Item number	How met?
1 (title and abstract)	(a) Page 3: Abstract includes “retrospective longitudinal study” (b) Page 3: Abstract
Introduction	
2 (background/rationale)	Page 5: Introduction includes scientific background and rationale for the study
3 (objectives)	Page 7: Objectives: Our study aimed to investigate the effect of combined antibiotics administered via enema infusion to eradicate *D. fragilis* and *B. hominis*.
Methods	
4 (study design)	Page 7: A retrospective, single centre longitudinal study during 2017-2018 was conducted on patients 18 years or older, who were positive for *D. fragilis*, *B. hominis* or both and were treated with triple antibiotics (furazolidone 0.9 g, nitazoxanide 3 g and secnidazole 3.6 g) infused over two consecutive days through rectal enema.
5 (setting)	Page 7: Dates: between January 2017 and December 2018. Page 7: Setting/location: All 54 patients who were positive for *D. fragilis*, *B. hominis* or both and were treated with triple antibiotics at our clinic (single centre, day hospital)
6 (participants)	Page 7: Eligibility criteria: Patients 18 years or older, who were positive for *D. fragilis*, *B. hominis* or both and requested treatment. Page 7: Methods of follow up: symptoms, Stool microscopy and polymerase chain reaction (PCR) testing on stool samples at three days, seven days and six weeks after the treatment monitored Patient progress.
7 (variables)	Page 8: Outcomes: symptoms, Stool microscopy and polymerase chain reaction (PCR) testing on stool samples were determined. Results from stool testing showed the presence or absence of parasite post-treatment. Page 8: Exposures: The treatment consisted of triple antibiotics (furazolidone 0.9 g, nitazoxanide 3 g and secnidazole 3.6 g) infused over two consecutive days through rectal enema. In the case of allergy the culprit drug was replaced by paromomycin 4.5 g or diloxanide furoate 4.5 g. Predictors: N/A for this study Potential confounders: previous treatment, concomitant medication Effect modifiers: N/A

	Diagnostic criteria: Stool microscopy and polymerase chain reaction (PCR) testing on stool samples were determined. Results from stool testing showed the presence or absence of parasite post-treatment.
8 (data sources/measurement)	Page 8: symptoms, Stool microscopy and polymerase chain reaction (PCR) testing on stool samples were determined pre- and post-treatment. Results from stool testing showed the presence or absence of parasite post-treatment.
9 (bias)	Efforts to address bias included: - Clear study objective - Explicit inclusion/exclusion criteria - Specified time for patient recruitment - Consecutive patient enrolment - Clinically relevant outcomes, collected prospectively - High follow up rate
10 (study size)	54 patients
11 (quantitative variables)	Page 8: Data was tabulated in Microsoft Excel from patients clinical records and descriptive statistics conducted. A Wilcoxon signed rank was used to assess the difference in symptoms and stool test results before and post-treatment. Statistical significance was set at $p<0.05$.
12 (Statistical methods)	(a) Data was tabulated in Microsoft Excel from patients clinical records and descriptive statistics conducted. Statistical differences between three or more sets of data were analysed using one-way analysis of variance and nonparametric technique, followed by Tukey's multiple comparison post-test if the *P* value was significant. Wilcoxon matched-pairs signed rank test was used to compare the differences between two sets of data. The association between categorical variables were determined using Chi-square and Fisher's exact test. *P* values of < 0.05 were considered significant. (b) N/A for this study (c) Mentioned in Table 2 (d) Mentioned in Table 2 (e) N/A to this study
Results	Page 8-10

13 (participants)	(a) Fifty-four (16 male, 38 female) patients with an age range of 21-81 years (median age 49 years) (b) Participants' non-compliance to complete the final stool testing
14 (descriptive data)	(a) Fifty-four (16 male, 38 female) patients with an age range of 21-81 years (median age 49 years) (b) N/A for this study
15 (Outcome data)	Out of 54 patients, 48 completed a final stool test for investigation of parasite eradication at six weeks post-treatment. Overall 79% of patients cleared the parasites from their faeces at six weeks. There was a significant reduction in abdominal discomfort, dizziness and blood in the stool at both seven days and six weeks post-treatment ($P < 0.040$).
16 (Main results reporting)	N/A for this study
17 (Other analyses)	N/A
Discussion	
18 (key results)	Page 11: First paragraph of discussion
19 (limitations)	The small sample size and inherent limitations of retrospective studies is recognised.
20 (interpretation)	Page 11-14: Discussion section
21 (generalisability)	The results showed a significant achievement in both parasitic eradication and improvement of clinical outcomes which points to the use of combination therapies with an alternative delivery as first line of therapy.
22 (funding)	Title page