivation-dependent antiamebic activity. This compound targets cysteine synthase, which is essential for the proliferation and antioxidative defence of *E. histolytica* trophozoites, and is implicated in various important biological processes, including attachment, motility, proliferation, and antioxidative defence (Mori et al., 2018).

5.2.4 Anisomycin and Prodigiosin

Anisomycin, an antibiotic isolated from *Streptomyces*, and prodigiosin, a natural red water-insoluble pigment isolated from *Serratia marcescens*, have already shown therapeutic effects over 50 years ago to treat amebiasis in small cohorts (Gonzalez Constandse, 1956; Ehrenkaufer et al., 2018). Ehrenkaufer and coworkers recently performed a screen of repurposed compounds against *E. histolytica*, anisomycin and prodigiosin were both able to kill MTZ-resistant parasites, while prodigiosin was active against mature cysts (Ehrenkaufer et al., 2018). This is the first compound showing efficacy against the dormant cyst form, which is highly resistant to environmental stresses and to the major drug used to treat amebiasis (MTZ).

Importantly, obatoclax an analogue of prodigiosin, which has been used in human clinical trials, had significant activity against both trophozoites and cysts. Development of this molecule as a therapeutic may be possible, given the established safety record in patients (Ehrenkaufer et al., 2020).

5.2.5 Auranofin

It is a gold-containing compound originally developed to treat rheumatoid arthritis that showed antiparasitic activity against *E. histolytica* (Debnath et al., 2013; Andrade and Reed, 2015). This compound targets the thioredoxin reductase in *E. histolytica*, thereby making the parasite sensitive to oxidative stress (Debnath et al., 2012). It also showed 10-fold better activity against *E. histolytica* than the standard drug MTZ. Unsurprisingly, auranofin was equally active against *G. lamblia*, both in *in vitro* and *in vivo* studies (Debnath et al., 2013; Tejman-Yarden et al., 2013), indicating the possibility of its use for a more general treatment of protozoan parasites. However, it presented side effects, such as abdominal pain, nausea, anaemia, and elevated liver enzymes, and its use is prohibited during pregnancy.

5.2.6 Disulfiram

This drug is an inexpensive orally administered drug used in the treatment of chronic alcoholism. It is rapidly metabolized to diethyldithiocarbamate (ditiocarb, DTC), which in the presence of zinc forms zinc diethyldithiocarbamate (ZnDTC). Gosh and colleagues demonstrated that the ZnDTC complex has a high *in vivo* activity against *E. histolytica* parasites (Ghosh et al., 2020). This metabolite of disulfiram showed the ability to inhibit COP9 signalosome, a critical upstream regulator of parasite protein degradation (Ghosh et al., 2020; Shirley et al., 2021).

5.3 Advances in Research for Novel Anti-*T. vaginalis* Activity Agents and FDA-Approved Drugs

5.3.1 Ixazomib and Carmaphycin-17

The proteasome is a multicatalytic proteinase complex, and proteasome inhibitors have also been shown to be toxic for other pathogens and useful in parasitic treatment (Khare et al., 2016). O´Donoghue and colleagues (2019) validated the proteasome as a drug target for the development of a novel class of trichomonacidal agents (O'Donoghue et al., 2019). They showed experimentally that two clinically approved anticancer drugs, ixazomib and carmaphycin-17 (CP-17), were active against vaginal trichomonad infections. In addition, CP-17 was able to overcome MTZ resistance in *T. vaginalis* and had significant *in vitro* and *in vivo* efficacy against trichomonads (O'Donoghue et al., 2019).

5.3.2 Benznidazole

In silico analyses classified 20 compounds as potentially active against *T. vaginalis*. From these, ipronidazole, dimetridazole and NTZ showed the highest cytocidal activity superior to MTZ and secnidazole, respectively. Besides, the *in vivo* assay revealed similar activity for benznidazole and MTZ, suggesting the former as a novel alternative in antitrichomonal therapy (Meneses-Marcel et al., 2018).

5.3.3 Disulfiram

The antiprotozoal activities of disulfiram have been studied over the past decade. Disulfiram, when complexed to divalent metal ions (such as zinc) has been demonstrated as an anti-parasitic agent against protozoan parasites *Trypanosoma, Leishmania, Giardia* and *E. histolytica*. It also appears to be similarly effective against *T. vaginalis.*

This drug has shown antitrichomonal activity, and its metabolite, ditiocarb, has been shown to be more effective than MTZ against both sensitive and resistant trichomonads (Bouma et al., 1998).

5.4 Advances in Research for Novel Anti-*Cryptosporidium* Activity Agents and FDA-Approved Drugs

5.4.1 FDA-Approved Drugs Phenotypic Screen

Even though promising targets and lead compounds have been identified, robust therapies to eliminate parasites are still lacking. Some of them (paromomycin, clarithromycin, azithromycin, rifaximin, rifabutin, and roxithromycin) (Shrivastava et al., 2017) have demonstrated limited potential when used in animal models, and all were ineffective in controlled trials in AIDS patients. Also, preclinical activity with miltefosine (originally developed as an anticancer drug) and clofazimine (leprosy drug) has been demonstrated, which had no efficacy in phase II studies in AIDS patients (Gavrilov, 1989; Croft et al., 2003; Sinkala et al., 2011; Huston, 2021).

Indeed, activity against an organism found by *in vitro* screening does not necessarily correlate to *in vivo* activity. Moreover, several aspects might explain unsuccessful drug discovery. One of the major bottlenecks to the development of specific anticryptosporidial drugs is the unavailability of a reproducible axenic *in vitro* culture system for *Cryptosporidium* spp., as well as being unable to genetically manipulate the organism.

The genomes of parasites have shown a limited biosynthetic capability that may be targets to identify promising new chemical entities. For example, there are approaches based on data mining of genome data to provide new insights into aspects of *Cryptosporidium* biology focused on novel targets. Recently, the identification of some ligands/inhibitors and parasite-specific molecules, such as parasite kinases; nucleic acid synthesis and processing; proteases; and lipid metabolism have paved the way for new therapies against *Cryptosporidium*, which is considered an attractive strategy. However, alternative salvage pathways could reduce the efficacy of many of these targets as one remarkable case demonstrated with the thymidylate synthase-dihydrofolate reductase target (Pawlowic et al., 2019)

5.4.2 Bumped Kinase Inhibitors

Protein kinases play essential roles in the biology of *Cryptosporidium* and can identify essential pathways to potential new targets. Bumped kinase inhibitors (BKIs) targeting calcium-dependent protein kinase 1 (CDPK1) have shown effect against *Cryptosporidium in vitro* and in mice. Pyrazolopyrimidine analogues inhibit *C. parvum* CDPK1 and block *C. parvum* growth in tissue culture *in vitro*. However, the effect on parasite growth was variable and did not correlate well with enzyme inhibition (Kuhlenschmidt et al., 2016), conversely as demonstrated by *Toxoplasma gondii*. It also has shown issue challenges related to cardiovascular toxicity, teratogenicity, and varying efficacy (Choi et al., 2020; Love and Choy, 2021). Another series of BKI compounds have been developed which inhibit CDPK. However, this class of drugs has presented significant adverse reactions because of inhibited binding to mammalian protein kinases (Wang et al., 2020).

Another, kinase target is phosphatidylinositol-4-OH kinase, which phosphorylates lipid molecules to participate in intracellular signalling and trafficking, and is a target for pyrazolopyridines. Screening a library of compounds with antiparasitic activity found pyrazolopyridine KDU731 as a promising anticryptosporidial drug candidate [(Cryptosporidium lipid kinase PI(4)K (phosphatidylinositol-4-OH kinase)] that is active against both *C. parvum* and *C. hominis* (Manjunatha et al., 2017; Funkhouser-Jones et al., 2020). However, safety and pharmacological preclinical evaluations are necessary in order to support the initiation of human clinical trials.

5.4.3 Inhibitors of Inosine Monophosphate Dehydrogenase

Another essential pathway in *Cryptosporidium* spp. is purine synthesis. Oxidoreductase inosine 5′-monophosphate dehydrogenase (IMPDH) is required for the conversion of adenosine into guanine nucleotides. IMPDH is a target for immunosuppressive, antiviral, and anticancer drugs. However, inhibitors of IMPDH have shown *in vitro* efficacy against *Cryptosporidium* spp (Jefferies et al., 2015).

5.4.4 tRNA Synthetase

tRNA synthetases comprise a family of enzymes that couple specific amino acid residues to selected tRNA for protein peptide synthesis. *Cryptosporidium* spp. are inhibited by a benzoxaborole (a 3-aminomethyl benzoxaborole, AN6426) targeting leucyl-tRNA synthetase. Despite the fact that homologous proteins exist in humans, this molecule has the potential for antimicrobial drug design, since structural and sequence divergences can be modified to enhance specificity and avoid toxicity (Palencia et al., 2016). Besides this one, quinazoline-based derivative shows potent activity against *Cryptosporidium* prolyl-tRNA synthetase (Jain et al., 2017); imidazopyridine derivatives are also potent inhibitors of *Cryptosporidium* methionyl-tRNA synthetase (Buckner et al., 2019). Similar results have been observed with cladosporin derivatives, also exhibiting potent activity against *Cryptosporidium* lysyl-tRNA synthetases (KRSs) (Baragana et al., 2019).

5.4.5 Cysteine Protease (Cryptopains)

Five genes coding cathepsin L-like proteases (cryptopains), a representative of clan CA, were identified in the *C. parvum* genome that are expressed during the sporozoite stage and are important for cell invasion and survival. The discovery that a cysteine protease inhibitor provides potent anticryptosporidial activity in an animal model of infection encourages the investigation and development of this class as a new (and urgently needed) therapy for cryptosporidiosis (Ndao et al., 2013).

6 ALTERNATIVE CHEMOTHERAPEUTIC AGENTS AGAINST PROTOZOAN PARASITES

6.1 Natural Compounds

Natural products remain an important source of biologically active substances and are an alternative source for parasitic

control. In this context, several research groups are focused on the isolation and identification of novel compounds with antimicrobial activity from plant and fungal extracts, aiming to use them in the discovery of new antiprotozoal drugs. A list of natural compounds with antiprotozoal activity is described in **Supplementary Table 1**.

6.2 Hybrid Compounds

Studies have shown that hybrid molecules display high protozoal activity, making them potentially promising agents for antiprotozoal therapy. Evaluation of nitazoxanide–N-methylbenzimidazole hybrid compounds showed strong activity *in vitro* against *G. intestinalis*, *E. histolytica* and *T. vaginalis*, especially with *E. histolytica*, where the IC_{50} values ranged between 3 and 69 nM (Soria-Arteche et al., 2013).

Other compounds with antiprotozoal properties are 1H-1,2,3-triazole-tethered metronidazole-isatin conjugates, which presented inhibitory activity against *T. vaginalis*, *Tritrichononas foetus*, *G. lamblia* and *E. histolytica in vitro*. Curiously, compounds with thiosemicarbazone moieties showed better results against *G. lamblia* and *E. histolytica* and were similar to MTZ against trichomonads (Kumar et al., 2018). Matadamas-Martínez and colleagues (2016) showed that a nitazoxanide-N-methyl-1H-benzimidazole hybrid molecule exhibited giardicidal activity at nanomolar concentrations and was more active *in vitro* than both MTZ and albendazole, and equipotent to NTZ. This compound induced ultrastructural changes and alterations in cytoskeleton proteins and in proteins that play an important role in the encystment process (Matadamas-Martinez et al., 2016). Further *in vitro* and *in vivo* studies revealed that the hybrid compound also exhibited broad activity against susceptible and resistant strains to albendazole and NTZ (Matadamas-Martínez et al., 2020).

MTZ-chalcone conjugates exhibited activity against MTZ-susceptible and resistant strains of *T. vaginalis* (Anthwal et al., 2014). 3-(3,5-Difluoro-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone and 3-(3-chloro-phenyl)-1-{4-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-phenyl}-propenone compounds were four times more potent than MTZ and safe against HeLa *in vitro*, being a candidate drug capable of overcoming the MTZ resistance of *T. vaginalis* (Anthwal et al., 2014).

6.3 Synthetic Compounds

The *in vitro* antiprotozoal activities of 2H-indazole derivatives have been reported for *E. histolytica, G. lamblia* and *T. vaginalis* at lower concentrations and are usually more potent compared to MTZ, appearing to be a good alternative (Perez-Villanueva et al., 2017; Rodriguez-Villar et al., 2021). In turn, 2′-hydroxychalcone showed trichomonicidal *in vitro* analysis: at 12.5 μM associated with MTZ (40 μM), a reduction of 95.31% in the trophozoite's viability was displayed after 24 hours of incubation (Das Neves et al., 2020). Furanyl *N*-acylhydrazone derivatives also presented activity against *T. vaginalis*, with IC_{50} values ranging from 1.69 μM to 1.98 μM (Alves et al., 2020).

MTZ thiosemicarbazones and ethyl- and methyl-quinoxaline-7-carboxylate 1,4-di-N-oxide derivatives showed a significant antiamoebic activity *in vitro*, with an IC_{50} of 0.56 μM and IC50 values ranging from 1.41 μM to 1.47 μM, respectively (Abid et al., 2008; Duque-Montano et al., 2013).

Muller and colleagues (2006) showed experimentally that NTZ-related thiazolide/thiadiazolide derivatives exhibited inhibitory activity *in vitro* against a *G. duodenalis* axenic culture and coculture with Caco2 cells (Muller et al., 2006). Another study revealed that 2-ethenyl- and 2-ethanyl-5-NI derivatives exhibited antigiardial activity without toxicity and were more potent that MTZ *in vitro*. Furthermore, they were more effective than MTZ in a murine giardiasis model (Valdez et al., 2009).

NTZ-related thiazolide/thiadiazolide compounds also exhibited *in vitro* inhibitory activity against *C. parvum*. Modifications of the NTZ chemical structure showed that anticryptosporidial activity is thought to be independent of the presence of a nitro group on the thiazole moiety, with IC_{50} lower than NTZ (Gargala et al., 2010).

Recently, *in vitro* and *in vivo* studies revealed that L-tert-leucyl thiazolide (aminoxadine), a soluble drug of tizoxanide (TIZ), possesses potency of cryptosporidiosis. *In vitro*, this compound dose-dependently inhibited *C. parvum* growth, and no toxicity was observed, since the IC_{50} for *C. parvum* (1.55 ± 0.21 μM) was at least 20-fold lower than the CC_{50} for HCT-8 cells. Surprisingly, in gerbils, a 5-day course of daily intramuscular aminoxanide treatment (100 mg/kg) resulted in a 72.5% oocyst excretion inhibition, statistically equivalent to 75.5% in rodents treated with a 4-fold lower oral dose of NTZ (Diawara et al., 2021). Until now, the only two injected drugs to inhibit *C. parvum* infection are aminoxanide and MMV665917, a new drug based on piperazine (Jumani et al., 2018).

6.4 Nanotechnology Against Protozoan Parasites

Nanotechnology-based drug delivery has emerged as a promising approach for several illnesses, including parasitic diseases, improving the ability to specifically target pathogens, penetrate barriers within the host to allow a drug to access areas of pathogen residence, reduce toxicity by lowering dose amount and frequency of administration, and increase the uptake of poorly soluble drugs (Sun et al., 2019). A summary of the types of nanoparticles susceptible to parasites is shown in **Table 2**.

7 CONCLUSION

Parasitic mucosa-associated protozoa cause severe health, social, and economic impacts, mainly in low-income settings that are resource constrained. Considering the scarcity drugs available to treat these diseases and the threat of resistant cases, the search for new therapeutics is urgently needed.

TABLE 2 | List of the types of nanoparticles susceptibility to protozoa parasites.

Type of nanoparticle	Drug	Parasite	Effect	Reference
Polymeric nanoparticles	Chitosan	*C. parvum*	Reduced the number of Cryptosporidium oocysts	(Ahmed et al., 2019)
Polymeric nanoparticles	Clofazimine	*C. parvum*	Increased solubility by 90 times	(Zhang et al., 2017; Feng et al., 2018)
Nano-suspension	Bupravaquone	*C. parvum*	Enhanced mucosal adsorption and targeting	(Lemke et al., 2010)
Polymeric nanoparticles	Nano-Nitazoxanide (NTZ)	*C. parvum*	decreased the number of parasites, been more effective at day 6 of treatment	(Sedighi et al., 2016)
Polymeric nanoparticles	antibody-engineered with Indinavir	*C. parvum*	were able to target C. parvum in infected cells	(Bondioli et al., 2011)
Polymeric nanoparticles	Silver	*C. parvum*	high concentrations are able to fully break the oocyst wall	(Cameron et al., 2016)
Polymeric nanoparticles	Cooper oxide and silver	*E. histolytica; C. parvum*	significant reduction for cysts viability	(Saad et al., 2015)
Polymeric nanoparticles	Silver, chitosan, and curcumin	*G. lamblia*	Highest effect was combining the three nanoform drugs. Parasite was eliminated from feces and intestine.	(Said et al., 2012)
Polymeric nanoparticles	Gold	*G. lamblia*	concentration of 0.3 mg/ml was effective to eliminated cysts	(Bavand et al., 2014)
Polymeric nanoparticles	Metronidazole	*G. lamblia*	completely eliminated cyst shedding and trophozoite count compared with Giardia-infected mice	(Madbouly et al., 2020)
Nano-emulsion	Micana cordifolia	*T. vaginalis*	anti-T. vaginalis activity was observed to be 100% at 1000 ppm	(Vazini, 2017)
Polymeric nanoparticles	Chitosan	*T. vaginalis*	showed a strong at 100 μg/mL	(Pradines et al., 2015)

However, despite considerable effort, no new novel drugs have been approved as a novel option for treatment. Also, there are no new compounds currently in clinical trials. Most of the targets and compounds were able to show some efficacy *in vitro*, *in vivo*, or both. Also, they have already proven effective in large animal models, although the compounds so far have not reached clinical application. Ahead, the huge challenge is to get the best candidates for clinical studies.

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KMR and HLCS contributed equally to this work and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2022.860442/full#supplementary-material

Supplementary Table 1 | List of natural compounds with antiprotozoa activity.

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