Revista Latinoamericana de Microbiología

Volumen *Volume* **43** **Número** *Number* **4** **Octubre-Diciembre** *October-December* **2001**

Artículo:

Clinical significance of the redefinition of the agent of amoebiasis

Revista Latinoamericana de MICROBIOLOGÍA
ALAM

REVIEW ARTICLE

Vol. 43, No. 4
October - December. 2001
pp. 183 - 187

Clinical significance of the redefinition of the agent of amoebiasis

Raj Lucas* and Jacqueline A Upcroft*

ABSTRACT. *Entamoeba histolytica* is the pathogenic species of *Entamoeba* that causes amoebic dysentery and other invasive disease. The morphologically similar species, *E. dispar,* is non-pathogenic and accounts for about 90% of the previously estimated 500 million *E. histolytica* infections world-wide. Because of the recent redefinition of *E. histolytica* and *E. dispar,* and the limited number of drugs available to treat amoebiasis, a new approach to treatment of individuals carrying these parasites is necessary. A meeting of eminent scientists has recently agreed that on no account should prophylaxis against amoebiasis be given, and no treatment without symptoms should be administered. The expense of treating asymptomatic individuals, both monetary and at the risk of over-use of precious drugs, does not appear to be justified. It would seem wise that we preserve currently effective anti-amoebic drugs and avoid the development of drug-resistant *E. histolytica.*

Key words: *Entamoeba histolytica, Entamoeba dispar,* metronidazole, amoebiasis, anti-amoebic drugs, metronidazole resistance.

RESUMEN. *Entamoeba histolytica* es la especie patógena de *Entamoeba* que causa la disentería amibiana y otras enfermedades invasivas. La especie morfológicamente similar *E. dispar* es no patógena y da razón del 90% de los estimados previamente 500 millones de infecciones a lo largo del mundo. Debido a la reciente redefinición de *E. histolytica* y *E. dispar,* y el limitado número de drogas disponibles para tratar la amibiasis, una nueva aproximación al tratamiento de individuos que albergan estos parásitos es necesaria. Una reciente reunión de científicos eminentes estuvieron de acuerdo en que en ningún caso la profilaxis contra amibiasis debe de ser dada y que ningún tratamiento sin síntomas debe ser administrado. El gasto del tratamiento de individuos asintomáticos, tanto monetario como por el riesgo de un sobreuso de las valiosas drogas, no parece estar justificado. Parece deseable que preservemos la actual efectividad de las drogas antiamibianas y evitemos el desarrollo de *E. histolytica* resistente a drogas.

Palabras clave: *Entamoeba histolytica, Entamoeba dispar,* metronidazol, amibiasis, drogas antiamibianas, resistencia al metronidazol.

INTRODUCTION

Invasive amoebiasis is one of the world's most prevalent and fatal infectious diseases. Primarily a problem of the developing world, around 500 million people are infected worldwide while 75,000 die of the disease annually. Behind malaria and schistosomiasis, amoebiasis ranks third on the list of parasitic causes of death worldwide.[1-3]

There are four species of the protozoan genus *Entamoeba* which are commonly found in the human gastrointestinal tract, 4 namely *E. coli, E. dispar, E. hartmanni* and *E. histolytica. E. histolytica* is the agent of invasive amoebiasis and hence the only one of medical importance.[5] The recent division of *E. histolytica* into non-pathogenic *E. dispar* and pathogenic *E. histolytica*,[6] has rekindled a long dormant hypothesis put forward by the French parasitologist, Emile Brumpt in 1925,[7] who suggested that there could be two morphologically identical, but genetically separate, species of *Entamoeba.* Recent mounting evidence and consensus of opinions indicates that he was correct. We now recognize *E. histolytica* as the only pathogenic species. While the implications of this development have been summarized in the WHO report of a meeting of experts,[8] the WHO/PAHO/UNESCO Consultation on Amebiasis,[9] and by Martinez-Polomo and Espinosa-Cantellо,[10] this review aims to present the background and implications for treatment of amoebiasis.

HISTORY

As far back as 1875, the Russian physician Fedor Lösch identified what he believed to be the causative agent (motile amoebae containing erythrocytes which he named *Amoeba coli*) of a case of dysentery but doubted its lone role in pathogenesis when it failed to produce disease in three of four dogs experimentally inoculated with it (see ref. 11). Kean,[12] however, states of the experiment at that time, that one of three dogs exhibited the disease from which amoebae were retrieved, thus almost completely fulfilling Koch's postulates. In 1886, while in Egypt, Robert Koch identified, and stained for the first time, amoebae from colonic and hepatic lesions but a Greek colleague of his, Stephanos Kartulis, failed to reproduce the disease in rabbits and guinea pigs (see ref. 11). In 1893 the German pair Quincke and Roos identified an important mode of transmission when they described 15 diseased patients who all shared the same drinking source. They also differentiated between one species of non-pathogenic (NP) amoeba which could not phagocytose erythrocytes and a pathogenic (P) one which could. The latter, described meticulously by Councilman and Lafleur was

* The Queensland Institute of Medical Research and the Medical School, University of Queensland, Herston Rd, Brisbane, Australia.

Rev Latinoam Microbiol 2001; 43 (4): 183-187

referred to as *E. dysenteriae* at the time (see ref. 11). The great protozoologist Schaudinn, omitting the work of Quinke and Roos, also described the differences between the two organisms and renamed them *E. histolytica* and *E. coli* (see ref. 12). A later study by Walker 13 demonstrated that oral ingestion of *E. dysenteriae* cysts by human volunteers led to invasive disease while *E. coli* cysts did not; a conclusive argument, one would think but one which was not given deserving attention, especially after the discovery of *Shigella* in 1898 as another cause of dysentery in man.

In 1925, Brumpt proposed that humans can be infected by two morphologically identical species of *Entamoeba* producing quadrinucleate cysts measuring 10 μm or greater in diameter;[7] the P organism was identified as *E. dysenteriae* and Brumpt named the NP one *E. dispar*. However, due to difficulties in proving the existence of two visually similar yet pathogenically different species, and because of human and animal studies suggesting that amoebae from asymptomatic carriers could produce disease,[14-16] Brumpt's hypothesis gained little favour. It took almost 70 years and the advent of modern biochemical techniques to re-establish Brumpt's idea.

In 1973, P and NP species were differentiated by their relative agglutinability with the lectin Con A.[17] This was followed in 1978 by the discovery that pathogenicity could also be correlated with the enzymatic profile of the organism. Sargeaunt and colleagues[18] used thin-layer starch-gel electrophoresis to separate isoenzyme variants of the glycolytic enzymes glucosephosphate isomerase (GPI), phosphoglucomutase (PGM) and L-malate: NADP oxidoreductase and classified 85 stocks of *E. histolytica* into four groups according to their enzyme profile. Enzyme Group II, which contained a faster migrating band of PGM, was found in all cases of clinical amoebiasis but was not found in any asymptomatic individuals, although Sargeaunt pointed out that in a larger study such persons (in a preclinical state of disease) may be found. Diamond and Clark[5] presented a host of data to confirm the existence of *E. histolytica* and *E. dispar*, and to honour the hypothesis put forward by Brumpt. Biochemically, they cited thousands of samples which correlated zymodeme class to pathogenicity.

Monoclonal antibodies have also provided much immunological evidence, demonstrating the difference in surface antigens between the two species[19,21] but genetic evidence has provided the most compelling argument, with DNA probes distinguishing between differing gene sequences and divergence in amino acid sequences.[22]

STRUCTURE AND LIFE-CYCLE OF *E. HISTOLYTICA*

E. histolytica exists in two forms: the motile and invasive trophozoite and an infective cyst. The diameter of the trophozoite varies between 10 and 60 μm; its variability is affected by changes in temperature, pH, osmolarity and redox potential, as well as feeding conditions actively invading amoebae tend to be larger.[4] The cyst has been far less studied than the trophozoite since encystation has proved difficult in axenic cultures. In the past, the presence of cysts in the stool has been used as a diagnostic tool for amoebiasis.

Cysts can remain viable outside the body for several days and infection usually occurs by ingestion of water or food contaminated by faecal matter. The cyst wall is dissolved in the upper gastrointestinal tract and the organism excysts in the terminal ileum, giving rise to 8 uni-nucleated trophozoites. Trophozoites of *E. histolytica* as the name suggests, are one of the most powerful tissue invaders known. Once penetration of the intestinal mucosa is achieved, dissemination to other organs, usually the liver, can occur. Trophozoites which dwell in the colon multiply, encyst and are passed in the stool from where further spread is possible.[4]

PATHOLOGY AND CLINICAL MANIFESTATIONS

E. histolytica causes pathology by invading intestinal epithelium and producing intestinal lesions which may later spread to extraintestinal sites. Four intestinal forms of amoebiasis have been described. Amoebic dysentery, also known as amoebic colitis, accounts for c.90% of intestinal amoebiasis. Clinical presentation is usually subacute and of less than one month's duration, with symptoms ranging from mild diarrhoea to classic dysentery.[3] Dysentery is an inflammatory condition of the (usually lower) intestine accompanied by abdominal pain, tenesmus and frequent stools containing both blood and mucus.[4] Fever and systemic manifestations are generally absent and the clinical course is moderate, with symptoms disappearing rapid with treatment. The remaining three forms tend to have a rapid course and are very severe conditions requiring immediate medical care. Fulminating amoebic colitis consists of widespread necrotic ulcerous lesions which may perforate and lead to peritonitis. Amoebic appendicitis is similar to its bacterial counterpart and amoeboma is a pseudotumoural lesion whose formation is associated with necrosis, inflammation and oedema of both mucosa and submucosa of the colon.[4] Extraintestinal amoebiasis, brought about by haematogenous spread of trophozoites, can infect the liver, brain, lung, skin and rarely genitourinary structures[3] but amoebic liver abscess is by far the most common complication. Single or multiple abscesses are formed by local necrosis and liquefaction.[23,24]

EPIDEMIOLOGY

While *E. histolytica* has been found in mammals such as cats, dogs and primates, man is thought to be the only important reservoir. Eichinger[25] goes as far as saying that there is no zoonotic reservoir of *E. histolytica* and the absence of having

Rev Latinoam Microbiol 2001; 43 (4): 183-187

to conform to multiple hosts has resulted in a simple life cycle involving only two stages. Amoebiasis has been described as a third world disease due to its high prevalence in poor countries. Low standards of hygiene and sanitation, in particular those related to crowding, contamination of food and water, and inadequate disposal of faeces, are all high risk factors for infection with *E. histolytica*.[4] While *E. histolytica* is found worldwide, the highest prevalence rates are in developing countries and regions such as the Indian subcontinent, parts of central and South America, and tropical regions of Africa.[3]

Amoebic liver abscess is more frequent in males than females[4,24] and 10 times more common in adults than children.[26] When prevalence of *Entamoeba* alone is considered, the 5-14 year age group was most affected as shown in a study in the Philippines where 1872 individuals were sampled, with a prevalence rate of 1% for *E. histolytica* and 7% for *E. dispar*.[27]

In Australia, *E. histolytica/dispar* has been found in 8% of 62 Aboriginal children examined in one study 28 but was not present in 1683 Western Australian Aboriginals.[29] One group found to have an increased prevalence of *E. histolytica/dispar* compared to the general population is homosexual males.[30] In their study of 128 Sydney homosexual men, Law et al.[30] found that 37% were infected with *E. histolytica* but that there was no evidence of pathogenicity, nor any association with GI symptoms. This led them to believe the organisms found were NP *(E. dispar)*, a conclusion supported by Sargeaunt[31] and Allason-Jones et al.[32] who found the majority of *E. histolytica* isolates from homosexual males to be of the NP zymodeme.

STOOL DETECTION TESTS

The diagnosis of intestinal amoebiasis primarily relies on the detection of parasites in the stools, but the epidemiological implications of *E. histolytica versus E. dispar* infection can be fully realized only if there are methods to distinguish the two species among large numbers of samples. The "simplest" (in terms of technology) form of detection is light microscopy. Gonzalez-Ruiz et al.[33] used the observation of erythrophagocytic trophozoites in the case of bloody diarrhoea as a marker of *E. histolytica* infection and found this method to be 100% specific (using isoenzyme electrophoresis as the reference).

Haque et al.[19] using a stool ELISA based on monoclonal antibodies to the galactose-specific adhesin of *E. histolytica*, found that this method was 97% specific and 100% sensitive in a small (12P and 22NP) number of samples. They also stated their disapproval of microscopy on the grounds that three or more separate stool samples were frequently required for detection.

Troll et al.[34] found that their PCR-based detection of *E. histolytica* and *E. dispar* had good sensitivity and specificity as long as fresh faecal samples were used. They propose the assay as a complementary test to microscopy in special patients such as pregnant women and the immunocompromised in whom it would be important to differentiate *E. histolytica* from *E. dispar*. Other reports of successful PCR assays include that of Rivera and colleagues[27] and Britten et al.[35] who successfully used defined primer sets to determine the prevalence of *E. histolytica* and *E. dispar* in formalin fixed stool specimens. Although inexpensive assays to distinguish *E. histolytica* and *E. dispar* in a clinical environment are not commercially available as yet, several tests are under development.[8,9,35]

ANTI-AMOEBIC DRUGS

Anti-amoebic drugs may be classified into three groups: luminal, tissue and mixed amoebicides. The drugs of choice belong to the latter and include metronidazole and their nitroimidazole derivative analogues, tinidazole and omidazole; these drugs are not only more effective therapeutically, than any others, but also have the advantage of oral administration.

The introduction of nitroheterocyclic drugs in the late 1950's and 1960's heralded a new era in the treatment of Gramm negative and positive bacteria and a range of pathogenic protozoan parasites. The antibiotic, azomycin (a 2-nitroimidazole compound) isolated in Japan from a streptomycete, was the first active nitroimidazole to be discovered[36] and acted as the main impetus for the systematic search for drugs with activity against anaerobic protozoa. This lead to the synthesis of the 5-nitroimidazole, metronidazole (1-??-hydroxyethyl-2-methyl-5-nitroimidazole), and the demonstration of activity against *Trichomonas vaginalis* by Cosar and Julou.[37] Subsequently metronidazole was shown to cure giardiasis,[38] amoebiasis[39] and *Balantidium*[40] infections. Metronidazole is now the most widely used drug in the treatment of anaerobic protozoan parasitic infections by *T. vaginalis*, *Giardia duodenalis* and *Entamoeba histolytica*.[41] It is remarkably safe compared with the toxic amoebicide, emetine,[42] and is the recommended drug to treat amoebiasis.

Metronidazole and the related nitroimidazole, tinidazole (which is not available in some countries), are also the only effective drugs for the treatment of trichomoniasis and are the drugs of choice to treat giardiasis.[43] In the latter cases clinical resistance to these drugs has been documented.[43-45]

Laboratory induced metronidazole resistance in *E. histolytica* has been reported where metronidazole-resistant *E. histolytica* was maintained indefinitely in medium containing 1.7 mg/l.[46] While serum concentrations of metronidazole reach a maximum of 17 mg/l with recommended treatment regimes the concentration within abscesses is likely to be less than this and for only a few days duration.

In the event of overt clinical resistance to metronidazole in the anaerobic protozoa, and while vaccine development,

Rev Latinoam Microbiol 2001; 43 (4): 183-187

at least for amoebiasis, is still in progress,[47] there is no acceptable alternative to treat either invasive amoebiasis, or *Trichomoniasis*, keeping in mind documented cross-resistance between currently used and experimental nitroimidazole drugs[41,48] and world-wide availability. Recent evidence indicates that once resistance among micro-organisms has developed, contrary to hopes and expectations, microbes will remain resistant long after doctors stop prescribing the drugs.[49] It is therefore imperative that every step is taken to, preserve our most precious drugs, metronidazole included.

Diloxanide furoate, diiodohydroxyquin and paromomycin are examples of luminal amoebicides with the well tolerated diloxanide furoate the mainstay for treatment of asymptomatic cyst carriers.[50,51] In the case of liver abscess, chloroquine which accumulates in high concentrations in tissues, may be used in addition to metronidazole.[52,53]

TREATMENT

All symptomatic patients with bloody stools containing motile trophozoites with ingested erythrocytes should be treated according to the severity of the disease. Severe cases may require surgery but for the majority of adult cases 7-10 days of 750 mg tid metronidazole will be sufficient.[53,54] Since metronidazole is routinely used to treat amoebiasis, on no account should it be used prophylactically.[55] Sargeaunt[56] is quite adamant that only *E. histolytica* is pathogenic and that the nonpathogenic *E. dispar* is stable, thus removing the need to treat *E. dispar* carriers. However, Diamond and Clark[5] caution against withholding treatment to asymptomatic individuals since *E. histolytica* cysts have been found in such persons. Gatti et al.[57] describe a case in Italy where they believe an asymptomatic Filipino housemaid transmitted the *E. histolytica* to the family employed by her, resulting in five cases of morbidity and one of mortality.

Similarly to, Sargeaunt, Burchard[58] advocates treatment only for *E. histolytica* but in the absence of differentiation advises all cyst-passers should be treated. Further, an analysis of the cost-effectiveness of treating *E. histolytica/E. dispar* cyst carriers was carried out in Mexico using a control (placebo) group and a treatment (metronidazole) group.[59] Results showed an absence of disease in the placebo group, an early acquisition of the carrier state in the treated group and a small difference in months free of the carrier state. The authors concluded that the high cost of treating these "patients" could be far better utilized in preventing amoebic disease. Cost in dollars cannot be the only consideration the cost of losing precious drugs, such as metronidazole, as a result of drug resistance to inappropriate or over-use must be avoided. If treatment of asymptomatic patients is considered necessary, diloxanide furoate should be used.

CONCLUSION

The redefinition of *E. histolytica* into two separate species, NP *E. dispar* and P *E. histolytica*, means that only patients carrying *E. histolytica* should be considered for treatment and that prophylactic treatment should not be given. Some experts go one step further and recommend no treatment without symptoms, including bloody stools and invasive disease. In light of the fact that there is only one family of drugs recommended for the treatment of amoebiasis and that cross resistance between drugs within this family is well documented, it would seem wise that we follow the latter recommendation. In the event of overt clinical resistance to the 5-nitroimidazole drugs in *E. histolytica* (and resistance to metronidazole has been induced in laboratory maintained *E. histolytica)* there is no safe alternative for treatment of the millions of sufferers of symptomatic amoebiasis.

REFERENCES

1. Walsh, J.A. 1984. Estimating the burden of illness in the Tropics. In: Warren KS, Mahmoud AAF, eds. Tropical and Geographical Medicine. New York: McGraw-Hill. 1073-85.
2. Walsh, J.A. 1986. Problems in recognition and diagnosis of amebiasis: Estimation of the global magnitude of morbidity and mortality. Rev. Infect. Dis. 8:228-38.
3. Li, E., Stanley, S.L. Jr. 1996. Protozoa: Amebiasis. In: Parasitic Diseases of the Liver and Intestines. Gastroenterol. Clin. N. Am. 25:471-92.
4. Martinez-Palomo, A., Espinosa-Cantellano, M. 1998. Intestinal amoebae. In: Cox FEG, Kreier JP, Wakeline D, eds. Topley and Wilson's Microbiology and Microbial Infections. Vol. 5, (9th ed.). 157-177.
5. Diamond, L.S., Clark, C.G. 1993. A redescription of *Entamoeba histolytica* Schaudinn, 1903 (Emended Walker, 1911) separating it from Entamoeba dispar Brumpt, 1925. J Euk. Micro. 40:340-4.
6. Clark, C.G. 1998. Amoebic disease: Entamoeba dispar, an organism reborn. Trans. R. Soc. Trop. Med Hyg. 92:361-4.
7. Brumpt, E. 1925. Étude sominaire de 1 "*Entamoeba dispar*" n sp Amibe à kystes quadrinucléés, parasite de l'homme. Bull. Acad Méd. (Paris) 94:943-52.
8. WHO news and activities. 1997. Entamoeba taxonomy. Bola. WHO. 75:291-2.
9. WHO/PAHO/UNESCO report. 1997. A consultation with experts on amoebiasis, Mexico City, Mexico 28-29 January 1997. Epidem. Bull./PAHO. 18:13-14.
10. Martinez-Palomo, A., Espinosa-Cantellano, M. 1998. Amoebiasis: new understanding and new goals. Parasitol. Today. 14: 1-3.
11. Guarner, V. 1990. History of amebiasis. In: Kretschmer RR, ed. Amebiasis. Infection and Disease by *Entamoeba histolytica*. USA: CRC Press Inc. 1-10.
12. Kean, B.H. 1988. A history of amebiasis. In: Ravdin JIR, ed. Amebiasis: Human Infection by *Entamoeba histolytica.* USA: Churchill Livingstone, New York. 1-10.
13. Walker, E.L., Sellards, A.W. 1913. Experimental entamoebic dysentery. Philipp. J Sci. Trop. Med. 8:253.
14. Kessel, J.F. 1928. Amoebiasis in kittens infected with amoebae from acute and "carrier" human cases and with the tetranucleate amoebae of the monkey and the pig. Am. J Hyg. 8: 311-55.
15. Meleny, H.E., Frye, W.W. 1936. The pathogenicity of *Endamoeba histolytica.* Trans. R. Soc. Trop. Med. Hyg. 29: 369-79.

16. Meleny, H.E., Frye, W.W. 1937. The pathogenicity of four strains of *Entamoeba histolytica* from Chicago. Am. J. Digest. Dis. Nutrit. 4:37-40.
17. Martinez-Palomo, A., Gonzales, R.A., de La Torre, M. 1973. Selective agglutination of pathogenic strains of *E. histolytica* induced by Con A. Nature New. Biol. 245:186-7.
18. Sargeaunt, P.G., Williams, J.E., Grene, J.D. 1978. The differentiation of invasive and non-invasive *Entamoeba histolytica* by isoenzyme electrophoresis. Trans. R. Soc. Trop. Med Hyg. 72:519-21.
19. Haque, R., Kress, K., Wood, S. et al. 1993. Diagnosis of pathogenic *Entamoeba histolytica* infection using a stool ELISA based on monoclonal antibodies to the galastose-specific adhesin. J. Infec. Dis. 167:247-9.
20. Marinets, A., Zhang, T., Guillén, N. et al. 1997. Protection against invasive amoebiasis by a single monoclonal antibody directed against a lipophosphoglycan antigen localized on the surface of *Entamoeba histolytica.* J. Exp. Med. 186:1557-65.
21. Petri, W.A. Jr, Jackson, T.F., Gathiram, V. et al. 1989. Pathogenic and nonpathogenic strains of *Entamoeba histolytica* can be differentiated by monoclonal antibodies to the galactose-specific adherence lectin. Infect. Immun. 58:1802-6.
22. Ortner, S., Clark, C.G., Binder, M., Scheiner, O., Wiedermann, G., Duchêne, M. 1997. Molecular biology of the hexokinase isoenzyme pattern, that distinguishes pathogenic *Entamoeba histolytica* from nonpathogenic *Entamoeba dispar.* Mol. Biochem. Parasitol. 86:85-94.
23. Panosian, C.B. 1988. Parasitic diarrhea. Infect. Dis. Clinics. N. Am. 2:685-703.
24. Seeto, R.K. 1999. Amebic liver abscess: epidemiology, clinical features, and outcome. West. J Med. 170:104-9.
25. Eichinger, D. Encystation of entamoeba parasites. Bio Essays 1997; 19:633-9.
26. Sepúlveda, B., Treviño-García, M.N. 1986. Clinical manifestations and diagnosis of amoebiasis. In: Martinez-Palomo A, ed. Amebiasis. Amsterdam: Elsevier. 169-88.
27. Rivera, W.L., Tachibana, H., Kanbara, H. 1998. Field study on the distribution of *Entamoeba histolytica* and *Entamoeba dispar* in the northern Philippines as detected by the polymerase chain reaction. Am. J Trop. Med Hyg. 59:916-21.
28. Best, J.C., Welch, J.S., Filippich, C., McPhee, L. 1976. Treatment of intestinal parasites in Australian aboriginal children. Med J Aust. 1:14-20.
29. Jones, H.I. 1980. Intestinal parasite infections in Western Australian Aborigines. Med J. Aust. 2:375-80.
30. Law, C.L., Walker, J., Qassim, M.H. 1991. Factors associated with the detection of *Entamoeba histolytica* in homosexual men. Int. J STD AIDS. 2:346-50.
31. Sargeaunt, P.G. 1987. The reliability of *Entamoeba histolytica* zymodemes in clinical diagnosis. Parasitol. Today. 3:40-43.
32. Allason-Jones, E., Mindel, A., Sargeaunt, P., Williams, P. 1986. *Entamoeba histolytica* as a commensal intestinal parasite in homosexual men. N. Engl. J Med. 315:353-6.
33. Gonzalez-Ruiz, A., Haque, R., Aguirre, A. et al. 1994. Value of microscopy in the diagnosis of dysentery associated with invasive *Entamoeba histolytica.* J Clin. Pathol. 47:236-9.
34. Troll, H., Marti, H., Weiss, N. 1997. Simple differential detection of *Entamoeba histolytica* and *Entamoeba dispar* in fresh stool specimens by sodium acetate-acetic acid-formalin concentration and PCR. J Clin. Microbiol. 35:1701-5.
35. Britten, D., Wilson, S.M., McNerney, R., Moody, A.H., Chiodini, P.L., Ackers, J.P. 1997. An improved colorimetric PCR-based method for detection and differentiation of *Entamoeba histolytica* and *Entamoeba dispar* in feces. J Clin. Microbiol. 35:1108-11.
36. Maeda, K., Osata, T., Umezawa, H. 1953. A new antibiotic, azomycin. J Antibiot. A 6:182.
37. Cosar, C., Julou, L. 1959. Activité de l'(hydroxy-2-éthyl)- 1 -méthyl-2-nitro-5 imidazole (8,823 R.P.) vis-à-vis des infections expérimentales *Trichomonas vaginalis.* Ann. Inst. Pasteur 96: 238-41.
38. Scheider, J. 1961. Traitment de la giardiase (lambliase) par le métronidazole. Bull. Soc. Path. Exot. 54:84-93.
39. Powell, S.J., Macleod, L., Wilmot, A.J., Elsdon-Dew, R. 1966. Metronidazole in amoebic dysentery and amoebic liver abscess. Lancet 2:1329-31.
40. Garcia-Leverde, A., Bonilla, L. 1975. Clinical trial with metronidazole in human balantidiasis. Am. J Trop. Med Hyg. 24:781-3.
41. Townson, S.M., Boreham, P.F.L, Upcroft, P., Upcroft, M. 1994. Resistance to the nitroheterocyclic drugs. Acta Tropica. 56:173-194.
42. Knight, R. 1980. The chemotherapy of amoebiasis. J. Antimicrob. Chemother. 6:577-93.
43. Upcroft, J.A., Upcroft, P. 1993. Drug resistance and *Giardia*. Parasitol. Today. 9:187-190.
44. Johnson, P.J. 1993. Metronidazole and drug resistance. Parasitol. Today 9:183-6.
45. Voolmann, T., Boreham PFL. 1993. Metronidazole resistant Trichomonas vaginalis in Brisbane. Med. J Aus. 159:490.
46. Samarawickrema, N.A., Brown, D.M., Upcroft. J.A., Thammapalerd, N., Upcroft, P. 1997. Involvement of superoxide dismutase and pyruvate: ferredoxin oxidoreductase in mechanisms of metronidazole resistance in *Entamoeba histolytica*. J Antimicrob. Chemother. 40: 833-40.
47. Stanley, S.L. Jr. 1997. Progress towards development of a vaccine for amoebiasis. Clin. Microbiol. Rev. 10:637-49.
48. Upcroft, J.A., Campbell, R.W., Benakli, K., Upcroft, P., Vanelle, P. 1999. Efficacy of new 5-nitroimidazoles against metronidazole-susceptible and –resistant *Giardia, Trichomonas,* and *Entamoeba spp.* Antimicrob. Agents Chemother. 43:73-6.
49. Morel, V. 1997. Antibiotic resistance: road of no return. Science. 278: 575-76.
50. Di Perri, G., Strosselli, M., Rondanelli, E.G. 1989. Therapy of entamoebiasis. J. Chemother. 1:113-22.
51. Qureshi, H., Ali, A., Baqai, R., Ahmed, W. 1997. Efficacy of a combined diloxanide-furoate-metronidazole preparation in the treatment of amoebiasis and giardiasis. J Int. Med. Res. 25:167-70.
52. Guarner, V. 1990. Treatment and prevention of amoebiasis. In: Kretschmer RR, ed. Amoebiasis: Infection and Disease by *Entamoeba histolytica*. USA: CRC Press Inc. 221-38.
53. Markell, E.K., Voge, M., John, D.T. 1992. Lumen-dwelling protozoa. In: Medical Parasitology. Seventh Edition. Philadelphia USA: Saunders WB. 22-41.
54. Freeman, C.D., Klutman, N.E., Lamp, K.C. 1997. Metronidazole: a therapeutic review update. Drugs. 54:679-708.
55. Harger, W.D., Rapp, R.P. 1992. Metronidazole. Obstet. Gynecol. Clin. N. Am. 19:497-510.
56. Sargeaunt, P.G. 1994. *Entamoeba histolytica* and *E. dispar*. Trans. R. Soc. Trop. Y Med. Hyg. 88:254.
57. Gatti, S., Cevini, C., Bruno, A., Novati, S., Scaglia, M. 1995. Transmission of *Entamoeba histolytica* within a family complex. Trans. R. Soc. Trop. Med Hyg. 89: 403-5.
58. Burchard, G.D. 1994. Therapy for malaria and amoebiasis. Immun. Infect. 22: 45-7.
59. Garduño-Espinosa, J., Martínez-García, M.C, Valadéz-Salazar, A., Padilla, G., Cedillo-Rivera, R., Muñoz, O. 1997. Cost-effectiveness analysis of treatment of *E. histolytica/E. dispar* cyst carriers. Arch. Med Res. 28: S293-4.

Correspondence to:

Jacqueline A Upcroft
Queensland Institute of Medical Research, PO Royal Brisbane Hospital, Qld 4029, Australia
E-mail: <jacquiU@qimr.edu.au>