5-Nitroimidazole refractory giardiasis effectively treated by secnidazole plus high-dose mebendazole or quinacrine. Matanzas, Cuba

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Background: *Giardiasis* is caused by the protozoan parasite *Giardia intestinalis*, which is found worldwide and affects an estimated 280 million people annually. ***Objective:*** To evaluate the effectiveness and tolerability of secnidazole combined with high-dose mebendazole for treatment of 5-nitroimidazole-resistant giardiasis. ***Method:*** Adults with microscopically verified Giardia intestinalis monoinfection attending a secondary level hospital in Matanzas City, Cuba were prospectively included in a cohort. A recently introduced treatment ladder consisting of metronidazole as first-line treatment, followed by secnidazole, tinidazole, secnidazole plus mebendazole and quinacrine as second-to fifth-line treatments, respectively, was used. Adverse events and treatment success were determined by questioning and microscopy on concentrated stool samples, respectively on days 3, 5 and 7 after the end of treatment. If *Giardia* intestinalis was detected on day 3, 5 or 7, then the infection was classified as refractory and no further microscopy was performed. ***Results:*** A total of 456 individuals were included. Metronidazole, 500 mg three times daily for 5 days, cured 248/456 (54%) patients. A single 2-g secnidazole dose as second-line treatment cured 50/208 (24%) patients. A single 2-g tinidazole dose as third-line treatment cured 43/158 (27%) patients. Three rounds of 5-nitroimidazole therapy therefore cured 341/456 (75%) patients. Secnidazole plus mebendazole (200 mg every 8 hours for 3 days) cured 100/115 (87%) of nitroimidazole refractory infections. Quinacrine cured the remaining 15 patients. All treatments were well tolerated. ***Conclusions:*** 5-Nitroimidazole refractory giardiasis was common, indicating that an alternative first-line treatment may be needed. Retreatment of metronidazole refractory giardiasis with an alternative 5-nitroimidazole was suboptimal, indicating cross-resistance. Mebendazole plus secnidazole were well tolerated and effective for the treatment of 5-nitroimidazole refractory Giardia intestinalis infection in this setting.

Key words: **clinical trial, drug therapy, *Giardia duodenalis*, mebendazole.**

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I. INTRODUCTION

Giardiasis is caused by the protozoan parasite *Giardia intestinalis* (synonymous with *Giardia lamblia* and *Giardia duodenalis*), which is found worldwide and affects an estimated 280 million people annually (1). When present, symptoms related to *G. intestinalis* infection include acute or chronic diarrhoea, with or without dehydration, abdominal pain, nausea, vomiting, bloating or malabsorption, and occasionally extra-intestinal manifestations and long-term consequences such as chronic fatigue (2). People of all ages are infected and giardiasis contributes significantly to the global burden of diarrhoeal disease, post-infectious chronic disorders and possibly stunting (2-4). The prevalence of *G. intestinalis* varies between 25% and 55% in Cuba despite the implementation of government initiatives to improve socioeconomic conditions, health, sanitation and water supplies (5-8).

Because of the lack of any effective and approved vaccine against giardiasis, prevention is based on measures that interrupt the biological cycle of the parasite and treatment with antiparasitic drugs (9,10). The 5-nitroimidazole compounds (metronidazole, secnidazole and tinidazole) are typically used as first-line treatment worldwide. However, therapeutic failures are common and recent studies indicate that 5-nitroimidazole refractory giardiasis is increasing (3,10,11). Re-treatment options include taking 5-nitroimidazoles for longer periods or at higher doses, treating with alternative drugs such as nitazoxanide, quinacrine, mebendazole, albendazole, furazolidone and paromomycin, or combining drugs with different modes of action (1,10,12).

In Cuba, giardiasis is typically treated with 5-nitroimidazoles. Failure rates with metronidazole and secnidazole were approximately 15% and 10%, respectively, in studies conducted in 2009 and 2010 (13,14). 5-Nitroimidazole refractory giardiasis appears to have increased in Matanzas during the last few years, though this has not been studied.

There is no recommended treatment ladder for 5-nitroimidazole refractory giardiasis but repeated treatment with different 5-nitroimidazoles is common. Both mebendazole and quinacrine have been shown to effectively cure approximately 85% of individuals when used as first-line treatment (9,15). A treatment ladder consisting of metronidazole, secnidazole, tindiazole, secnidazole + mebendazole and finally quinacrine as first-to fifth line treatments, respectively, was therefore instituted at the Faustino Pérez Hernández hospital in Cuba in January 2017.

The aim of this study was to evaluate the effectiveness and tolerability of repeated 5-nitroimidazole treatment (as this was commonly used) and standard-dose secnidazole combined with high-dose mebendazole for the treatment of 5-nitroimidazole refractory giardiasis.

II. METHODS

a. Study design, participants, and setting

From January 2017 to October 2018 a prospective, observational cohort study was carried out at the hospital Faustino Pérez Hernández. This is a secondary-level hospital located in Matanzas City, the capital of the province Matanzas in Cuba. Patients from all over the province with or without a referral note from their general practitioners attend the hospital.

Patients with signs and/or symptoms suggestive of gastrointestinal infection referred to the parasitology outpatient clinic had stools examined for *G. intestinalis*. Stool examination was part of the routine hospital services and consisted of microscopic examination of faecal wet-mount samples and examina-

tion after Ritchie concentration. Microscopy was performed at the parasitology laboratory of the Centre of Hygiene, Epidemiology and Microbiology. Routine PCRs for detection of G. *intestinalis* were not available. Patients treated for giardiasis according to a treatment ladder and systematic follow up implemented in January 2017 were eligible for inclusion.

Inclusion criteria were: monoinfection with *G. intestinalis* identified by microscopic examination of faecal wet-mount samples and/or after Ritchie concentration, age 19-70 years, and absence of other gastrointestinal parasites, medical co-morbidities, allergies or drug intolerances.

Exclusion criteria were: a history of hypersensitivity to any 5-nitroimidazole or benzimidazole compound, participation in another clinical trial, pregnancy, assessed as unlikely to attend all required follow-up examinations and unwillingness to give informed consent. In practical terms, patients assessed as unlikely to attend all the required follow-up examinations comprised patients living far from the hospital and those unable to come due to work commitments.

b. Treatment

The treatment ladder implemented at the hospital was as follows. First-line treatment was metronidazole, 500 mg every 8 hours for 5 days. Second-line treatment was a single 2-g dose of secnidazole and third-line treatment was a single 2-g dose of tinidazole. Patients with recurrent giardiasis despite treatment with metronidazole, secnidazole and tinidazole were treated with a single 2-g dose of secnidazole given on the first day of treatment combined with 200 mg mebendazole three times daily for 3 days starting at the same time as secnidazole (fourth-line treatment). The treatment for patients failing three rounds of nitroimidazole monotherapy as well as mebendazole plus secnidazole combination therapy was 100 mg quinacrine thrice daily for 5 days (fifth line treatment). Tablets containing 250 mg metronidazole, 500 mg secnidazole, 500 mg tinidazole, 100 mg mebendazole or 100 mg quinacrine were obtained from Reynaldo Gutierrez Pharmaceutical (Havana, Cuba).

c. Compliance

Verbal instructions about drug administration, follow-up visit attendance and hygiene measures recommended to reduce the risk of re-infection were given to patients. Intake of all first doses were observed at the hospital. Patients took their prescribed medications and referral notes to their family doctors. Family doctors supervised metronidazole, mebendazole and quinacrine intake according to standard routine practice in Cuba. Cuban family doctors live in the communities they serve, enabling repeated home visits. The presence of any of the following was considered evidence of treatment non-compliance: failure to attend a follow-up visit, reporting having missed one or more prescribed doses.

d. Follow up

Follow up consisted of visits on days 3, 5 and 7 after the end of each treatment during which symptoms were elicited by questioning using a standardized questionnaire and faecal samples were collected for analyses. Instructions on how to collect the stool samples were provided in writing to the patients. Presence or absence of *G. intestinalis* in faecal samples was assessed as described above (7-9). If *G. intestinalis* was detected on day 3, 5 or 7, then the infection was classified as refractory and no further

microscopy was done.

e. Evaluation of safety profile

Patients were encouraged to report all symptoms regardless of suspected causal relationship to treatments taken. Adverse events were defined as signs or symptoms that did not exist before or that

became more pronounced following the start of therapy. Serious adverse events were defined as any life-threatening, disabling or incapacitating event, events requiring hospitalization, and death. Patients

were asked about the presence or not of the following symptoms: abdominal pain, nausea, vomiting, yellowish colouration of urine, bitter taste, dizziness and any other symptom at each visit.

f. Outcomes

The primary outcome was cure, defined as the absence of *G. intestinalis* trophozoites or cysts in any of the three post-treatment faecal specimens. The secondary outcome was treatment tolerability assessed by frequency and severity of adverse events.

g. Ethics

The Research and Ethics Committee of Faustino Pérez Hernández Hospital (institutional review board) and by the institutional review board from the Centre of Hygiene, Epidemiology and Microbiology, Matanzas City, Cuba (reference code 13-18) approved the study. Treatments, follow up and laboratory services were free of charge for all patients. No financial incentives were offered. Treatments were assigned according to departmental guidelines. Treatments, follow up and monitoring of adverse events were part of routine quality assurance. The only intervention that this study entailed was accessing this data.

h. Data management and statistical analysis

Parasitological responses and adverse events were recorded on case record forms by attending physicians and checked by the physician responsible for the outpatient clinic. Data were collected prospectively and analysed using STATA 12 software. No power calculation was done because the data represent routine treatment of a cohort of patients. However, the study aimed to include at least 100 patients treated with the combination of mebendazole plus secnidazole. The significances of observed treatment outcomes were not compared statistically because the groups were not comparable. The non-parametric test for trend was used to compare the days of recurring parasites as described below. A significance level of 0.05 was used.

III. RESULTS

Between January 2017 and October 2018, 624 adults of both sexes sought treatment for giardiasis and 456 patients with a median age of 32 (range 19-69) years were included. The flow of patients through the study is shown in Fig 1. Baseline characteristics reported by infected patients before each treatment are shown in Table 1.

Treatment outcomes are shown in Fig 2. Initial treatment with metronidazole for 5 days cured 54% (95% CI 50%-59%) of patients (n= 248/456). The 208 patients in whom *G. intestinalis* recurred were treated with secnidazole, which cured a further 50 individuals (24%; 95% CI 18%-30%). The remaining 158 patients were treated with tinidazole, which cured a further 27% (95% CI 20%-35%) of patients (n= 43 of 158). In total, the three rounds of 5-nitroimidazole treatment thus cured 75% (95% CI 71-79) of patients (n= 341/456). The 115/456 (25%) patients that were not cured were treated with secnidazole plus three days of mebendazole that cured 87% (95% CI, 79-93) of patients (n = 110/115). The remaining 15 patients were cured by quinacrine.

The proportions of *G. intestinalis* detected on days 3, 5 or 7 are shown in Fig 3 and were compared using the non-parametric test for trend. Refractory giardiasis was significantly more commonly detected on days 3 versus 5 versus 7 after treatment, irrespective of treatment ($p < 0.001$). As duration of treatment differed, days 3, 5 and 7 after the end of treatment differed between treatments, preventing statistical comparisons between them.

The frequency of adverse events is shown in Table 2. Adverse events were all mild, transient and self-limited, and did not require discontinuation of treatment or additional medication. The frequency of reported adverse events did not differ significantly between patients taking secnidazole alone compared with secnidazole and mebendazole. Similarly, the frequency of reported symptoms did not differ significantly between patients taking quinacrine and any other drug nor when compared with the 5-nitroimidazole as a group.

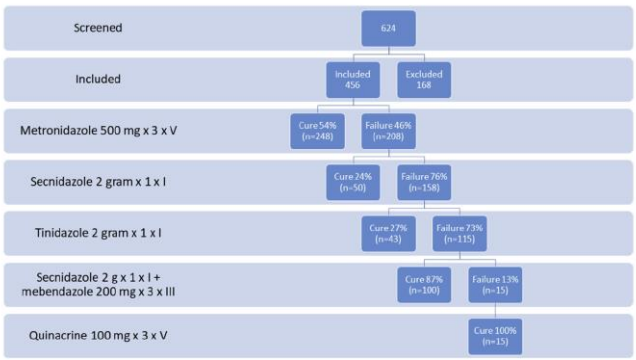

Fig. 1. Flow diagram. Causes for exclusion were as follows: not deemed likely to complete follow up ($n = 95$), age >70 years ($n = 24$), pregnancy ($n = 22$), hypersensitivity ($n = 8$), unwilling to give informed consent ($n = 5$). The overall 5-nitroimidazole treatment success rate was 75% (341/456).

Table 1
Baseline characteristics and symptoms of patients before each round of treatment

Treatment given	Metronidazole	Secnidazole	Tinidazole	Secnidazole + mebendazole	Quinacrine
Number	456	248	208	115	15
Median age (range), years	32 (19–69)	36 (22–72)	39 (23–71)	42 (21–69)	29 (22–67)
Sex (male: female)	183:273	87:161	78:130	44:71	6:9
Median weight (range), kg	73 (65–82)	70 (63–79)	71 (65–80)	73 (65–78)	70 (64–74)
Abdominal pain, *n* (%)	218 (48%)	99 (40%)	71 (34%)	34 (30%)	5 (33%)
Diarrhoea, *n* (%)	160 (35%)	74 (30%)	60 (29%)	28 (24%)	4 (27%)
Bloating, *n* (%)	186 (41%)	95 (38%)	62 (30%)	25 (22%)	3 (20%)
Nausea, *n* (%)	96 (21%)	44 (18%)	19 (9%)	11 (10%)	3 (20%)
Fatigue, *n* (%)	80 (17%)	28 (11%)	17 (8%)	6 (5%)	1 (7%)
Weight loss, *n* (%)	79 (17%)	32 (13%)	16 (8%)	5 (4%)	1 (7%)

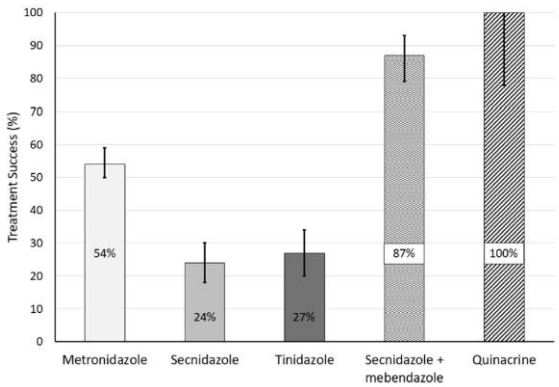

Fig. 2. Treatment outcomes after treatment with metronidazole, secnidazole, tinidazole, secnidazole plus mebendazole, and quinacrine. The error bars represent 95% CI.

Table 2
New or more severe symptoms since start of treatment elicited by active questioning on days 3, 5 and 7 after end of treatment

	Metronidazole	Secnidazole	Tinidazole	Secnidzole + mebendazole	Quinacrine
Number	456	208	158	115	15
Abdominal pain, *n* (%)	142 (31%)	40 (19%)	28 (18%)	25 (22%)*	4 (27%)†
95% CI	27%–37%	14%–25%	12%–25%	15%–30%	8%–55%
Nausea, *n* (%)	97 (21%)	33 (16%)	24 (15%)	19 (17%)*	4 (27%)†
95% CI	18%–25%	11%–22%	10%–22%	10%–25%	8%–55%
Vomiting, *n* (%)	81 (18%)	31 (15%)	22 (14%)	19 (16%)[a]	3 (20%)[b]
95% CI	14%–22%	10%–20%	9%–20%	10%–25%	4%–48%
Bitter taste, *n* (%)	37 (8%)	12 (6%)	8 (5%)	0	0
95% CI	6%–11%	3%–10%	2%–10%		
Yellowish coloration of urine, *n* (%)	24 (5%)	8 (4%)	3 (2%)	0	0
95% CI	3%–8%	2%–7%	0.4%–5%		

[a] The frequency of reported symptoms did not differ significantly between patients taking secnidazole alone compared with secnidazole and mebendazole.
[b] The frequency of reported symptoms did not differ significantly between patients taking quinacrine and any other drug nor when compared with the 5-nitroimidazoles, as a group.

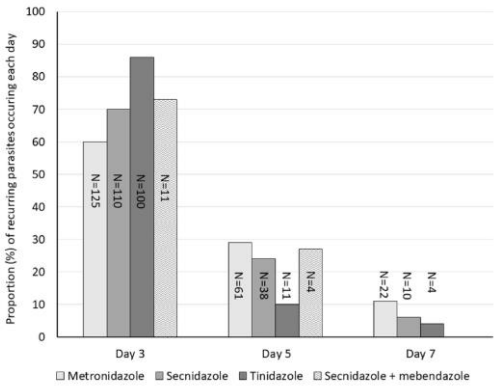

Fig. 3. Proportion of *Giardia duodenalis* detected on days 3, 5 and 7 after end of treatment with metronidazole, secnidazole, tinidazole and secnidazole plus mebendazole. The number of treatment failures in each column are shown as *n* = xxx.

The 46% frequency of metronidazole refractory giardiasis is considerably higher than the 15% found in a randomized clinical trial carried out in Matanzas in 2009 (14). Patients treated for *G. intestinalis* infection in the past month were excluded from the 2009 study, which probably accounts for part of the difference. However, attending physicians reported that most patients in the current study had not recently received treatment for *G. intestinalis* suggesting that the frequency of refractory giardiasis has increased in line with international data (11). Moreover, another clinical trial conducted in a similar setting in Havana, in 2005-2006, after excluding previously treated patients, found only 8% frequency of secnidazole refractory giardiasis, possibly supporting an increased prevalence of 5-nitroimidazole-resistant giardia (13). Irrespective of the exact frequency, the results of the present study confirm the impression that 5-nitroimidazole refractory giardiasis is a considerable problem in Matanzas and indicate that the first-line treatment needs to be assessed in treatment-naive patients.

Treatment with secnidazole and tinidazole cured an additional 24% and 27% of patients that failed metronidazole treatment, respectively. The efficacy of secnidazole and tinidazole when used for treatment of *G. intestinalis* that survived treatment with metronidazole was therefore approximately half that of metronidazole on mainly treatment-naive parasites, suggesting a degree of cross-resistance, as previously found in vitro (16,17). However, the ability of repeated nitroimidazole treatment to cure a proportion of patients suggests that cross-resistance is not complete, in line with previous data. Nevertheless, the poor efficacy of repeated 5-nitroimidazole treatment indicates that this. common practice should be avoided and that an effective treatment ladder is needed.

None of the six different classes of drugs in use for the treatment of giardiasis are universally effective and the optimal treatment for 5-nitroimidazole refractory giardiasis is unknown (12). The 87% efficacy found in the 115 patients treated with mebendazole plus secnidazole is therefore encouraging and suggests that the combination can be a highly effective treatment option for 5-nitroimidazole refractory

giardiasis. Mebendazole was chosen because it has previously been shown to be effective and well tolerated for treatment-naive giardiasis in Cuba. It was combined with secnidazole based on data showing that albendazole plus a 5-nitroimidazole was effective in the treatment of 5-nitroimidazole refractory giardiasis and specifically, that the combination of albendazole and metronidazole was far more effective than albendazole alone (11). Nevertheless, the results need to be confirmed in studies on 5-nitroimidazole refractory giardiasis from other parts of the world.

The secnidazole plus mebendazole combination was also chosen due to availability and low cost in Cuba as well as differing mechanisms of action (9). Secnidazole affects electron transport whereas mebendazole appears to exert its antigiardial effects by interaction with tubulin in the cytoskeleton (13). The different modes of action of these drugs may reduce the probability of resistance developing. However, cross-resistance between albendazole and metronidazole has been shown in vitro and combining a failing drug class with a new drug class may not be optimal. Moreover, the data supporting combining mebendazole with a 5-nitroimidazole is based on small studies as discussed above. It would therefore be of value to assess mebendazole monotherapy as well as other treatments such as a 5-day chloroquine treatment regimen, nitazoxanide and quinacrine (that have all been shown to be effective and well tolerated in Cuba) for treatment of 5-nitroimmidazole refractory giardiasis (9,15).

The adverse events, abdominal pain, nausea and vomiting found with secnidazole plus mebendazole did not differ significantly from secnidazole monotherapy, were moderate, transient and self-limiting, in line with previous studies (1,3,9,13). Mebendazole is poorly absorbed from the intestine, generally well tolerated and safe even at the higher dose used in this study (9,13). Similarly, secnidazole has been extensively used at this dosage and shown to be well tolerated. The combination therefore appears to be well tolerated.

To summarize, in a cohort of 456 adult Cuban patients the frequency of metronidazole refractory giardiasis was 48% and repeated treatment with tinidazole and secnidazole was suboptimal. High-dose mebendazole plus secnidazole was well tolerated and cured 87% (100/115) and quinacrine was well tolerated and cured 100% (15/15) of patients with 5-nitroimidazole refractory giardiasis.

IV. CONCLUSIONS

Repeat treatment with 5-nitroimidazoles should be avoided and the combination of mebendazole plus secnidazole is an effective and well-tolerated treatment option for 5-nitroimidazole treatment failures. Treatment with quinacrine is safe and effective.

REFERENCES

1. Einarsson E, Ma'ayeh S, Svard SG. An up-date on *Giardia* and giardiasis. Curr Opin Microbiol. 2016; 34:47-52.
2. Robertson LJ, Hanevik K, Escobedo AA, Morch K, Langeland N. Giardiasis e why do the symptoms sometimes never stop? Trends Parasitol 2010; 26:75-82.
3. Lalle M, Hanevik K. Treatment-refractory giardiasis: challenges and solutions. Infect Drug Resist. 2018; 11:1921333.

4. Rogawski ET, Bartelt LA, Platts-Mills JA, Seidman JC, Samie A, Havt A, et al. Determinants and impact of Giardia infection in the first 2 years of life in the MAL-ED Birth Cohort. J Pediatr Infect Dis Soc. 2017; 6:153-60.

5. Rivero LR, Fernandez FA, Robertson LJ. Cuban parasitology in review: a revolutionary triumph. Trends Parasitol. 2008; 24:440-8.

6. Escobedo AA, Canete R, Nunez FA. Prevalence, risk factors and clinical feature associated with intestinal parasitic infections in children from San Juan y Martinez, Pinar del Rio, Cuba. West Indian Med J. 2008; 57:377-82.

7. Cañete R, Campos Y, Valdes R, Rodriguez P. Prevalence and factors associated with intestinal parasitic infection among school children from Jagüey Grande Municipality in Matanzas Province, Cuba. West Ind Med J. 2017; 66:361-6.

8. Cañete R, Diaz MM, Avalos Garcia R, Laud Martinez PM, Manuel Ponce F. Intestinal parasites in children from a day care centre in Matanzas City, Cuba. PLoS One. 2012:e51394.

9. Cañete R, Brito K, Brito I, Semper A, Gonzalez ME. Effectiveness and tolerability of 3-day mebendazole treatment of Giardia duodenalis infection in adults and children: two prospective, open-label phase IV Trials. Curr Ther Res Clin Exp. 2018; 89:43e7.

10. Ansell BR, McConville MJ, Ma'ayeh SY, Dagley MJ, Gasser RB, Svard SG, et al. Drug resistance in Giardia duodenalis. Biotechnol Adv. 2015; 33:888e901.

11. Nabarro LE, Lever RA, Armstrong M, Chiodini PL. Increased incidence of nitroimidazole-refractory giardiasis at the hospital for tropical diseases, London: 2008-2013. Clin Microbiol Infect. 2015; 21:791-6.

12. Carter ER, Nabarro LE, Hedley L, Chiodini PL. Nitroimidazole-refractory giardiasis: a growing problem requiring rational solutions. Clin Microbiol Infect. 2018; 24:37-42.

13. Almirall P, Escobedo AA, Ayala I, Alfonso M, Salazar Y, Cañete R, et al. Mebendazole compared with secnidazole in the treatment of adult giardiasis: a randomised, no-inferiority, open clinical trial. J Parasitol Res. 2011;2011. 636857.

14. Cañete R, Rodriguez P, Mesa L, Brito K, Prior A, Guilhem D, et al. Albendazole versus metronidazole in the treatment of adult giardiasis: a randomized, double-blind, clinical trial. Curr Med Res Opin. 2012; 28:149-54.

15. Cañete R, Escobedo AA, Gonzalez ME, Almirall P. Randomized clinical study of five days therapy with mebendazole compared to quinacrine in the treatment of symptomatic giardiasis in children. World J Gastroenterol. 2006; 12:6366-70.

16. Leitsch D. Drug resistance in the microaerophilic parasite giardia lamblia. Curr Trop Med Rep. 2015; 2:128-35.

17. Tejman-Yarden N, Millman M, Lauwaet T, Davids BJ, Gillin FD, Dunn L, et al. Impaired parasite attachment as fitness cost of metronidazole resistance in *Giardia lamblia*. Antimicrob Agents Chemother. 2011; 55:4643-51.