In vitro efficacy study of Ornidazole against clinical isolates of *E. histolytica* and *E. bangladeshi*

A Dissertation submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Masters of Pharmacy.

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Declaration by the Research Candidate

I, Nabeela Zaman, hereby declare that this dissertation, entitled **"*In vitro* efficacy study of Ornidazole against clinical isolates of *E. histolytica* and *E. bangladeshi*"** submitted to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Masters of Pharmacy, is a genuine & authentic research work carried out by me under the guidance of Professor Dr. Sufia Islam, Department of Pharmacy, East West University, Dhaka. The contents of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any Degree or Diploma of Fellowship.

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Acknowledgement

All praise is for Almighty for all the bounties granted to me and only with His guidance and help this achievement has become possible.

It is my pleasure and proud privilege to express my heartiest regards and gratitude to my respected teacher and supervisor Prof. Dr. Sufia Islam, Department of Pharmacy, East West University, for her expert supervision, constructive criticism, valuable advice, optimistic counseling, constant support and continuous backup and encouragement throughout every phase of the project as well as to prepare this dissertation.

My Special Thanks and profound gratitude to Prof. Dr. Chowdhury Faiz Hossain, Chairperson, department of Pharmacy, East West university for giving me the opportunity to obtain this project and providing his valuable support for me.

Numerous thanks to ICDDR,B lab officers and other staffs for their help and assistance, friendly behavior and earnest co-operation which enabled me to work in a very congenial and comfortable ambience.

Finally, I am very much grateful to my beloved family for their love and supporting me during the period of this research and I also mentioned a lot of thanks to my thesis partner Evana Afrose for her kind contribution to my journey.

Dedication

This Research Paper is dedicated to

My beloved parents

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Abstract

Entamoeba histolytica and *Entamoeba Bangladeshi* both parasites are the member of *Entamoeba* group. *E. bangladeshi* is a novel species which is identified very recently and *E. histolytica* is a common parasite which is the main cause of amoebiasis. The drugs of choice for invasive amoebiasis are tissue active agents, like metronidazole, Ornidazole and chloroquine or the more toxic emetine derivatives, including dehydroemetine. Ornidazole is derived from 5-nitroimdazole which kill the trophozoites by alterations in the protoplasmic organelles of the amoeba. The aim of the study is to determine the efficacy of Ornidazole against clinical isolates of *E. histolytica* and *E. bangladeshi.* The clinical isolates of *E. histolytica* and *E. bangladeshi* were treated with Ornidazole at different concentrations (0.96, 0.48, 0.24, 0.12, 0.06, 0.03 μg/ml). Drug sensitivity assay of the samples was carried out by using microtiter plates containing 100μl of parasite suspension (1×10^6 parasites/ml). Plates were incubated at 37ºC. After 4 hours the viable parasites were counted by haemocytometer under microscope. Viable counts of the *E. histolytica* and *E. bangladeshi* in each concentration of drugs were compared with the control. Result shows that after treatment with Ornidazole, the cell count of *E. bangladeshi* is higher than *E. histolytica* when both parasites are compared with the control ($p<0.05$). There is also a significant difference in percent cell inhibition between the two clinical isolates. The percent cell inhibition of *E. histolytica is* 30% whereas only 18% cell inhibiton is observed for *E. bangladeshi.* We conclude that Ornidazole is sensitive against *E. bangladeshi*. However, the efficacy of Ornidazole to inhibit the parasites is significant in *E. histolytica* than *E. bangladeshi*. In this study the parasite inhibition was occurred in a dose dependent manner.

Key words: *Entamoeba histolytica*; *Entamoeba Bangladeshi;* amoebiasis; emetine derivatives; trophozoites; protoplasmic; microtiter plates; haemocytometer; incubation;

Chapter One

Introduction

1.1 Background

Amoebiasis is a disease caused by the parasite *Entamoeba histolytica*. Hippocrates who described a patient with fever and dysentery first recognized it as a deadly disease. With the application of a number of new molecular biology-based techniques, tremendous advances have been for the diagnosis, natural history, and epidemiology of amoebiasis. On a global basis, amoebiasis affects approximately 50 million persons each year resulting in 100,000 deaths. Amoebiasis is also very common in Bangladesh. Hippocrates first identified *E histolytica* around 300 B.C. by describing a patient with dysentery and fever. Developments came later in 1855 when it was suggested that the disease might have a parasitic origin. Finally, *Entamoeba histolytica* was identified from a stool sample in 1875 by FredorLosch (also known as FedorLesh). Throughout the world, amoebiasis is the second leading cause of death from a parasitic disease. Although it is the second leading cause of death from parasitic diseases, about 90% of the people exposed to *E. histolyticca* are asymptomatic or report very mild symptoms (Petri, 2003).

1.2 Parasites

A parasite is an organism that lives on or in a host organism and gets its food from or at the expense of its host. There are three main classes of parasites that can cause disease in humans: protozoa, helminthes and ectoparasites (Robert, 2015). Among them protozoa are microscopic, one-celled organism that can be free living or parasitic in nature. They are able to multiply in humans, which contributes to their survival and also permits serious infections to develop from just a single organism (Paulin, 2014).

1.3 Amoeba

Amoeba often called amoeboid. It is a type of cell which has the ability to alter its shape. An amoeba is any of several tiny, one-celled protozoa in the phylum (or primary division of the

animal kingdom) Sarcodina. The well-known type Amoeba Proteus live in freshwater and salt water, in soil, and as parasites in moist body parts of animals. They are composed of cytoplasm (cellular fluid) divided into two parts: a thin, clear, gel-like outer layer that acts as a membrane (ectoplasm); and an inner, more watery grainy mass (endoplasm) containing structures called organelles. Amoebas may have one or more nuclei, depending upon the species. It is found on decaying bottom vegetation of freshwater streams and ponds (Visvesvara et al., 2007).

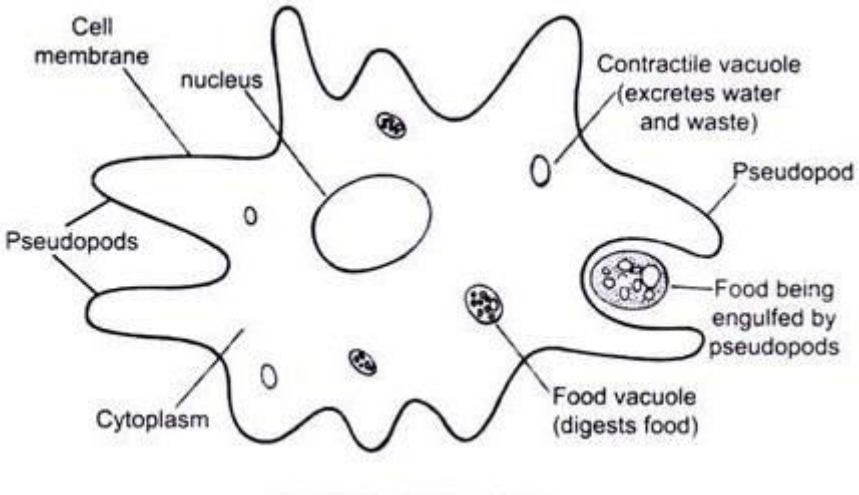

Figure 1.1: Amoeba

There are many varieties of free-living amoeba, but only four genera have been causally associated with disease in humans. These are *Acanthamoeba*s, *Balamuthia mandrillaris* (the only know species of *Balamuthia*), *Naegleriafowleri* (sometimes considered not to be an amoeba at all, but more closely related to *Leishmania*and *Trypanosoma*) and *Sappiniapedata*. They are distinct from the more famous *Entamoeba histolytica* (an obligate anaerobic parasite which can cause amoebiasis, amoebic dysentery and amoebic liver abscesses). *Acanthamoeba*s and *B. mandrillaris* are opportunistic pathogens causing infections of the CNS, lungs, sinuses and skin, mostly in immunocompromised humans. *B.*

mandrillaris is also associated with disease in immunocompetent children, and *Acanthamoeba*s pp. cause a sight-threatening keratitis, mostly in contact lens wearers. *N. fowleri* causes an acute and fulminating meningoencephalitis in immunocompetent children and young adults. A few human cases of encephalitis caused by *Sappiniadiploidea* have been described (Petri and Tanyuskel, 2003).

1.4 Distribution

These organisms are ubiquitous and found worldwide. Acanthamoebas pp. are found in soil, dust, air and water (eg, swimming pool, domestic and sewage), ventilation and air conditioning systems. They have been isolated in hospitals, medicinal pools, dental treatment units, dialysis machines and contact lenses. They have also been found in mammalian cell cultures, human nostrils and throats and human and animal brain, skin, and lung tissues. In cell cultures they are commonly contaminants. This is how they were discovered in the 1950s - they grew on cell cultures grown for the polio vaccine. Acanthamoebas pp. can also be found in fish and have been isolated from the nasal and throat mucosa of healthy humans. B. mandrillarishas not been isolated from the environment but has been isolated from autopsy specimens of infected humans and animals. N. fowleri is also ubiquitous and found in soil and warm fresh water. Sappinias pp. is found in soil and tree bark. Both Acanthamoebas pp. and B. mandrillariscan act as hosts for other bacterial infections - eg, legionellosis (Gurvinder, 2014).

1.5 Life cycle of Amoeba

Reproduction in amoeba is a periodic process taking place at intervals. Reproduction in amoeba chiefly occurs by asexual method, i.e., by binary fission, multiple fission and sporulation.

(i) Binary fission

It is the most common mode of reproduction. In this process, the whole body divides into two daughter amoebae by mitosis. The division involves nuclear division (karyokinesis) followed by division of cytoplasm (cytokinesis) (fig. 9.8). This takes place during favourable conditions.

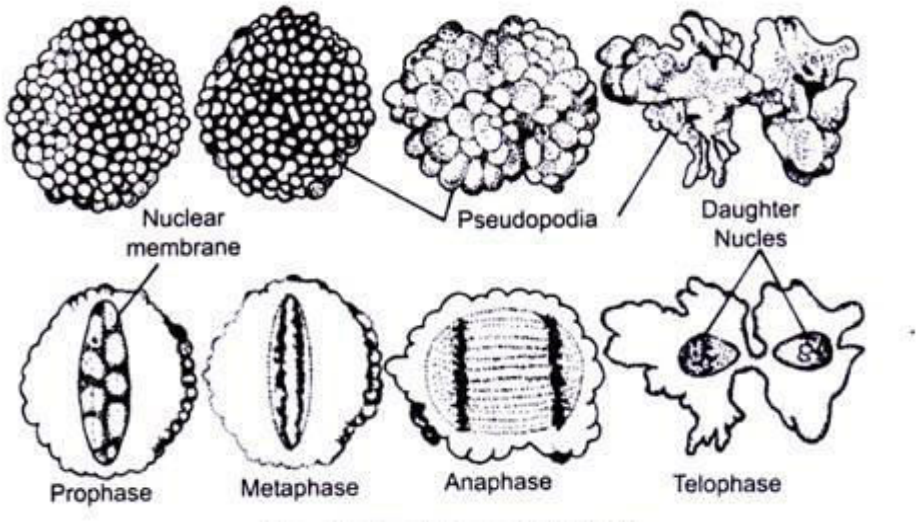

Figure 1.2: Binary fission in Amoeba

(ii) Sporulation

Under un-favourable conditions amoeba reproduces by formation of spores internally. It starts with the breakdown of nuclear membrane and release of chromatin blocks into the cytoplasm. Each chromatin blocks acquires a nuclear membrane and becomes a small daughter nuclei. The newly formed nuclei get surrounded by cytoplasm to form amoebulae. The peripheral cytoplasmic layer of amoebulae forms a tough and resistant spore-membrane or spore case (fig. 9.9). About 200 such spores are formed inside a single parent amoeba. Finally the body of parent amoeba disintegrates to release the spores. The spore

remain inactive for some time and on getting favourable conditions each spore forms a young amoeba.

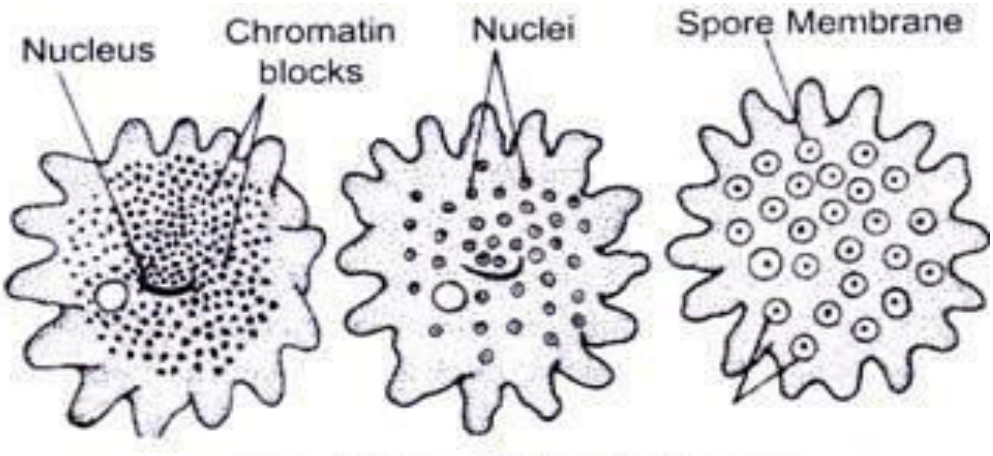

Figure 1.3: Sporulation in Amoeba

(iii) Multiple fission

In un-favourable conditions, amoeba divides by multiple fission. It withdraws its pseudopodia, becomes spherical and secretes three layered cyst around itself. Its nucleus undergoes repeated mitosis division forming 500- 600 daughter nuclei. Each daughter nucei gets surrounded by mass of cytoplasm and divides into minute amoebulae. On getting favourable conditions the cyst ruptures to release the amoebulae which soon grows into adult amoeba.

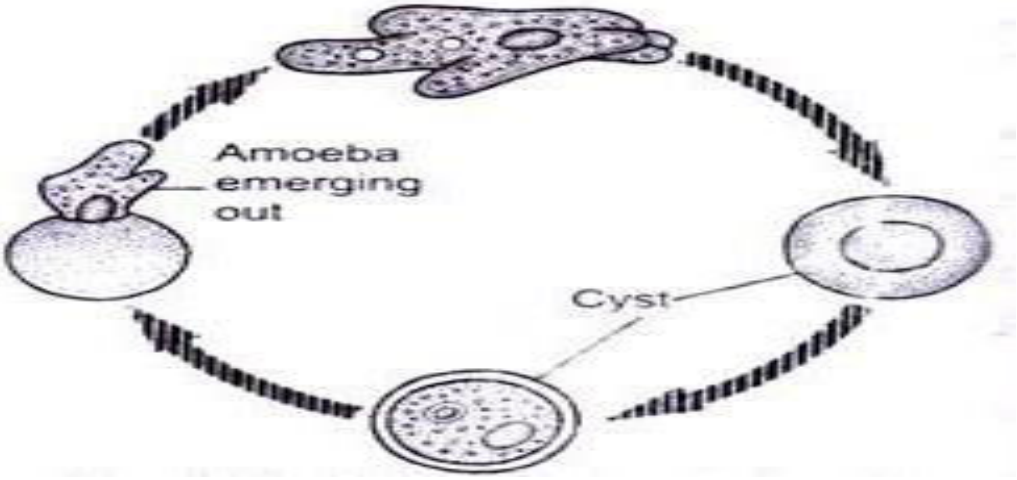

Figure: 1.4 Multiple fission in Amoeba

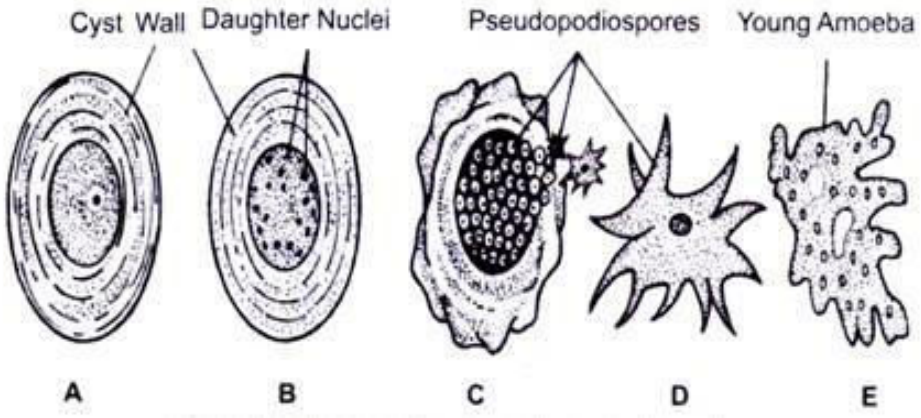

Figure 1.5: Stages of multiple fission in Amoeba

1.6 Regeneration

Amoeba has tremendous power of regeneration. If it is cut into small pieces, each piece regenerates into a new amoeba, however, a piece without nuclear fragment does not regenerate (Medhekar 2016).

1.7 Amoebiasis

Amoebiasis is an infection of intestine caused by a parasite *Entamoeba histolytica*. *It* remains an important health problem in tropical countries where sanitation infrastructure and health are often inadequate. This disease can present with no, mild, or severe symptoms. Only about 10% to 20% of people who are infected with *Entamoeba histolytica* become sick from the infection. It can affect anyone, although it is more common in people who live in tropical areas with poor sanitary conditions (Roger, 2016).

Amoebiasis is a common infection of the human gastro-intestinal tract. Amoebiasis is more closely related to poor sanitation and socioeconomic 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. In 1969, WHO defined amoebiasis, a condition in which a patient harbouring the organism *Entamoeba histolytica* in the bowel. Culture of *Entamoeba histolytica* is a long and laborious process. There are three basic types of culture systems of *Entamoeba histolytica*: xenic, in which the parasite is grown in the presence of an undefined flora; monoxenic, in which the parasite is grown in the presence of a single additional species; and axenic in which the parasite is grown in the absence of any other metabolizing cells (Farrar et al., 2013).

1.8 Etiology

Amebiasis is a parasitic infection caused by the protozoal organism *E. histolytica*, which can give rise both to intestinal disease (eg, colitis) and to various extraintestinal manifestations, including liver abscess (most common) and pleuropulmonary, cardiac, and cerebral dissemination. The genus Entamoeba contains many species, some of which (ie, *E. histolytica*, *Entamoeba dispar*, *Entamoeba moshkovskii*, *Entamoeba polecki*, *Entamoeba coli*, and *Entamoeba hartmanni*) can reside in the human interstitial lumen. Of these, *E. histolytica* is the only one definitely associated with disease; the others are considered nonpathogenic. Studies have recovered *E. dispar* and *E. moshkovskii* from patients with

gastrointestinal (GI) symptoms, but whether these species cause these symptoms remains to be determined. Although *E. dispar* and *E. histolytica* cannot be differentiated by means of direct examination, molecular techniques have demonstrated that they are indeed 2 different species, with E dispar being commensal (as in patients with HIV infection) and E histolytica pathogenic. It is currently believed that many individuals with Entamoeba infections are actually colonized with *E. dispar*, which appears to be 10 times more common than E histolytica; however, in certain regions (eg, Brazil and Egypt), asymptomatic *E. dispar* and *E. histolytica* infections are equally prevalent. In Western countries, approximately 20%-30% of men who have sex with men are colonized with *E dispar. E histolytica* is transmitted primarily through the fecal-oral route. Infective cysts can be found in fecally contaminated food and water supplies and contaminated hands of food handlers. Sexual transmission is possible, especially in the setting of oral-anal practices (anilingus). Poor nutrition, through its effect on immunity, has been found to be a risk factor for amebiasis (Dhawan 2017).

1.9 *Entamoeba histolytica*

Entamoeba histolytica is a pseudopod forming, anaerobic parasitic amoebozoa and a part of the genus Entamoeba. Predominantly infecting humans and other primates causing amoebiasis, *Entamoeba histolytica* is estimated to infect about 50 million people worldwide (Weedall and Hall, 2011).

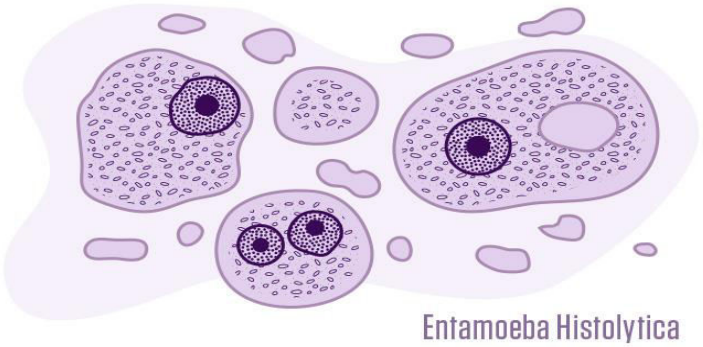

Figure 1.6: Microscopic image of *Entamoeba histolytica*

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 due to this infection. *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 per cent of the infections. Poverty, ignorance, overcrowding, poor sanitation and malnutrition favour transmission and increased disease burden. Prevalence varies from country to country and within a country. *Entamoeba histolytica* infections can be detected through fecal microscopy, culture, PCR, and antigen detection (Ryan & Ray, 2004).

1.10 Scientific classification

Kingdom	Protista
Subkingdom	Protozoa
Phylum	Sarcomastigophora
Class	Lobosea
Order	Amoebida
Family	Entamoebida
Genus+Species	*Entamoeba histolytica*

1.11 Life cycle

Entamoeba histolytica is a monogenetic parasite as its life cycle is completed in a single host i.e., man. Three distinct morphological forms exist in its life cycle. - Trophozoite, Pre-cystic stage and Cystic stage (Saritha, 2015).

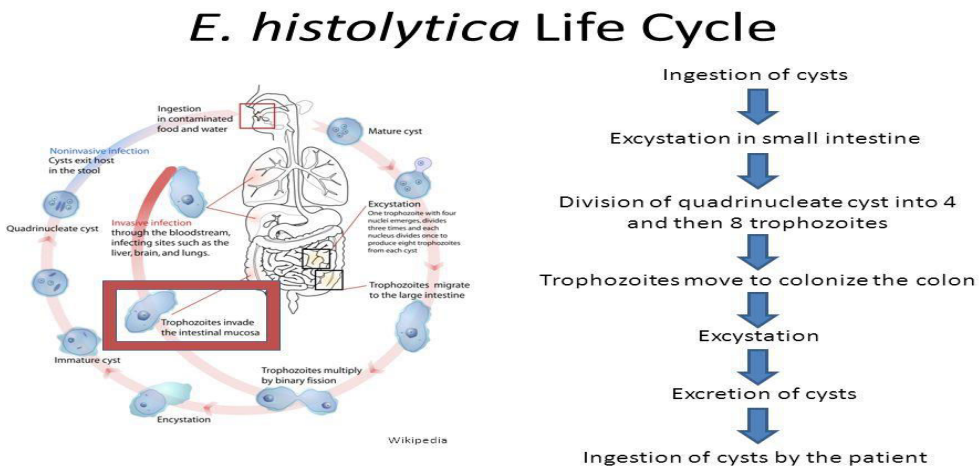

Figure 1.7: Life cycle of *E. histolytica*

1.12 Transmission

A matured quadrinucleate cyst of *Entamoeba histolytica* is the infective stage of the parasite. Transmission of *Entamoeba histolytica* from one person to another occurs due to ingestion of these cysts. Fecal contamination of edible substances and drinking water are the primary 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 agent to carry infective cysts to the food and drink. Sometimes drinking water supply contaminated with infected faces give rise to epidemics.

(b) Oral-rectal contact:

Sexual transmission by oral-rectal contact is also one of the modes of transmission, specially among male homosexuals (Sodeman, 1996).

1.13 Structure

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

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

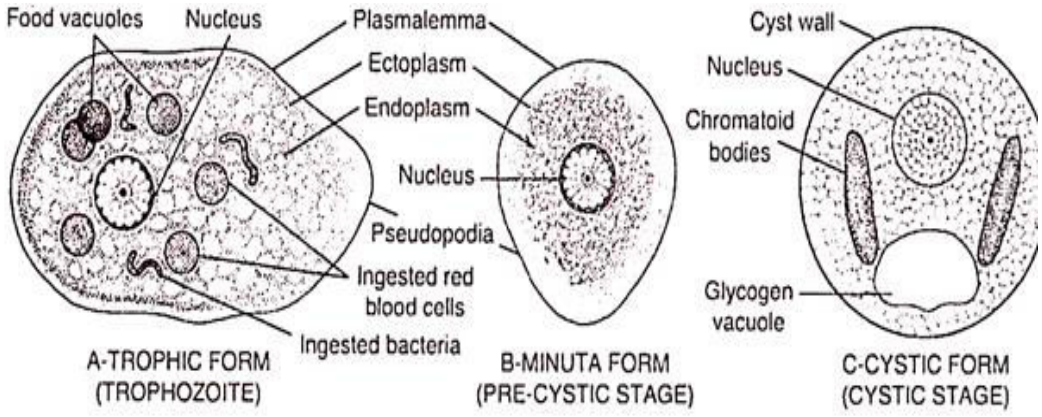

Figure 1.8: Stages of *Entamoeba histolytica*

1. Trophozoite stage

Trophozoite is the motile, feeding and pathogenic stage of *Entamoeba histolytica*. It measures about 20-30 microns in diameter. Trophozoite is surrounded by the limiting membrane called plamalemma.

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 *Entamoeba histolytica*. It is relatively smaller in size, measuring about 10-20 micrometers.

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

encystation which is a means to tide over the unfavourable conditions that the parasite is going to encounter while that the parasite is going to encounter while passing to a new host (Sodeman, 1996).

1.14 Geographical distribution

Entamoeba histolytica is an enteric protozoan parasite with worldwide distribution. But it is more common in tropical and sub-tropical countries. In India it occasionally takes an epidemic form. It is estimated that about seven to eleven per cent of the population in India suffers from its infection cells (Lauren et al., 2016).

1.15 Excystation

When the quadrinucleate cyst enters in the ileum of the small intestine of the new host, the process of excystation begins. Excystation is the process of transformation of cysts to the trophozoites. It occurs in the intestinal lumen of the host. The parasite at this stage moves into the caecum of the host's large intestine, get attached to the epithelial 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).

1.16 Pathology

The lesions produced by *E. histolytica* are primarily intestinal, and secondary extra intestinal. 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 stools containing blood, mucous and necrotic mucosa accompanied by acute abdominal pain, tenderness and fever. Chronic amoebiasis is characterized by recurrent attacks of dysentery with gastrointestinal disturbance. In extra intestinal amoebiasis, the liver is invaded chiefly, resulting amoebic hepatitis or liver abscess. An enlarged, tender liver, with

pain in upper right hipochondrium, characterizes it. Less frequently, lung abscess, splenic abscess, brain abscess or cutaneous amoebic lesions are seen (Vinod, 2015).

1.17 *Entamoeba bangladeshi*

In 2010–2011, during analysis of feces positive for Entamoeba organisms by microscopy or culture but negative for *Entamoeba histolytica*, *E. dispar*, and *E. moshkovskii* by PCR, a new species was identified, which was named *Entamoeba bangladeshi* nov. sp. in recognition of the support of the Bangladesh community for this research. By light microscopy, we detected no apparent differences between *Entamoeba bangladeshi* and *Entamoeba histolytica*. The physical resemblance between *Entamoeba histolytica* and *Entamoeba bangladeshi* is notable because direct microscopic examination of fecal samples is still used as a diagnostic tool in areas to which these species are endemic to detect *Entamoeba histolytica* parasites. (Haque R, Mondal D, 2009). To further characterize *Entamoeba bangladeshi*, it has the ability to established in xenic 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 *Entamoeba histolytica* and *E. dispar.* Tests negative in *Entamoeba histolytica* ELISA and in species-specific PCRs. Currently only identifiable by its small subunit ribosomal RNA gene sequence (Stensvold CR, 2011).

1.18 Treatment of amoebiasis

In endemic areas, asymptomatic infections are not treated. In nonendemic areas, however, asymptomatic infection should be treated; luminal agents that are minimally absorbed by the GI tract (eg, paromomycin, iodoquinol, and diloxanidefuroate) are best suited for such therapy. This recommendation is based on 2 arguments: first, that invasive disease may develop, and second, that shedding of E histolytica cysts in the environment is a public health concern (Vinod, 2015). Infection is primarily treated by instituting antiamoebic therapy. The drugs of choice for invasive amoebiasis are tissue active agents, like

metronidazole, Ornidazole and chloroquine or the more toxic emetine derivatives, including dehydroemetine. Ornidazole is derived from 5-nitroimdazole which kill the trophozoites by alterations in the protoplasmic organelles of the amoeba. (Thomson, 2006).

1.19 Ornidazole

Ornidazole is a nitroimidazole antiprotozoal agent used in ameba and trichomonas infections. It is partially plasma-bound and also has radiation-sensitizing .Ornidazole is rapidly absorbed from the GI tract and peak plasma concentrations of about 30 mgm per ml have been achieved within 2 hours of a single dose of 1.5 gm, falling to about 9 mgm per ml after 24 hours and 2.5 mgm per ml after 48 hours. The plasma elimination half-life is 12-14 hours. Less than 15% is bound to plasma proteins. Ornidazole is widely distributed in body tissues and fluids, including the cerebrospinal fluid. It is extensively metabolized in the liver (95%) and excreted in the urine, mainly as conjugates and metabolites, and to a lesser extent in the faeces. Billiary excretion is important in the elimination of Ornidazole and its metabolite. Ornidazole is equally effective as Metronidazole. Plasma protien binding is <15%. And metabolism is reported Hepatic. Its plasma half-life is 12-14 hrs. The molecular weight of Ornidazole is 219.625 g/mol. It is metabolized via the liver, excreted in the Urine and feces. 85% of single oral dose is eliminated with 5 days urine (63%) and feces (22%) (Thomson, 2006).

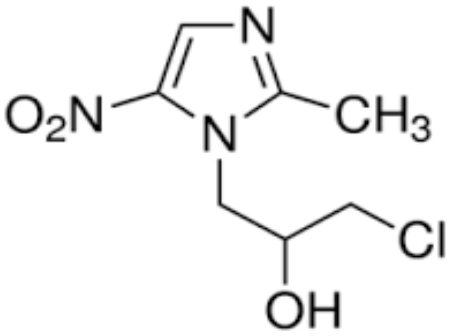

Figure 1.9: Structure of Ornidazole

1.20 Pharmacology of Ornidazole

Ornidazole is rapidly absorbed from the GI tract and peak plasma concentrations of about 30 mgm per ml have been achieved within 2 hours of a single dose of 1.5 gm, falling to about 9 mgm per ml after 24 hours and 2.5 mgm per ml after 48 hours. The plasma elimination half-life is 12-14 hours. Less than 15% is bound to plasma proteins. Ornidazole is widely distributed in body tissues and fluids, including the cerebrospinal fluid. It is extensively metabolized. in the liver (95%) and excreted in the urine, mainly as conjugates and metabolites, and to a lesser extent in the faeces. Billiary excretion is important in the elimination of Ornidazole and its metabolites (Thomson, 2006).

1.21 Mechanism of action of Ornidazole

Ornidazole is a nitro imidazole which has broad spectrum cidal activity against protozoa and some anaerobic bacteria. Its selective toxicity to anaerobic microbes involves

1. Drug enters the cell by diffusion,

2. Nitro group of drug is reduced by redox proteins present only in anaerobic organisms to reactive nitro radical which exerts cytotoxic action by damaging DNA and other critical biomolecules.

3. DNA helix destabilization &strand breakage has been observed (Thomson, 2006).

1.22 Metronidazole

Metronidazole is an antibiotic that is used to treat a wide variety of infections. It works by stopping the growth of certain bacteria and parasites. This antibiotic treats only certain bacterial and parasitic infections. It will not work for viral infections (such as common cold, flu). Using any antibiotic when it is not needed can cause it to not work for future

infections. Metronidazole may also be used with other medications to treat certain stomach/intestinal ulcers caused by a bacteria (H. pylori) (Lauren et al., 2016).

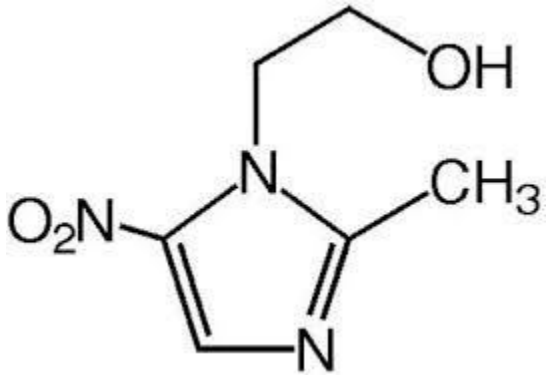

Figure 1.10: Structure of metronidazole

1.23 Pharmacology of Metronidazole

Metronidazole, a synthetic antibacterial and antiprotozoal agent of the nitroimidazole class, is used against protozoa such as *Trichomonas vaginalis*, amebiasis, and giardiasis. Metronidazole is extremely effective against anaerobic bacterial infections and is also used to treat Crohn's disease, antibiotic-associated diarrhea, and rosacea. Metronidazole is a synthetic nitroimidazole derivative with antiprotozoal and antibacterial activities. Although its mechanism of action is not fully elucidated, un-ionized metronidazole is readily taken up by obligate anaerobic organisms and is subsequently reduced by low-redox potential electron-transport proteins to an active, intermediate product. Reduced metronidazole causes DNA strand breaks, thereby inhibiting DNA synthesis and bacterial cell growth (NCI term browser, 2016).

1.24 Mechanism of action of Metronidazole

Metronidazole is of the nitroimidazole class. It inhibits nucleic acid synthesis by disrupting the DNA of microbial cells. This function only occurs when metronidazole is partially reduced, and because this reduction usually happens only in anaerobic cells, it has relatively little effect upon human cells or aerobic bacteria (Lauren et al., 2016).

Chapter Two

Literature Review

2.1 *In vitro* sensitivity of different brands of antiamoebic drugs (Metronidazole tablets) against clinical isolates of *Entamoeba histolytica* in bangladesh

This study has shown that *In vitro* sensitivity of different metronidazole tablets from Bangladeshi pharmaceuticals against clinical isolates of *E. histolytica.* Metronidazole tablets of 12 different brands were randomized from some big and small pharmaceuticals according to their business. The parasite count was adjusted to $3x10^6$ parasites mL^{-1} in a medium. *In vitro* drug sensitivity assay of the samples was carried out by using microtiter plates after treatment with different concentrations of metronidazoles. The viable parasites were counted by haemocytometer. No statistical significant difference was observed in terms of viable parasites with the metronidazole tablets from three big pharmaceuticals at the concentration of 2.3, 3.5 and 4.6 μM when compared with the standard metronidazole. The brands from some big pharmaceuticals showed *In vitro* sensitivity against *E. histolytica* (Sarker et al., 2008).

2.2 *In vitro* studies on the sensitivity of local *Entamoeba histolytica* to anti-amoebic drugs

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. Locally isolated strains from thirty hosts were 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 the MIC of ornidazole, metronidazole and tinidazole. Metronidazole was superior to that of dehydroemetine but

was not significantly different among ornidazole, metronidazole and tinidazole (Chintana et al., 1986).

2.3 *In vitro* activity of antiamoebic drugs against clinical isolates of *Entamoeba histolytica* and *Entamoeba dispar*

This 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 (15 *E. histolytica* and 30 *E. dispar*) 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* (HM1: IMSS) was assessed by nitro blue tetrazolium (NBT) reduction assay after exposure to various concentrations of each drug. The results showed that all clinical isolates had a higher IC_{50} compared to reference strain to all the four drugs. *E. histolytica* isolates appeared to be more susceptible compared to *E. dispar* isolates and the reference strain of *E. histolytica* after treatment with metronidazole, chloroquine, emetine and tinidazole (Bansal et al., 2004).

2.4 Study of combination regimens of anti-amoebic drugs for the treatment of amoebic dysentery caused by *E. histolytica*

This study determined the sensitivity of the combination regimens of anti-amoebic drugs against clinical isolates of *E. histolytica*. The clinical isolates of *E. histolytica* were treated with metronidazole, ornidazole, metronidazole+ornidazole, secnidazole, metronidazole + secnidazole, tinidazole, and metronidazole+tinidazole at different concentrations (12, 6, 3 &1.5 mg/ml). Drug sensitivity assay of the samples was carried out by using microtiter plates containing 50 µl of parasite suspension (3×106 parasites/ml). Plates were incubated at 37ºC. After 4 hours the viable parasites were counted by haemocytometer under

microscope. Viable counts of the *E. histolytica* in each concentration of drugs were compared to the control. Result showed that combination of metronidazole and ornidazole (1.5 mg/ml) inhibit the growth of *E. histolytica* and it has found significantly different when compared with the control ($p<0.05$). Combination of tinidazole and metronidazole at the concentration of 6 and 12mg/ml has also found statistically significant ($p<0.05$) to inhibit the growth of *E. histolytica* when compared with the control. At the concentration of 3 mg/ml, only tinidazole was significantly different when compared with the control to inhibit the growth of *E. histolytica* (Suki 2015).

2.5 *Entamoeba bangladeshi*: An insight

Molecular tools have the potential to differentiate microscopically similar gut micro-eukaryotes that may have significantly different relationships with the human host. Using broad range *Entamoeba* primers to amplify a section of the eukaryotic 18S small subunit ribosomal RNA gene a novel member of the *Entamoeba* family (*Entamoeba bangladeshi*) has recently been identified. Primers directed against a small subunit rRNA region conserved throughout the *Entamoeba* family were used to amplify a variable section of the gene, and its sequence confirmed that *E. bangladeshi* was a novel species that was most similar to the other members of the *Entamoeba* family, which infect humans, *E. histolytica* and *E. dispar*. The goal of this review is to place this species in the context of what is already known about this genus and to discuss the tools and data needed to elucidate the host-microbe relationship (Gilcrist et al., 2014).

2.6 Entamoeba bangladeshi nov. sp., Bangladesh

Feces from both diarrheal 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 amoebic trophozoites and cysts. In 2010–

2011, during analysis of feces, a new species was identified 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* nov.sp. in recognition of the support of the Bangladesh community for this research. The incidence and effect of infection in infants by the newly recognized species *E. bangladeshi* await future epidemiologic studies (Royer et al., 2012).

Chapter Three

Objective

3.1 Objective of the Study

The objective of the study is to compare the efficacy of Ornidazole at different concentration against *Entamoeba histolytica* and *Entamoeba bangladeshi*

Chapter Four

Materials and Method

4.1 Active ingredients of Ornidazole

The active ingredient (API) of Ornidazole tablet was collected from Drug International Limited of Bangladesh. The physical appearance, name of the manufacturer, batch number, manufacturing date, expiry date, manufacturing license number, D.A.R. number were properly checked for the active ingredient.

4.2 *E. histolytica* and *E. bangladeshi*

The cells of parasites were collected from parasitology laboratory of ICDDR, B.

4.3 Period and place of the study

This investigation was performed in the parasitology laboratory of ICDDR, B during September 2016 to February 2017.

4.4 Preparation of stock solution

0.96µg/ml of Ornidazole solution was prepared. For this preparation, at first 0.96 µg of active ingredient was weighed and then distilled water was filled to make it 1 ml. After preparation of the stock solution it was stored in the refrigerator.

4.5 Preparation of parasitic cells

Clinical isolates of *E. histolytica* and *E. bangladeshi* were harvested from 24 hours old cultures and suspended in a LYI-S-2 medium. The Axenic medium (LYI-S-2) consists of liver digest, yeast extract, iron, serum. The parasite count was adjusted to 1×10^6 parasites/ml in medium 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 previously sterilized, and was thereby rid of contaminating organisms. The isolation process was performed in the Parasitology Laboratory, Laboratory Sciences Division, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b).

4.6 Apparatus and instruments

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

1. Analytical balance

An analytical balance is a class of balance designed to measure small mass in the sub-milligram range. The measuring pan of an analytical balance (0.1 mg or better) is inside a transparent enclosure with doors so that dust does not collect and so any air currents in the room do not affect the balance's operation.

Figure 4.1: Analytical Balance

2. Microscope

A microscope is an instrument used to see objects that are too small to be seen by the naked eye. Microscopic means invisible to the eye unless aided by a microscope.

Figure 4.2: Microscope

3. 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. Micropipettes differ in size and volume dispensed and depending on those particular aspects they also require specific pipette tips.

Figure 4.3: Micropipette

4. Microtips

A microtiter plate 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 4.4: Microtips

5. Vortex machine

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. Most vortex mixers are designed with 2 or 4-plate formats, have variable speed settings ranging from 100 to 3,200 rpm, and can be set to run continuously, or to run only when downward pressure is applied to the rubber piece.

Figure 4.5: Vortex Machine

6. Incubator

An incubator is a device used to grow and maintain microbiological cultures or cell cultures. 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.

Figure 4.6: Incubator

7. Microtiter plate

A microtiter plate 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.

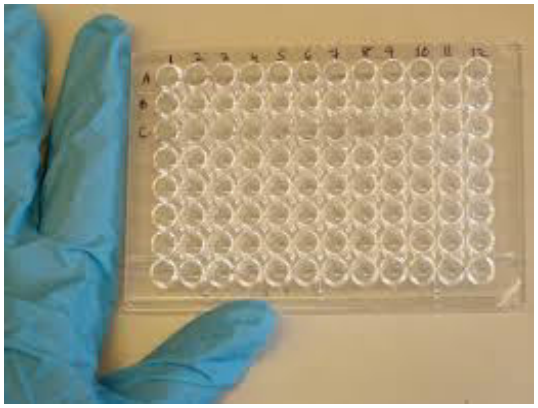

Figure 4.7: Microtiter Plate

8. Haemacytometer

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 4.8: Haemocytometer

4.7 Procedure

- Drug sensitivity assay of the samples was carried out by using microtiter plates. In wells (A-1) to (F-1), except (C-1) 100 µl medium was added. In the whole procedure B-1 was empty.
- Then in (C-1), 100 µl Ornidazole stock solution was added and serial dilutions of the drugs were performed from {(C-1) to (F-1)}.
- All the plates were mixed properly.

- 100 µl of the medium from the well (F-1) was discarded to maintain the equality of the concentration of the drugs.
- Further 100 µl of parasite suspension (1×10^6 parasites/ml) was added to all the wells {(A-1) to (F-1)}.
- The final concentrations of the drugs were 0.96, 0.48, 0.24, 0.12, 0.06 and 0.03 mg/ml.
- Well (A-1) was control. It contains only media and cells.
- Then plastic strip was used to cover the plate.
- Plates were incubated at 37ºC for 4 hours.
- After the incubation period the plate was taken from the incubator.
- Then the viable parasites of each well were counted by haemocytometer under microscope.

This whole procedure was performed for both *E.histolytica* and *E. bangladeshi*.

4.8 Statistical analysis

Data were analyzed with Prism 6 (GraphPad Software, La Jolla, CA, USA). One way analysis of Variance with a post hoc HolmeSidak multiple comparisons test was used for data analysis. Statistical significance was assigned to P values <0.

Chapter Five

Results

5.1 Determination of viable cell count of *Entamoeba histolytica* and *Entamoeba bangladeshi* after treatment with Ornidazole

The study was conducted with active ingredient of Ornidazole manufactured by Drug International Limited. The *in vitro* sensitivity of Ornidazole against *Entamoeba histolytica* and *Entamoeba bangladeshi* was observed after treating with different concentration of Ornidazole. The different concentration were 0.96, 0.48, 0.24, 0.12, 0.06 and 0.03 µg/ml.

5.1.1 Mean viable cell count of *Entamoeba histolytica* and *Entamoeba bangladeshi* after treatment with 0.96µg/ml of Ornidazole.

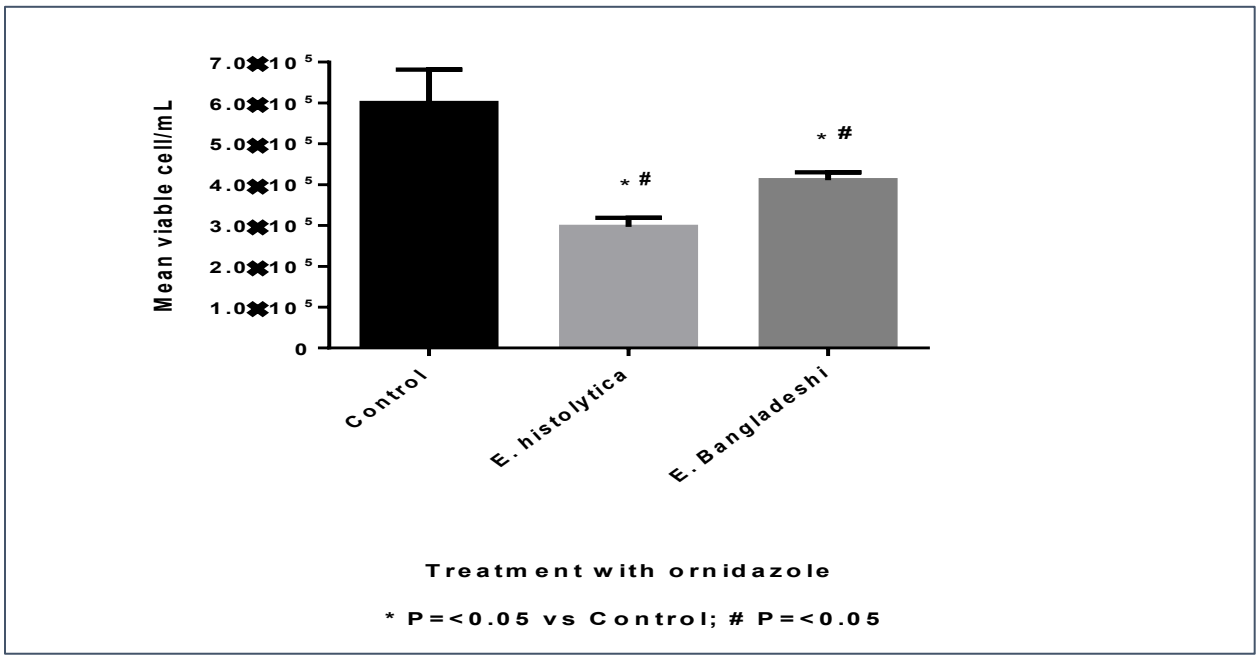

Figure 5.1: Mean viable cell count of *Entamoeba histolytica* and *Entamoeba bangladeshi*after treatment with Ornidazole (0.96µg/ml)

The bar diagram shows the viable cell count from clinical isolates of *Entamoeba histolytica* and *Entamoeba bangladeshi* after treatment with Ornidazole. The incubation period was 4

hour. The viable cell count of *Entamoeba bangladeshi* is significantly higher (p= <0.05) when it was compared with cell count of *Entamoeba histolytica*. The viable count of *E. histolytica* in control was $6.0X10^5$ cell/ml. The viable cell counts of *E. histolytica* after treatment with Ornidazole was $2.96X10^5$ cell/ml. However, the viable cell count of *E. bangladeshi* after treatment with Ornidazole was $4.11X10^5$ cell/ml.

5.1.2 Mean viable cell count of *Entamoeba histolytica* and *Entamoeba bangladeshi* at 0.48µg/ml of Ornidazole after 4 hours of incubation

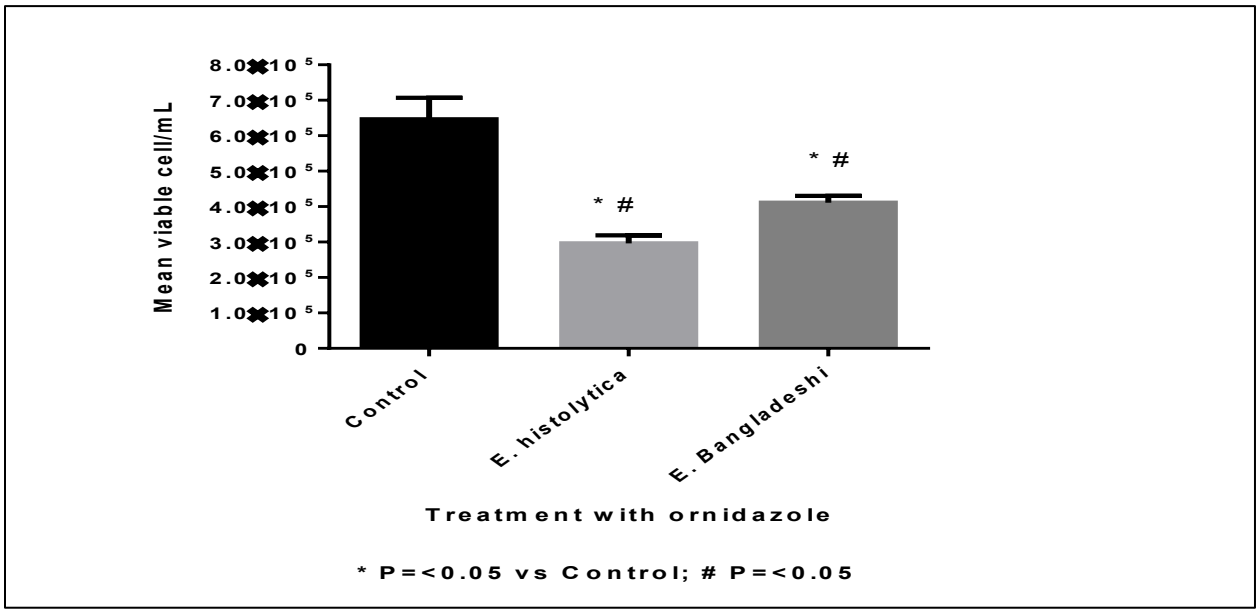

Figure 5.2: Difference of viable cell count between *Entamoeba histolytica* and *Entamoeba bangladeshi*

The bar diagram shows the statistical sigficance among the viable cell count from clinical isolates of *Entamoeba histolytica* and *Entamoeba bangladeshi* after treatment with Ornidazole. When the two parasites were treated with 0.48 µg/ml drug concentration, *Entamoeba bangladeshi* is significantly greater (p= <0.05) than the standard *Entamoeba*

histolytica in terms of viable cell count. The viable count of *E. histolytica* in control was $6.0X10^5$ cell/ml. The viable cell counts of *E. histolytica* after treatment with Ornidazole was $3.25X10^5$ cell/ml. However, the viable cell count of *E. bangladeshi* after treatment with Ornidazole was $4.5X10^5$ cell/ml.

5.1.3 Mean viable cell count of *E. histolytica* and *E. bangladeshi* at 0.24 µg/ml of Ornidazole after 4 hours of incubation

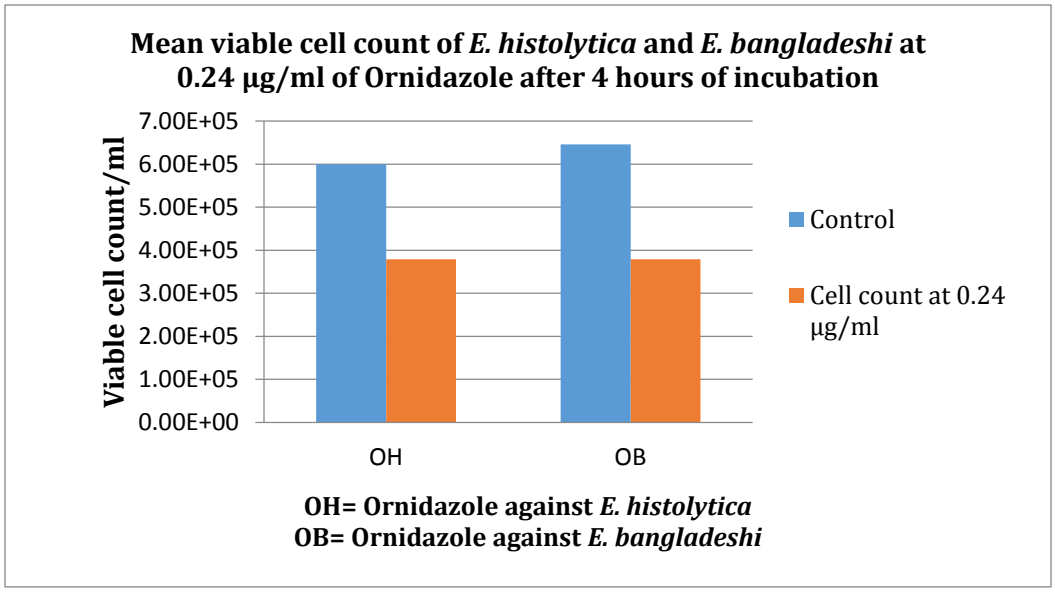

Figure 5.3: Difference of viable cell count between *E. histolytica* and *E. bangladeshi*

The bar diagram shows the viable cell count from clinical isolates of *Entamoeba histolytica* and *Entamoeba bangladeshi* after treatment with Ornidazole at 0.24 µg/ml. The incubation period was 4 hour. Mean viable cell count of both parasites were also compared with their control individually. In the above figure, the mean viable cell count of *E. histolytica* in control was $6.0X10^5$ cell/ml and after treatment with drug at 0.24 µg/ml the viable cell count of *E. histolytica* was observed $3.79X10^5$ cell/ml. In case of *E. bangladeshi* the cell count in control was $6.46X10^5$ cell/ml and after treatment with drug it was $4.96X10^5$

cell/ml. The mean viable cell count of *Entamoeba bangladeshi* is significantly higher (p= <0.05) when it was compared with cell count of *Entamoeba histolytica*.

5.1.4 Viable cell count of *E. histolytica* and *E. bangladeshi* at 0.12µg/ml of Ornidazole after 4 hours of incubation

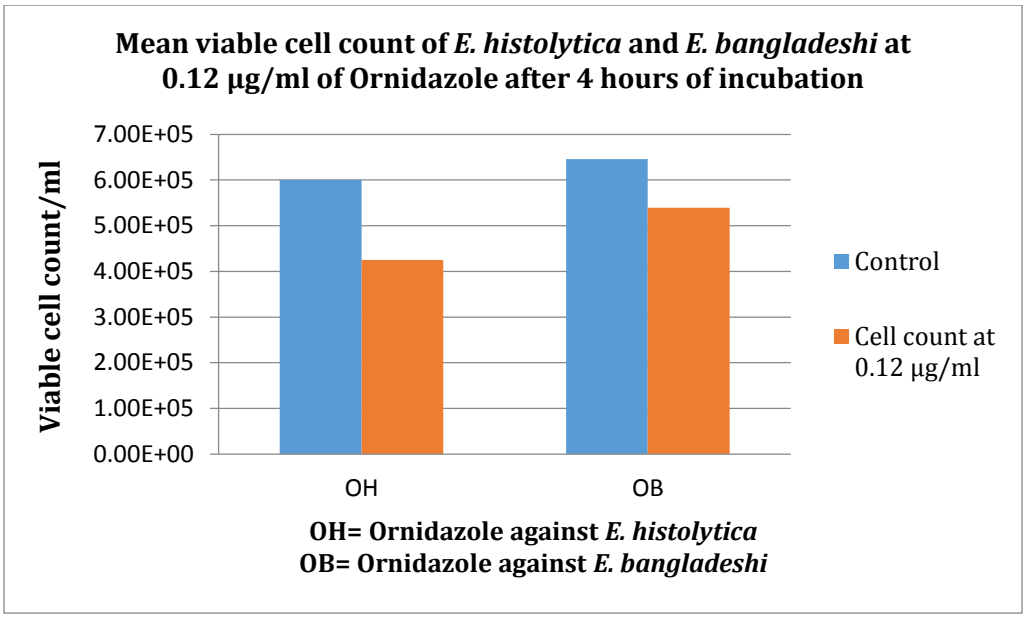

Figure 5.4: Difference of viable cell count between *E. histolytica* and *E. bangladeshi*

The bar diagram shows the viable cell count of *Entamoeba histolytica* and *Entamoeba bangladeshi* after treatment with Ornidazole at 0.12 µg/ml.after 4 hours of incubation the mean viable cell count of both parasites were also compared with their control individually. In the above figure, the mean viable cell count of *E. histolytica* in control was $6.0X10^5$ cell/ml whereas the viable cell count of *E. histolytica* was observed $3.79X10^5$ cell/ml after treatment with Ornidazole at 0.12 µg/ml. In case of *E. bangladeshi* the cell count in control was $6.46X10^5$ cell/ml and after treatment with drug it was $4.96X10^5$ cell/ml. The mean viable cell count of *Entamoeba bangladeshi* is significantly higher (p= <0.05) when it was compared with cell count of *Entamoeba histolytica.*

5.1.5 Viable cell count of *E. histolytica* and *E. bangladeshi* at 0.06µg/ml of Ornidazole after 4 hours of incubation

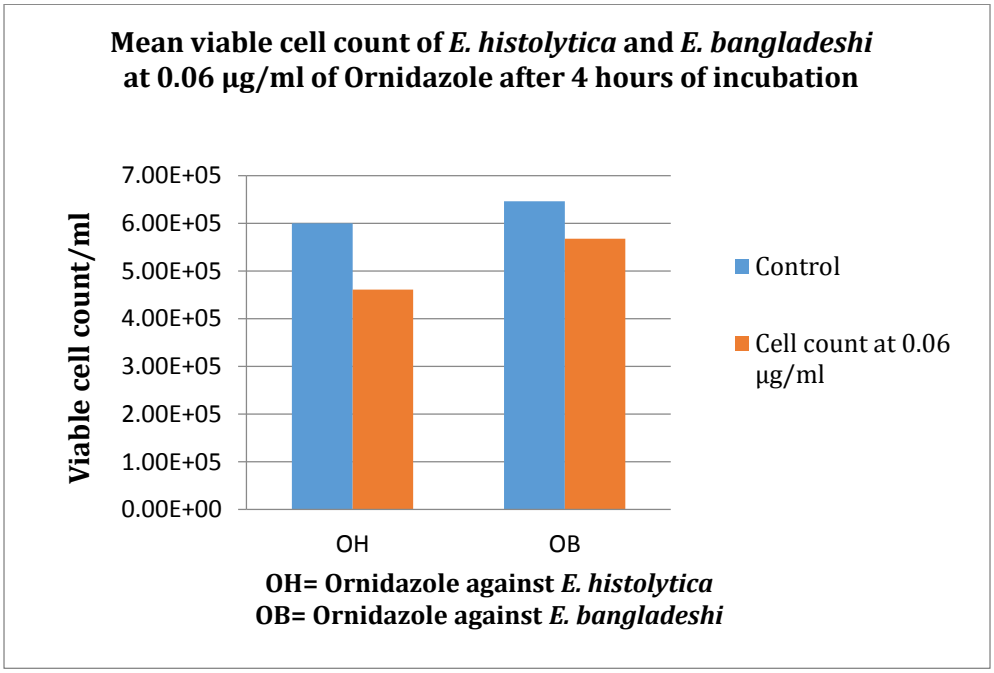

Figure 5.5: Difference of viable cell count between *E. histolytica* and *E. bangladeshi*

The above figure shows the viable cell count of *Entamoeba histolytica* and *Entamoeba bangladeshi* after treatment with Ornidazole at 0.06 µg/ml. The incubation period was 4 hours after that period the mean viable cell count of both parasites were also compared with their control individually. In the above bar diagram, the mean viable cell count of *E. histolytica* in control was $6.0X10^5$ cell/ml whereas the viable cell count of *E. histolytica* was observed $4.61X10^5$ cell/ml after treatment with Ornidazole at 0.06 µg/ml. In case of *E. bangladeshi* the cell count in control was $6.46X10^5$ cell/ml and after treatment with drug it was $5.68X10^5$ cell/ml. The mean viable cell count of *Entamoeba bangladeshi* is significantly higher ($p = <0.05$) when it was compared with cell count of *Entamoeba histolytica.*

5.1.6 Viable cell count of *E. histolytica* and *E. bangladeshi* at 0.03µg/ml of Ornidazole after 4 hours of incubation

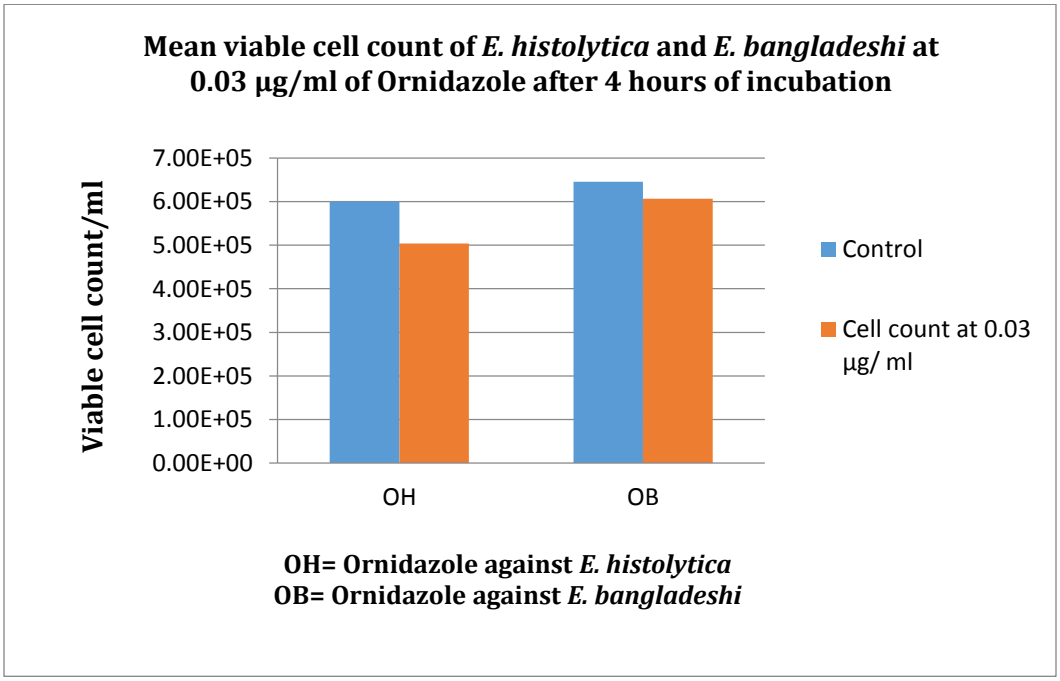

Figure 5.6: Difference of viable cell count between *E. histolytica* and *E. bangladeshi*

The above figure shows the viable cell count of *Entamoeba histolytica* and *Entamoeba bangladeshi* after treatment with Ornidazole at 0.03 µg/ml. The incubation period was 4 hours after that period the mean viable cell count of both parasites were also compared with their control individually. In the above bar diagram, the mean viable cell count of *E. histolytica* in control was $6.0X10^5$ cell/ml whereas the viable cell count of *E. histolytica* was observed $5.04X10^5$ cell/ml after treatment with Ornidazole at 0.03 µg/ml. In case of *E. bangladeshi* the cell count in control was $6.46X10^5$ cell/ml and after treatment with drug it was $6.07X10^5$ cell/ml. The mean viable cell count of *Entamoeba bangladeshi* is significantly higher ($p = <0.05$) when it was compared with cell count of *Entamoeba histolytica.*

5.2 Efficacy of different concentration of Ornidazole

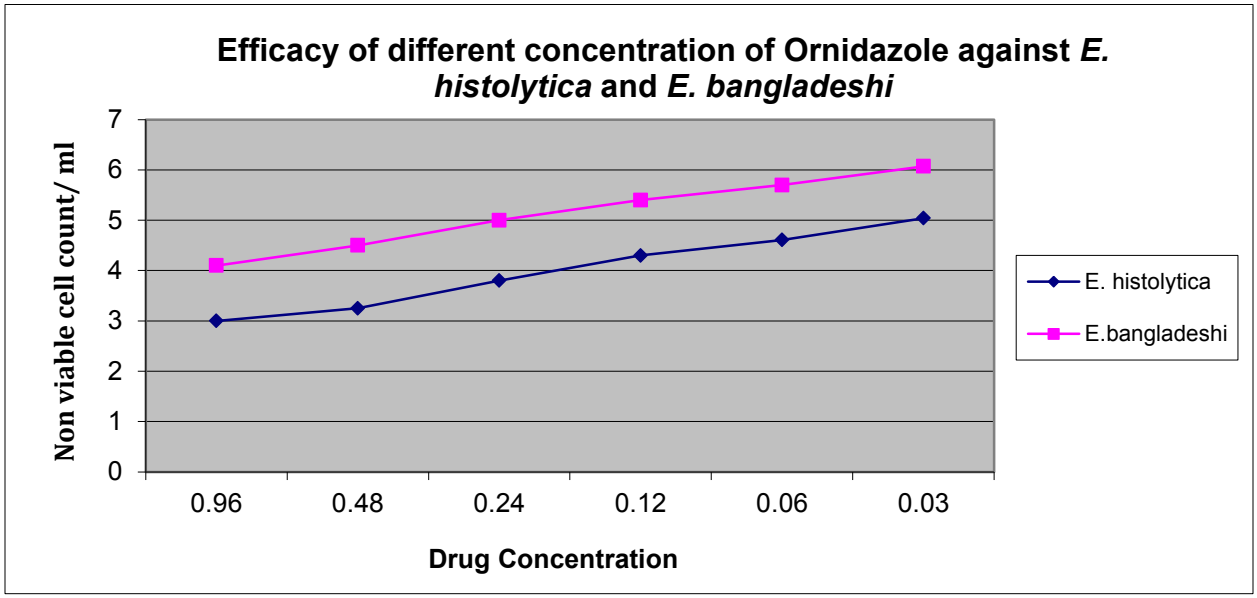

Figure 5.7: Non-viable cell inhibition of *Entamoeba histolytica*and *Entamoeba bangladeshi* by different concentration of Ornidazole

Figure 5.7 shows the non-viable cell countof *E. histolytica* and *E. bangladeshi* after treatment with different concentrations of Ornidazole after 4 hours incubation. The non viable cell count of *E. histolytica* is 3.04, 2.75, 2.21, 1.75, 1.39, and 0.96 after treatment with 0.96, 0.48, 0.24, 0.12, 0.06, 0.03 µg/ml of Ornidazole respectively. Similarly the viable cell count of *E. bangladeshi* is 2.35, 1.96, 1.5, 1.07, 0.78, 0.39 after treatment with 0.96, 0.48, 0.24, 0.12, 0.06, 0.03 µg/ml of Ornidazole respectively. The figure shows that inhibition of parasite is occurred in a dose dependent manner. Cell inhibition is maximum at the highest concentration of Ornidazole.

Table 5.3 *In vitro* sensitivity of Metronidazole based on viable counts of *E. histolytica* after 4 hours incubation (n=7)

Concentration (µg/ml)	*Entamoeba histolytica*(Mean±SD)	*Entamoeba bangladeshi* (Mean±SD)	P value (P<0.05)
0	6±0.8	6.46±0.60	Significant
0.96	2.96±0.22	4.11±0.11	Significant
0.48	3.25±0.29	4.50±0.25	Significant
0.24	3.79±0.27	4.96±0.22	Significant
0.12	4.25±0.32	5.39±0.28	Significant
0.06	4.61±0.38	5.68±0.35	Significant
0.03	5.04±0.49	6.07±0.35	Significant

Significant difference was observed.

Values are expressed as Mean ±SD (n=7). *p<0.05 is used as level of significance. After 4 hours incubation, when the concentration of Ornidazole is 0.03 µg/ml the viable count of *E.* is 5.04 and *Entamoeba bangladeshi*was 6.07. When the concentration is 0.06 µg/ml, the viable count of *Entamoeba histolytica*and *Entamoeba bangladeshi*is 4.61 and 5.68 respectively. The viable count of *Entamoeba histolytica*and *Entamoeba bangladeshi*is4.25 and 5.39 respectively after treatment with 0.12 µg/ml Ornidazole. In the same manner, the viable cell count of *Entamoeba histolytica* is 3.79, 3.25 and 2.96 at 0.24, 0.48 and 0.96 µg/ml of Ornidazole. When *Entamoeba bangladeshi* is treated with Ornidazole at 2.96 at 0.24, 0.48 and 0.96 µg/ml concentration, 4.96, 4.50 and 4.11 viable cell count is observed.

5.4 Efficacy of different concentration of Ornidazole

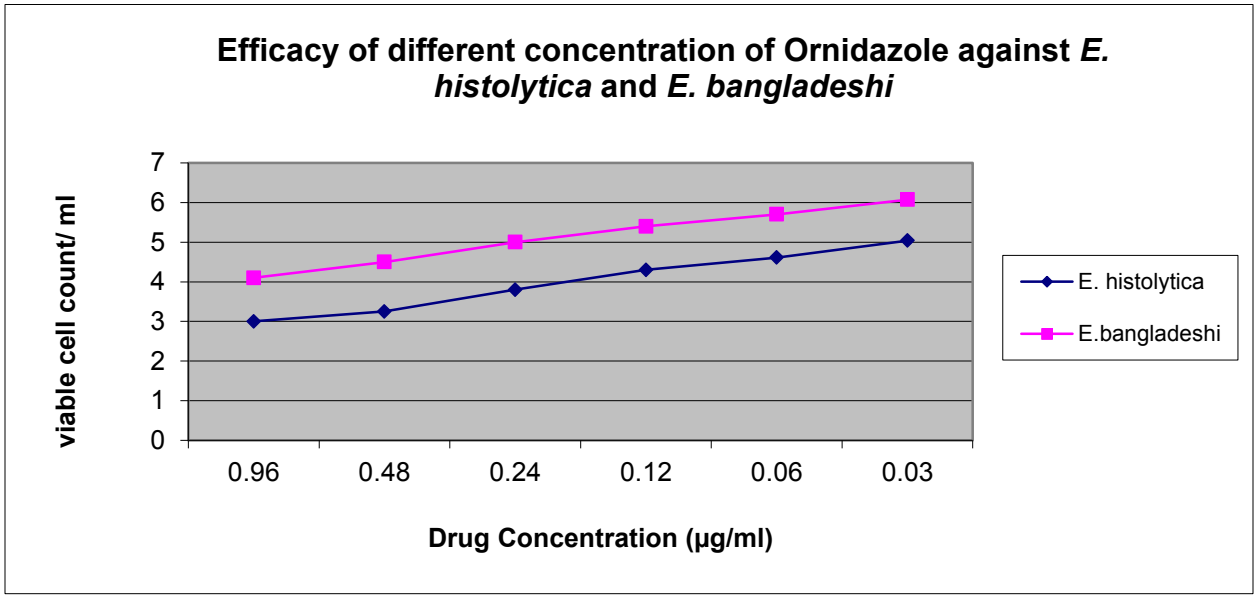

Figure 5.8: Viable cell inhibition of *Entamoeba histolytica*and *Entamoeba bangladeshi* by different concentration of Ornidazole

Figure 5.8 shows the viable cell countof *E. histolytica* and *E. bangladeshi* after treatment with different concentrations of Ornidazole after 4 hours incubation. The viable cell count of *E. histolytica* is 2.96, 3.25, 3.79 4.25 4.61, 5.04 after treatment with 0.96, 0.48, 0.24, 0.12, 0.06, 0.03 µg/ml of Ornidazole respectively. Similarly the viable cell count of *E. bangladeshi* is 4.11, 4.5, 4.96, 5.39 5.68, 6.07 after treatment with 0.96, 0.48, 0.24, 0.12, 0.06, 0.03 µg/ml of Ornidazole respectively. The figure shows that inhibition of parasite is occurred in a dose dependent manner. Cell inhibition is maximum at the highest concentration of Ornidazole.

5.5 Percentage cell inhibition of parasites after treatment with Ornidazole by IC50 calculation

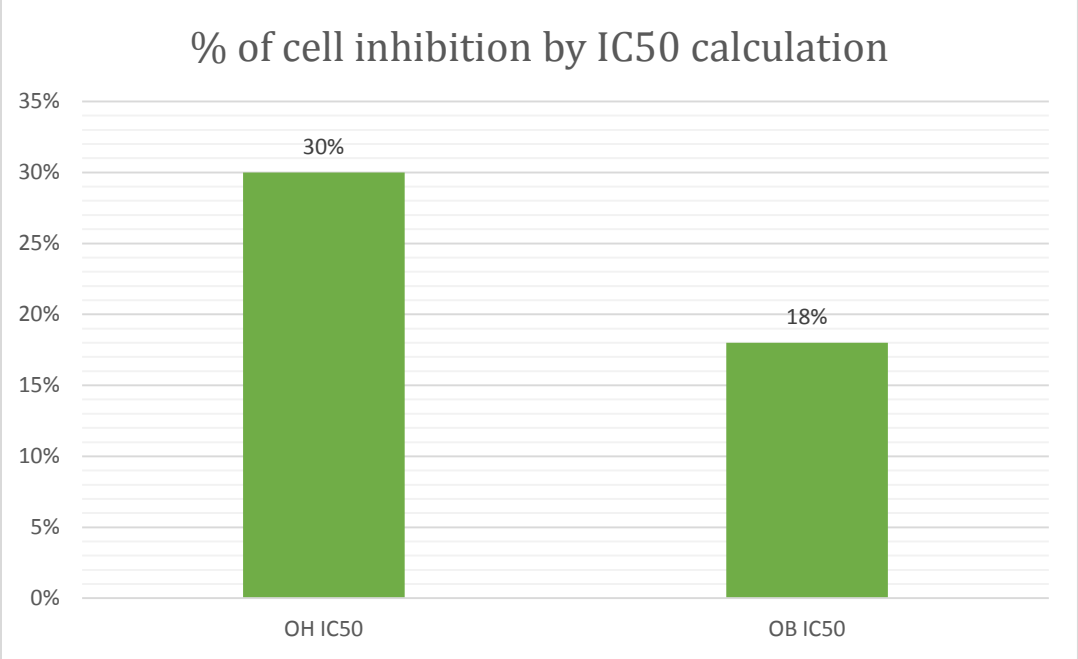

Figure 5.9: Percentage cell inhibition of *Entamoeba histolytica* and *Entamoeba bangladeshi* after treatment with Ornidazole

The percentage cell inhibition is calculated from an IC50 software named "Very Simple IC50 Tool Kit". From the bar diagram it has been observed that the percentage cell inhibition of *Entamoeba histolytica* and *Entamoeba bangladeshi* is 30% and 18% respectively. That means the percentage cell inhibition of *Entamoeba histolytica* is significantly greater than *Entamoeba bangladeshi* when they both are treated with different concentration of Ornidazole. So from the above figure it can be summarized that the efficacy of Ornidazole is greater in *Entamoeba histolytica* than *Entamoeba bangladeshi.*

Chapter six

Discussion & Conclusion

Discussion and Conclusion

Entamoeba histolytica is a protozoan pathogen and is best known for its ability to produce amoebic dysentery and liver abscess. Amoebiasis is most prevalent in developing countries with inadequate sanitation.

In the present study the *E. histolytica* and *E. bangladeshi* clinical isolates maintained by *in vitro* cultivation in axenic medium were subjected to drug susceptibility tests against antiamoebic drug Ornidazole. The objective of the study is to determine the therapeutic efficacy of anti-amoebic drug against clinical isolates of *E. histolytica* and *E. bangladeshi*.

The clinical isolates of parasites were treated with Ornidazole at different concentrations. The concentrations of Ornidazole are 0.96, 0.48, 0.24, 0.12, 0.06 and 0.03 µg/ml in this present experiment. 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 0.96 µg/ml of Ornidazole. The highest count has been seen in the control group.

It has been shown from the study that the viable cell count of *Entamoeba bangladeshi* is significantly higher ($p = <0.05$) than *Entamoeba histolytica* at 0.96 µg/ml and 0.48 µg/ml of drug concentrations. It has been also found that the inhibition of parasite is occurred in a dose dependent manner. Cell inhibition is maximum at the highest concentration of Ornidazole.

It has been observed from the study that the percent cell inhibition of *E. histolytica* is 30% whereas *E. bangladeshi* is only 18%.

Sarker et al. reported *in vitro* sensitivity of different metronidazole tablets from Bangladeshi pharmaceuticals against clinical isolates of *E. histolytica.* Metronidazole tablets of 12 different brands were randomized from some big and small pharmaceuticals. The parasite count was adjusted to $3x10^6$ parasites mL^{-1} in a medium. The viable parasites were counted by haemocytometer. No statistical significance was observed in terms of viable parasites with the metronidazole tablets from three big pharmaceuticals at the

concentration of 2.3, 3.5 and 4.6 μM when compared with the standard metronidazole (Sarker et al., 2008). In our study we adjusted the parasite count to $1x10^6$ cells/ml. Metronidazole and Ornidazole both are from imidazole group. We carried out our study by using Ornidazole which shows same sensitivity against *E. histolytica* after 4 hours of incubation. In our study we used six different concentrations of Ornidazole which were 0.96, 0.48, 0.24, 0.12, 0.06 and 0.03 μg/ml.

Another study determined the sensitivity of the combination regimens of anti-amoebic drugs against clinical isolates of *E. histolytica*. The clinical isolates of *E. histolytica* were treated with metronidazole, Ornidazole, metronidazole+Ornidazole, secnidazole, metronidazole + secnidazole, tinidazole, and metronidazole+tinidazole tablets at different concentrations (12, 6, 3 &1.5 mg/ml). After 4 hours of incubation the viable parasites were counted and the counts of the *E. histolytica* for each concentration of drugs were compared to the control. In this case significant differences had been found (Suki, 2015). In our study, we used active ingredient of Ornidazole and carried out the same procedure to find out the sensitivity of the drug. When we compared the viable cell counts of *E. histolytica* with the control, we also observed significant differences among them.

In 2010–2011, during analysis of feces, a new species was identified 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* nov. sp. in recognition of the support of the Bangladesh community for this research (Royer et al., 2012). Future epidemiological studies are needed to find out the incidence and the effect of infection in infants by the newly recognized species of *E. bangladeshi*. As *E. bangladeshi* is a novel species we do not know whether it is pathogenic or non pathogenic and no drug sensitivity studies have been conducted to determine the efficacy of the drug against this parasite. In our study we have determined the sensitivity of Ornidazole at different concentration against *E. bangladeshi* and *E. histolytica.*

In a study Bansal et al., reorted *in vitro* susceptibility of clinical isolates of *E. histolytica* and *E. dispar* to metronidazole, chloroquine, emetine and tinidazole. The results showed that all

clinical isolates had a higher IC_{50} compared to reference strain to all the four drugs. *E. histolytica* isolates appeared to be more susceptible as compared to *E. dispar* isolates and the reference strain of *E. histolytica* (Bansal et al., 2004). In our study, we observed percent cell inhibiton of *E. histolytica* and *E. Bangladeshi* after treatment with Ornidazole. IC_{50} calculation was done in our experiment. In our study the IC_{50} of *Entamoeba histolytica* and *Entamoeba bangladeshi* is 30% and 18% respectively. The effect of anti-amoebic drug (Ornidazole) in this study for the treatment of amoebic dysentery caused by *E. bangladeshi* (if *E. bagladeshi* would be pathogenic) may be an innovative treatment option against amoebiasis. This study may help to make awareness among the physicians and consumers to establish new regimen of drug.

Chapter seven

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