GIARDIASIS TREATMENT: AN UPDATE WITH A FOCUS ON REFRACTORY DISEASE

Kristine Mørch and Kurt Hanevik

Purpose of review
Giardiasis remains a common cause of diarrhea and intestinal enteropathy globally. Here we give an overview of clinical treatment studies and discuss potential mechanisms and molecular targets for in-vitro testing of drug resistance.

Recent findings
Giardia is a cause of disease both in diarrheal and nondiarrheal cases. The prevalence of treatment refractory giardiasis is increasing. Recent studies reveal 5-nitroimidazole refractory infection occurs in up to 50% of cases. Mechanisms of drug resistance are not known. Placebo controlled studies of drug efficacy, taking the self-limiting course of giardiasis into account, has not been reported. No randomized controlled trials of treatment of refractory infection have been performed the last 25 years. Based on the clinical studies reported, combination treatment with a 5-nitroimidazole and a benzimidazole is more effective than repeated courses of 5-nitroimidazole or monotherapies in refractory cases. Quinacrine is effective in refractory cases, but potentially severe side effects limit its use.

Summary
A combination of a 5-nitroimidazole and albendazole or mebendazole, and quinacrine monotherapy, are rational choices in nitroimidazole refractory infections, but randomized controlled studies are needed. Further research into more recent clinical isolates is necessary to uncover mechanisms for the increase in metronidazole refractory giardiasis observed during the last decade.

Keywords
5-nitroimidazoles, albendazole, Giardia, paromomycin, quinacrine

INTRODUCTION

Giardiasis is a protozoan infection of the small bowel, frequently responsible for traveler’s diarrhea [1,2], and for complications from chronic or repeated infections [3,4].

The infectious cysts can survive for months in the environment, explaining why the infection is closely associated with unsafe water. In low-income and middle-income countries (LMICs), the incidence is very high and in some areas close to 100% of children have had one or more Giardia infections before the age of 2 years [3,5,6]. In high-income countries, Giardia is a frequent cause of waterborne outbreaks [7], as in Bergen, Norway in 2004 where approximately 2500 people were treated for giardiasis [8].

In a nonendemic area, clinical infection in all age groups range from asymptomatic carrier state to severe abdominal pain, diarrhea, vomiting, flatulence, malabsorption, anorexia and weight loss, with a fraction of patients developing postinfectious irritable bowel disease and chronic fatigue [8–11].

Histological findings in over 500 cases of giardiasis found normal appearance of the duodenal mucosa in the majority of cases; only a minority of cases revealed duodenal inflammation and mild villous shortening on light microscopy [12]. However, in previously unexposed adults, protracted infection has been associated with duodenal...
inflammation [13,14] and increased risk of postinfectious complications [15].

In low-resource settings, *Giardia* infection is common both in diarrheal and non diarrheal cases, and is associated with chronic diarrhea [16]. It mainly occurs in children, and a serious manifestation is its contribution to malnutrition and stunting of growth following early or repeated infections [3,17–20]. Two large multicenter studies of diarrhea cause in LMIC reported that *Giardia* was more prevalent in controls than in diarrheal cases [21,22], leading to speculations about a potential negative association between *Giardia* and acute infectious diarrhea. However, after adjusting for metronidazole exposure in diarrhea cases in the MAL-ED study [3], a negative association between *Giardia* and diarrhea was not significant.

A small experimental study among adults challenged with *Giardia* revealed that establishment of infection was dose dependent and shedding was self-limited in 85% of individuals, but 15% of individuals developed chronic shedding [23]. The mean duration of infection in spontaneously cured cases was almost 3 weeks. It is rational to treat giardiasis both in endemic and nonendemic areas, to prevent complications, chronic disease and spread of the infection.

The first-line treatment of giardiasis is the 5-nitroimidazoles, but there are reports of high incidence of nitroimidazole refractory cases of giardiasis [24,25**]. Although antimicrobial resistance is an increasing problem in giardiasis, in-vitro susceptibility testing methods to help clinicians guide targeted treatment is not available. This review provide an overview of clinical studies of anti-*Giardia* treatment and suggest choice of treatment in refractory cases, and discuss potential mechanisms and molecular targets for future in-vitro testing of drug resistance.

**TREATMENT OF GIARDIASIS**

Until a few decades ago, *Giardia lamblia* was considered an innocent bystander in the microbial gut flora, but after the pathogenicity of the parasite was recognized, different classes of drugs have been used [26*]. Current therapies, with different availability across countries, include nitroimidazole derivatives (metronidazole, tinidazole, secnidazole and ornidazole), benzimidazoles (albendazole, mebendazole), nitazoxanide, furazolidone, quinacrine, chloroquine and paromomycin.

**Current treatment options**

Randomized controlled trials (RCTs) of drugs used in first-line treatment of giardiasis are listed in Table 1. Efficacy is evaluated by stool microscopy posttreatment in all the studies. Study designs are heterogeneous regarding study population, dose and duration of treatment, time until follow-up and number of stool samples examined.

**5-Nitroimidazoles**

5-Nitroimidazoles are the most frequently used first-line drugs. These drugs become activated after entering the parasite, and kill the microorganism by release of reactive, toxic, partially reduced intermediates [67]. Tinidazole, secnidazole and ornidazole have long half-life, and cure rates above 90% are reported when single-dose regimens of 1–2 g in adults and 30 mg/kg (secnidazole), 20–40 mg/kg (ornidazole) and 50 mg/kg (tinidazole) in children, are given (Table 1). These nitroimidazoles are better tolerated than metronidazole. Metronidazole has a shorter half-life, and longer courses are needed to achieve similar efficacy; 250 mg three times daily (tid) for 7 days or 500 mg tid for 5 days in adults, and 5–7 mg/kg tid for 5–7 days in children. Reported side effects are gastrointestinal discomfort, anorexia, metallic taste, disulfiram-like effect, headache, vertigo, insomnia, irritability, neuropathy, seizures, rash, leukopenia, hepatitis and pancreatitis (references in Table 1).

**Benzimidazoles**

Albendazole and mebendazole are widely used antihelmintic drugs, but treatment efficacy in giardiasis is variable (Table 1). The benzimidazoles exert their function by binding to, and preventing microtubule transport and assembly, and may also induce oxidative stress in the parasite [68].

Albendazole 400 mg in adults, and 10 mg/kg in children, single dose for 5 days, show 83–96% efficacy, while shorter duration is less effective. Efficacy for mebendazole vary greatly (14–95%), both for single-dose treatment and for dosage 200 mg tid up to 7 days. Benzimidazoles are usually well tolerated; reported side effects are nausea, vomiting, diarrhea and abdominal pain.

**KEY POINTS**

- Giardiasis is an important cause of diarrhea and malabsorption.
- Clinical resistance against 5-nitroimidazoles is an increasing problem.
- A 5-nitroimidazole in combination with a benzimidazole, and quinacrine monotherapy, are effective second and third-line treatment options.
- Mechanisms of clinical drug resistance are yet not known.
### Table 1. Randomized controlled studies evaluating anti-Giardia treatment

<table>
<thead>
<tr>
<th>Study Region</th>
<th>Age group</th>
<th>Population</th>
<th>Nitroimidazoles</th>
<th>Benzimidazoles</th>
<th>Anthelminthics</th>
<th>Amino-glycoside</th>
<th>Acridine</th>
<th>Nitrofurans</th>
<th>Place/Ref.</th>
<th>Follow-up (days)</th>
<th>Dose/Dur</th>
<th>Ef %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leningrad [27]</td>
<td>A</td>
<td>60</td>
<td>56</td>
<td>200 tid/7</td>
<td>73</td>
<td>300 tid/10</td>
<td>73</td>
<td>200 tid/10</td>
<td>73</td>
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<td></td>
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<tr>
<td>Leningrad [28]</td>
<td>A</td>
<td>45</td>
<td>56</td>
<td>200 tid/7</td>
<td>73</td>
<td>300 tid/10</td>
<td>73</td>
<td>200 tid/10</td>
<td>73</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>India [29]</td>
<td>A</td>
<td>175</td>
<td>14</td>
<td>200 tid/7</td>
<td>73</td>
<td>300 tid/10</td>
<td>73</td>
<td>200 tid/10</td>
<td>73</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Bangladesh [30]</td>
<td>A</td>
<td>3</td>
<td>2</td>
<td>8</td>
<td>6</td>
<td>15</td>
<td>6</td>
<td>15</td>
<td>6</td>
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<tr>
<td>Egypt [31]</td>
<td>A</td>
<td>200</td>
<td>14</td>
<td>1500 tid/7</td>
<td>73</td>
<td>300 tid/10</td>
<td>73</td>
<td>200 tid/10</td>
<td>73</td>
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<tr>
<td>Brazil [32]</td>
<td>C</td>
<td>25</td>
<td>20</td>
<td>1500 tid/7</td>
<td>73</td>
<td>300 tid/10</td>
<td>73</td>
<td>200 tid/10</td>
<td>73</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Bangladesh [33]</td>
<td>C</td>
<td>103</td>
<td>114</td>
<td>116</td>
<td>115</td>
<td>10</td>
<td>125 tid/5</td>
<td>97</td>
<td>1500 tid/7</td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Croatia [34]</td>
<td>C</td>
<td>75</td>
<td>14</td>
<td>200 tid/7</td>
<td>73</td>
<td>300 tid/10</td>
<td>73</td>
<td>200 tid/10</td>
<td>73</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Iran [35]</td>
<td>C</td>
<td>68</td>
<td>17</td>
<td>1200 tid/5</td>
<td>73</td>
<td>300 tid/10</td>
<td>73</td>
<td>200 tid/10</td>
<td>73</td>
<td></td>
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<tr>
<td>Turkey [36]</td>
<td>C</td>
<td>267</td>
<td>21</td>
<td>1500 tid/7</td>
<td>73</td>
<td>300 tid/10</td>
<td>73</td>
<td>200 tid/10</td>
<td>73</td>
<td></td>
<td></td>
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<tr>
<td>Cuba [37]</td>
<td>C</td>
<td>126</td>
<td>3–5–10</td>
<td>250 tid/5</td>
<td>73</td>
<td>300 tid/10</td>
<td>73</td>
<td>200 tid/10</td>
<td>73</td>
<td></td>
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<td></td>
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<tr>
<td>Cuba [38]</td>
<td>C</td>
<td>146</td>
<td>7</td>
<td>225 tid/5</td>
<td>73</td>
<td>300 tid/10</td>
<td>73</td>
<td>200 tid/10</td>
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<tr>
<td>Cuba [39]</td>
<td>C</td>
<td>122</td>
<td>3–5–7</td>
<td>200 tid/5</td>
<td>73</td>
<td>300 tid/10</td>
<td>73</td>
<td>200 tid/10</td>
<td>73</td>
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</tbody>
</table>

**Notes:**

- **Age group:** A = Adult, C = Child
- **Population:** N = Number of patients
- **Nitroimidazoles:** Metronidazole, Tinidazole, Ornidazole
- **Benzimidazoles:** Secnidazole, Albendazole, Mebendazole
- **Anthelminthics:** Paromomycin, Quinacrine
- **Amino-glycoside:** Furazolidone, Nitazoxanide, Chloroquine
- **Acridine:** Dose/Dur: Ef%
- **Nitrofurans:** Place/Ref.
- **Follow-up (days):** Days
- **Dose/Dur:** Dose/Duration
- **Ef %:** Efficiency %
### Table 1 (Continued)

| Place/Ref. | Population | Age group | N   | Follow-up (days) | Dose/Dur a | Ef % | Dose/Dur b | Ef % | Dose/Dur c | Ef % | Dose/Dur d | Ef % | Dose/Dur e | Ef % | Dose/Dur f | Ef % | Dose/Dur g | Ef % | Dose/Dur h | Ef % | Dose/Dur i | Ef % | Dose/Dur j | Ef % |
|------------|------------|-----------|-----|-----------------|-------------|------|-------------|------|-------------|------|-------------|------|-------------|------|-------------|------|-------------|------|-------------|------|-------------|------|
| Asia [53]  | C          | 120       | 30  | 200* bid/5      | 60          | 100  | 2000* bid   | 52   | 2000* bid   | 86   | 2000* bid   | 95   | 2000* bid   | 100  | 2000* bid   | 100  |
| Iran [54]  | C          | 148       | 20  | 125 bid/5       | 100         | 100  | 250 bid/7   | 77   | 250 bid/7   | 95   | 250 bid/7   | 95   | 250 bid/7   | 95   | 250 bid/7   | 95   |
| USA [55]   | C          | 45        | 42–84 | 125 bid/7       | 87         | 87   | 150 bid/7   | 97   | 150 bid/7   | 97   | 150 bid/7   | 97   | 150 bid/7   | 97   | 150 bid/7   | 97   |
| Brazil [56]| A + C      | 172       | 21  | 125 bid/7       | 87         | 87   | 150 bid/7   | 97   | 150 bid/7   | 97   | 150 bid/7   | 97   | 150 bid/7   | 97   | 150 bid/7   | 97   |
| USA [57]   | C          | 22        | 28  | 250 bid/7       | 87         | 87   | 250 bid/7   | 97   | 250 bid/7   | 97   | 250 bid/7   | 97   | 250 bid/7   | 97   | 250 bid/7   | 97   |
| Mexico [58]| C          | 82        | 3   | 63–250 bid/10   | 96         | 96   | 250 bid/10  | 96   | 250 bid/10  | 96   | 250 bid/10  | 96   | 250 bid/10  | 96   | 250 bid/10  | 96   |
| Cuba [59]  | C          | 256       | 21  | 250 bid/7       | 80         | 80   | 250 bid/7   | 80   | 250 bid/7   | 80   | 250 bid/7   | 80   | 250 bid/7   | 80   | 250 bid/7   | 80   |
| Mexico [60]| C          | 82        | 3   | 100 bid/3       | 80         | 80   | 100 bid/3   | 78   | 100 bid/3   | 78   | 100 bid/3   | 78   | 100 bid/3   | 78   | 100 bid/3   | 78   |
| Cuba [61]  | C          | 137       | 10  | 250 bid/7       | 91         | 91   | 250 bid/7   | 91   | 250 bid/7   | 91   | 250 bid/7   | 91   | 250 bid/7   | 91   | 250 bid/7   | 91   |
| Egypt [62] | A          | 11 + place| 7–10 | 250 bid/7       | 91         | 91   | 250 bid/7   | 91   | 250 bid/7   | 91   | 250 bid/7   | 91   | 250 bid/7   | 91   | 250 bid/7   | 91   |
| Mexico [63]| C          | 51        | 14  | 200 bid/3       | 42         | 42   | 200 bid/3   | 42   | 200 bid/3   | 42   | 200 bid/3   | 42   | 200 bid/3   | 42   | 200 bid/3   | 42   |
| Peru [64]  | C          | 110       | 7–10 | 125–250 bid/5   | 75         | 75   | 125–250 bid/5| 75   | 125–250 bid/5| 75   | 125–250 bid/5| 75   | 125–250 bid/5| 75   | 125–250 bid/5| 75   |
| Peru [65]  | C          | 32 + place| Clinical | 200 bid/3       | 71         | 71   | 200 bid/3   | 71   | 200 bid/3   | 71   | 200 bid/3   | 71   | 200 bid/3   | 71   | 200 bid/3   | 71   |
| Cuba [66]  | C          | 165       | 7–10 | 500 bid/5       | 62         | 62   | 500 bid/5   | 62   | 500 bid/5   | 62   | 500 bid/5   | 62   | 500 bid/5   | 62   | 500 bid/5   | 62   |

A, adults; bid, twice daily; C, children; Dur, duration; Ef, efficacy; Ns, not stated; plac, placebo; qd, four times daily; sd, single dose; sign, significant; tid, three times daily.

*Response assessed by stool microscopy. Duration of follow-up in days.

Dosages in milligram (mg), duration in days. Single dose given for 1 day, if other is not stated.

mg/kg.

mg/kg/day divided into three doses.

Adjusted to pediatric dosage.
Aminoglycoside
The oral aminoglycoside paromomycin is the drug of choice for giardiasis in pregnant women, because it is poorly absorbed and has no systemic effect [69]. Paromomycin inhibits the protein synthesis in Giardia [70]. Although this is the only anti-Giardia drug considered completely safe during early pregnancy, few clinical studies of treatment efficacy have been reported (Table 1). In a controlled study from Cuba among 256 children, efficacy was 92% compared with 80% for metronidazole [59], while a small case series from 1962 using 15 mg/kg/day for 5 days reported 40% efficacy [71].

Acridine
Quinacrine is an effective anti-Giardia drug (Table 1), although its exact mechanism of action in Giardia is not known [72]. Two studies among children treated for 5 days report 100% efficacy. However, quinacrine has bothersome and potentially severe side effects. In a study reporting 77% efficacy, severe vomiting explained most of the treatment failures in small children [55]. In a study from Cuba reporting 84% efficacy, nausea, vomiting, discoloration of skin and headache was significantly more common than in the metronidazole group [52]. Yellow discoloration of skin is a common but harmless and self-limiting side effect [52,54,55], while neuropsychiatric disturbances ranging from restlessness, nightmares and insomnia to seizures and psychoses are rare but feared complications [73–75].

Furazolidone
Furazolidone has shown high efficacy in studies of first-line therapy, although no RCTs are reported since 1980s (Table 1). It is a prodrug which release damaging intermediates when its nitro group is reduced [76]. The drug is contraindicated in glucose-6-phosphate-dehydrogenase (G6PD) deficiency and in neonates, due to risk of hemolytic anemia.

Nitroimidazoles
Nitroimidazoles are used as second-line therapy, although some experience high treatment failure (Table 1). Furazolidone has shown high efficacy in studies of first-line therapy, although no RCTs are reported since 1980s (Table 1). It is a prodrug which release damaging intermediates when its nitro group is reduced [76]. The drug is contraindicated in glucose-6-phosphate-dehydrogenase (G6PD) deficiency and in neonates, due to risk of hemolytic anemia.

Nitazoxanide
Nitazoxanide is the only available drug for Cryptosporidium, and like metronidazole it is also activated by reduction of its nitro group and inhibits metabolic enzymes [77]. In controlled studies of giardiasis, it has shown efficacies between 44 and 91% (Table 1). It may cause some gastrointestinal discomfort but is usually well tolerated.

Chloroquine
Chloroquine is effective against nonfalciparum malaria and rheumatic disorders. Its mechanism of action against giardiasis is not known, but it may limit the trophozoite adherence to the intestinal wall. Its effect against Giardia has been investigated in RCTs in Cuba; in a large study among children, efficacy was 86% compared with 91% for tinidazole and 62% for albendazole [66]. Reported side effects were bitter taste and gastrointestinal problems, similar to for tinidazole. Chloroquine may potentially cause prolonged QT time, and hemolytic anemia in G6PD deficiency [78].

Treatment of refractory giardiasis
Drug trials have usually shown efficacy above 90% for the nitroimidazoles (Table 1); however, treatment refractory disease is an increasing problem. In a study from England, nitroimidazole failure increased from 15% (n = 8/53) in 2008 to 40% (n = 35/87) in 2013, and among travelers from India treatment failure occurred in as much as 50% [24]. The association between location of contracting infection and nitroimidazole failure, in four clinical studies, is shown in Table 2. As much as 46% failure

<table>
<thead>
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</thead>
<tbody>
<tr>
<td>Asia</td>
<td>214</td>
<td>56/143 (39)</td>
<td>12/36 (33)</td>
<td>10/35 (29)</td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>101</td>
<td>51/101 (50)</td>
<td>nk</td>
<td>nk</td>
<td></td>
</tr>
<tr>
<td>Africa</td>
<td>97</td>
<td>9/47 (19)</td>
<td>4/32 (12)</td>
<td>3/18 (17)</td>
<td></td>
</tr>
<tr>
<td>Europe</td>
<td>34</td>
<td>6/23 (26)</td>
<td>3/4 (75)</td>
<td>0/7 (0)</td>
<td></td>
</tr>
<tr>
<td>Latin America</td>
<td>51</td>
<td>1/17 (6)</td>
<td>2/23 (9)</td>
<td>1/11 (9)</td>
<td></td>
</tr>
<tr>
<td>Cuba</td>
<td>456</td>
<td>208/456 (46)</td>
<td>nk</td>
<td>nk</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>852</td>
<td>208/456 (46)</td>
<td>72/230 (31)*</td>
<td>21/95 (22)</td>
<td>14/71 (20)</td>
</tr>
</tbody>
</table>

*aIncrease during study period: 2008: 8/53 (15%), 2011: 15/73 (21%), 2012: 23/99 (23%), 2013: 35/87 (40%).
was reported after metronidazole 500 mg tid for 5 days in a large cohort from Cuba in 2018 [25].

Although there are few studies and number of cases are small, treatment failure among European travelers seemed to be more common after travel to Asia than to Africa and Latin America (Table 2).

A few clinical, mainly small, studies are available providing evidence for treatment efficacy in refractory cases (Table 3). Observational studies show that another class of drug, combination therapy, or repeated courses with increased dose/duration of the same drug is used with varying efficacy.

Quinacrine is effective in almost all cases, but due to availability problems and potentially severe side effects, the drug is normally preferred only when other treatment options fail. Both quinacrine in combination with a 5-nitroimidazole for up to 3 weeks, and quinacrine monotherapy for as short as 3 days, were effective in reported studies (Table 3).

### Table 3. Clinical studies of treatment in nitroimidazole refractory infections

<table>
<thead>
<tr>
<th>Study place and year (Ref.)</th>
<th>Design and number of cases, N</th>
<th>Study drugs</th>
<th>Cure ratea, N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italy 1995 [81]</td>
<td>RCT adults (20)</td>
<td>ABZ 400 mg bid + MTZ 250 mg tid 7 days or ABZ 400 mg bid 7 days</td>
<td>9/10 (90) / 2/10 (20)</td>
</tr>
<tr>
<td>United States 2001 [82]</td>
<td>Case series, adults (6)</td>
<td>MTZ 250–750 mg tid or TDZ 2 g qd + QNC 100 mg tid 2–3 weeks</td>
<td>5/6 (83)</td>
</tr>
<tr>
<td>Norway 2008 [83]</td>
<td>Prospective treatment ladder, adults (38)</td>
<td>ABZ 400 mg + MTZ 250 mg bid 7 days / PRM 500 mg tid 7 days / QNC 100 mg + MTZ 500–750 mg tid 2–3 weeks</td>
<td>30/38 (79) / 3/6 (50) / 3/3 (100)</td>
</tr>
<tr>
<td>Spain 2010 [84]</td>
<td>Retrospective case series, adults and children (10)</td>
<td>One combination for 5–10 days Nitroimidazole + ABZ + PRM Nitroimidazole + QNC</td>
<td>10/10 (100)</td>
</tr>
<tr>
<td>Spain 2013 [79]</td>
<td>Retrospective case series, adults (14)</td>
<td>PRM 500 mg tid 8 days / ABZ / QNC 100 mg tid 7 days</td>
<td>0/3 (0) / 0/2 (0) / 14/14 (100)</td>
</tr>
<tr>
<td>Spain 2013 [85]</td>
<td>Prospective cohort, adults and children (3)</td>
<td>QNC 100 mg tid 5 days</td>
<td>3/3 (100)</td>
</tr>
<tr>
<td>Israel 2014 [86]</td>
<td>Retrospective case series, adults (12)</td>
<td>ABZ / NTZ / QNC / PRM</td>
<td>4/10 (40) / 1/4 (25) / 2/2 (100) / 1/1 (100)</td>
</tr>
<tr>
<td>England 2015 [24]</td>
<td>Retrospective audit (73)</td>
<td>TDZ / MTZ / TDZ + PRM / TDZ + ABZ / TDZ + NTZ / ABZ / NTZ / PRM / QNC / QNC + ABZ / QNC + TDZ</td>
<td>23/39 (60) / 2/11 (18) / 1/1 (100) / 12/20 (60) / 1/2 (50) / 2/8 (25) / 9/18 (50) / 1/3 (33) / 7/7 (100) / 2/2 (100)</td>
</tr>
<tr>
<td>Spain 2017 [80]</td>
<td>Prospective observational study, adults and children (14)</td>
<td>QNC 100 mg or 8 mg/kg/day tid 5 days</td>
<td>14/14 (100)</td>
</tr>
<tr>
<td>Cuba 2019 [25**]</td>
<td>Prospective treatment ladder, adults (208)</td>
<td>SCZ 2 g sd / TDZ 2 g sd / SCZ 2 g sd + MBZ 200 mg tid 3 days / QNC 100 mg tid 3 days</td>
<td>50/208 (24) / 43/158 (27) / 100/115 (87) / 15/15 (100)</td>
</tr>
</tbody>
</table>

**ABZ, albendazole; bid, two times daily; MTZ, metronidazole; NTZ, nitazoxanide; PRM, paromomycin; QNC quinacrine; RCT, randomised controlled trial; SCZ, secnidazole; sd, single dose; TDZ, tinidazole; TDZ, tinidazole; MBZ, mebendazole; tid, three times daily.**

* Cure was evaluated by microscopy, except in the study from England where both PCR and microscopy were used.

*Severe immunodeficiency in n = 5/6.
Combination of drugs from different classes also seems to be an effective second-line option. Albendazole in combination with metronidazole was effective in 90% in a small RCT in Italy [81], and 79% effective in a prospective treatment ladder study in Norway [83]. The combination of secnidazole and mebendazole was effective in 89% in a large treatment ladder study in Cuba, while a repeated course of 5-nitroimidazole after initial metronidazole treatment cured only 24–27% [25**].

Albendazole monotherapy seem to be less effective, although number of cases reported are small (Table 3).

**DRUG RESISTANCE IN GIARDIASIS**

Antimicrobial resistance is defined by WHO as ‘the ability of a microorganism (like bacteria, viruses and some parasites) to stop an antimicrobial (such as antibiotics, antivirals and antimalarials) from working against it. As a result, standard treatments become ineffective, infections persist and may spread to others’ [87]. There are no defined molecular-resistance mechanisms in *Giardia* yet, thus the term treatment refractory is better suited to describe treatment failure, as there is no doubt that also the hosts’ combined immune defenses play a role in eradicating the parasite.

Treatment refractory clinical infections have mostly been observed against the 5-nitroimidazole drug metronidazole [24]. To exert its antibiotic function, metronidazole needs to be activated by intracellular reduction of its nitro group, creating hyper-reactive intermediates, disrupting the microorganism by excessive oxidative stress [72]. Four enzymes have been identified in the metabolism of *Giardia* that seem capable of activating metronidazole by partial reduction into toxic hyper-reactive intermediates; nitroreductase 1 [88], pyruvate:ferredoxin oxido-reductase (PFOR) 1 and 2 [89,90] and the thiol-cycling associated enzyme thioredoxin reductase (TrxR) [89,91]. This partial reduction only occurs under anaerobic or microaerophilic conditions.

Inducing resistance in *vitro*, by gradually increasing drug concentrations in growth media of a small fraction of *Giardia* strains that can be cultured, is quite easy. Most research on resistance mechanisms have been performed on such laboratory-strains, isolated from infected humans decades ago [92]. Significantly, the drug resistance seen in laboratory-induced strains is reduced or lost if the trophozoites pass through a cyst stage [93]. It is unknown how important this nontransmissible resistance is in clinical practice, but the rapidly increasing resistant infections seen, indicates the presence of new heritable traits conferring metronidazole resistance, or the ability to rapidly develop it. Typing of metronidazole resistant isolates in two studies shows that several subgroups of assemblage A and B isolates are represented among resistant strains [80,94].

**Elucidating mechanisms of drug resistance**

Methods for determining resistance in *Giardia* are not easily available. Only a small fraction of clinical isolates will grow in available liquid culture media, with assemblage A more often being successful than assemblage B parasites [95]. Strains will grow at different speeds making measurement of growth inhibition in drug dilutions very difficult to standardize, even for the few isolates that are culturable [96,97].

Compared with bacteria, identifying genetic markers of resistance in an early eukaryote, binucleate, functionally tetraploid organism such as *Giardia* is challenging. A relatively high degree of genetic diversity is seen in the metronidazole activating genes [89], both between isolates and in the up to four alleles of each gene. This offers *Giardia* substantial potential for variability to tweak its metabolism and for selection of well adapted variants.

Studies analyzing both genes and gene expression in metronidazole resistant laboratory strains and their metronidazole-susceptible ancestor, find a quite broad and variable adaptive response in the resistant isolates [98]. There seem to be both post-transcriptional and posttranslational alterations [67,93,99], and *Giardia* thereby seem to have several ways it may develop tolerance to metronidazole.

A frequent finding in the few existing studies in metronidazole-resistant laboratory strains has been downregulation of nitroreductase 1 [98–100]. One isolate has been shown to harbor a nonsense mutation in transcripts of this gene, inferring that it may have substantially lower levels of this metronidazole-activating enzyme [98]. Results from genetic analysis of the other metronidazole metabolizing enzymes have been less consistent, but indicates a role for nitroreductase 2, PFOR, and TrxR and flavin mononucleotide-dependent oxidoreductases in resistance against metronidazole [67].

In a study examining protein expression in *Giardia* strains made gradually resistant *in vitro* against metronidazole and nitazoxanide, no specific trait or marker was identified [101*]. Rather, it seemed that each of the three resistant strains had found their own strategy for tolerating high level of the drugs.

Thus, we can conclude that despite the carefully designed gene and protein expression studies in
cultured isolates we still have very limited knowledge regarding the ways *Giardia* protects itself from drugs such as metronidazole. However, laboratory-induced resistant strains gathered decades ago may be quite different from the metronidazole refractory isolates now circulating. The rapidly increasing resistance reported [24,25] suggest that there are genetic changes that help the parasite overcome the toxic effects of metronidazole, but further research is needed in clinical isolates.

CONCLUSION

Nitroimidazole failure in up to 50% is reported in giardiasis, both among travelers and in high endemic countries. Repeated courses of nitroimidazoles, and monotherapy with a drug with another mode of action, seem to be less effective than combination therapy. A combination of a 5-nitroimidazole and albendazole or mebendazole, and 100% efficacy after quinacrine as the last step. The largest prospective study of treatment refractory giardiasis reported up to now.

Research in drug resistant *Giardia* laboratory strains have not yet succeeded in identifying mechanisms, or markers, of resistance but show that *Giardia* strains possess a varied armamentarium of adaptations. Further research into more recent clinical isolates seems necessary to uncover mechanisms for the emerging metronidazole refractory cases.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as: of special interest **of outstanding interest

26. Good quality treatment ladder study from Cuba. Metronidazole resistance 46%, low efficacy of repeated courses of nitroimidazoles, high cure rate after nitroimidazole in combination with mebendazole, and 100% efficacy after quinacrine as last step. The largest prospective study of treatment refractory giardiasis reported up to now.
28. Extensive review of drugs under investigation and potential targets for anti-giardial agents.
32. Good quality treatment ladder study from Cuba. Metronidazole resistance 46%, low efficacy of repeated courses of nitroimidazoles, high cure rate after nitroimidazole in combination with mebendazole, and 100% efficacy after quinacrine as last step. The largest prospective study of treatment refractory giardiasis reported up to now.
100. Muller J, Muller N. Nitroreductases of bacterial origin in Giardia lamblia: potential role in detoxification of xenobiotics. Microbiologyopen 2018; 8:e904.

The article examines protein expression profiles in laboratory-induced resistant strains and their susceptible ancestor strain. No distinct pattern was found. Rather, examination of enzymes involved in nitroreduction and detoxification of nitro radicals, NO or O₃, suggests the use of different strategies for each drug and each strain.