In vitro susceptibility of *Blastocystis hominis* isolated from patients with irritable bowel syndrome

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ABSTRACT

This study aims to determine the growth pattern and *in vitro* susceptibility of clinical isolates of *Blastocystis hominis* to different concentrations of metronidazole, furazolidone and ciprofloxacin. Stool specimens from 25 consecutive patients with irritable bowel syndrome presenting to the gastroenterology department of Aga Khan University Hospital between January and May 2003 are examined by microscopy and cultured for *B. hominis*. Drug susceptibility assays are performed for metronidazole, furazolidone, and ciprofloxacin using final concentrations of 0.01 mg/mL and 0.1 mg/mL. The effect of the drugs is assessed after *B. hominis* culture for 48 h. With furazolidone and metronidazole, 68 % (17/25) and 60 % (15/25) of *B. hominis* isolates, respectively, failed to grow at drug concentrations of both 0.01 mg/mL and 0.1 mg/mL. However, ciprofloxacin failed to suppress growth completely at both concentrations. *B. hominis* resistance to furazolidone, metronidazole and ciprofloxacin at 0.01 mg/mL was 32 % (8/25), 40 % (10/25) and 100 % (25/25), respectively. *B. hominis* isolates varied in their degree of susceptibility to the three drugs studied, being greater with furazolidone than with metronidazole, and complete resistance with ciprofloxacin.

KEY WORDS: Blastocystis hominis. Ciprofloxacin. Diarrhea. Furazolidone. Metronidazole.

Introduction

Blastocystis hominis is a common human parasite found in the large intestine. Infection occurs worldwide but is found commonly in the tropics and in developing countries. The pathogenic potential of *B. hominis* in the human intestine is controversial because the organism has been found in both symptomatic and asymptomatic individuals.

Metronidazole is an antimicrobial drug with high activity against anaerobic bacteria and protozoa. Indications for use include *Trichomonas vaginitis*, *Entamoeba histolytica* and *Giardia lamblia* infection. Furazolidone is a bactericidal agent used primarily in the treatment of giardiasis. The drug is also useful in treating diarrhea caused by other infective organisms. Ciprofloxacin is active against both Gram-positive and Gram-negative bacteria, and its use includes the treatment of infections of the gastrointestinal tract such as typhoid fever.

Ciprofloxacin is largely absorbed from the small intestine and is excreted mostly unchanged through the kidneys (55 %) and in faeces (40%). Ciprofloxacin and other fluoroquinolones have become the drugs of first choice in empirical therapy for moderate-to-severe travellers' diarrhoea in adults,[1] and have been used to treat chronic diarrhea caused by *Isospora belli* and *Cyclospora cayetanensis* in human immunodeficiency virus (HIV)-infected patients who cannot tolerate trimethoprim-sulfamethoxazole.[2]

The drug of choice for treating infections caused by these anaerobic protozoa is metronidazole. However, resistance to this agent has been demonstrated in *E. histolytica*, *G. intestinalis* and *T. vaginalis* in both *in vivo* and *in vitro* conditions.[3] Studies have examined the *in vitro* effect of metronidazole and furazolidone against *B. hominis* but little data exist for ciprofloxacin, which is used increasing for enteric pathogens[4].

This study aims to evaluate the *in vitro* effect of three antibiotics in two different concentrations on the *B. hominis* parasite counts at 48 h using a reproducible method described previously. The strains of the parasite used are isolated from the stool specimens obtained from patients with irritable bowel syndrome (IBS).

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Materials and methods

This prospective study was conducted at the Aga Khan University Hospital, Karachi, Pakistan. Stool specimens were collected from 25 (14 males and 11 females) consecutive IBS patients at the gastroenterology department between January and May 2003. History of antibiotic use was taken and stool microscopy and culture for *B. hominis* were performed as described previously.[5]

Microscopy
Briefly, approximately 2 mg faeces was thoroughly emulsified on a glass slide in one drop of physiological saline and covered with a coverslip. A similar preparation was prepared in Lugol's iodine on another slide. Each was examined under low power (x10) and high dry (x 40) objectives.

Culture
Approximately 50 mg faeces was inoculated into 5 mL Jones' medium (without starch), which supports good parasite growth. Cultures were incubated at 37°C and examined after 24, 48, 72 and 96 h. If no *B. hominis* were identified

by the end of this period then the specimen was regarded as negative. The sediment was examined under both low power (x10) and high dry (x40) objectives.

Drugs
Aqueous working solutions of 1 mg/mL metronidazole, ciprofloxacin and furazolidone were prepared and added to the medium to give final concentrations of 0.01 mg/mL and 0.1 mg/mL.

Drug susceptibility assay
In vitro susceptibility assays were performed using a method described previously.[6] Briefly, 250 µL culture containing 250,000 *B. hominis* was added to the medium in each tube and incubated for 48 h at 37°C with each of the two concentrations of metronidazole, furazolidone or ciprofloxacin described above. The effect of the drug was assessed after 48-h incubation by carefully removing 4 mL supernatant medium from each tube without disturbing the culture pellet at the bottom that contained the *B. hominis*. The sediment containing the parasite was then agitated gently to obtain a uniform distribution and *B. hominis* were counted in a Neubauer chamber. The percentage increase or decrease in growth between the control (medium containing *B. hominis* but no drug) and the test tubes was calculated for each strain.

Statistical analysis
Results are expressed as mean ± standard deviation (SD) for *B. hominis* count and number (percentage). Univariate analysis was performed using analysis of variance to compare the effect of drugs. Post-hoc Tukey's test was used for multiple comparisons among the different drugs. These analyses were carried out using the SPSS statistical software (release 10.0.0, standard version; SPSS, 1989–99).

Results

Furazolidone
In 68 % (17/25) of cases, *B. hominis* did not grow in culture with furazolidone at a concentration of 0.01 mg/mL and 0.1 mg/mL (Fig. 1). In 32 % (8/25), the drug suppressed *B. hominis* growth by means of 19 ± 6 and 39 ± 12 at concentrations of 0.01 mg/mL and 0.1 mg/mL, respectively (Figs. 2–4). Thus, by increasing the concentration of furazolidone from 0.01 mg/mL to 0.1 mg/mL, mean inhibition increased by a factor of two.

Metronidazole
In 60 % (15/25) of cases, *B. hominis* did not grow in cultures containing metronidazole at either concentration (Fig. 1). In 40 % (10/25) of cases, it suppressed growth of *B. hominis* by means of 54 ± 15 and 19 ± 6 at concentrations of 0.1 mg/mL and 0.01 mg/mL, respectively (Figs. 2–4). Thus, by increasing the concentration of metronidazole from 0.01 mg/mL to 0.1 mg/mL, mean inhibition increased by a factor of three.

Ciprofloxacin
Ciprofloxacin failed to completely suppress the growth of *B. hominis* isolates (Fig. 1). At concentrations of 0.1 mg/mL and 0.01 mg/mL, ciprofloxacin suppressed the growth of *B. hominis* isolates by means of 240 ± 46 and 47 ± 7, respectively (Fig 2–4). Thus, by increasing the concentration of ciprofloxacin from 0.01 mg/mL to 0.1 mg/mL, mean inhibition increased by a factor of five.

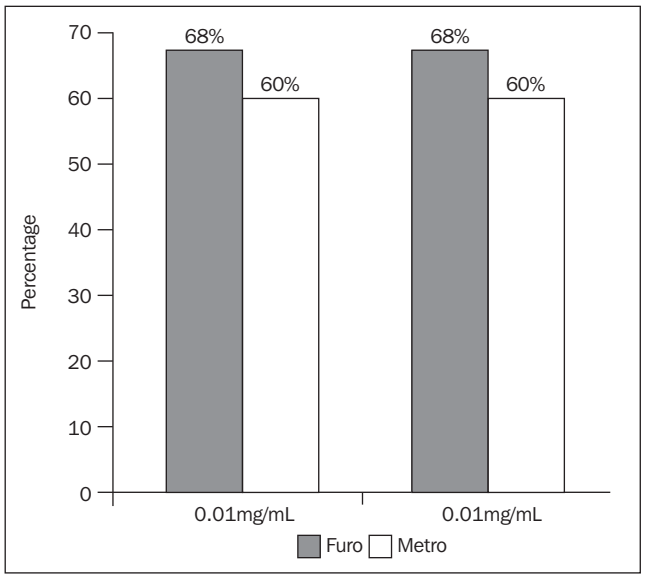

Fig. 1. Effect of 0.01 mg/mL furazolidone (Furo) and metronidazole (Metro) on *B. hominis*.

Comparison of the drugs
The effect of ciprofloxacin at 0.01 mg/mL on *B. hominis*, when compared to baseline growth, was not significant (P = 0.999). At a similar dose level, metronidazole and furazolidone gave P values of 0.014 and <0.001, respectively. At a doses of 0.1 mg/mL, ciprofloxacin showed a significant effect compared to baseline (P <0.001) but not when compared to the effect of metronidazole and furazolidone (P = 0.999). Metronidazole and furazolidone at concentrations of 0.01 mg/mL and 0.1 mg/mL showed significantly better effect compared to baseline growth and ciprofloxacin at 0.01 mg/mL, but not to each other (Figs. 2–4).

Discussion

B. hominis has been associated with diseases in both immunocompetent and immunocompromised subjects.[1,7] These patients are treated with metronidazole when *B. hominis* is suspected of causing disease. Currently, metronidazole is the drug of choice for treating protozoal infection.

Furazolidone is another drug commonly used for the same indication. However, *in vitro* efficacy of ciprofloxacin against *B. hominis* has not been established or compared with that of metronidazole and furazolidone.

In the present study, the viable cell count method described previously provided a reliable means to determine the activity of the three drugs against clinical isolates of *B. hominis*.[6] The ability of *B. hominis* isolates to grow at a concentration of 0.01 mg/mL demonstrated that these drugs had no effect on the parasite and that they were resistant to the drugs (32 % to furazolidone, 40 % to metronidazole and 100 % to ciprofloxacin). This is consistent with previous reports in which *B. hominis* trophozoites and cysts demonstrated resistance to metronidazole.[8,9]

The results of the present study are consistent with

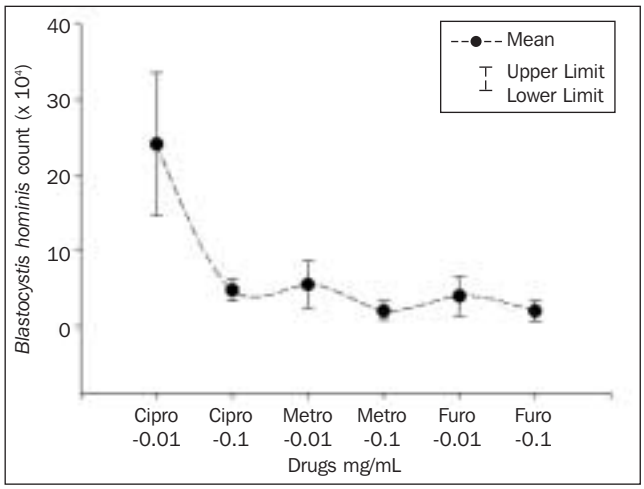

Fig. 2. Effect of ciprofloxacin (Cipro), furazolidone (Furo) and metronidazole (Metro) in different concentrations on *B. hominis*.

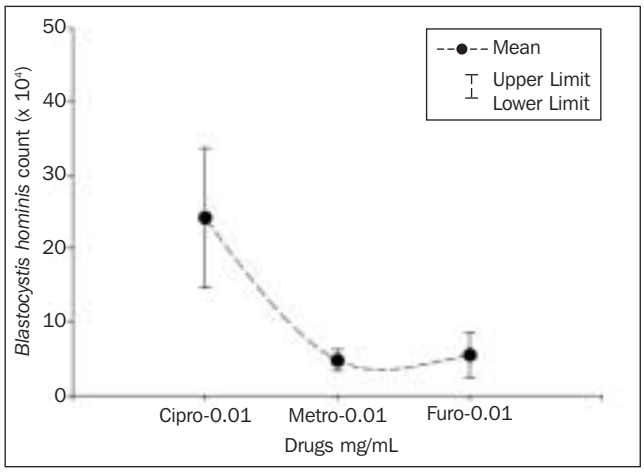

Fig. 3. Effect of 0.01 mg/mL ciprofloxacin (Cipro), furazolidone (Furo) and metronidazole (Metro) on *B. hominis*.

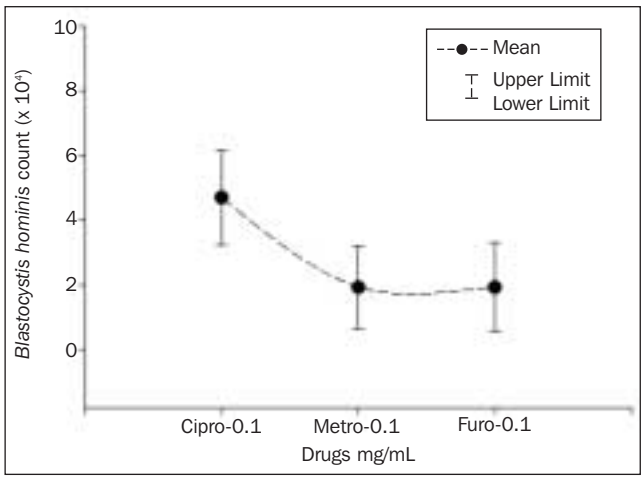

Fig. 4. Effect of 0.1 mg/mL ciprofloxacin (Cipro), furazolidone (Furo) and metronidazole (Metro) on *B. hominis*.

Haresh *et al*., who demonstrated metronidazole resistance in Indonesian and Malaysian isolates of *B. hominis*.[8] Possible mechanisms for apparent failure include indiscriminate use of these drugs in society, inadequate dosage, patient non-compliance and inactivation of the drug by the normal bacteria flora.

In the present study, metronidazole and furazolidone proved most potent against *B. hominis*, while ciprofloxacin showed a dose-dependent inhibitory effect on the growth of the trophozoites. However, the nitrofuran furazolidone showed a more consistent *in vitro* blastocidal effect than did metronidazole.

The *B. hominis* strains used in the present study were not stored and thus reflected the parasites' natural susceptibilities. Metronidazole-resistant *B. hominis* infection does occur and treatment with furazolidone may prove more effective in such cases. If a protozoal cause of diarrhoea is suspected, ciprofloxacin use should be reviewed if symptoms persist.

In conclusion, this study showed that *B. hominis* isolates vary in their susceptibility to metronidazole, furazolidone and ciprofloxacin. Clearly, further research is needed to find a drug that will eliminate *B. hominis* efficiently

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