Effect of Metronidazole and Secnidazole against clinical isolates of *E. Bangladeshi* collected from Mirpur area, Dhaka city, Bangladesh

A Dissertation Submitted to the Department of Pharmacy, East West University, as the Partial Fulfillment of the Requirements for the Degree of Master of Pharmacy.

Submitted by

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Declaration by the Research Candidate

I, Md. Ariful Islam, hereby declare that this dissertation entitled “Effect of Metronidazole and Secnidazole against clinical isolates of *E. Bangladeshi* collected from Mirpur area, Dhaka city, Bangladesh” the Department of Pharmacy, East West University, in partial fulfillment for therequirement of the Degree of Master of Pharmacy, is an authentic research work done byme under the guidance of Professor Dr. Sufia Islam, Department of Pharmacy, East WestUniversity, Dhaka Bangladesh. The content of this dissertation in full or in parts, have notbeen submitted to any other Institution or University for the award of any Degree or anyDiploma of Fellowship.

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Certificate by the Supervisor

This is to certify that the dissertation, entitled "Effect of Metronidazole and Secnidazole against clinical isolates of *E. Bangladeshi* collected from Mirpur area, Dhaka city, Bangladesh" is a bonafide research work done under our guidance and supervision by Md. Ariful Isam (ID # 2016-3-79-003), in partial fulfillment for the requirement of the Degree of the Master of Pharmacy.

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Dedication

This research paper is dedicated to my Beloved Parents

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Abstract

Infectious diseases continue the major cause of morbidity and mortality worldwide including Bangladesh. *Entamoeba histolytica* is the etiological agent of amoebic dysentery and amoebic liver abscess (ALA). Worldwide, 40–50 million symptomatic cases of amoebiasis occur annually and 70,000 to 100,000 deaths are due to this infection. In amoebiasis Metronidazole, a 5-nitroimidazole drug is the drug of choice for intestinal disease caused by *Entamoeba histolytica*. In 2010-2011 a new species of Entamoeba named *E. bangladeshi* has been identified, which was positive for Entamoeba organism but negative for *E.histolytica*, *E. dispar*and *E. moshkovskii* by PCR. It was found in the feces of children living in Mirpur, Dhaka, Bangladesh. Till now no studies have been carried out to determine the Sensitivity of Metronidazole and Secnidazole against this new species. Therefore, the objective of the study is to determine the sensitivity of Metronidazole and Secnidazole against clinical isolates of *E. bangladeshi*. The clinical isolates of *E. Bangladeshi* were treated with Metronidazole and Secnidazole at different concentrations (2.56, 1.28, 0.64, 0.32, 0.16, 0.08, and 0.04 μg/ml). Drug sensitivity assay of the samples was carried out by using microtiter plates containing 100 μg/ml of parasite. Plates were incubated at 37^0C. After 4 hours incubation, the viable parasites in Control were $6.3X10^6$ and$7.05X10^6$ for testing the Metronidazole and Secnidazole respectively. Viable cell counts of *E. Bangladeshi* were $3.2X10^6$, $4.5X10^6$ when different concentrations of Metronidazole (2.56 and 1.28 μg/ml) were used. Viable cells counts of *E. Bangladeshi* were $3.15X10^6$ and $3.65X10^6$ when different concentrations of Secnidazole (2.56 and 1.28 μg/ml) were used. Inhibition of parasite occurred in a dose dependent manner when treated with metronidazole and secnidazole. Cell inhibition was maximum at the highest concentration of individual treatment (Metronidazole and Secnidazole). The results indicate that, Entamoeba isolates in Bangladesh do not seem to be resistant to the commonly used antiamoebic drugs. Metronidazole drug may be a useful treatment to inhibit the growth of *E. bangladeshi.* Further study is needed to explore different drugs at different concentrations and time intervals to determine the in vitro sensitivity against *E. bangladeshi*.

1.1 Background Information

Amebiasis is caused by *Entamoeba histolytica,* a protozoan that is found worldwide. The highest prevalence of amebiasis is in developing countries where barriers between human feces and food and water supplies are inadequate though most cases of amebiasis are asymptomatic, dysentery and invasive extraintestinal disease can occur. Amebic liver abscess is the most common manifestation of invasive amebiasis, but other organs can also be involved, including pleuropulmonary, cardiac, cerebral, renal, genitourinary, peritoneal, and cutaneous sites. In developed countries, amebiasis primarily affects migrants from and travelers to endemic regions, men who have sex with men, and immunosuppressed or institutionalized individuals.*E histolytica* is transmitted via ingestion of the cystic form (infective stage) of the protozoa. Viable in the environment for weeks to months, cysts can be found in fecally contaminated soil, fertilizer, or water or on the contaminated hands of food handlers. Fecal-oral transmission can also occur in the setting of anal sexual practices or direct rectal inoculation through colonic irrigation devices.Diarrheal diseases have a major effect on global health, particularly the health of malnourished children. The enteric parrasites *Entamoeba histolytica* and *E.bangladeshi* potential causes of diarrheal disease in children.[Tricia L. Royar at all]

1.2 Amoebiasis

Amoebiasis, also known amoebic dysentery, is an infection caused by any of the amoebas of the Entamoeba group. Symptoms are most common during infection by *Entamoeba histolytica.* Amoebiasis can be present with no, mild, or severe symptoms. Symptoms may include abdominal pain, diarrhea, or bloody diarrhea. Complications can include inflammation of the colon with tissue death or perforation, which may result in amoebiasis is a common infection of the human gastro-intestinal tract. Amoebiasis is more closely related to poor sanitation and socio economic status than to climate. It has worldwide distribution. It is a major health problem in China, South East and West Asia and Latin America, especially Mexico and Bangladesh (Sherris Medical Microbiology (4th ed.).In 1969, WHO defined amoebiasis, a condition in which a patient harbouring the organism

Entamoeba histolytica in the bowel. Culture of *E. histolytica* is a long and laborious process.There are three basic types of culture systems of *E. histolytica*, xenic, in which the parasiteis grown in the presence of an undefined flora; monoxenic, in which the parasite is grownin the presence of a single additional species; and axenic in which the parasite is grown inthe absence of any other metabolizing cells (Weedall and Hall, 2011) eritonitis. People affected may develop anemia due to loss of blood. [Vinod K Dhawan& MD. FACPA]

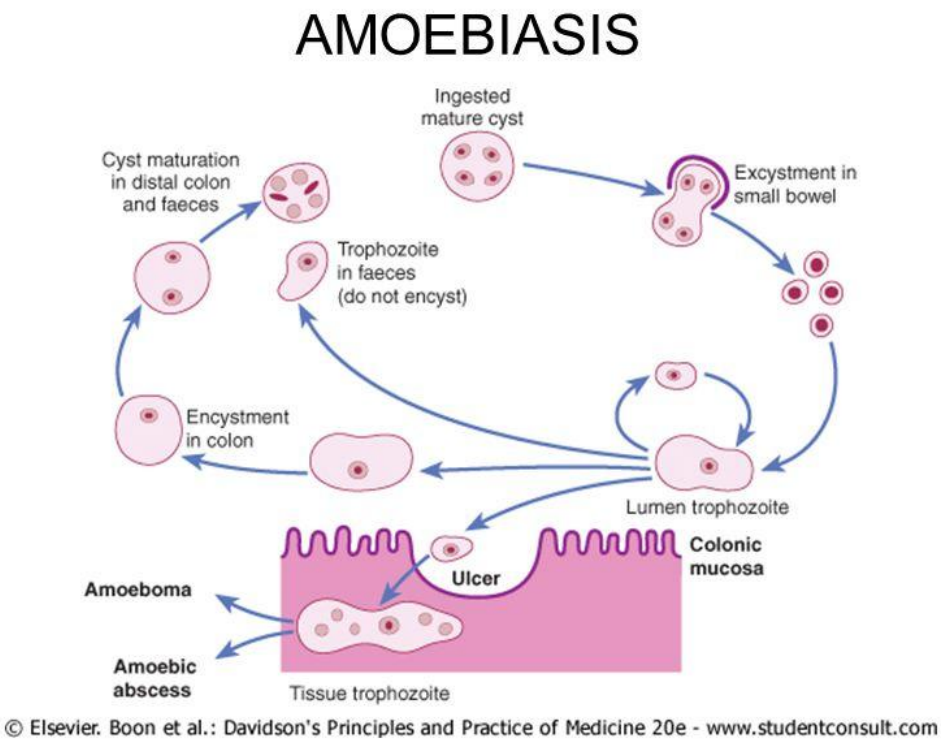


Figure:Amoebiasis

1.3 Amoeba

The term "amoeba" refers to simple eukaryotic organisms that move in a characteristic crawling fashion. However, a comparison of the genetic content of the various amoebae shows that these organisms are not necessarily closely related. Amoeba is a genus of single-celled amoeboids in the family Amoebidae. The type species of the genus is Amoeba proteus, a common freshwater organism, widely studied in classrooms and laboratories. There are many varieties of free-living amoeba, but only four genera have been causallyassociated with disease in humans such as *E. histolytica*is the cause of amoebiasis or amoebic dysentery, Naegleriafowleri (the "brain-eating amoeba") is a fresh-water native species that can be fatal to humans if introduced through the nose. Acanthamoeba can cause amoebic keratitis and encephalitis in humans, Balamuthi amandrillaris is the cause of (often fatal) granulomatous amoebic meningoencephalitis. Theyare distinct from the more famous *Entamoeba histolytica* (an obligate anaerobic parasite which can cause amoebiasis, amoebic dysentery and amoebic liver abscesses).

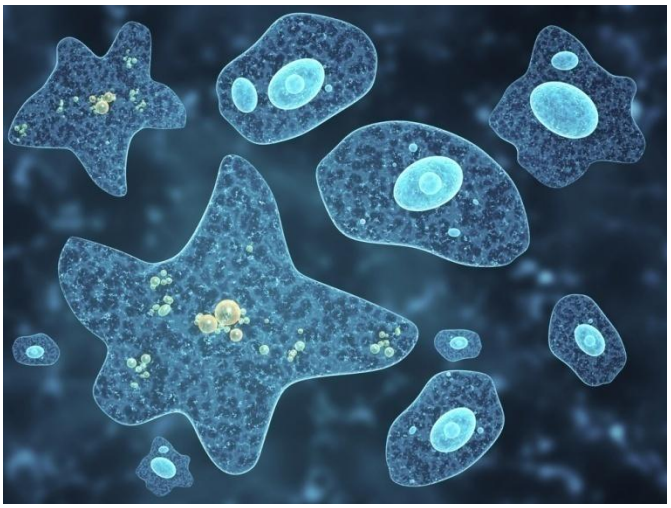


Figure: amoeba

Acanthamoebas and *B. mandrillaris* are opportunistic pathogens causing infections of the CNS, lungs, sinuses and skin, mostly in immune compromised humans. B. mandrillarisis also associated with disease in immune competent children, and Acanthamoebas pp. causea sight-threatening keratitis, mostly in contact lens wearers. N. fowlerica uses an acute and fulminating meningo encephalitis in immune competent children and young adults (Jeon,Kwang, 1973).

1.4 Amoeba and human disease

There are at least six species of amoeba, in the broader sense of amoebozoa, whichare parasitic in humans. However, most of them have an impact on the body characterized as "nonpathogenic intestinal amoeba infection." That means that these parasites are classified as ones that never make people sick or harm the body, even in people with weak immune systems. For example, *Entamoeba coli, Entamoeba dispar,* and *Entamoeba hartmanni*are considered harmless parasites. *Entamoeba histolytica*, however, is a parasitic amoebozoa that negatively impacts the human body, resulting in the infectious disease known as amebiasis. It is most commonin developing countries that have poor sanitary conditions. Mild amoebiasis is associated with stomach pain and stomach cramping, but a severe form is amoebic dysentery, whichcan cause stomach pain, fever, and bloody stools. In the worse cases, it can spread to other parts of the body, including the brain and lungs (UXL Encyclopedia of Science, 2002).

1.5 *Entamoeba histolytica*

*Entamoeba histolytic*a is an anaerobic parasitic amoebozoa, part of the genus Entamoeba. Predominantly infecting humans and other primates causing amoebiasis, *E. histolytica* is estimated to infect about 50 million people worldwide. Previously, it was thought that 10% of the world population was infected, but these figures predate the recognition that at least 90% of these infections were due to a second species, *E. dispar*. Mammals such as dogs and cats can become infected transiently, but are not thought to contribute significantly to transmission.

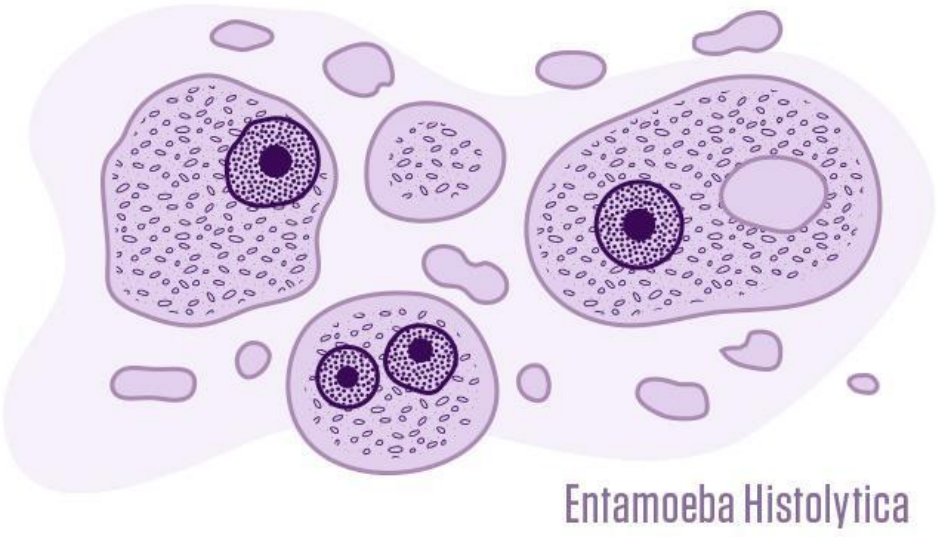


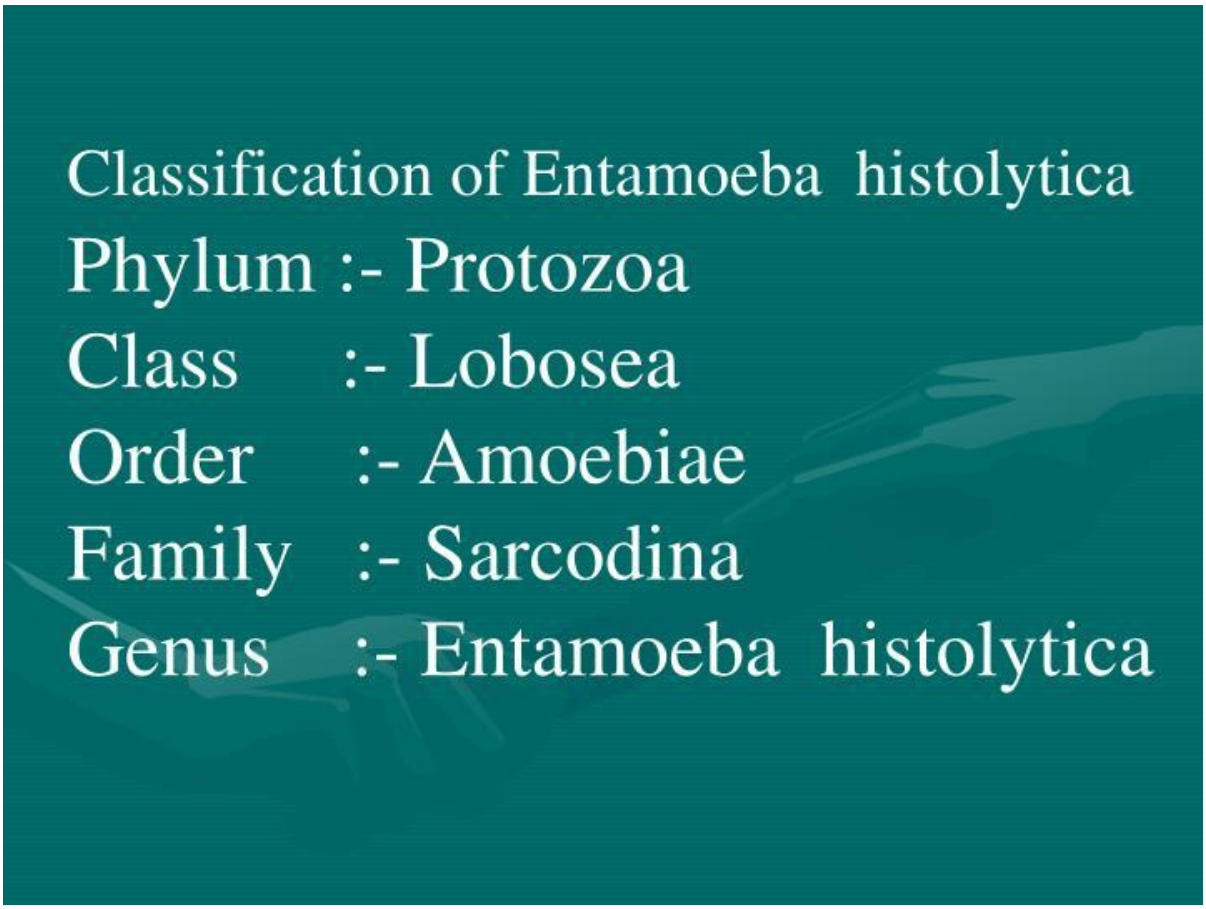
Figure: Microscopic image of E. histolytica

*Entamoeba histolytica*is an anaerobic parasitic amoebozoa, part of the genus Entamoeba. There are at least six species of Entamoeba that can infect the human gut but only *E.histolytica* causes disease. Predominantly infecting humans and other primates causing amoebiasis, *E. histolytica is* estimated to infect about 50 million people worldwide. *Entamoebahistolytica* is the etiological agent of amoebic dysentery and amoebic liverabscess (ALA). Worldwide, 40–50 million symptomatic cases of amoebiasis occur annually and 70,000 to 100,000 deaths due to this infection (Walsh JA et al., 1968). *Entamoeba histolytica* associated with high morbidity and mortality continues to be a major public health problem throughout the world. Asymptomatic individuals account for almost 90 percent of the infections. Poverty, ignorance, overcrowding, poor sanitation and malnutritionfavor transmission and increased disease burden. Prevalence varies from country tocountry and within a country (Lauren, 2016).

1.6 Species of Entamoeba

The genus Entamoeba contains many species, six of which *(Entamoeba histolytica, Entamoebadispar, Entamoeba moshkovskii, Entamoeba polecki, Entamoeba coli,* and *Entamoeba hartmanni*) reside in the human intestinal lumen. *Entamoeba histolytica*is the causative agent of amebiasis and is considered a leading parasitic cause of death worldwide in humans. Although recent studies highlight the recovery of *E. dispar* and *E. moshkovskii* from patients with gastrointestinal symptoms, there is still no convincing evidence of acausal link between the presence of these two species and the symptoms of the host (Ryan& Ray, 2004). New approaches to the identification of *E. histolytica* are based on detection of *E. histolytica*-specific antigen and DNA in stool and other clinical samples. Several molecular diagnostic tests, including conventional and real-time PCR, have been developed for the detection and differentiation of *E. histolytica*, *E. dispar*, and *E. moshkovskii* in clinical samples (Fotedar et al., 2007).

1.7 Scientific classification of E. histolytica



1.8 Distribution of *E. histolytica*

E. histolytica is cosmopolitan in distribution, but is more common in tropical andsub-tropical countries. In India it occasionally takes an epidemic form. It isestimated that about seven to eleven per cent of the population in India suffers fromits infection. The prevalence of Entamoeba infection is as high as 50% in areas of Central and South America, Africa, and Asia. *E. histolytica* seroprevalence studies in Mexico revealed that more than 8% of the populations were positive (Caballero-Salcedo, 1994). In endemic areas, as many as 25% of patients may be carrying antibodies to *E. histolytica* as a result of prior infections, which may be largely asymptomatic. The prevalence of asymptomatic *E. histolytica* infections seem to be region-dependent; in Brazil, for example, it may be as high as 11% (Tengku SA,Norhayati M, 2011).

1.9 Life cycle of *E. histolytica*

E. histolytica is a monogenetic parasite as its life cycle is completed in a single hosti.e., man. Three distinct morphological forms exist in its life cycle. Trophozoite, Precysticstage and Cystic stage (Sherris Medical Microbiology (4th ed.).

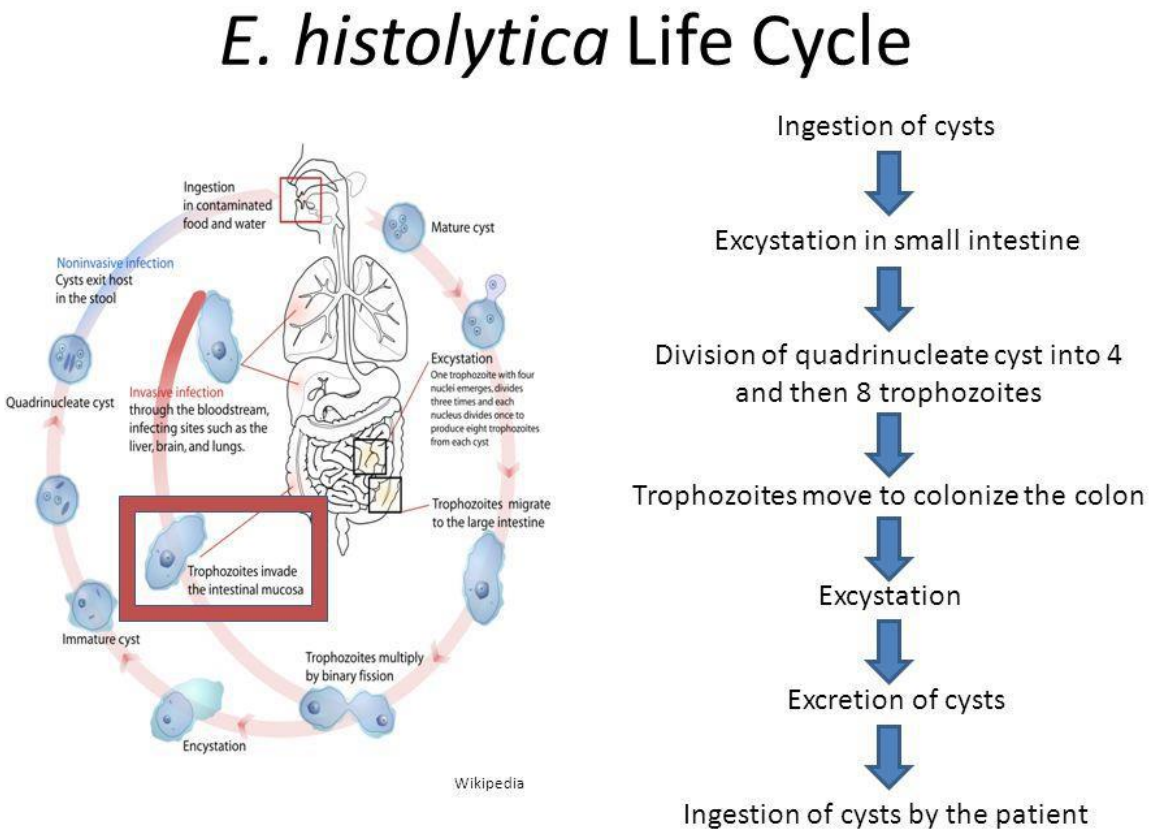


Figure 1.3: Life cycle of E. histolytica

1.10 Stages of *E. histolytica*

The life cycle of *Entamoeba histolytica*, includes three stages ,

1. Trophozoite stage,
2. Precystic stage and
3. Cystic stage.

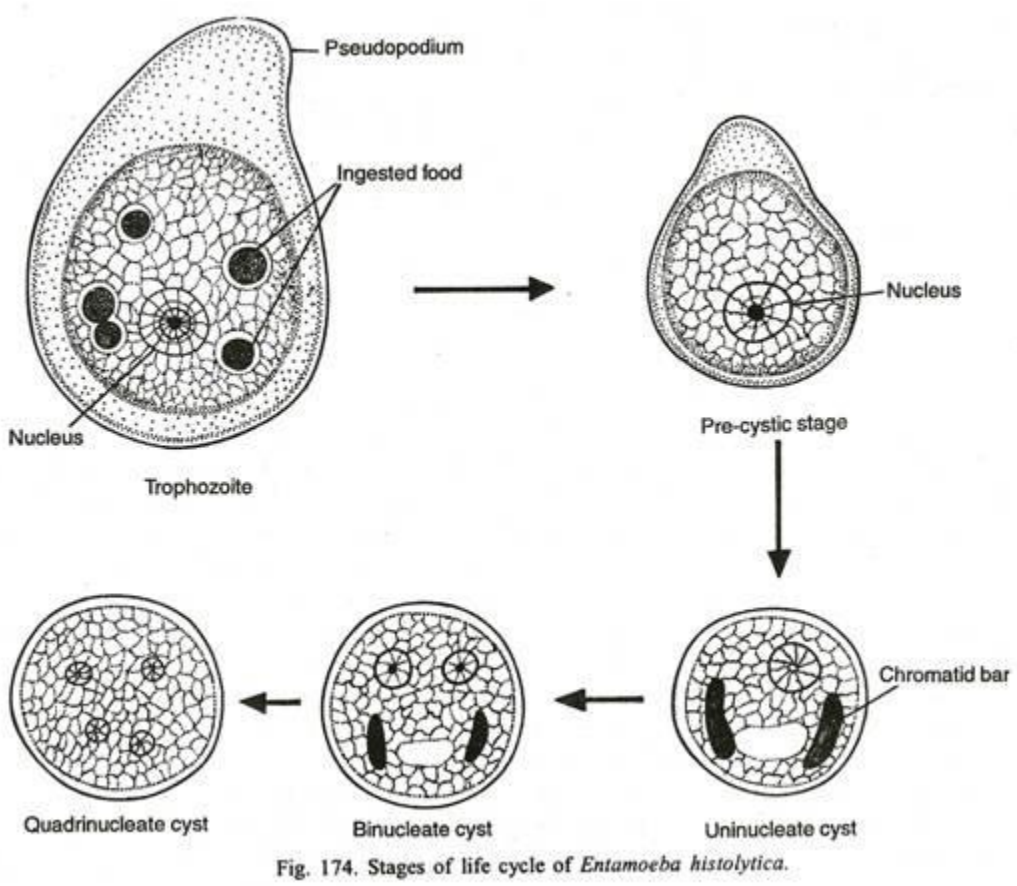


Fig. 174. Stages of life cycle of *Entamoeba histolytica*.

Figure 1.4: Stages of E. histolytica

1. Trophozoite stage

Trophozoite is the motile, feeding and pathogenic stage of *E. histolytica*. It measures about20-30 microns in diameter. Trophozoite is surrounded by the limiting membrane called plamalemma (William and Sodeman, 1996).

2. Precystic stage

Some of the daughter amoebae that are entered into the lumen of intestine develop transform into precystic stage. It is the nonmotile, nonfeeding and nonpathogenic stage of *E. histolytica*. It is relatively smaller in size, measuring about 10-20 micrometers (William and Sodeman, 1996).

3. Cystic stage

It is found in the lumen of the large intestine. It is round in shape and is surrounded by a thin, delicate and highly resistant wall. The precess of development of cyst wall is called encystations which is a means to tide over the unfavorable conditions that the parasite is going to encounter while that the parasite is going to encounter while passing to a new host (William and Sodeman, 1996).

1.11 Mode of infection

A matured quadrinucleate cyst of *E. histolytica* is the infective stage of the parasite. Transmission of *E. histolytica* from one person to another occurs due to ingestion of these cysts. Fecal contamination of edible substances and drinking water are theprimary cause of infection. Following are the mode of transmission of this parasite.

(a) Fecal-oral route

In majority of cases infection takes place through intake of contaminated uncooked vegetables and fruits. Insect vectors like flies, cockroaches and rodents act as agentto carry infective cysts to the food and drink. Sometimes drinking water supply contaminated with infected faces gives rise to epidemics (Saritha, 2015).

(b) Oral-rectal contact

Sexual transmission by oral-rectal contact is also one of the modes of transmission,especially among male homosexuals (Saritha, 2015).

1.12 Excystation

The process of excystation begins when the quadrinucleate cyst enters in the ileumof the small intestine of the new host. Excystation is the process of transformation of cysts to the trophozoites. It occurs in the intestinal lumen of the host. The cyst wallgets dissolved by the neutral or alkaline intestinal juice. The nucleus inside the cystdivides once again to form eight daughter nuclei.

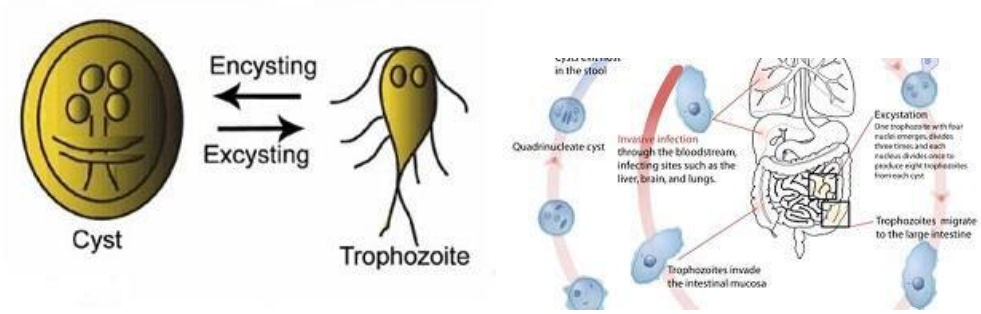


Figure 1.5: Excytation process

Certain amount of cytoplasm surrounds each of the nuclei to form 8 trophozoites. Theparasite at this stage moves into the caecum of the host's large intestine, get attached to theepithelial cells of the large intestine, produces necrosis by proteolytic ferment (cytolysin) and enters into the mucosa and sub-mucosa layers by means of their own mobility action (Saritha, 2015).

1.13 Pathophysiology of *E. histolytica*

The lesions produced by E. histolytica are primarily intestinal and secondary extraintestinal. The intestinal lesions are confined to the large intestine, frequently cecal and sigmoidorectal regions. The typical flask like primary ulcer to large nacrotic areas is produced. In acute amoebiasis, there are severe dysentery in numerous small stoolscontaining blood, mucous and necrotic mucosa accompanied by acute abdominal pain,tenderness and fever. Chronic amoebiasis is characterized by recurrent attacks ofdysentery with gastrointestinal disturbance. In extra intestinal amoebiasis, the liver is invaded chiefly, resulting amoebic hepatitis or liver abscess. An enlarged, tender liver, withpain in upper right hipochondrium, characterizes it. Less frequently, lung abscess, splenicabscess, brain abscess or cutaneous amoebic lesions are seen Ingested cysts of *E. histolytica* excyst in the small intestine are showing in figure below. Trophozoites are carried to the colon, where they mature and reproduce. The parasite maylead a commensal existence on the mucosal surface and in the crypts of the colon.Successful colonization depends on factors such as inoculum size, intestinal motility, transittime, the presence or absence of specific intestinal flora, the host's diet and the ability ofthe ameba to adhere to the colonic mucosal cells. The ameba adherence molecule has been identified as a lectin which can bind to either of two common carbohydrate components of cell membrane, galactose and N-acetyl glactoseamine. Binding to colonic mucins blocks adherence to mucosal cells. Depletion of mucus results in binding to the mucosa, anessential step in the development of the disease. If amebas pass down the colon they encystunder the stimulus of desiccation, and then are evacuated with the stool (William and Petri1993).

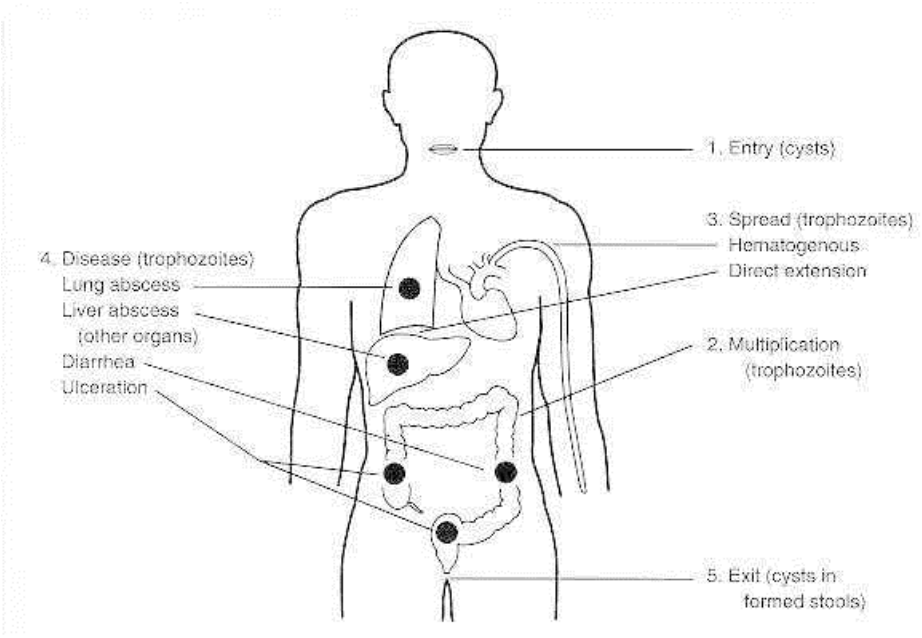


Figure 1.6: Pathophysiology of E.histolytica

1.14 *Entamoeba bangladeshi*

Diarrheal diseases have a major effect on global health, particularly the health of malnourished children. The enteric parasites *Entamoeba histolytica and E. moshkovskii* are potential causes of diarrheal disease in children. For the past 20 years, it has been studying Entamoeba infections in children from the urban slum of Mirpur in Dhaka, Bangladesh. *E.histolytica* infections can be detected through fecal microscopy, culture, PCR, and antigen detection. Microscopy and culture have limited specificity because several species of Entamoeba, which vary in their pathogenic potential, have morphologically similar cysts and trophozoites.

In 2010–2011, during analysis of feces which are positive for Entamoeba organisms by microscopy or culture but negative for *E. histolytica, E. dispar, and E. moshkovskii* by PCR, a

new species was identified, which have named *Entamoeba Bangladeshi*nov.sp. in recognition of the support of the Bangladesh community for this research. Feces from bothdiarrheal and surveillance specimens were collected from a cohort of children living in Mirpur. A total of 2,039 fecal samples were examined microscopically (0.9% saline smear)and/or by fecal culture for amebic trophozoites and cysts. One hundred forty-nine (7%) ofthe samples were positive by microscopy and/or culture for an Entamoeba parasite with both cysts and trophozoites that closely resembled those of *E. histolytica , E. moshkovskii, and E. dispar* (Royer TL, 2012). By light microscopy, there were no apparent differences between *E. Bangladeshi and E.histolytica.* The physical resemblance between *E. histolytica* and *E. Bangladeshi* is notablebecause direct microscopic examination of fecal samples is still used as a diagnostic tool inareas to which these species are endemic to detect E. histolytica parasites (Haque R,Mondal D, 2009). To further characterize *E. bangladeshi*, it has the ability to established inxenic culture, and it displayed the ability to grow at 37°C and 25°C, a characteristic shared with *E. Moshkovskii* and E. ecuadoriensis but that distinguishes it from *E. histolytica* and E. dispar. Tests negative in *E. histolytica* ELISA and in species-specific PCRs. Currently onlyidentifiable by its small subunit ribosomal RNA gene sequence (Stensvold CR, 2011).

1.15 Antiamoebic drugs

Infection is primarily treated by instituting antiamoebic therapy. The detection of *Entamoeba Bangladeshi* is an important goal of the clinical microbiology laboratory. This is because amebiasis is presently one of the three most common causes of death from parasitic disease. Drugs of choice for invasive amoebiasis are issue active agents, like metronidazole, Ornidazole and chloroquine or the more toxic emetine derivatives, including dehydroemetine. Metronidazole is derived from 5- nitroimdazole which kill the trophozoites by alterations in the protoplasmic organelles of the amoeba. Nitroimidazole therapy leads to clinical response in approximately 90% of patients with mild-to-moderate amebic colitis. Because intraluminal parasites are not affected by nitroimidazoles, nitroimidazole therapy for amebic colitis should be followed by treatment with a luminal agent (eg, paromomycin or diloxanidefuroate) to prevent a relapse. Amebic liver abscess of up to 10 cm can be cured with metronidazole without drainage.

Clinical deferve scence should occur during the first 3-4 days of treatment. Failure of metronidazole therapy may be an indication for surgical intervention. Chloroquine has also been used for patients with hepatic amebiasis. Dehydroemetine (available from the Centers for Disease Control and Prevention [CDC] has been successfully used but, because of its potential myocardial toxicity, is not preferred. Broad-spectrum antibiotics may be added to treat bacterial super infection in cases of amoebic colitis and suspected perforation. Bacterial co-infection of amebic liver abscess has occasionally been observed (both before and as a complication of drainage), and adding antibiotics to the treatment regimen is reasonable in the absence of a prompt response to nitroimidazole therapy (Stanley, 2003).

1.16 Metronidazole

Metronidazole is an antibiotic that is used to treat a wide variety of infections which marketed under the brand name Flagyl among others. It works by stopping the growth of certain bacteria and parasites (Brayfield, 2014). Metronidazole (1-(2-hydroxyethyl)-2- methyl-5-nitroimidazole) is the drug of choice for the treatment of infections caused by anaerobic or microaerophilic microorganisms and has been used in clinical practice for >25 years. The molar mass of metronidazole is 171.15 g/mol. Metronidazole began to be commercially used in 1960 in France. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system (WHO 2015). It is effective for giardiasis, trichomoniasis, and amebiasis. It is the drug of choice for a first episode of mild-to-moderate Clostridium difficile colitis. Metronidazole is available by mouth, as a cream, and intravenously (Cohen, 2010)

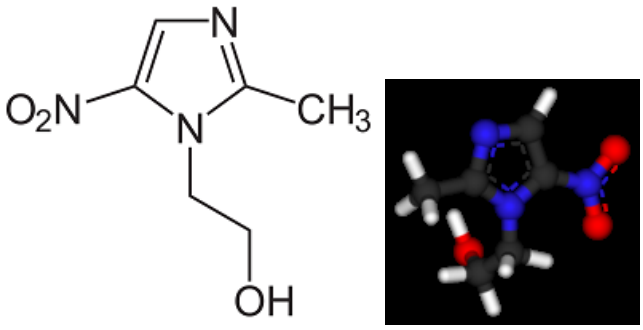


Figure 1.7: Chemical and 3D structure of Metronidazole

For the treatment of anaerobic infections and mixed infections, surgical prophylaxis requiring anaerobic coverage, Clostridium difficile-associated diarrhea and colitis, Helicobacter pylori infection and duodenal ulcer disease, bacterial vaginosis, Giardia lamblia gastro-enteritis, amebiasis caused by *E. histolytica*, acne rosacea (topical treatment), and Trichomonas infections (McEvoy, 2006).

1.17 Pharmacokinetics of Metronidazole

Metronidazole appears in cerebrospinal fluid, saliva, and breast milk in concentrations similar to those found in plasma. Less than 20% is protein bound. It is absorbed rapidly and completely if also given through orally. It is distributed very well into mucosa, saliva, bone and CSF. It is metabolized in liver. Routes of elimination are via urine (60% to 80%) and feces (6% to 15%). Renal Clearance is approximately 10 mL/min per 1.73 m2. The plasma ½ life of metronidazole is about 8 hours (Thomson, 2006).

1.18 Metabolism of Metronidazole

Approximately 30-60% of an oral or IV dose of metronidazole is metabolized in the liver by hydroxylation, side-chain oxidation, and glucuronide conjugation. The major metabolite, 2-hydroxy metronidazole, has some antibacterial and antiprotozoal activity. Four other nitro-group-containing metabolites have been identified, each derived from side-chain oxidation of ethyl and/or methyl group. They include 1- acetic acid-2-methyl-5-nitroimidazole and 1-(2-hydroxyethyl)-2-carboxylic acid-5- nitroimidazole salt. The liver is the main site of metabolism, and this accounts for over 50% of the systemic clearance of metronidazole. The 2 principal metabolites result from oxidation of side chains, a hydroxy derivative and an acid.

1.19 Mechanism of action of Metronidazole

Metronidazole is of the nitroimidazole class. It inhibits nucleic acid synthesis by disrupting the DNA of microbial cells (Eisenstein, 2007). Metronidazole can enter the cell through passive diffusion, where a nitro group is subsequently reduced to reactive cytotoxic nitro radicals by reduced ferredoxin or flavodoxin. Ferredoxin and flavodoxin function as electron acceptors of pyruvate. ferredoxinoxidoreductase, hydrogenase, and some other enzymes found specifically in microaerophilic bacteria and protozoan parasites. Under aerobic conditions, the nitro radicals can be oxidized by oxygen, which leads to futile cycling and detoxification of the drug. During this reaction, superoxide radical anions are formed that may also be toxic for the anaerobic organisms (Thomson, 2006). Metronidazole is a prodrug. Unionized metronidazole is selective for anaerobic bacteria due to their ability to intracellularly reduced metronidazole to its active form. This reduced metronidazole then covalently binds to DNA, disrupt its helical structure, inhibiting bacterial nucleic acid synthesis and resulting in bacterial cell death. Treatment with Metronidazole is usually very effective (Brayfield, A, 2014).

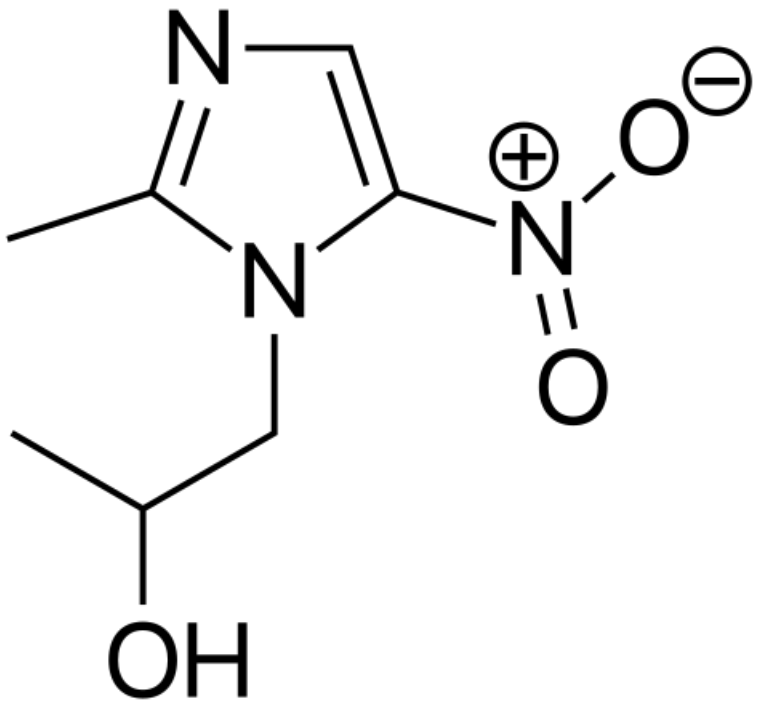
1.20 Biological Half-Life of Metronidazole

The plasma half-life of metronidazole is reported to be 6-8 hours in adults with normal renal and hepatic function. The plasma half-life of metronidazole is not affected by changes in renal function; however, the half-life may be prolonged in patients with impaired hepatic function. It has been observed from a study that half-life of metronidazole averaged 18.3 hours in adults with alcoholic liver disease and impaired hepatic function, (range: 10.3-29.5 hours) (McEvoy, 2006).

1.21 Secnidazole

Secnidazole (trade names Flagentyl, Sindose, Secnil, Solosec) is a nitroimidazole anti-infective. Effectiveness in the treatment of dientamoebiasis has been reported. It has also been tested against Atopobiumvaginae. On the United States, secnidazole is approved for the treatment of bacterial vaginosis in adult women.[3]

Secnidazole is structurally related to the commonly used 5-nitroimidazoles metronidazole and tinidazole. These drugs share a common spectrum of activity against anaerobic micro-organisms and they appear particularly effective in the treatment of amoebiasis, giardiasis, trichomoniasis and bacterial vaginosis.



1.22 Pharmacokinetics of secnidazole

It is of Synthetic origin and belongs to Nitroimidazole. It belongs to Amebicides pharmacological group.The Molecular Weight of Secnidazole is 185.20. Renal Excretion accounts for 50 % and plasma half life is 20 hr.

Indications

Secnidazoleis primary indicated Amoebiasis, Giardiasis, Trichomoniasis, Urethritis, Vaginitis.

Contraindications

Secnidazole is contraindicated in conditions like Hypersensitivity to any component of product.

Side Effects

The severe or irreversible adverse effects of Secnidazole, which give rise to further complications include Leucopenia.

The symptomatic adverse reactions produced by Secnidazole are more or less tolerable and if they become severe, they can be treated symptomatically, these include Dizziness, Abdominal pain, Urticaria, Glossitis, StomatitisX, Taste disturbances, Paresthesias.

Literature review

2.1 In vitro activity of antiamoebic drugs against clinical isolates of *Entamoebahistolytica* and Entamoebadispar

It has been actively exhibit something that the amoebiasis is a major public health problem in tropical and subtropical countries. Although a number of antiamoebic agents are used for its treatment, yet the susceptibility data on clinical isolates of *Entamoeba histolytica* and *Entamoeba dispar* are not available. Therefore, the present study was aimed to assess the in vitro susceptibility of clinical isolates of *E. histolytica* and *E. dispar* to metronidazole, chloroquine, emetine and tinidazole. A total of 45 clinical isolates were maintained in polyxenic cultures followed by monoxenic cultures. In vitro drug sensitivity (IC_{50}) of clinical isolates and standard reference strain of *E. histolytica* was assessed by nitro blue tetrazolium (NBT) reduction assay after exposure to various concentrations of each drug. The results indicate that till date, *Entamoeba* isolates in India do not seem to be resistant to the commonly used antiamoebic drug. [Devendra et al,2004]

2.2 Tinidazole and metronidazole in the treatment of intestinal amoebiasis.

The study has been shown that sixty adult patients with symptomatic intestinal amoebiasis and with *Entamoeba histolytica* present in stools were allocated at random to treatment with tinidazole or metronidazole, both administered in a dose of 2 g once daily for 3 consecutive days. The treatment period was extended in patients with stools positive for *Entamoeba histolytica* on the day following the last treatment day. Fifty-six patients, 29 on tinidazole and 27 on metronidazole, completed the trial as per the protocol. Twenty-eight patients (96.5%) on tinidazole and 15 (55.5%) on metronidazole were cured. Parasitological cure with partial relief of symptoms was obtained in 1 (3.5%) and 5 (18.5%) patients on tinidazole and metronidazole, respectively. Seven patients (26%) on metronidazole were treatment failures. Treatment had to be extended beyond 3 day in 53% of patients (8/15) on metronidazole as opposed to 11% (3/28) on tinidazole (p less than 0.01). The total number of side-effects, their severity, and the types were more in the metronidazole group. No toxic effects due to either drug were recorded.

Tinidazole provided significantly higher cure rates than metronidazole in the treatment of symptomatic intestinal amoebiasis (p less than 0.01), and was better tolerated than metronidazole. [Swami B& Lavakusulu D,1997]

2.3 In vitro activity of nitazoxanide and related compounds against isolates of Giardia intestinalis, *Entamoeba histolytica* and *Trichomonasvaginalis*

It has been shown that the activities of the *N*-(nitrothiazolyl) salicylamidenitazoxanide and its metabolite tizoxanide were compared with metronidazole *in vitro* in microplates against six axenic isolates of *Giardia intestinalis*. Tizoxanide was eight times more active than metronidazole against metronidazole-susceptible isolates and twice as active against a resistant isolate. In 10 axenic isolates of *Entamoebahistolytica*, while tizoxanide was almost twice as active as metronidazole against more susceptible isolates, it was more than twice as active against less susceptible isolates. Fourteen metronidazole-susceptible isolates of *Trichomonasvaginalis* were 1.5 times more susceptible to tizoxanide, which was nearly five times as active against resistant isolates. Two highly metronidazole-resistant isolates retained complete susceptibility to tizoxanide, and one moderately resistant isolate showed reduced susceptibility. In all three organisms, nitazoxanide results paralleled those of tizoxanide. Analogues lacking the reducible nitro-group had similar low activities against susceptible *G. intestinalis*, *E. histolytica* and *T. vaginalis*, indicating that nitro-reduction and free radical production was a probable mode of action. Nitazoxanide and its metabolite tizoxanide are more active *in vitro* than metronidazole against *G. intestinalis*, *E. histolytica* and *T. vaginalis*. Although, like metronidazole, they depend on the presence of a nitro-group for activity, they retain some activity against metronidazole-resistant strains, particularly of *T. vaginalis*. [SullaymanAdagu et al, 2002]

2.4 In Vitro Effect of NitazoxanideAgainst *Entamoebahistolytica*, Giardia intestinalis and *Trichomonasvaginalis* Trophozoites

It demonstrated thatnitazoxanide, a 5-nitrothiazolyl derivative, is effective in the treatment of a broad range of parasitic infections. In vitro, it is active against several protozoa, including Cryptosporidiwnparvum, Blastocystishominis, and Giardia intestinalis. The objective of this study was to determine the in vitro effect of nitazoxanide on the growth and morphology of three anaerobic protozoa (*Entamoeba histolytica*, *Giardia intestinalis*, and *Trichomonasvaginalis*) and to compare these effects with those of metronidazole and albendazole. A subculture method was used to determine the concentrations required to inhibit growth by 50% or 90% (IC50 and IC90). Nitazoxanide exhibited IC50 and IC90 values of 0.017 and 0.776 μg/ml respectively, against E. histolytica, 0.004 and 0.067 μg/ml against G. intestinalis, and 0.034 and 2.04 6 μg/ml against T. vaginalis. Based on the IC90 values, nitazoxanide was more toxic than metronidazole and albendazole against E. histolytica; albendazole and nitazoxanide were more toxic than metronidazole against G. intestinalis; and metronidazole was the most toxic drug against T. vaginalis. The effects of nitazoxanide on trophozoite ultrastructure of all three parasites included cell swelling and distorted cell shape, a redistribution of vacuoles, plasma membrane damage, and the formation of extensive empty areas in the cytoplasm of the protozoa. [ROBERTO et al, 2002]

2.5 Metronidazole

It has been on view that the nitroimidazole antibiotic metronidazole has a limited spectrum of activity that encompasses various protozoans and most Gram-negative and Gram-positive anaerobic bacteria. Metronidazole has activity against protozoans like *Entamoeba histolytica*, *Giardia lamblia* and *Trichomonasvaginalis*, for which the drug was first approved as an effective treatment.

Anaerobic bacteria which are typically sensitive are primarily Gram-negative anaerobes belonging to the Bacteroides and Fusobacterium spp. Gram-positive anaerobes such as peptostreptococci and Clostridia spp. are likely to test sensitive to metronidazole, but resistant isolates are probably encountered with greater frequency than with the Gram-negative

anaerobes. Gardnerellavaginalis is a pleomorphic Gram-variable bacterial bacillus that is also susceptible to metronidazole.

Metronidazole has played an important role in anaerobic-related infections. Advantages to using metronidazole are the percentage of sensitive Gram-negative anaerobes, its availability as oral and intravenous dosage forms, its rapid bacterial killing, its good tissue penetration, its considerably lower chance of inducing C. difficile colitis, and expense. Metronidazole has notable effectiveness in treating anaerobic brain abscesses. [Collin D et al, 2012]

2.6 In vitro studies on the sensitivity of local *Entamoebahistolytica*to anti-amoebic drugs.

I would be necessary to seen that the in vitro activity of drugs, namely dehydroemetine, ornidazole, metronidazole and tinidazole were determined against the locally isolated strains of *E. histolytica* in Thailand. The test was performed in liquid monophasic medium, i.e. liver marmite serum medium. In all, locally isolated strains from thirty hosts studied, the minimal inhibitory concentration (MIC) for dehydroemetine ranged from 0.125 to 1 microgram/ml, ornidazole ranged from 0.0625 to 0.25 microgram/ml, metronidazole ranged from 0.0625 to 0.125 microgram/ml, and tinidazole ranged from 0.0625 microgram/ml to 0.25 microgram/ml. The MIC of dehydroemetine was significantly different from ornidazole, metronidazole and tinidazole. Metronidazole was superior to that of dehydroemetine but was not significantly different among ornidazole, metronidazole and tinidazole.[Chintana T et al, 1986]

Objective of the Study

The objective of the study is to investigate the efficacy & the effect of Metronidazole & Secnidazole drugs against *E. Bangladeshi* at different concentrations. Therefore, the present study was aimed to find out the in vitro sensitivity test of Metronidazole &Secnidazole of clinical isolates of *Entamoeba bangladeshi*.

3.1**Significance of the study**

In the present study *in vitro* sensitivity of Metronidazole & Secnidazole against *E. bangladeshi* were maintained by *in vitro* cultivation in axenic medium and were subjected to drug sensitivity tests against antiamoebic drugs. Several studies have been carried out to determine the sensitivity of Metronidazole and Secnidazole against *E. bangladeshi*. However, no studies have been shown in the literature about the effectiveness of the antiamoebic drugs against the novel species of *E. Bangladeshi.* Therefore, the effective use of anti-amoebic drugs against clinical isolates of E. *Bangladeshi* was tested in this study.

Materials and Methods

4.1 Clinical isolation of cell

Clinical isolates of *E. Bangladeshi* were harvested from 24 hours oldcultures and suspended in a LYI-S-2 medium. Axenic medium (LYI-S-2) consists of liverdigest, yeast extract, iron, serum. The parasite count was adjusted to 1×106 parasites/ml inmedium by haemocytometer (Mukhopadhyay, R.M, et al., 1996; Bansal, D. et al., 2004).Isolation is usually achieved by growing the species in an environment that was previouslysterilized, and was thereby rid of contaminating organisms. This isolation was performedin the Parasitology Laboratory, Laboratory Sciences Division, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b).

4.2 Sample collection

The drug Metronidazole & Scenidazolerow materials collected from Incepta Pharmaceutical Industry. Theparasites were collected from the research department of ICDDR'B in Mohakhali, Dhaka. This investigation was performed in the parasitology Laboratory of ICDDR'B during thetime of june 2017 to january 2018.

4.3 Preparation of antimicrobial drug

1. 5.12µg of metronidazole & Secnidazole was weighted carefully by using a calibrated analytical took balance
3. The calculated amount of sample was dissolved in 1ml distilled water.
4. The solution was mixed by shaking carefully.
5. Then the stock solution was stored in refrigerator.

4.4 Apparatus and Instruments

1) Eppendrof
2) Falcone tube
3) Micropipettes
4) Microtips
5) Beaker
6) Microtiter plate
7) Analytical balance
8) Vortex machine
9) Haemocytometer
10) Microscope
11) Incubator
12) Autoclave
13) Laminar flow
14) Trypan blue reagent

4.5 Incubator

An incubator is a device used to grow and maintain microbiological cultures or cell ultures. The incubator maintains optimal temperature, humidity and other conditions such as the carbon dioxide (CO2) and oxygen content of the atmosphere inside. Incubators are essential for a lot of experimental work in cell biology, microbiology and molecular biology and are used to culture both bacterial as well as eukaryotic cells.

Figure4.1: Incubator

4.6 Microscope

A microscope (from the Ancient Greek: "small" and "to look" or "see") is an instrument used to see objects that are too small to be seen by the naked eye. Microscopy is the science of investigating small objects and structures using such an instrument. Microscopic means invisible to the eye unless aided by a microscope.

Figure4.2: Microscope

4.7 Haemocytometer

The haemocytometer is a device usually used and originally designed to count blood cells or parasite cells. The haemocytometer was invented by Louis-Charles Malassez and consists of a thick glass microscope slide with a rectangular indentation that creates a chamber. This chamber is engraved with a laser-etched grid of perpendicular lines. The device is carefully crafted so that the area bounded by the lines is known, and the depth of the chamber is also known. It is therefore possible to count the number of cells or particles in a specific volume of fluid, and thereby calculate the concentration of cells in the fluid overall.

Figure 4.3: Haemocytometer

4.8 Autoclave

An autoclave is a pressure chamber used to carry out industrial processes requiring elevated temperature and pressure different from ambient air pressure. Autoclaves are used in medical applications to perform sterilization. Many autoclaves are used to sterilize equipment and supplies by subjecting them to high-pressure saturated steam at 121 °C (249 °F) for around 15–20 minutes depending on the size of the load and the contents. The autoclave was invented by Charles Chamberl and in 1879.

Figure 4.4: Autoclave

4.9 Microtiter plate

A microtiter plate (spelled Microtiter is a registered trade name in the United States) or microplate or microwell plate or multiwell, is a flat plate with multiple "wells" used as small test tubes. The microplate has become a standard tool in analytical research and clinical diagnostic testing laboratories.

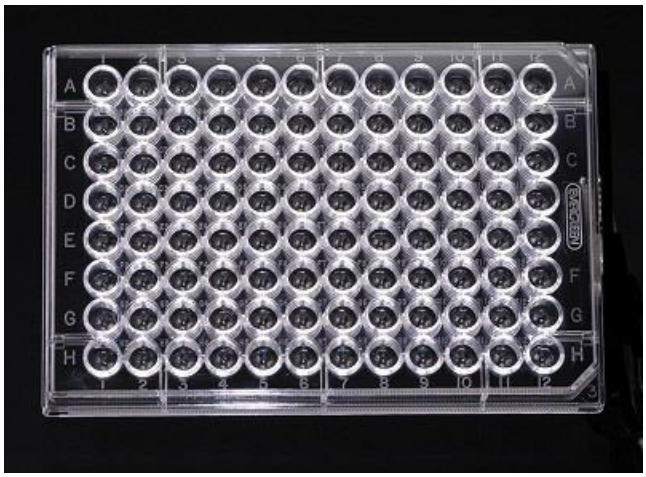


Figure 4.5: Microtiter plate

A vortex mixer, or vortexer, is a simple device used commonly in laboratories to mix small vials of liquid. It consists of an electric motor with the drive shaft oriented vertically and attached to a cupped rubber piece mounted slightly off-center. As the motor runs the rubber piece oscillates rapidly in a circular motion. When a test tube or other appropriate container is pressed into the rubber cup (or touched to its edge) the motion is transmitted to the liquid inside and a vortex is created. Most vortex mixers are designed with 2 or 4- plate formats, have variable speed settings ranging from 100 to 3,200 rpm.

Figure 4.6: Vortex machine

Vortex mixers are quite commonplace in bioscience laboratories. In cell culture and microbiology laboratories they may be used to suspend cells. In a biochemical or analytical laboratory they may be used to mix the reagents of an assay or to mix an experimental sample and a dilutant.

4.11 Micropipette

Micropipettes are utilized in the laboratory to transfer small quantities of liquid, usually down to 0.1 uL. They are most commonly used in chemistry, biology, forensic, pharmaceutical, and drug discovery labs, among others.

Figure 4.7: Micropipette

4.12 In vitro drug sensitivity assay procedure

1. Drug sensitivity assay of the samples was carried out by using microtiterplates. In wells (A-1) to (F-1), 100 µl medium was added except (B-1) and (C-1)
2. Then in (C-1), 100 µl Metronidazole & Secnidazole stock solution was added and serial
3. dilutions of the drugs were performed down {(C-1) to (F-1)}.
4. Then the plate was mixed properly.
5. After that 100 µl of the medium from the well (F-1) was discarded to maintain the equality of the concentration of the drugs.
6. Further 100 µl of parasite suspension (1×106 parasites/ml) was added to all

the wells {(A-1) to (F-1)} except (B-1).

7. The final concentrations of the drugs were 2.56, 1.28, 0.64, 0.32, 0.16, 0.08, 0.04 µg/ml.
8. Well (A-1) was control (contains only media and cells only).
9. Then plastic strip was used to cover the plate.
10. Plates were incubated at 37ºC for 4 hours.
11. After 4 hours the plate was taken from the incubator.
12. Then the viable parasites were counted by haemocytometer under microscope in each of the wells.

The entire procedure was carried out for both parasites *E.bangladeshi*.

Results

5.0 Determination of viable cell count of *E. Bangladeshi* after treatment with Metronidazole

The study was conducted with the active ingredient of Metronidazole & Secnidazole collected from Incepta Pharmaceutical Ltd. The in vitro sensitivity of Metronidazole was carried outagainst clinical isolates of *E. Bangladeshi* by using different concentrationsof the drug.

5.1 Mean viable cell count of*E. Bangladeshi*after 4hours incubation of Metronidazole (2.56µg/ml)

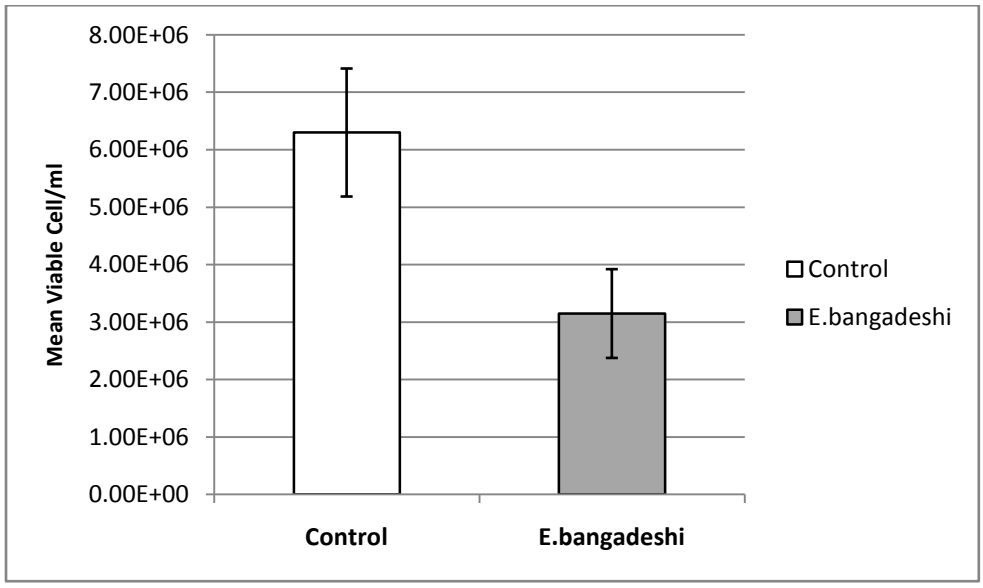


Figure 5.1.1: Mean viable cell counts of *E. bangladeshi*after treatment with Metronidazole

Figure 5.1.1shows the viable cell count of *E. Bangladeshi* at the concentration of 2.56 µg/ml after treatment with Metronidazole. The incubation periodwas 4 hours at 37^0C. The mean viable count of *E. Bangladeshi* in control was $6.3X10^6$ parasite/ml. The mean viable cellcount of *E. Bangladeshi* was $3.2X10^6$ parasites/ml at the concentration of 2.56µg/ml after treating with Metronidazole. Viable cell count is reduced in the treatment groups when compared with the control.

5.2 Mean viable cell count of *E. Bangladeshi* after 4hours incubation of Metronidazole (1.28µg/ml)

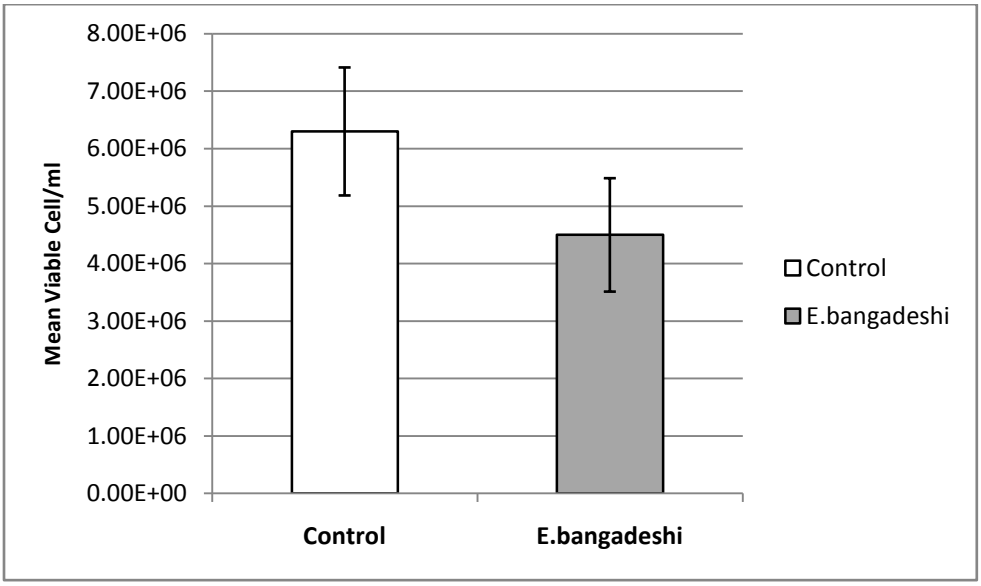


Figure 5.1.2: Mean viable cell counts of *E. Bangladeshi* after treatment with Metronidazole

Figure 5.1.2 shows the viable cell count of *E. Bangladeshi* at the concentration of 1.28µg/ml after treatment with Metronidazole. The cell was incubated for 4 hours at with 37^0C Metronidazole drug. The mean viable count of *E. Bangladeshi* in control was $6.3X10^6$ parasite/ml. The mean viable cell count of the *E. Bangladeshi* was $4.5X10^6$ at the concentration

of 1.28µg/ml after treating with Metronidazole. Viable cell count is reduced in the treatment groups when compared with the control.

5.3 Mean viable cell count of *E. Bangladeshi* after 4 hours incubation of Metronidazole (0.64g/ml)

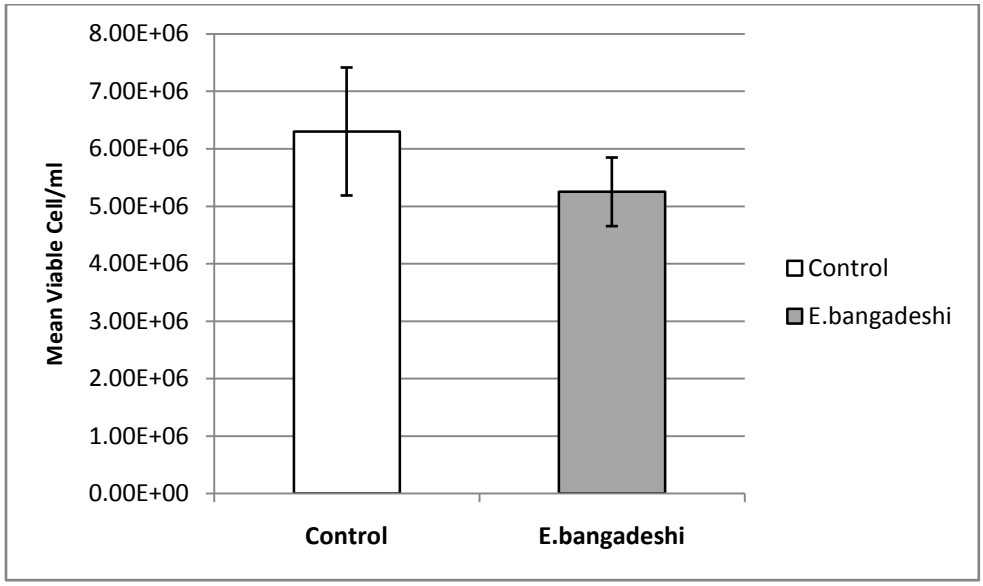


Figure 5.1.3: Mean viable cell counts of*E. Bangladeshi* after treatment with Metronidazole

Figure 5.1.3 shows the viable cell count of *E. Bangladeshi* at the concentration of 0.64µg/ml after 4 hours incubation with Metronidazole. The incubationtemperature was 37^{0}C. The mean viable count of *E. Bangladeshi* in control was 6.3X10^{6}parasite/ml. The mean viable cell *E. Bangladeshi* was5.3X10^{6} parasites/ml at the concentration of 0.64µg/ml after treating with Metronidazole..Viable cell count is reduced in the treatment groups when compared with the control.

5.4 Mean viable cell count of*E. Bangladeshi* after 4hours incubation of Metronidazole (0.32 µg/ml)

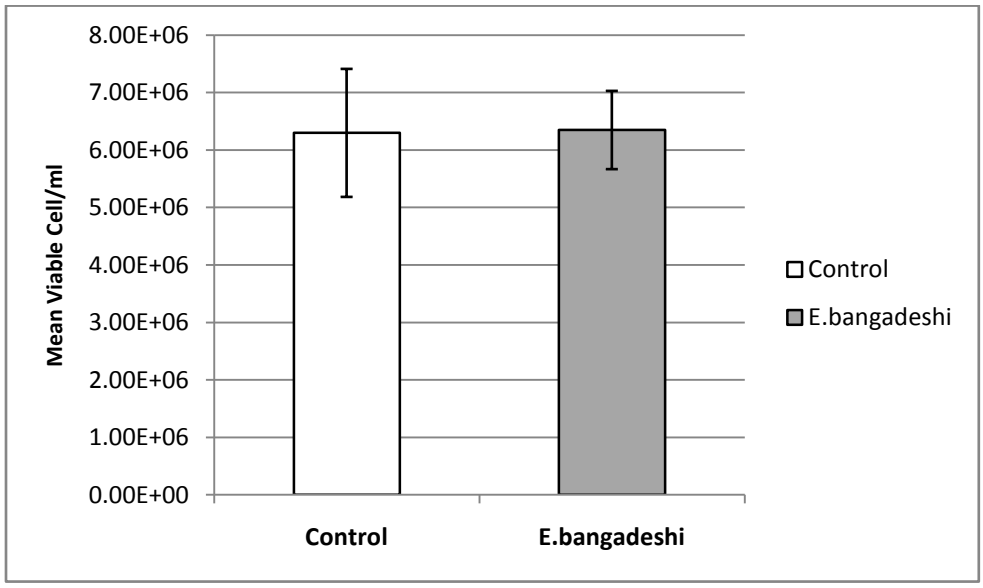


Figure 5.1.4: Mean viable cell counts of *E. Bangladeshi* after treatment with Metronidazole

Figure 5.1.4 shows the viable cell count of*E. Bangladeshi* at the concentration of 0.32 µg/ml after treated with Metronidazole. The incubation period was 4hours at 37^{0}C. The mean viable count of *E. Bangladeshi* in control was 6.3X10^{6}. The mean viable cell count of *E. Bangladeshi* was 6.4X10^{6} parasites/ml after treating with Metronidazole atthe concentration of 0.32µg/ml. Viable cell count is increased in the treatment groups when compared with the control.

5.5 Mean viable cell count of *E. Bangladeshi* after 4 hours incubation of Metronidazole (0.16µg/ml)

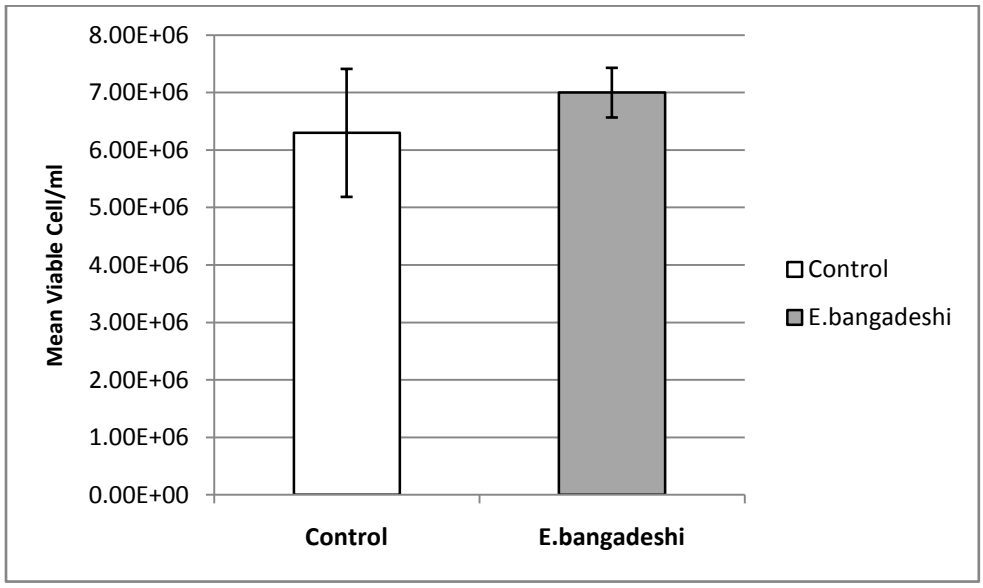


Figure 5.1.5: Mean viable cell counts of *E. Bangladeshi* after treatment with Metronidazole

Figure 5.1.5 shows the viable cell count of *E. Bangladeshi* at the concentration of 0.16µg/ml after incubation with Metronidazole. The incubation period was 4 hours at 37^{0}C. The mean viable count of *E. Bangladeshi* in control was $6.3X10^{6}$ parasite/ml. The mean viable cell count of *E. Bangladeshi* was $7X10^{6}$ parasites/ml at the concentration of 0.16µg/ml after treating with Metronidazole. Viable cell count is increased in the treatment groups when compared with the control.

5.6 Mean viable cell count of *E. Bangladeshi* after 4 hours incubation of Secnidazole (2.56µg/ml)

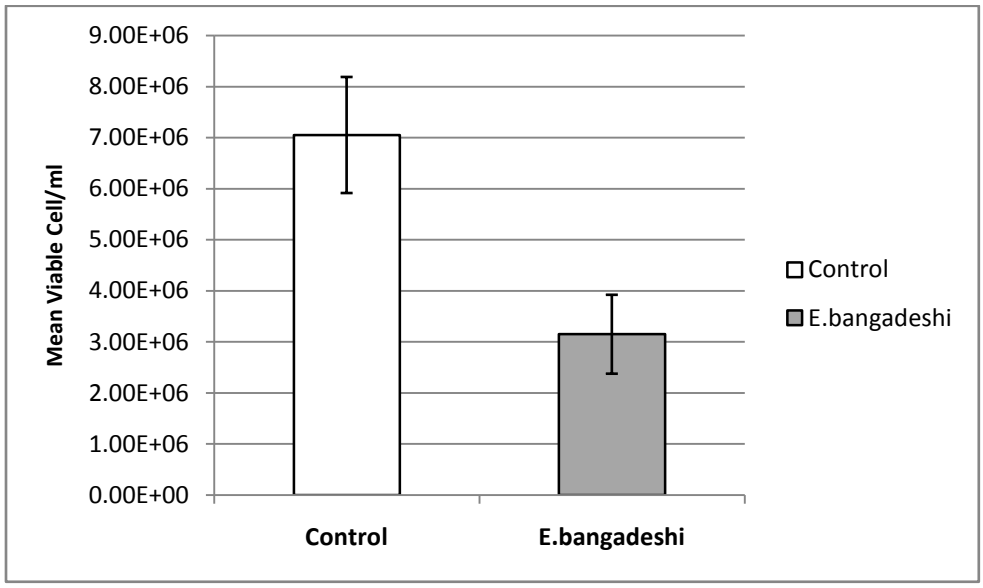


Figure 5.2.1: Mean viable cell counts of*E. Bangladeshi* after treatment with Secnidazole

Figure 5.2.1 shows the viable cell count of *E. Bangladeshi* at the concentration of 2.56µg/ml after incubation with Secnidazole. The incubation period was 4 hours at 37^{0}C. The mean viable count of *E. Bangladeshi* in control was $7.05X10^{6}$ parasite/ml. The mean viable cell count of *E. Bangladeshi* was $3.15X10^{6}$ parasites/ml at the concentration of 2.56 µg/ml after treating with Metronidazole.Viable cell count is reduced in the treatment groups when compared with the control.

5.7 Mean viable cell count of *E. Bangladeshi* after 4 hours incubation of Secnidazole (1.28µg/ml)

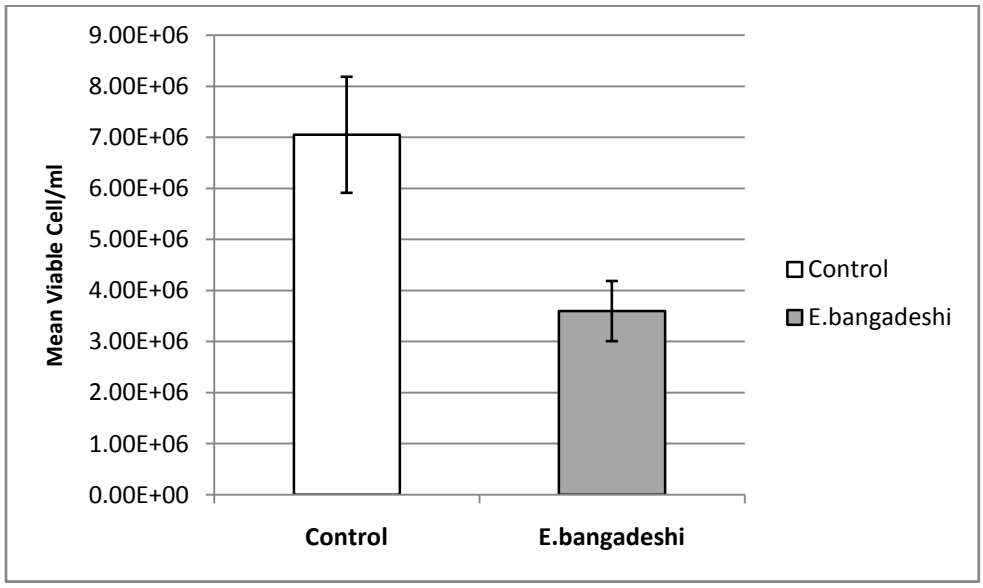


Figure 5.2.2: Mean viable cell counts of *E. Bangladeshi* after treatment with Secnidazole

Figure 5.2.2 shows the viable cell count of *E. Bangladeshi* at the concentration of 1.28µg/ml after incubation with Secnidazole. The incubation periodwas 4 hours at 37^{o}C. The mean viable count of *E. Bangladeshi* in control was 7.15X10^{6} parasite/ml. The mean viable cell count of *E. Bangladeshi* was 3.65X10^{6} parasites/ml at the concentration of 1.28µg/ml after treating with Metronidazole. Viable cell count is reduced in the treatment groups when compared with the control.

5.8 Mean viable cell count of *E. Bangladeshi* after 4 hours incubation of Secnidazole (0.64µg/ml)

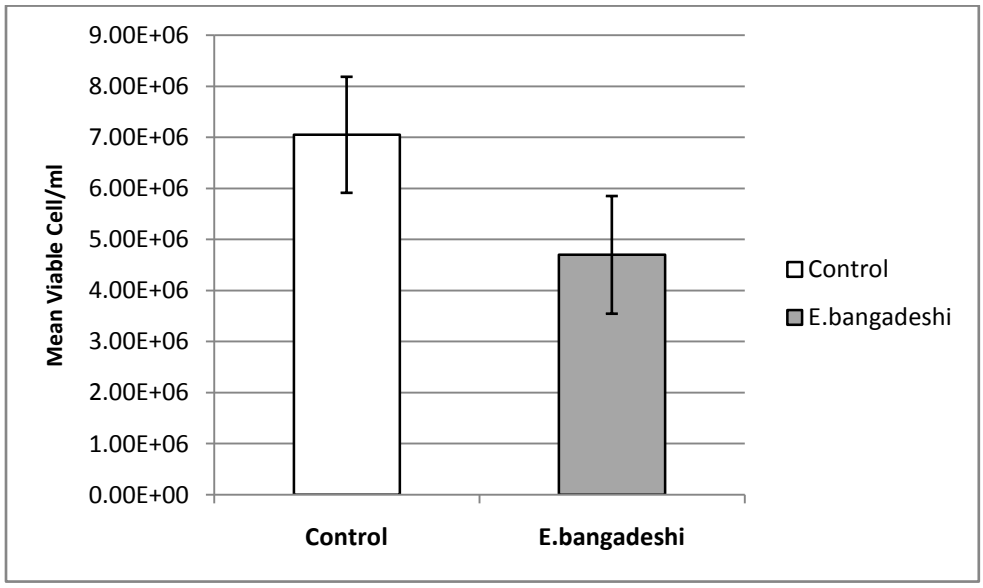


Figure 5.2.3: Mean viable cell counts of*E. Bangladeshi* after treatment with Secnidazole

Figure 5.2.3 shows the viable cell count of *E. Bangladeshi* at the concentration of 0.64µg/ml after incubation with Secnidazole. The incubation periodwas 4 hours at 37^{o}C. The mean viable count of *E. Bangladeshi* in controlwas7.05X10^{6} parasite/ml. The mean viable cellcount of *E. Bangladeshi* was 4.70X10^{6} parasites/ml at the concentration of 0.08 µg/ml after treating with Metronidazole. Viable cell count is reduced in the treatment groups when compared with the control.

5.9 Mean viable cell count of *E. Bangladeshi* after 4 hours incubation of Secnidazole (0.32µg/ml)

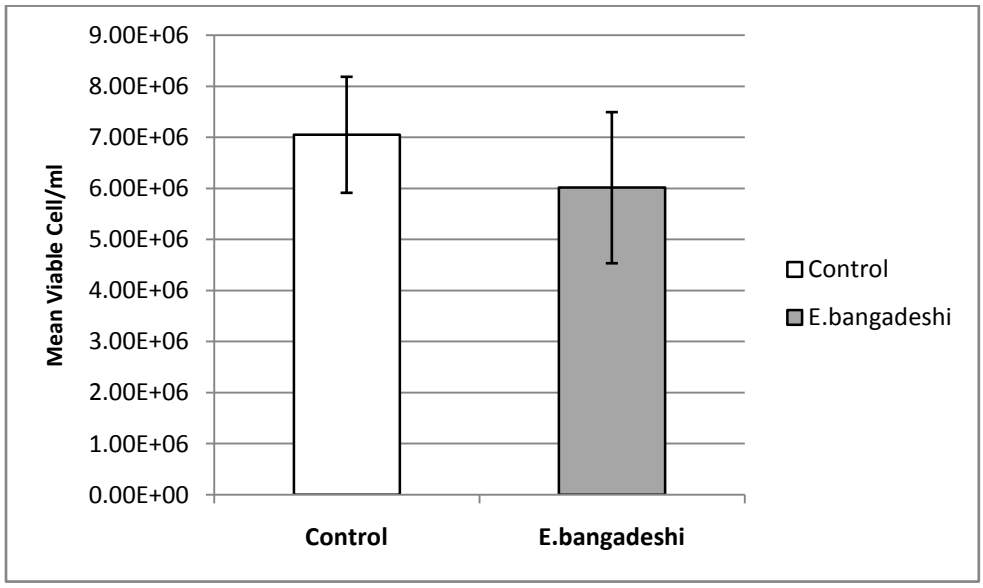


Figure 5.2.4: Mean viable cell counts of *E. Bangladeshi* after treatment with Secnidazole

Figure 5.2.4 shows the viable cell count of *E. Bangladeshi* at the concentration of 0.32µg/ml after incubation with Secnidazole. The incubation periodwas 4 hours at 37°C. The mean viable count of *E. Bangladeshi* in controlwas7.05X10^6 parasite/ml respectively. The mean viable cellcount of *E. Bangladeshi* was 6.02X10^6 parasites/ml at the concentration of 0.32µg/ml after treating with Metronidazole.Viable cell count is reduced in the treatment groups when compared with the control.

5.10 Mean viable cell count of *E. Bangladeshi* after 4 hours incubation of Secnidazole (0.16µg/ml)

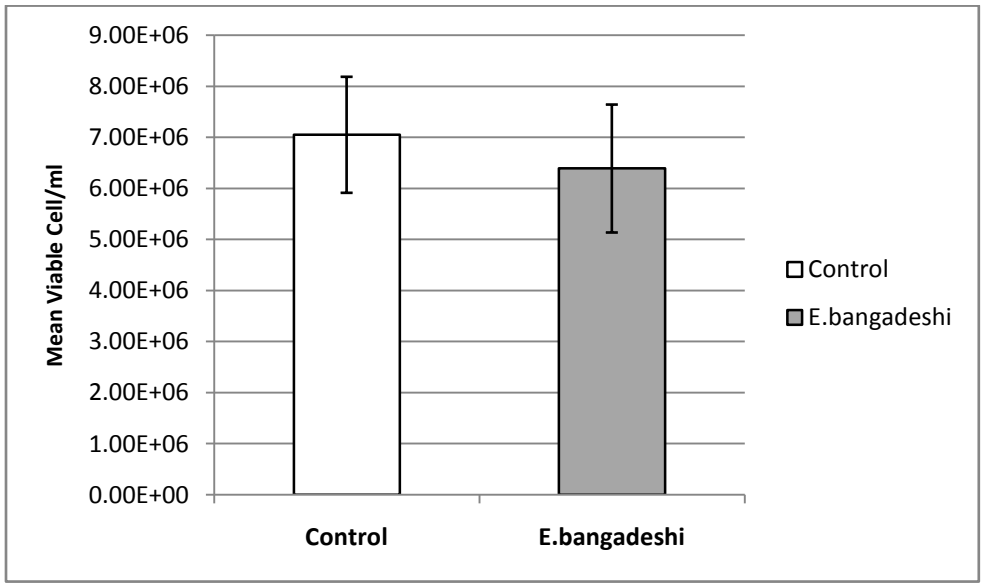


Figure 5.2.5: Mean viable cell counts of *E. Bangladeshi* after treatment with Secnidazole

Figure 5.2.5 shows the viable cell count of *E. Bangladeshi* at the concentration of 0.16µg/ml after incubation with Secnidazole. The incubation period was 4 hours at 37°C. The mean viable count of *E. Bangladeshi* in control was $7.05X10^6$ parasite/ml respectively. The mean viable cell count of *E. Bangladeshi* was $6.39X10^6$ parasites/ml at the concentration of 0.16µg/ml after treating with Metronidazole. Viable cell count is reduced in the treatment groups when compared with the control.

6.1 Table: In vitro sensitivity based on viable counts of E. Bangladeshi after 4 hours incubation

Drug concentration	**Metronidazoe against *E.bangladeshi***	**Secnidazole against *E.bangladeshi***
Control	$6.3X10^6$	$7.05X10^6$
2.56 μg/ml	$3.2X10^6$	$3.15X10^6$
1.28 μg/ml	$4.5X10^6$	$3.65X10^6$
0.64 μg/ml	$5.3X10^6$	$4.70X10^6$
0.32 μg/ml	$6.4X10^6$	$6.02X10^6$
0.16 μg/ml	$7X10^6$	$6.39X10^6$

The incubation period was 4 hours. After 4 hours of incubation, the viable parasite counts in Control were $6.3X10^6$ and$7.05X10^6$ for Metronidazole and Secnidazole respectively. The clinical isolates of *E.bangladeshi* were treated with Metronidazole and Secnidazole drugs at different concentrations. Viable counts of E. Bangladeshi were $3.2X10^6$, $4.5X10^6$ when different concentrations of Metronidazole (2.56 and 1.28 μg/ml) were used. Viable counts of E. Bangladeshi were $3.15X10^6$ and $3.65X10^6$ when different concentrations of Secnidazole (2.56 and 1.28 μg/ml) were used. The number of viable counts is decreased when the concentrations of drugs are increased. The lowest number of viable count of both parasites was found when they were treated with 2.56 μg/ml of either Metronidazole or Secnidazole. The viable cell counts of *E. bangladeshi* were different at different drug concentrations.

Discussion and Conclusion

Entamoeba histolytica produces amebic dysentery and liver abscess. Although amoebiasis (symptomatic infection with *E. histolytica*) is mostly prevalent in developing countries within adequate sanitation, the presence of endemic foci in developed countries, the speed of modern travel, and the current prevalence of amebic infection in the homosexual community make the treatment of this infection a matter of concern for all physician. In 2010-2011 a new species was indentified from the feces of children which were named *Entamoeba bangladeshi* in recognition of the community of Bangladesh for this research.

In the present study *in vitro* sensitivity of Metronidazole & Secnidazole against *E. Bangladeshi* were maintained by *in vitro*cultivation in axenic medium and were subjected to drug sensitivity tests against antiamoebic drugs. Several studies have been carried out to determine the sensitivity of Metronidazole and Secnidazole against E. histolytica. However, no studies have been shown in the literature about the effectiveness of the antiamoebic drugs against the novel species of *E. Bangladeshi.* Therefore, the effective use of anti-amoebic drugs against clinical isolates of E. Bangladeshi was tested in this study.

Different concentrations Metronidazole & Secnidazole were used against *E. bangladeshi*. The concentrations of Metronidazole and Secnidazole drugs were 2.56μg/ml1.28μg/ml, 0.64μg/ml, 0.32μg/ml, 0.16μg/ml, 0.08μg/ml in this present experiment. The incubation period was 4 hours. After 4 hours of incubation, the viable parasite counts in Control were $6.3X10^6$ and$7.05X10^6$ for Metronidazole and Secnidazole respectively. The clinical isolates of *E.bangladeshi* were treated with Metronidazole and Secnidazole drugs at different concentrations. Viable counts of E. Bangladeshi were $3.2X10^6$, $4.5X10^6$ when different concentrations of Metronidazole (2.56 and 1.28 μg/ml) were used. Viable counts of E. Bangladeshi were $3.15X10^6$ and $3.65X10^6$ when different concentrations of Secnidazole (2.56 and 1.28 μg/ml) were used. The number of viable counts is decreased when the concentrations of drugs are increased. The lowest number of viable count of both parasites was found when they were treated with 2.56 μg/ml of either Metronidazole or Secnidazole. The viable cell counts of *E. Bangladeshi* were different at

different drug concentrations. It also shows that the inhibition of parasite is occurred in a dose dependent manner which means cell inhibition is maximum at the highest concentration of Metronidazole and Secnidazole. In another study a significant difference in parasite counts has been observed between the combination and mono therapy of antiamoebic drugs (suki, 2015; unpublished). In our study we use Metronidazole and Secnidazole to carry out the same procedure to find out the sensitivity of the drug against a new species. Here we compared the viable cell counts of *E. Bangladeshi* with the control.

This new species was identified in 2010–2011 during the analysis of feces which was positive for Entamoeba organisms by microscopy or culture but negative for *E. histolytica, E. dispar*,and *E. moshkovskii* by PCR. This new species is named *Entamoeba Bangladeshi*. in recognition of the support of the Bangladesh community for this research. (Royer et al., 2012).

As *E. Bangladeshi* is a new novel species we do not know its pathogenicity. Moreover, Metronidazole and Secnidazole were effective against this parasite. In our study we have determined the sensitivity of Metronidazole & Secnidazole at different concentrations against *E.bangladeshi*.

In conclusion the present study suggests that both Metronidazole & Secnidazole is sensitive for *E. Bangladeshi*. However, there is insufficient evidence to draw conclusions regarding the efficacy of Metronidazole drugs against *E. bangladeshi*. Further study is needed to draw a more evidence-based conclusion of effectiveness of antiamoebic drugs against the new parasite *Entamoeba bangladeshi*.The results indicate that till date, *Entamoeba* isolates in Bangladesh do not seem to be resistant to the commonly used antiamoebic drugs.

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