

## *2. REVIEW OF LITERATURE*

## HISTORICAL PERSPECTIVE

The earliest record of amoebiasis most probably came from the Sanskrit document 'Brigu-samhita', written about 1000 BC, refers to bloody, mucus diarrhea (Vaidya and Ray, 1982). Amoebiasis was recognized by Hippocrates (460–377 BC) as a deadly disease (Encyclopedia Americana, 1970). Later the Old Testament and Huang Ti's Classic in Internal Medicine (140–87 BC) made reference to dysentery (Kean, 1988). In the second century AD, Galen (Galenus of Pergamon, 129BC–200AD) and Celsus (25BC–50AD) described liver abscesses that were probably amoebic, and the works of Aretaeus, Archigenes, Aurelanus and Avicenna toward the end of the first millennium gave good accounts of both dysentery and hepatic involvement (Scott, 1939). Assyrian and Babylonian texts from the Library of King Ashurbanipal refer to blood in the feces, in the Tigris Euphrates basin before the sixth century BC and also the hepatic and perianal abscesses described in both Epidemics and Aphorisms in the Corpus Hippocratorum suggested the presence of amoebiasis (Bray, 1996; Labat, 1954; Jones and Whithington, 1953).

The relation between amoebic dysentery and liver abscesses was described first by Budd in 1857 (Budd, 1857). The amoeba *E. histolytica* was discovered by Friedrich Lösch in 1873 in Russia (Lösch, 1975) Lösch was the first to describe the milestones of *E. histolytica* and amoebiasis, and he also established the relationship between the parasite and the disease in dogs experimentally infected with amoebae from humans. Stephanos Kartulis, found amoeba in intestinal ulcers in patients suffering from dysentery in Egypt in 1885 and 1896 and noted that he never found

amoeba from dysenteric cases. He also showed that cats could be infected with amoeba per rectum and developed dysentery (Kartulis, 1886).

The delineation of amoebic liver abscess and colitis were described by Osler and his colleagues in 1890.

Councilman and Lafleur in 1891, reported the pathology of amoebiasis at the end of the 19th century, and it is of much valid today (Councilman and Lafleur, 1891). Quincke and Roos in 1893 described the cyst form (Quincke and Roos, 1893), and Schaudinn in 1903 named *E. histolytica* and differentiated it from *Entamoeba coli*.

As amoebiasis became widespread in the developed world, there were numerous records of 'bloody flux' in Europe, Asia, Persia and Greece in middle ages (Kiple, 1993). The disease appears to have been introduced into the New World by Europeans sometime in the 16th century (Crosby, 1986) and with the later development of European colonies and increased world trade; there are numerous clear descriptions of both the intestinal and hepatic forms of amoebiasis. In the 19th century, both intestinal and hepatic amoebiasis was prevalent in India (Annersley, 1828).

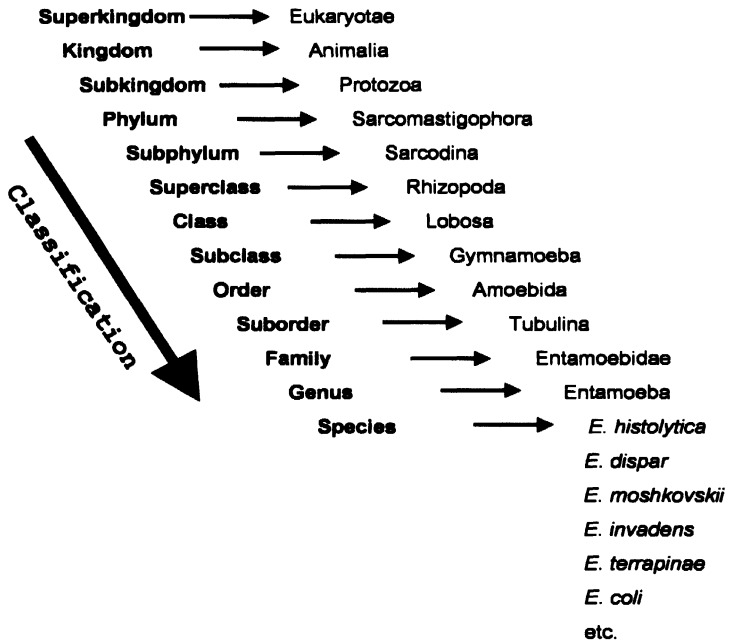
*E. histolytica* was first successfully established in culture by Boeck and Drbohlav in 1925 in a diphasic egg slant medium called as Locke's egg serum medium which they had developed for isolation of intestinal flagellates (Boeck and Drbohlav, 1925), followed by another medium described by Robinson and Jones (Robinson, 1968; Jones, 1946). The first axenic cultivation was established by Diamond in 1961 (Diamond, 1982, Diamond et al., 1978).

**WHAT IS NEW IN THE UNDERSTANDING OF THE AMOEBA?**

Earlier it was Brumpt in 1925 who proposed that *E. histolytica* and *E. dispar* were two distinct species and suggested that they should be named as pathogenic and nonpathogenic species (Brumpt, 1925). After a period of more than 70 years only, the suggestion was considered and the World Health Organization (WHO) (WHO, 1997) has recognized *E. histolytica* and *E. dispar* as two distinct species (Diamond and Clark, 1993; Sargeant et al., 1978; Sargeant and Williams, 1979; Strachan et al., 1988; Tannich et al., 1989) based on genetic, biochemical, and immunological studies (**Figure 1**).

The cyst and trophozoite stages of *E. dispar* are similar in their morphology to those of *E. histolytica* when seen under the light microscope, except in rare cases where *E. histolytica* trophozoite may have ingested RBC (Gonzalez-Ruiz et al., 1994). Similarly another species of amoeba, *Entamoeba moshkovskii* has also been documented in the year 1998 in human stool specimens which is also similar in morphology to that of *E. histolytica* / *E. dispar* (Haque et al., 1998b). *E. moshkovskii* has so far rarely been shown to colonize humans; however, the organism appears to be ubiquitous in anoxic sediments. Although the early isolations of this species were from sewage, *E. moshkovskii* can also be found in environments ranging from clean riverine sediments to brackish coastal pools (Clark and Diamond, 1991a). *E. moshkovskii* is osmotolerant, can be cultured at room temperature, and is resistant to emetine, all characteristics that distinguish it from *E. histolytica* and *E. dispar* (Dreyer, 1969; Entner, 1965; Richards, 1966; Clark and Diamond, 1991a).

**Figure 1**  
**Complete taxonomy of *Entamoeba***



*E. histolytica* is a harmful amoeba causing amoebic colitis and liver abscess, whereas both *E. dispar* and *E. moshkovskii* are non-pathogenic harmless commensals and till now they have never been reported to cause any intestinal or extra-intestinal disease in humans (Parija, 2006). The demonstration of look -alike *E. dispar* and *E. moshkovskii* in stool specimens has led to rethinking on the diagnosis, epidemiology, and treatment of the infections caused by *E. histolytica*.

Differentiation of *E. histolytica* / *E. dispar* / *E. moshkovskii* complex in stool samples is not reliable on the basis of microscopy alone (Diamond and Clark, 1993; Krogstad et al., 1978; Tannich et al., 1989; Gonzalez-Ruiz et al., 1994). Diagnosis, treatment and epidemiological report of most of the previous infections as *E. histolytica* infections based on microscopic examination only can be considered as defective and misleading. In reality, many of these organisms mistaken as pathogenic *E. histolytica* were probably genetically distinct non-pathogenic *E. dispar* (Tannich et al., 1989) or *E. moshkovskii*. In addition, these subjects might have been treated unnecessarily with anti-amoebic drugs, as the infection with *E. dispar* and *E. moshkovskii* does not warrant anti-amoebic chemotherapy (Kasper et al., 2005; Parija, 2006).

In the era of evidence based medicine, the differential diagnosis of *E. histolytica* form *E. histolytica* like amoeba is of great importance because it allows the clinician to identify and treat *E. histolytica* infection early in the community to avoid personal and public health problem. This will also avoid the unnecessary treatment of patients with anti-amoebic drugs. Amoebic copro-antigen and Deoxy-ribose nucleic acid (DNA) detection are the recent tools that allow differentiation

of *E. histolytica* from look-alike amoebae such as *E. dispar* and *E. moshkovskii* (Acuña-Soto et al., 1993; Britten et al., 1997; Evangelopoulos et al., 2000; Haque et al., 1998b; Rivera et al., 1999; Rivera et al., 1996; Sanuki et al., 1997; Verweij et al., 2000; Zaman et al., 2000).

## THE LIFE CYCLE AND BIOLOGY

The life cycle of *E. histolytica* consists of two stages: cysts and trophozoites. *E. histolytica* cysts measure 10–16 µm in diameter and typically contain four nuclei. The cysts are resistant to acidification, chlorination and desiccation, and are capable of surviving in a moist environment for several weeks. Cysts can remain alive outside the host for weeks or months, especially under moist conditions (Markell et al., 1999), but are rapidly destroyed at temperatures under -5°C and over 40°C (Ivey, 1980). Cysts are transmitted by the ingestion of faecally contaminated food or water. Cysts are not invasive, but trophozoites can penetrate the gastrointestinal mucosa (Clark et al., 2000). During excystation in lumen of the small intestine (terminal ileum), nuclear division followed by cytoplasmic division, give rise to eight trophozoites (Katz et al., 1989).

Trophozoites measure about 20–40 µm in diameter and contain a single nucleus with a central karyosome. The cytoplasm frequently contains many red blood cells (RBCs) that have been ingested. The amoebae reside in the lumen of the caecum and large intestine, where they adhere to the colonic mucus and epithelial layers. The adherence is due to the interaction of a d-galactose- (d-gal-) or *N*-acetyl- d-

galactosamine- (GalNAc-) (McCoy et al., 1994; Ravdin et al., 1985; Chadee et al., 1987). Approximately, 90% of individuals infected with *E. histolytica* are asymptotically colonized (Chadee et al., 1987). In approximately 10% of cases, adherence of the amoeba and subsequent lysis of the colonic epithelium, mediated by the Gal/GalNAc-specific lectin, initiates invasion of the colon by trophozoites. Once the intestinal epithelium is crossed, extra-intestinal spread to the peritoneum, liver and other sites may follow. Trophozoites are easily destroyed, usually within minutes in the external environment. Re-encystation of the trophozoites occurs as it migrates down and out of the colon, resulting in the excretion of cysts in the faeces and continuation of the life cycle (Figure 2).

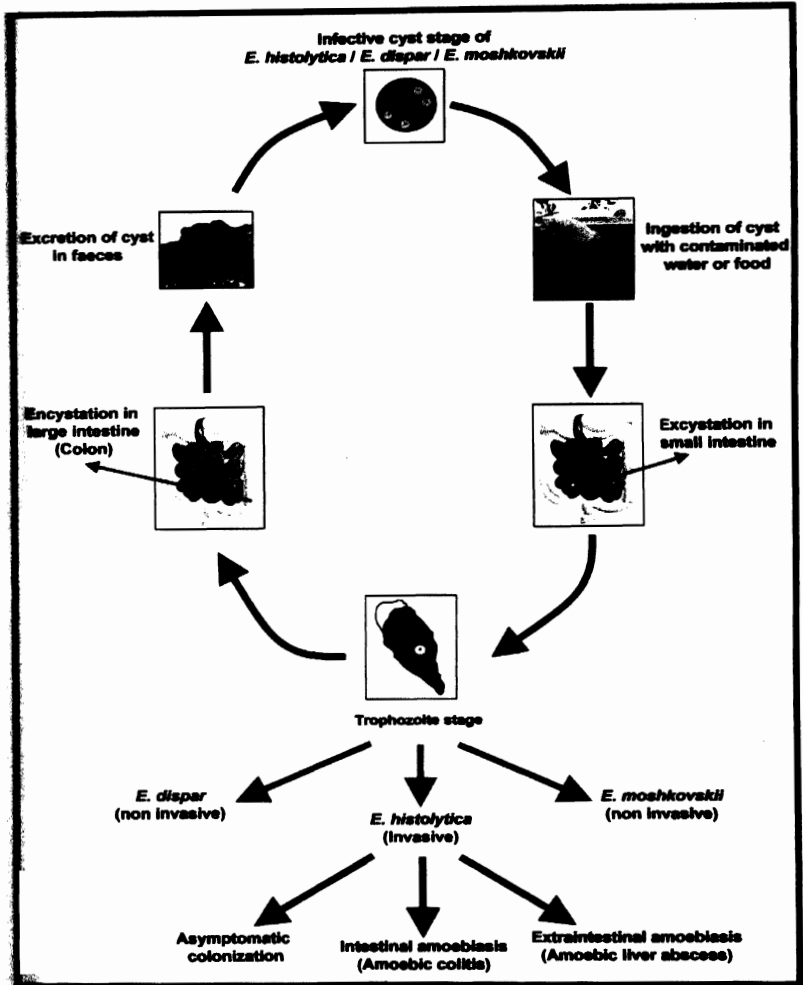
## **CLINICAL FEATURES**

Amoebiasis caused *E. histolytica* by can manifest either as intestinal amoebiasis or as an extra-intestinal amoebiasis.

### **1. Intestinal amoebiasis**

The incubation period of intestinal amoebiasis varies from 1 to 4 weeks (Healy and Garcia, 1995), but can vary, ranging from a few days to even months or years (Garcia and Bruckner, 1997). The spectrum of intestinal amoebiasis may ranges from asymptomatic to transient intestinal inflammation to a fulminant colitis with an array of manifestations that may include toxic megacolon and peritonitis (Kasper et al., 2005).

Figure 2



***Asymptomatic colonization***

The symptoms of *E. histolytica* infection are absent or very mild in the majority (90%) of cases (Gatti et al., 2002; Jackson et al., 1985). These patients have normal findings; usually there is no history of blood in the stool samples. Trophozoite without ingested RBCs may be demonstrated by microscopy (Garcia and Bruckner, 1997). Surprisingly, in most of the individuals infected asymptotically with *E. histolytica*, but not *E. dispar*, show the presence of serum antibodies against the parasite (Abd-Alla et al., 1998). Although infection with *E. dispar* is much more common than *E. histolytica*, but till now, *E. dispar* has never been reported to cause colitis or ALA.

There is still ambiguity regarding the true prevalence of patients infected with *E. histolytica* asymptotically (Krogstad et al., 1978). This is because the diagnosis of intestinal amoebiasis in many countries still, is commonly carried out by microscopic examination of stool samples for *E. histolytica/E. dispar*. Only 10 % of patients infected with *E. histolytica* are symptomatic and remaining are asymptomatic (Gathiram and Jackson., 1987). Systemic anti-amebic immune response is present in symptomatic *E. histolytica* intestinal infections, but asymptomatic *E. dispar* intestinal infections do not show any evidence of disease or a serum anti-amebic antibody response (Gathiram and Jackson., 1987). Patients with asymptomatic colonization of *E. histolytica* , however , need to be treated by anti-amoebic chemotherapy (Faust et al., 1970). This is because these patients are potential carrier of cyst and may spread infection in the community or may themselves suffer from colitis later on (Fritsche and Smith, 2001).

***Symptomatic colonization (Amebic colitis and dysentery)***

Symptoms associated with symptomatic *E. histolytica* colonization are abdominal pain or tenderness and diarrhea (watery, bloody, or mucous) and one-third of patients suffer from fever (Parija, 2006; Fuchs et al., 1988). The colonic findings in intestinal amoebiasis can range from thickening of the intestinal mucosa to classical flask-shaped ulceration found mostly in the cecum or appendix or near the ascending colon region, but rarely found in the sigmoidorectal region (Parija, 2006; Garcia and Bruckner, 1997).

The occurrence of amoebic colitis with toxic megacolon is the most dangerous complication of intestinal amoebiasis. Toxic megacolon is characterized by an acute dilatation of the colon, which occurs in approximately 0.5% of cases. If the toxic megacolon is not promptly diagnosed and treated it can prove fatal and the patient can die of sepsis (Garcia, 1990; Garcia and Bruckner, 1997). Intestinal amoebiasis can also manifest as “amoeboma”, a condition due to the formation of granular tissue extending into the lumen of the intestine. This condition can easily be misinterpreted as carcinoma of the colon, and fistula formation.

**2. Extra-intestinal amoebiasis**

Amoebic liver abscess (ALA) is the most common extra-intestinal manifestation of amoebiasis affecting the liver. ALA is more common in adults than in children. ALA occurs most commonly in the age group of 20 to 45 years. It has been noted infrequently at the extremes of age and is seven to nine time more common in

males. The manifestation of ALA may be as an acute process or as a chronic indolent disease.

Abdominal pain, fever, and anorexia are the main presenting features of acute ALA. Abdominal pain is usually moderate and is localised in the right upper quadrant or to the epigastrium. Symptoms of diffuse abdominal pain, pleuritic chest pain, and radiation of right upper quadrant pain to the right shoulder are common. In cases of left lobe abscesses epigastric pain is most common. In ALA fever is mostly of moderate degree, but high fever with chills is suggestive of bacterial super infection. Cough with or without expectoration and pleuritic chest pain is also observed in ALA. In most patients the duration of acute illness and symptoms last for less than 2 weeks.

Clinical jaundice may develop in one-third of patients during the course of illness. Large abscess, multiple abscesses or an abscess situated at the portal hepatic can cause severe icterus (Data et al., 1973). Appearance of jaundice can lead to problem in the diagnosis of ALA because it brings in the possibilities of intra-hepatic obstruction or viral hepatitis (Sharma and Ahuja, 2003). Up to 80% of patients may show tender hepatomegaly. A left lobe abscess may manifest as toxæmia, deep jaundice, and encephalopathy. Extra-intestinal amoebiasis has occasionally been described in the lung, brain, skin, and genitals (Kasper, 2005).

## **EPIDEMIOLOGY OF AMOEBIASIS**

Amoebiasis occurs worldwide but is more prevalent in the tropics. It is estimate that *E. histolytica* causes between 34 to 50 million symptomatic infections annually (Walsh, 1986). Amoebiasis results in 100,000 deaths per year, making it one of the three most common causes of death from parasitic disease as stated by WHO (Walsh, 1986; WHO, 1997). Prior to description of *E. dispar* and *E. moshkovskii*, amoebiasis caused by so called pathogenic *E. histolytica* was known as a 10% disease because it has been estimated that approximately, 500 million people or 10 percent of world's population are infected with *E. histolytica*. 10 percent of these infected people or 50 million people each year suffer from active amoebic disease; 10 percent of which (50,000 to 1, 00, 000 people) die every year (Parija, 2006). The mortality due to amoebiasis is mainly because of extra-intestinal amoebiasis especially ALA (Kasper et al., 2005).

Humans are the known primary reservoir of *E. histolytica* infection (Katz et al., 1989). Chronically infected humans, who remain asymptomatic are carriers and are main source of transmission. Infection spreads when the stool infected with cyst contaminates fresh food and water. Oral-anal sexual contact is the other mode of transmission of infection. There is an ambiguity regarding the zoonotic transmission of the amoebae from dogs, monkeys, and probably pigs (Beaver et al., 1984; Belding, 1952; Krauss et al., 1997). Arthropods such as cockroaches and house flies are also capable of transmitting *E. histolytica* infection (Ivey, 1980; Walsh, 1988).

Although *E. histolytica* infection occurs worldwide, morbidity and mortality are greatest in Central and South America, Africa and India subcontinent (Petri, 1996) (Figure 3).

*E. dispar* has been documented from most places of the world (Acun˘a-Soto et al., 1993; Britten et al., 1997; Diamond and Clark, 1993; Evangelopoulos et al., 2000; Ortner et al., 1995; Rivera et al., 1999; Rivera et al., 1996; Sanuki et al., 1997; Tanyuksel et al., 2001; Verweij et al., 2000). *E. dispar* has been reported from few pockets in India like New Delhi (Baveja et al., 1990), Kolkata (Mukherjee et al., 1992), Chandigarh (Mukhopadhyay et al., 2002; Bansal et al., 2004) and from our laboratory at JIPMER, Puducherry (Parija and Khairnar, 2005) (Figure 4).

Human isolates of *E. moshkovskii* to date have been documented from North America, Italy, South Africa, Bangladesh and India and they have never been associated with disease (Parija and Khairnar, 2005; Clark and Diamond, 1991a; Haque et al., 1998b) (Figure 5). Our laboratory at JIPMER, has reported for the first time, the presence of *E. moshkovskii* in human stool specimens in India (Parija and Khairnar, 2005).

Estimation of prevalence of intestinal amoebiasis based on detection and identification of *E. histolytica* in stool specimens by microscopy alone, therefore, is not reliable. There are many question marks raised on the true epidemiology of amoebiasis and the biggest question is "just how common is *E. histolytica*". The concept of epidemiology of amoebiasis needs revision with the reporting of new look-alike species of amoeba such as *E. dispar* and *E. moshkovskii*. It is now being

Figure 3

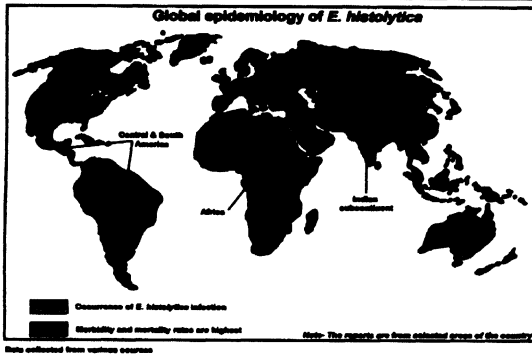


Figure 4

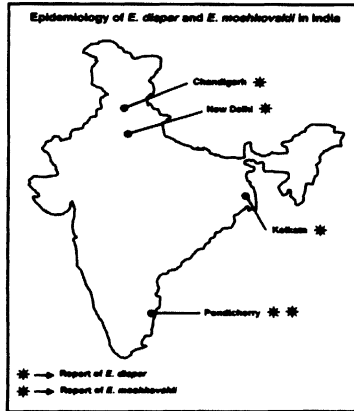
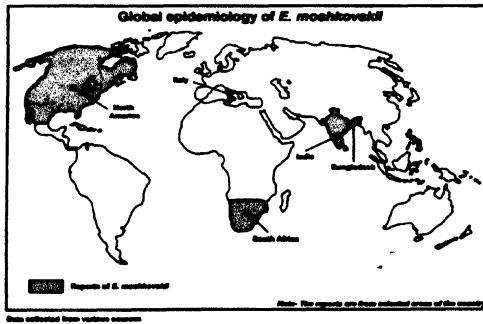


Figure 5



suggested that majority of 500 million people who were earlier believed to harbor *E. histolytica* in their intestinal tract may be harbouring commensal and non-pathogenic *E. dispar* or possibly *E. moshkovskii*.

### **PATHOGENICITY OF *E. HISTOLYTICA***

The ability of *E. histolytica* to invade tissue makes it a unique among the intestinal amoebae parasitizing humans (Jackson et al., 1985). There is still ambiguity regarding the factors that control the invasiveness of *E. histolytica*. Cysteine proteinases, amoebapore and Gal/GalNAc-inhibitable lectin are the few virulence factors of *E. histolytica* that have been well studied in recent times.

*E. histolytica* cysteine proteinases act by degrading host proteins and facilitate attachment of the amoebae to the intestinal mucosa by degrading mucus and debris. The enzyme also stimulates host cell proteolytic cascades. The enzyme, cysteine proteinases can be possibly targeted for treatment of amoebiasis due to their potential role in promoting invasion (Keene et al., 1990; Leippe et al., 1992; Luaces and Barrett, 1988; Que and Reed, 2000).

The amoebapore is stored in cytoplasmic granules, and is released when target cell comes in contact with trophozoite. The amoebapore forms ion channels in the membranes of both eukaryotic cells and phagocytosed bacteria. The amoebapore may be directly responsible for the cytolysis of host cells by the parasite (Leippe et al., 1991; Leippe et al., 1992).

The Gal/GalNAc-binding lectin plays an important role in the pathogenesis of amoebiasis. It facilitates parasite cell adherence, complement resistance, contact-dependent cytotoxicity, actin polymerization and capping, and endocytosis. The role of amoebic lectin in adhesion and cytolysis was first documented by Ravdin and Guerrant (Ravdin and Guerrant, 1981). Use of monoclonal antibodies raised against the Gal/GalNAc-binding can reduce both adherence and cytotoxicity of *E. histolytica in-vitro*; this proves the vital role played by Gal/GalNAc lectins in the pathogenicity of *E. histolytica* (Petri et al., 1990; Saffer and Petri, 1991b). The cytopathic effect of amoebic trophozoites on the host tissue was shown to be blocked by the addition of Gal/GalNAc or galactose (Guerrant et al., 1981; Ravdin et al., 1980).

The Gal/GalNAc-lectin has sequence analogy and antigenic cross-reactivity to CD59. The CD59 is a human leukocyte antigen that prevents the assembly of the complement C5b–C9 membrane attack complex (Braga et al., 1992). The complement anaphylatoxins C3a and C5a are rapidly degraded by amoebic cysteine proteinases (Reed et al., 1995). The enzyme degrades secretory IgA and serum IgG thereby providing protection against opsonisation. The immunogenicity of Gal/GalNAc-binding lectin also makes it a potential vaccine candidate against amoebiasis (McCoy et al., 1994; Ramakrishnan et al., 1996; Saffer and Petri, 1991a).

The tissue invasion by *E. histolytica* is presumably facilitated by proteolytic enzymes such as collagenase, neutral proteases and cysteine proteases (Que and Reed, 1997). *E. histolytica* has been demonstrated to perform contact-dependent

extra-cellular killing of neutrophils, macrophages, and erythrocytes (Petri et al., 2002). The intestinal mucous layer serves as a protective barrier to invasion by inhibiting amoebic trophozoite adherence to the underlying epithelium and by slowing trophozoite motility, but when the trophozoite penetrates the intestinal mucous layer it results in colitis (Chadee et al., 1987). The human colonic mucin layer may play a protective role by preventing the host cell from undergoing cytolytic activity by neutralizing the binding epitopes on the lectin during attachment (Tanyuksel and Petri, 2003). *E. histolytica* can also prevent both the macrophage respiratory burst and antigen presentation by class II major-histocompatibility complex (MHC) molecules (Ankri, 2002). Hepatic infection occurs when the colonic trophozoites ascend via the portal vein and invade the parenchyma (Mortele et al., 2004).

The mucosal IgA response against the carbohydrate- recognition domain of the Gal/GalNAc lectin of *E. histolytica* imparts immunity to infection with *E. histolytica*. A study conducted for a period more than a year on children with this response had 86% fewer new infections than children without this response (Haque et al., 2002). In ALA patients cell-mediated immune response has been shown to provide protection against *E. histolytica* which is characterized by lymphocyte proliferation and lymphokine secretion that is amoebicidal *in-vitro* (Salata et al., 1986). One study found that in patients with ALA, the prevalence of the class II MHC haplotype HLA-DR3 is increased by a factor of more than three, suggesting a role of CD4+ T-cell function in the outcome of the disease (Arellano et al., 1996).

The asymptomatic intestinal colonization is undoubtedly common, but it is noteworthy that the acquired immunodeficiency syndrome pandemic has not led to increases in cases of invasive amoebiasis (Fontanet et al., 2000). Interestingly, one study conducted on the murine model of amoebic colitis states that the depletion of CD4+ T cells decreases the severity of the disease (Haupt et al., 2002).

### **LABORATORY DIAGNOSIS OF AMOEBIASIS**

The laboratory diagnosis plays a very important role for accurate diagnosis, treatment and epidemiology of amoebiasis. The diagnosis is essential not only for patients with symptoms of amoebiasis but also for the ninety percent of asymptomatic patients colonised with *E. histolytica*. This is because such asymptomatic carriers of *E. histolytica* can spread the infection in the community, especially in developing countries where sanitation is poorly practiced (Choudhuri et al., 1991).

The laboratory diagnosis of amoebiasis can be made by microscopy, culture and isoenzyme, antibody detection, antigen detection and nucleic acid based diagnostic tests. During the last few years, there has been a vast development in the development and evaluation of molecular diagnostic tests to detect *E. histolytica*.

#### **1. Microscopy**

Since a long diagnosis of amoebiasis is based on examination of trophozoite/cyst of *Entamoeba* by light microscopy.

The presence of haematophagous trophozoite of *E. histolytica* in stool is suggestive of an active invasive amoebiasis (Gonzalez-Ruiz et al., 1994). Fresh stool specimens that contain mucus, pus, and trace amounts of blood are more likely to contain *E. histolytica* trophozoite. It is difficult to visualize the nuclei of trophozoite in wet mounts (Proctor, 1991). Products of degenerated eosinophils called as "Charcot-Leyden crystals" and clumped RBCs can be seen in a wet mount preparation (Garcia and Bruckner, 1997). High level of experience and skill is required for definitive diagnosis of intestinal amoebiasis (Walsh, 1986) otherwise inadequate training and inexperience may lead to misdiagnosis (Garcia and Bruckner, 1997).

The characteristic motility of *E. histolytica* in fresh stool wet mount preparation is usually in a linear non random fashion, with the clear hyaline ectoplasm flowing to form blunt-ended pseudopodia, which is followed by flowing in of the endoplasm containing the nucleus (Proctor, 1991). If a delay in examination of fresh stool specimen is expected then it recommended to preserve the stool specimen in a fixative such as polyvinyl alcohol or kept at 4°C in refrigerator. Occasionally, it is possible to see motile trophozoites even after 4 hours at 4°C (Ravdin, 1995; Walsh, 1986). In unfixed stool specimens the trophozoites disintegrate rapidly (Proctor, 1991).

Stool specimens can be examined either unstained with 0.9% saline or stained with Lugol's or D'Antoni's iodine. Nucleus of *E. histolytica* is perfectly visible when

stained with Iodine stains. The chromatoid bodies appear same in both stained and unstained preparations (Proctor, 1991).

There are several other stains, including Giemsa, methylene blue, Choroazole black E, Wright's, and iodine-trichrome stains available for staining and for permanent smear preparation. Wheatley's trichrome staining or one of the modified iron hematoxylin stains can be used for routine use in the diagnosis of *E. histolytica*/*E. dispar* (Garcia, 1990; Garcia and Bruckner, 1997; Proctor, 1991; Walsh, 1986). Our laboratory at JIPMER has shown lacto phenol cotton blue (LPCB) as an effective staining technique for detection of *Entamoeba* trophozoite and cyst in stool specimens by light microscopy (Parija and Prabhakar, 1995, Parija, 1998a). D'Antoni's iodine stain is much better than saline or buffered methylene blue for examination of cyst stage of *E. histolytica* while saline and buffered methylene blues are equally good for examination of *E. histolytica* trophozoites (Shetty and Prabhu, 1988).

*E. histolytica* is morphologically similar to *E. dispar* and *E. moshkovskii* (Clark and Diamond 1991a; Edman et al., 1990; Tannich et al., 1992). The comparative morphology and the characteristics of different intestinal amoebas such as *E. histolytica* / *E. dispar* / *E. moshkovskii*, *E. coli*, *E. hartmanni*, *E. Polecki*, *Endolimax nana*, *I. bu`tschlii* and *Dientamoeba fragilis* is depicted in **figure 6** and **table 1**.

The only way *E. histolytica* can be distinguished from *E. dispar* and *E. moshkovskii* by microscopy is the erythrophagocytosis (i.e. trophozoite with

Figure 6

Comparison of amoeba morphology							
	<i>Entamoeba histolytica</i> / <i>Entamoeba dispar</i> / <i>Entamoeba moeshkovskii</i>	<i>Entamoeba hartmanni</i>	<i>Entamoeba coli</i>	<i>Entamoeba polecki</i> <sup>a</sup>	<i>Endolimax nana</i>	<i>Iodamoeba butschlii</i>	<i>Dientamoeba fragilis</i> <sup>b</sup>
Trophozoite							
Precyst						No precyst stage	No precyst stage
Cyst							No cyst stage

<sup>a</sup> Rarely found in humans, mostly of animal origin

<sup>b</sup> Flagellate, but found commonly in intestine with other amoebae

0 5 10 μm  
Scale

ingested RBCs). A study conducted *in-vitro* condition, revealed that *E. histolytica* has a significantly higher phagocytic rate of ingested RBCs than do the nonpathogenic *Entamoeba* species like *E. invadens* and *E. moshkovskii* (Trissl et al., 1978). The erythrophagocytosis is considered to be a definitive diagnosis for *E. histolytica* infection but, unfortunately this is a rare finding in diagnostic set up (Garcia and Bruckner, 1997; Gonzalez-Ruiz et al., 1994). In the absence of erythrophagocytosis the *E. histolytica*/*E. dispar*/*E. moshkovskii* complex can not be distinguished by light microscopy. There are reports claiming that *E. dispar* is also capable of ingesting RBCs *in-vitro* (Haque et al., 1995; Ackers, 2002).

There are some major limitations to microscopy; the sensitivity and specificity of microscopy is less (Garcia and Bruckner, 1997; Markell et al., 1997). In fact under the best of circumstances the sensitivity of light microscopy is only 60% (Krogstad et al., 1978) (Table 2); The result of microscopy may be adversely affected by the factors like lack of well-trained microscopists, delay in delivery of specimen to the laboratory (motility can cease and trophozoites can lyse within 20 to 30 min), difficulty in differentiating non-motile trophozoites from polymorphonuclear leukocytes, macrophages, and tissue cells, administration of anti-amoebic antibiotics before specimen collection, interfering substances such as laxatives, antacids, cathartics like magnesium sulfate, anti-diarrheal preparations like kaolin or bismuth, enemas; lack of preservation of stool specimens with fixatives (polyvinyl alcohol, Schaudinn's fluid, merthiolate-iodine-formalin, sodium acetate-acetic acid-formalin and 5 or 10% formalin);

Table 1

Morphology, pathogenicity, virulence factors, prevalence and treatment of intestinal amoebae *							
Characteristics	<i>E. histolytica</i> , <i>E. dispar</i> and <i>E. moshkovskii</i>	<i>E. coli</i>	<i>E. hartmanni</i>	<i>E. politii</i>	Endolimax name	<i>Disentariae fragilis</i> †	<i>Iodamoeba butschlii</i>
Trophozoites (size, nucleus, and movement)	15–20 µm; single nucleus; actively motile cytoblastic protrusions, quickly finger shaped pseudopodium	20–25 µm; single nucleus; slow movement, short and blunt pseudopodium	8–10 µm; single nucleus; non-successive	15–20 µm; single nucleus; motility resembles <i>E. coli</i>	7–9 µm; single nucleus, blunt and hyaline pseudopodium, slow movements	7–12 µm; mono- and binucleate; hyaline broad leaf like serrated margins; successive motility	8–14 µm; single nucleus, slow movement, non-successive, hyaline pseudopodium
Cysts (size, nucleus)	13–15 µm; mature cyst has four nuclei, immature cyst has one or two nuclei	15–25 µm; mature cyst has eight nuclei, rarely sixteen or more nuclei	6–8 µm; mature cyst has 4 nuclei; immature cyst has 1 or 2 nuclei; two nucleated cysts very common	10–15 µm; single nucleus, very rarely binucleate or quadrinucleate	6–8 µm; four nuclei	No cyst stage	10–12 µm; single nucleus
Appearance of trophozoites	Blinded trophozoites show fine, uniform granules of peripheral chromatin, and small central karyosome in nucleus; ingested RBC ( <i>E. dispar</i> and <i>E. moshkovskii</i> are similar to <i>E. histolytica</i> trophozoites, except ingested RBC)	Nucleus with irregular cluster of peripheral chromatin; large, irregular, eccentric karyosome	Nucleus structure similar to <i>E. histolytica</i> ; ingested bacteria; cytoplasm finely granular	Nucleus with subtle central karyosome, with fine granules of peripheral chromatin, finely granular cytoplasm; ingested bacteria	Nucleus with large karyosome; no peripheral chromatin	High percentage of binucleate trophozoites; nuclei without peripheral chromatin	Large central karyosome, granular cytoplasm
Appearance of cysts	Uniform size in having both karyosome and peripheral chromatin, typical nuclear structure, chromatoidal bars with squared or rounded ends	Typical nuclear structure, silver shaped or irregular chromatoidal	Typical nuclear structure, chromatoidal bars with rounded or squared ends	Mononucleate; large central karyosome; inclusion masses, chromatoidal bars with pointed or angular ends	Chromatin, four nuclei with large karyosomes and no peripheral chromatin	No cyst stage	Large karyosome, eccentric nucleolus granules (mask nucleus), large compact glycogen, no peripheral chromatin
Pathogenicity	<i>E. histolytica</i> is pathogenic, but <i>E. dispar</i> and <i>E. moshkovskii</i> are nonpathogenic	Non pathogen	Non pathogen	Non pathogen *	Non pathogen	Non pathogen *	Non pathogen *
Virulence factors	Cytotoxic proteins, aminoglycoside Oat/GalNAc-inhibitable lectin (Except <i>E. dispar</i> and <i>E. moshkovskii</i> are nonpathogenic)						
Prevalence	<i>E. histolytica</i> and <i>E. dispar</i> (prevalent in most part of the world) <i>E. moshkovskii</i> reported only from North America, Italy, South Africa, Bangladesh and India	Most part of the world	Most part of the world	Most part of the world *	Most part of the world	Most part of the world	Most part of the world
Treatment with antiamoebic chemotherapy	Required	Not required	Not required	Not required	Not required	Not required	Not required

\* Data collected from various sources

† *D. fragilis* is a flagellate but resembles other intestinal amoebae

\* Occasionally reported to cause diarrhoeal illness in humans

\* Reported in humans from Papua New Guinea, Cambodia, Venezuela and Vietnam

and presence of other look alike amoeba (*E. dispar* and *E. moshkovskii* are identical and *E. coli* and *E. hartmanni* are closely similar in appearance to *E. histolytica*) (Garcia and Bruckner, 1997; Krogstad et al., 1978; Abd-Alla et al., 1998).

## 2. Culture and isoenzymes

*E. histolytica* was first cultivated by Boeck and Drbohlav in a diphasic egg slant medium. The National Institutes of Health (NIH) modification of Locke-egg medium has been used in some research laboratories. Robinson medium (Robinson, 1968) and TYSGM-9 (Diamond, 1982) are more often used for xenic cultivation of *E. histolytica*. TYI-S-33 (Diamond et al., 1978) medium designed by Diamond is one of the most widely used axenic media.

The isoenzyme patterns to differentiate “pathogenic” and “nonpathogenic” *Entamoeba* obtained from amoebic culture lysates were widely used (Gathiram and Jackson, 1990; Haque et al., 1990; Sargeant, 1987; Sargeant et al., 1982; Sargeant et al., 1978; Scaglia et al., 1991). There are a total of 24 different zymodemes reported, composed of 21 zymodemes from human isolates (9 *E. histolytica* and 12 *E. dispar*) and 3 zymodemes from experimental culture amoebic strains (Blanc et al., 1989; Sargeant, 1985; Sargeant et al., 1988). Except two zymodemes all other zymodemes appear to be reliable due to contributions of the zymodeme pattern from bacterial contaminant in the xenic culture (Jackson and Suparsad, 1997).

If only most stable zymodemes bands are considered, then only three remain for *E. histolytica* (II, XIV and XIX) and one for *E. dispar* (I) (Clark et al., 2002). The genetic difference between hexokinase of *E. histolytica* from *E. dispar* is sufficient enough to make zymodeme analysis a useful tool in their differentiation (Ortner et al., 1995). Culturing *E. histolytica* from stool or liver abscess pus specimen and

performing the isoenzyme analyses are mostly unsatisfactory and not useful in routine laboratory practice (Sehgal et al., 1995);

The disadvantages with culture and isoenzyme analysis are; maintaining protozoa in culture is more labor-intensive and difficult than maintaining bacteria in culture. Maintaining *Entamoeba* in culture is primarily a research tool rather than diagnostic tool (Clark and Diamond, 2002). Additionally the non pathogenic species of amoeba *E. dispar* is shown to be capable of growing in both xenic and axenic culture (Markell et al., 1999). Therefore, for *Entamoeba* species identification by culture and isoenzyme analysis, the risk of one species outgrowing the other in cultures of specimens from mixed infections can not be excluded (Evangelopoulos et al., 2000). The over growth of some undesired organisms like *Blastocystis hominis* in culture, can often lead to suppression of *E. histolytica* growth in culture and can lead to false negative result of *E. histolytica* (Clark and Diamond, 2002). The main disadvantage of isoenzyme technique is that the culture followed by isoenzyme analysis takes long time usually from days to weeks to give report and it is labour intensive.

### **3. Antibody detection**

Techniques used for serum amoebic antibody detection include IHA (Parija et al., 1989; Knobloch and Mannweiler, 1983; Shetty et al., 1988; Stevens et al., 1979), complement fixation (CF) (Knobloch and Mannweiler, 1983; Lotter et al., 1993), counter immuno electrophoresis (CIE) (Parija and Karki, 1999; Bapat and Bhawe, 1990; Garcia et al., 1982; Krupp, 1974; Restrepo et al., 1996; Sheehan et al., 1979; Shetty et al., 1988, Stevens et al., 1979), indirect fluorescence assay (IFA) (Garcia

et al., 1982; Jackson et al., 1984; Tanyuksel et al., 1995; Welch et al., 1986), amoebic gel diffusion test (Jackson et al., 1984), latex agglutination (Cummins et al., 1994; Haider and Fayyaz, 1978; Lotter et al., 1993; Lotter et al., 1995), carbon-immunoassay (CIA) (Parija et al., 1987) and ELISA (Agundis et al., 1996; Attia et al., 1995; Braga et al., 1996; Kelsall et al., 1994; Knobloch and Mannweiler, 1983; Lotter et al., 1993; Lotter et al., 1995; Braga et al., 1996; Pal et al., 1996; Tandon, 1981) with variable sensitivity and specificity .

### ***ELISA***

ELISA is the most frequently used test for diagnosis of patients with ALA and intestinal amoebiasis by demonstration of serum anti-lectin antibodies (Gathiram and Jackson, 1987; Haque et al., 2000; Ravdin et al., 1990). The sensitivity of detection of specific antibodies to *E. histolytica* in serum is reported to be nearly 100%, which holds good for the diagnosis of ALA (Knobloch and Mannweiler, 1983; Ravdin et al., 1990; Zengzhu et al., 1999). Serum anti-lectin immunoglobulin G (IgG) antibodies can be demonstrated within 1 week after the onset of symptoms in patients with amoebic colitis and ALA; with a value over 95% (Abd-Alla et al., 1992; Ravdin et al., 1990) (**Table 2**).

Detection of anti-amoebic IgG antibodies in serum does not indicate whether the infection is recent or old (Pillai and Kain, 2000). Examination of anti-amoebic antibodies of class IgG, IgM, and IgA in a person living in an area of endemicity can explain when the infection occurred (Abd-Alla et al., 2002).

Diagnosis of amoebiasis is generally based on the presence of anti-lectin IgG (which appears later than 1 week after onset of symptoms) or on the existence of positive *E. histolytica* IgM antibodies (especially during the first week of amoebic colitis) (Abd-Alla et al., 1998). In a study conducted on hundred amoebic colitis patients, their anti-lectin IgM and anti-lectin IgG levels were measured by ELISA, and their sensitivities for the period of first week was found to be 45.1 and 5.6% respectively. After a period longer than 1 week it was observed that the levels of anti-lectin IgM and anti-lectin IgG increased to 79.3 and 93.1%, respectively (Abd-Alla et al., 1998). It is important to diagnose recently acquired infection accurately especially for clinical management of patients with invasive amoebiasis (Kraoul et al., 1997).

### **IHA**

Antibody detection by IHA can be very useful in the diagnosis of invasive amoebiasis especially ALA when used in conjunction with the appropriate clinical syndrome (Parija et al., 1988; Garcia and Bruckner, 1997; Kasper et al., 2005). The serum antibody cut off titer for diagnosis of amoebiasis is usually  $\geq 1:128$  (Parija et al., 1988; Khan et al., 1989; Tsai et al., 2006; Hira et al., 2001; Sexton et al., 1974). One study reports that the sensitivity of IHA was found to be 72.4% in patients with ALA during first and second weeks after the onset of symptoms, but it was 86.9% at the end of third week (Knobloch and Mannweiler, 1983). The average antibody concentration by IHA starts to decrease by the sixth month (Knobloch and Mannweiler, 1983; Kasper et al., 2005). In our laboratory, 41 (82%) of 50 patients with ALA were positive by IHA. Three sera (12%) from other

parasitic and miscellaneous controls gave false-positive reactions by IHA. The positive and negative predictive values of IHA were reported to be 93.1 and 83.9%, respectively (Parija and Karki, 1999). In one study, IHA could detect invasive amoebiasis in 18 patients out of 22 patients which included 13 with amebic colitis by histopathological techniques and 9 with ALA by imaging techniques (Hung et al., 1999). Another study has compared the sensitivity and specificity of three tests for the detection of anti-amoebic antibodies: IHA, latex agglutination, and ELISA. They found the respective values for these tests to be 97.6, 90.7, and 93% sensitivity and 97, 95, and 100% specificity (Kraoul et al., 1997).

### *CIE*

In a study, a total of 110 serum samples which included 30 patients with ALA, 30 patients with amebic colitis, and 50 control serum samples were studied by both CIE and ELISA. Among patients with amoebic colitis, the CIE did not detect serum anti-amoebic antibody in any of the patients, whereas ELISA was positive for serum anti-amoebic antibodies 10% of patients. Both CIE and ELISA assays gave negative results with control group sera. CIE and ELISA assays done on ALA patient sera gave 66.6% and 90% positive results respectively. The study concluded that CIE assay was less sensitive than ELISA assay for diagnosis of ALA (Restrepo et al., 1996).

In another study conducted on a total of 153 cases of intestinal amoebiasis patients; 27 sera from 84 patients with early-confirmed cases (positive by microscopy for *E.*

*histolytica*) and 12 sera from 69 patients with nonearly- confirmed cases (negative by microscopy for *E. histolytica*) were positive for anti-amoebic antibodies by using CIE. This study also included a total of 59 cases of ALA, of which CIE was positive in 20 out of 30 proved cases of ALA and in 4 of the 29 unproved cases. In addition, CIE was negative for sera from patients with non-amoebic illness (n = 48), normal healthy controls (n = 100) and asymptomatic cyst passers (n = 75) (Bapat and Bhawe, 1990).

One study reports the sensitivity of CIE to be 100% and 25 % for detecting anti-amoebic antibody in sera of 7 patients with extra-intestinal amoebiasis and 8 patients with asymptomatic intestinal amoebiasis respectively (Sheehan et al., 1979). According to this study the sensitivity of CIE test was found to be high in cases of invasive amoebiasis, but the sensitivity was low in cases of intestinal amoebiasis. The CIE was also reported to be more time-consuming than ELISA.

It has been reported that the anti-amoebic antibodies can become detectable by CIE in blood within 5 days (the seropositive rate was 66.7%) after the onset of clinical symptoms. The anti-amoebic antibody titers increased rapidly and in approximately 2 weeks reached the maximum (on day 11, the seropositive rate was 100%) (Samrejrongroj and Tharavanij, 1985).

Reports claim that it is possible to detect anti-amoebic antibody by CIE and agarose gel diffusion assays up to six months or more (Jackson et al., 1984; Juniper et al., 1972; Stanley et al., 1984). The gel diffusion precipitin test has been reported

to detected anti-amoebic antibodies for even as long as 4 years following infection (Patel and DeRidder, 1989).

### **CIA**

The CIA as a method for serodiagnosis of ALA was developed and evaluated for the first time in our laboratory at JIPMER (Parija et al., 1987). The novel immunoassay relies on a specific reaction between the carbon particles or the India ink and anti-amoebic antibodies coated on surface of *E. histolytica*; being mediated by the complement. The reaction is visualized by simple light microscopy. The CIA was performed, in parallel with the IHA test and ELISA on 25 sera from ALA patients, 25 sera from non-amoebic liver diseases and 50 from healthy blood donors. The CIA could detect anti-amoebic antibodies in serum of 22 (88%) of ALA patients when compared with that of IHA (92%) and ELISA (100%). No false positivity was observed amongst the non-amoebic liver disease sera by CIA, which was similar to IHA and ELISA. However, a false positive on 2 (4 %) sera of healthy blood donors was noted by this assay in contrast to none demonstrated by IHA and ELISA. The CIA required inexpensive easily available reagents, minimum technical expertise and an ordinary light microscope; which could be made available to any Primary health centre.

The disadvantage associated with antibody detection for the diagnosis of amoebiasis is that in majority of developing countries amoebiasis is endemic; most of the people with intestinal amoebiasis have been exposed to *E. histolytica* in some stage of their life (Kasper et al., 2005). The serum anti-amoebic antibodies

continue to remain positive for years even after eradication of the *E. histolytica* infection (Kasper et al., 2005). This makes definitive diagnosis of amoebiasis by antibody detection difficult because of the inability to distinguish past from current infection (Caballer et al., 1994; Gathiram and Jackson, 1987).

Antibody based serological tests may be of more help especially in developed countries where *E. histolytica* infection is not common (Ohnishi and Murata, 1997; Walderich et al., 1997; Weinke et al., 1989). The best strategy for diagnosis of amoebiasis will be the combination of antibody detection tests with detection of the parasite antigen or DNA (Haque et al., 1998a).

#### **4. Antigen detection**

A single Immuno-chromatographic strip coated with monoclonal antibodies specific for *E. histolytica* / *E. dispar* antigen (29 kDa) and for antigens of *Giardia lamblia* (Pillai and Kain, 1999) and *Cryptosporidium parvum* has been designed by Triage parasite panel (BIOSITE Diagnostics, San Diego, Calif.) (Garcia et al., 2000).

The sensitivity and specificity of Triage was reported to be 96 and 99.1%, respectively, for *E. histolytica*/*E. dispar* in 99 stool specimens compared to a stool ova and parasite examination by microscopy (Garcia et al., 2000). The Triage has been reported to have a maximum specificity of 100%, but when compared to the Alexon ProSpecT ELISA the specificity of Triage was low (68.3%) (Pillai and Kain, 1999). Both Alexon ProSpecT ELISA and Triage panel are unable to

distinguish *E. histolytica* from *E. dispar*. When compared to microscopy the sensitivity and specificity of the ProSpecT ELISA was reported to be 73.5 and 97.7%, respectively, for detecting *E. histolytica* / *E. dispar* (Jelinek et al., 1996).

In present scenario the use of monoclonal antibodies in antigen-based ELISA has become the most favored method of choice. For example, the *E. histolytica* test II an antigen based ELISA kit designed by TechLab, Blacksburg, Va., which uses monoclonal antibodies against the Gal/GalNAc-specific lectin of *E. histolytica* and Optimum S kit designed by Merlin Diagnostika, Bornheim-Hersel, Germany which uses monoclonal antibodies against the serine-rich antigen of *E. histolytica* are reported to be capable of detecting *E. histolytica* specifically.

There are other antigenic components which have been targeted for *E. histolytica* detection include the use of a monoclonal antibody against a lysine-rich surface antigen (Petri and Singh, 1999), a lipophosphoglycan (Mirelman et al., 1997), a salivary 170-kDa adherence lectin antigen (Abd-Alla et al., 2000), and an uncharacterized antigen (Wonsit et al., 1992) but these are still restricted as research module. The sensitivity and specificity of '*Entamoeba* test' for detecting *E. histolytica*/*E. dispar* complex and '*E. histolytica* test II' for detecting *E. histolytica* specifically was found be 80 to 99% and 86 to 98%, respectively (Haque et al., 1997; Haque et al., 1995). The overall correlation between results of the TechLab antigen detection test and PCR from stool specimens for detecting *E. histolytica* infection was found to be 94 % (Haque et al., 1998a).

Different types of clinical specimens other than stool have been evaluated for detecting amoebic antigens which include saliva, serum, and liver abscess fluid. The Gal/GalNAc lectin in the sera of patients with ALA was detected by using the TechLab *E. histolytica* test II kit (Hughe and Petri, 2000; Haque et al., 2000). The adherence lectin antigen getting secreted in saliva of ALA patients was first reported by Abd-Alla et al (Abd-Alla et al., 2000) using ELISA. The sensitivity of this assay was found to be 22% (Table 2). A polyclonal antibody based ELISA designed to capture amoebic antigen in pus specimens of ALA patients showed positivity of 97.6% (Zengzhu et al., 1999).

In our laboratory at JIPMER counter-current immunoelectrophoresis (CIEP) test was evaluated for detection of amoebic antigen in the serum of ALA patients (Parija and Karki, 1999). The CIEP test detected amoebic antigen in the sera of 38 (76%) of 50 ALA patients but, it failed to detect antigen in 12 (24%) patients with ALA found positive for anti-amoebic antibodies by the IHA test.

Antigen-based diagnostic tests have several significant advantages over other methods currently used for diagnosis of amoebiasis:(i) some of the antigen based diagnostic methods such as ELISA which detect coproantigen, are able to detect *E. histolytica* specifically; (ii) more number of samples can be tested in ELISA format; (iii) The detection of antigen in serum makes more sense than antibody detection because the antigenemia is a mark of presence of parasite in the body; and (iv) Moreover, unlike antibody the antigen does not persist in blood for long period after elimination of the parasite.

The antigen detection in clinical specimens has certain limitations, such as; only fresh or frozen clinical specimens are required because the antigen gets denatured in short period. Another limitation with antigen detection in stool specimen is that at present this technique can detect *E. histolytica* and *E. dispar* but cannot detect *E. moshkovskii*.

### 5. Nucleic acid based diagnostic PCR test

PCR based methods for the diagnosis of amoebiasis is gradually becoming popular and more preferred over the conventional methods like microscopy and culture because of its high sensitivity and specificity. The PCR based test can detect and differentiate *E. histolytica* / *E. dispar* / *E. moshkovskii* complex by exploiting the differences in their genomes (Haque et al., 1998a; Parija and Khairmar 2005). This will help to specifically detect the pathogenic *E. histolytica* from those of look-alike non-pathogenic amoebas such as *E. dispar* and *E. moshkovskii*.

There are several reports claiming the successful application of PCR on stool specimens for the diagnosis of intestinal amoebiasis (Acuña-Soto et al., 1993; Britten et al., 1997; Evangelopoulos et al., 2000; Haque et al., 1998a; Rivera et al., 1996; Rivera et al., 1999, Verweij et al., 2000). One study shows that nested PCR amplification of ribosomal ribose nucleic acid (RNA) (rRNA) genes is more sensitive than antigen detection when cultured parasites are used as the source of DNA and antigen (Mirelman et al., 1997). However, in the real-world scenario where the parasite has to be identified directly in stool specimens, the nested PCR

and ELISA had shown comparable sensitivities as 87% (Table 2) and 85% respectively (Haque et al., 1998a).

Two separate nested PCR studies, one reported by International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh (Ali et al., 2003) and another in our laboratory at Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER) hospital, Puducherry, India (Parija and Khairnar, 2005) on stool sample targeting 16S rRNA gene were found to distinguish accurately between the infections caused by *E. histolytica*, *E. dispar* and *E. moshkovskii*. But these three species, *E. histolytica*, *E. dispar* and *E. moshkovskii* were identified separately with a separate nested PCR for each species.

A multiplex PCR strategy for the simultaneous detection and differentiation of both *E. histolytica* and *E. dispar* in stool samples by using two pairs of species specific primers in a single reaction mixture has been reported (Nunez et al., 2001). This novel approach had 94% sensitivity and 100% specificity. This approach revealed that *E. histolytica* and *E. dispar* mixed infection rate was 24.5% in the Mexican children studied. An improved PCR solution hybridization enzyme-linked immuno assay technique has been developed and is suggested to have an important role in the study of the complex ecology of amoebiasis (Aguirre et al., 1995; Britten et al., 1997; Britten et al., 1997; Verweij et al., 2000).

Only few real-time PCR assays for the specific detection of *E. histolytica* had been published: a Light Cycler assay utilizing hybridization probes to detect amplification of the 18S rRNA gene (Blessmann et al., 2002) and two TaqMan

assays targeting the 18S rRNA gene (Verweij et al., 2004) and the episomal repeats (Verweij et al., 2003), respectively. One study (Qvarnstrom et al., 2005) has compared all the three real-time PCR assays along with a SYBR Green-based assay using previously published primers (Clark and Diamond, 1992).

PCR can also prove to be very helpful for diagnosis of ALA when aspirated pus is available (Zaman et al., 2000). There are few reports of the application of PCR on liver abscess pus specimens for the diagnosis of ALA (Tachibana et al., 1992; Zaman et al., 2000; Haque et al., 1998a; Zengzhu et al., 1999).

PCR technique has some disadvantages; PCR is susceptible to cross-contamination (because of its high sensitivity even a minute quantity of contamination is enough for PCR to give false positive results); false-negative results are also a major problem with PCR this may be due to inhibitors of DNA polymerase present in clinical specimens (Evangelopoulos et al., 2000). The method applied for DNA extraction and designing of primers are key to a successful PCR diagnosis. The use of spin columns can bring down the amount of PCR inhibitors in DNA extract and thus improve the PCR diagnosis (Verweij et al., 2000). The inclusion of internal amplification control (IAC) in PCR is a good strategy to avoid false negative results in diagnostic setup (Hoorfar et al., 2003; Hoorfar et al., 2004).

The PCR test is usually more time consuming than enzyme-linked immuno assay, is technically complex, and is costly (Haque et al., 1998a). However, it is felt that as the time progresses PCR will also become affordable and a part of routine diagnosis in clinical setup in developing countries. However, PCR has the potential

to become the “gold standard” by which other diagnostic techniques can be estimated (Tanyuksel and Petri, 2003). PCR is a powerful tool to explore the area of genetic polymorphism in *Entamoeba* (Barker, 1994).

Table 2

Comparison of sensitivities of different tests for diagnosis of amoebiasis \*

Diagnostic test	Amoebic colitis	Amoebic liver abscess
<b>Microscopy</b>		
Stool	<80%	10-40%
Liver abscess pus	NA <sup>a</sup>	<25%
<b>Culture with isoenzyme analysis</b>		
	Lower than antigen or PCR tests	<25%
<b>Antigen detection (ELISA)</b>		
Stool	>80%	~80%
Liver abscess pus	NA <sup>a</sup>	100% (before treatment)
Serum	65% (early)	~75% (late) - 100% (first 3 days)
Saliva	>85% (< 1 week), >80% (> 1 week)	70% ; >22% (convalescent)
<b>Antibody detection</b>		
Serum (RIA)	70% (acute), >80% (convalescent)	70-80% (acute), >80% (convalescent)
Serum (ELISA)	>90% (< 1 week), 100% (> 1 week)	100% (regardless of duration of illness)
Saliva (ELISA)	>18% (< 1 week), >83% (> 1 week)	92% (regardless of duration of illness)
<b>DNA detection (PCR)</b>		
Stool	>87%	Not done
Liver abscess pus	NA <sup>a</sup>	NA <sup>a</sup>

\* Data collected from reference (Haque et al., 2003, Abd-Alla et al., 2000; Tanyuksel and Petri, 2003)

<sup>a</sup> NA, not available

## DIAGNOSTIC POTENTIAL OF OTHER BODY FLUIDS LIKE URINE AND SALIVA AS CLINICAL SPECIMENS

Collection of blood for serum is an invasive procedure, and the procedure requires technical expertise and disposable syringes (Parija, 1998b). If the method is not

carried out under stringent conditions then it is associated with the risk of acquiring needle borne infections such as hepatitis B virus and human immunodeficiency virus (HIV).

Therefore, of late much interest has been shown towards the use of urine as a specimen alternate to the blood for the diagnosis of some parasitic infections including malaria, schistosomiasis, kala-azar, cystic echinococcosis and neurocysticercosis (Parija, 1998b). Urinary antigen for cystic echinococcosis (CE) and neurocysticercosis has been reported for the first time from our laboratory at JIPMER, Puducherry, India (Parija et al., 1997; Parija et al., 2004). Our laboratory has developed for the first time a counter-current immunoelectrophoresis (CIEP) and co-agglutination (Co-A) to detect the hydatid antigen excreted in the urine for the diagnosis of CE (Parija et al., 1997; Ravinder et al., 2000), and Co-A to detect cysticercus antigen in the urine for the diagnosis of neurocysticercosis (Parija et al., 2004).

Detection of DNA in urine by PCR has been employed for the diagnosis of *Toxoplasma gondii*, *Neisseria gonorrhoeae*, *Borrelia burgdorferi*, *Mycobacterium tuberculosis*, *Mycobacterium leprae* and *Chlamydia trachomatis* infections (Crotchfelt et al., 1997; Pleyer et al., 2001; Fuentes et al., 1996; Aceti et al., 1999; Parkash et al., 2004). Some studies have also shown that the kidney barrier in rodents and humans is permeable to DNA molecules large enough to be analyzed by standard genetic methodologies (Botezatu et al., 2000; Su et al., 2004). A very rough estimate based on a study (Botezatu et al., 2000) indicates that; 0.5–2% of the free DNA that passes through the bloodstream crosses the kidney barrier and is

excreted in the urine. To the best of our knowledge till now there is no report available on detection of *Entamoeba* DNA in the urine for the diagnosis of ALA.

Like urine, saliva can also be collected non-invasively. In the last decade saliva has been used as a clinical specimen for the diagnosis of infectious diseases. Salivary antibody detection was found to be useful for the diagnosis of bacterial infections caused by *Helicobacter pylori*, *Shigella* and *Borrelia burgdorferi* (Loeb et al., 1997; Schultz et al., 1992; Schwartz et al., 1991) and various viral infections such as hepatitis A, hepatitis B, hepatitis C, measles, mumps, rubella, rotavirus, dengue, parvovirus B 19 and HIV (Parry et al., 1989; El-Medany et al., 1999; Thieme et al., 1994; Jayashree et al., 1988; Cuzzubbo et al., 1998; Rice and Cohen, 1996; Malamud, 1992). Salivary antibody detection has also been studied for the diagnosis of some parasitic infections caused by *Toxoplasma gondii*, *Schistosoma mansoni*, *Taenia solium* and *Entamoeba histolytica* (Loyola et al., 1997; Garcia et al., 1995; Ramos et al., 1997).

Saliva has also been used for antigen detection in the diagnosis of pneumococcal pneumonia (Krook et al., 1986), hepatitis B virus, measles, mumps and rubella (Chaita et al., 1995; Friedman, 1982; Perry et al., 1993; Brown et al., 1994). There is only one report, till date on the detection of salivary lectin antigen of the parasite *E. histolytica* for the diagnosis of ALA with a sensitivity and specificity of 22% and 97.4% respectively (Abd-Alla et al., 2000).

The reports on the use of saliva for DNA detection for the diagnosis of infectious diseases, however, are limited (LaDuca et al., 1998; Miller et al., 2006; Pozo and

Tenorio, 1999; Crepin et al., 1998; Li et al., 1995). The PCR has been used to facilitate diagnosis of viral infections such as Epstein-Barr, cytomegalovirus, human herpes viruses 6, 7, and 8 and rabies by using saliva (LaDuca et al., 1998; Miller et al., 2006; Pozo and Tenorio, 1999; Crepin et al., 1998). The PCR has also been evaluated for detection of *H. pylori* associated peptic ulcer, by demonstration of *H. pylori* DNA in saliva (Li et al., 1995). However, report on the detection of *Entamoeba* DNA in the saliva for the diagnosis of ALA is still lacking.

### **ENTAMOEBIA GENOME PROJECT AN OVERVIEW**

During past 30 years, the tools of molecular biology have greatly increased the understanding the biology and pathogenesis of *E. histolytica*. The recent publication of the *E. histolytica* genome (Loftus et al., 2005) provides an opportunity for new insights into the biology of *E. histolytica*, and for better understanding of the pathogen in far more details. *Entamoeba* genome sequencing has made a high impact on the understanding of the biology and evolution of this parasite genus (Samuel and Stanley, 2005). The completion of genome sequencing of *Entamoeba histolytica*, however, is still awaited (Loftus et al., 2005).

National Institute of Allergy and Infectious Diseases (NIAID) is funding The Institute of Genomic Research (TIGR) to implement a large scale sequencing project of *E. histolytica*. Because of the medical importance of amoeba and their unique biology, the goal of this project is to determine 99% of the genomic

sequence of *E. histolytica* strain HM1: IMSS analyze and annotate the data and provide ready equal access to the sequence information and analysis.

The Wellcome Trust has recently funded the Sanger Institute Pathogen Sequencing Unit, in collaboration with Graham Clark at the London School of Hygiene and Tropical Medicine, to undertake whole genome shotgun sequencing of *E. histolytica*. A collaborative approach is now underway at both centers to achieve significantly higher sequence coverage of the *Entamoeba* genome.

In addition, the Biotechnology and Biological Sciences Research Council (BBSRC) has funded the Sanger Institute Pathogen Sequencing Unit, in collaboration with Graham Clark at the London School of Hygiene and Tropical Medicine, to undertake whole genome shotgun sequencing of *E. dispar*, and *E. moshkovskii* among others. The sequence data for *E. dispar*, *E. moshkovskii* and others are available at websites <http://www.sanger.ac.uk/Projects>.

The gene organization of *E. histolytica* seems quite distinct from that of other eukaryotes. Although the structure of *E. histolytica* chromosomes is not yet known completely, electrokaryotypic analysis suggests that the chromosomes range in size from 0.3 to 2.2 Mb and gives a total haploid genome size of approximately 20 Mb (Willhoeft and Tannich, 1999). A complete sequence map of the ribosomal DNA (rDNA) episome has been successfully completed (Blanc et al., 1989; Sehgal et al., 1994). The *E. histolytica* circular DNA is 24.5 kb (Sehgal et al., 1994; Bhattacharya et al., 1998). This sequence has proved quite useful for genotyping of the different enteric amoeba (Clark and Diamond., 1993; Tannich et al., 1992).

The *E. histolytica* genome is ~24 Mb in size and split into 14 chromosomes. A whole genome shotgun sequence (8X coverage) has been produced in collaboration with TIGR and assembled into 888 scaffolds. This sequence data has been published (Loftus et al., 2005) and completion is now in progress. The draft genome sequence with preliminary automated annotation can be accessed from GeneDB (<http://www.genedb.org/>). The sequence data can be downloaded by File Transfer Protocol (FTP).

As part of the comparative *Entamoeba* sequencing project 20,000 sequences from *E. dispar* and 10,000 sequences from *E. moshkovskii* have been produced and the raw sequence reads can be downloaded by FTP and Basic Local Alignment Search Tool (BLAST) searching. The knowledge about the organization of genes of known virulence factors such as amebapores (Leippe and Herbst, 2004), cysteine proteinases (Bruchhaus et al., 2003) and lectins (Petri et al., 2002) of *E. histolytica* can lead to a better understanding of their role in disease.

The *Entamoeba* genome sequencing project has already changed the way in which the *Entamoeba* research is done today, and is proving to be a useful resource for investigators who are interested in studying the *Entamoeba* at gene level in coming years.

## **MUTATION DETECTION AND ITS SIGNIFICANCE**

Mutations are caused by changes in the genetic materials such as DNA or RNA. The mutations can result either spontaneously or can be induced artificially. They also differ in the way that they impact evolution. Mutations, when accidental, often lead to the malfunction or death of a cell. The majority of mutations have no significant effect on the organism, because DNA repair is able to revert most changes before they become permanent mutations. Moreover, many organisms have mechanisms for eliminating otherwise permanently mutated somatic cells. In some cases even if a change becomes permanent mutation it does not affect the phenotype such a mutation is called as "silent mutation".

To function correctly, each cell depends on thousands of proteins to function in the right places at the right times. Changes in DNA caused by mutation can cause errors in protein sequence, creating partially or completely non-functional proteins. When a mutation alters a protein that plays a critical role in the body, a medical condition can result. A condition caused by mutations in one or more genes is called a genetic disorder (Hacia et al., 1996).

A very small percentage of all mutations actually have a positive effect (Anthony et al., 1999). These mutations lead to new versions of proteins that help an organism and its future generations better adapt to changes in their environment. For example, a beneficial mutation could result in a protein that protects the organism from a new strain of bacteria (Anthony et al., 1999).

Technological development continues to take place in the field of mutation analysis. In the past few years, the necessity to screen amplified DNA products to

identify mutations or polymorphisms has assumed increasing importance in studying the genetics of organisms. The term “mutation analysis” refers to the identification of changes in DNA.

Detecting mutations in DNA (or RNA) requires various combinations of (a) physical mapping, (b) cloning or amplifying DNA, (c) screening methods to identify DNA changes involving one to a few nucleotide bases, (d) electrophoretic separation of DNA fragments, and (e) sequencing. The DNA sequencing is the gold standard in terms of DNA mutation analysis. There are two types of mutation analysis performed which require completely different and rarely overlapping methods. The first is the more difficult search for a mutation in a piece of DNA for the first time, “unknown mutations” and the second one is the search for previously identified mutations, “known mutations”. However, most of the time, the number of genes and samples are too large to make sequencing a practical diagnostic approach (Nataraj et al., 1999).

A number of screening methods are used for detecting “unknown mutations”. These include single strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), restriction fragment length polymorphisms (RFLP) and low-stringency single specific primer PCR (LSSP-PCR) etc.

### **1. Single strand conformational polymorphism (SSCP)**

SSCP technique is widely used for the large scale screening of unknown mutations as well as polymorphisms. In the last 10 years, SSCP rapidly gained popularity as

the most straightforward and versatile methods to screen for DNA alterations (Nataraj et al., 1999), to detect DNA polymorphisms and mutations at multiple places in DNA fragments (Orita et al., 1989). The SSCP as a mutation scanning technique is more often used to analyze the polymorphisms at single loci, especially when used for medical diagnoses (Sunnucks et al., 2000). Variations of the SSCP technique have been successfully used to examine the genes contributing to the multidrug resistance of *Mycobacterium tuberculosis* (Heym et al., 1994).

The SSCP analysis was originally described by Orita et al in 1989 (Orita et al., 1989). The general idea is to take a small PCR product, denature it, and electrophorese it through a non-denaturing polyacrylamide gel. Thus, as the PCR product moves into and through the gel, it will regain secondary structure that is sequence dependent (similar to RNA secondary structure). The mobility of the single-stranded PCR products will depend upon their secondary structure. Therefore, PCR products that contain substitutional sequence differences as well as insertions and deletions will have different mobilities. The major advantage of SSCP is that many individual PCR products may be screened for variation simultaneously. Most researchers use SSCP to reduce the amount of sequencing necessary to detect new alleles at loci of interest (Sweetman et al., 1992) or to better estimate allele frequencies of populations (Widjojoatmodjo et al., 1994).

The uses of SSCP is mainly twofold .First, the SSCP is used to screen PCR products of genes that researchers intend to sequence for phylogenetic analysis. The SSCP screening allows researchers to determine (1) if the gene contains sufficient polymorphism that sequencing will be worthwhile, (2) which portion of

the gene is most polymorphic, (3) what level of intra-specific variation exists, and (4) if there is polymorphism among multicopy genes within individuals (e.g. rDNA). Second, SSCP is a much more efficient method of obtaining information about levels of polymorphisms within anonymous nuclear loci than the restriction enzyme protocol originally described (Karl et al. 1992). The advantages of using SSCP in this context are: (1) the PCR amplicons are smaller and thus easier to amplify, (2) because the amplicons are only 200–300 bp, the entire sequence needed for PCR primer design can be obtained from a single sequencing reaction, (3) similar amounts of information are obtained by simply running the amplicons on 2 SSCP gels (e.g. one with glycerol and one without), rather than doing 50 restriction digests and assuming that the restriction sites are independent (a problematic assumption when dealing with a 1 kb amplicon), and (4) when polymorphisms are detected, the amplicons are of optimal size to sequence using an ABI automated sequencer (so getting sequence level information is easy).

Both ethidium bromide and silver-staining are used, rather than radiation to detect SSCP alleles. The SSCP was first designed to detect mutations in oncogenes and allelic variations in the human genome (Orita et al., 1989). Since then, The SSCP has been extensively applied in biomedical research and it has been refined and tuned as per the requirement of researchers (Jordon et al., 1999). The technique has played a role in bacterial typing (Widjojoatmodjo et al., 1994) and in *Salmonella* studies (Karl et al., 1992).

A study has documented presence of a 482 base pair gene fragment from samples of amoebae *E. histolytica* and *E. dispar* amplified by PCR. The amplification

products of fragments from the 2 species of amoebae showed differences in mobility in non-denaturing polyacrylamide gel, probably due to sequence-dependent conformational alterations in the DNA fragments. The study suggests that this method permits *E. histolytica* and *E. dispar* to be distinguished with greater sensitivity and rapidity (Gomes et al., 1999).

The disadvantage of SSCP technique is that it has low sensitivity (60–90%) for detecting mutations (Sheffield et al., 1993) and also it requires extensive optimization.

## **2. Denaturing gradient gel electrophoresis (DGGE)**

DGGE is another technique used to detect mutation in the PCR amplified gene fragments (Muyzer et al., 1993). DGGE electrophoresis can detect differences in the melting behavior of small DNA fragments (200-700 bp) that differ by as little as a single base substitution. When a DNA fragment is subjected to an increasingly denaturing physical environment, it partially melts. As the denaturing conditions become more extreme, the partially melted fragment completely dissociates into single strands. Rather than partially melting in a continuous zipper-like manner, most fragments melt in a step-wise process. Discrete portions of the fragment suddenly become single-stranded within a very narrow range of denaturing conditions and a mobility shift is observed. The rate of mobility of DNA fragments in acrylamide gels changes as a result of sequence variation.

## **3. Restriction fragment length polymorphism (RFLP)**

RFLP analysis is a powerful technique for the characterization of DNA at the molecular level. Although RFLP is not the preferred method for today's research, but it was the technology that began the dramatic explosion in genetic technology by allowing the field to locate genes for Mendelian disorders like Huntington's disease and cystic fibrosis (<http://www.bioon.com/experiment/mob17/62153>). Direct detection of point mutations by restriction mapping is an application of RFLP technology is a useful tool being evaluated in diagnostics.

Molecular methods enable the differentiation of morphologically similar organisms for use in classification. DNA sequence to be analyzed must be ubiquitous and conserved in the organisms of interest; the sequence must at the same time be variable enough so that species specific markers can be identified. Genes that satisfy these criteria include those encoding the small and large subunit ribosomal RNAs.

The method "riboprinting" was first described by this name in 1991, which combines the restriction site polymorphism analysis with the rDNA amplification method of Medlin (Medlin et al., 1988) to study sequence variation in the small subunit rDNA indirectly (Clark and Diamond, 1991b). This method was originally developed by Clark with the goal of studying relationships among species of *Entamoeba*, but the same approach has been used successfully in a number of taxonomically diverse protist and fungal genera (Clark, 1997b; Clark and Pung, 1994; Brown and De Jonckheere, 1994; Jerome and Lynn, 1996). There are few reports in which riboprinting have been applied to study genetic diversity in

*Entamoeba* species (Clark and Diamond, 1997; Clark and Diamond, 1992; Clark, 1997a; Mukhopadhyay et al., 2002). The same approach was also developed independently by mycologists (Vilgalys and Hester, 1990) and bacteriologists, where it is also known as ARDRA (amplified ribosomal DNA restriction analysis) (Vanechoutte et al., 1992).

In the ribotyping method, the PCR amplified rDNA is digested with restriction enzymes and the fragments are separated in agarose gels along with a DNA size marker. The gels are then stained and documented to provide a permanent record. Some of the restriction sites fall in conserved regions and some in variable regions and this allow sequence polymorphisms to be detected as differences in the restriction fragment sizes. Species often share one or more comigrating fragments for each restriction enzyme. Since the number of comigrating fragments decrease as the gene sequences diverge, ribotyping is also used to estimate genetic distances among species (Nei and Li, 1979). When more than 10 enzymes each with a four base recognition sequence is used 10-15% of the gene sequence is sampled without the necessity of any cloning or sequencing of the DNA (Clark, 1997a).

Ribotyping is thus a method that does not require large amounts of starting material. It is quick, it is reproducible and it uses tools that can be found in almost every molecular biology laboratory. Since only 10-15% of the gene sequence is sampled by this indirect method, significant variation can be present and will go undetected unless divergence falls within restriction sites, and is the main disadvantages of RFLP ribotyping. Similarly, if two restriction sites fall close

together the resulting fragment will not be visible in the agarose gels and, if the sites are very close, the corresponding reduction in size of the adjacent restriction fragment may be too small to be resolved or noticed. It is known that some species defined by reproductive isolation have small subunit rDNAs that are identical in sequence (Clark, 1992). Therefore, identical riboprints do not indicate that the organisms necessarily belong to the same species.

#### **4. Low-stringency single specific primer PCR (LSSP-PCR)**

LSSP-PCR is an extremely simple PCR-based technique that detects single or multiple mutations in gene-sized DNA fragments. A purified DNA fragment is subjected to PCR using high concentrations of a single specific oligonucleotide primer, large amounts of *Taq* polymerase, and a very low annealing temperature. Under these conditions the primer hybridizes specifically to its complementary region and nonspecifically to multiple sites within the fragment, in a sequence-dependent manner, producing a heterogeneous set of reaction products resolvable by electrophoresis. The complex banding pattern obtained is significantly altered by even a single-base change and thus constitutes a unique "gene signature." LSSP-PCR can be used either as screening or diagnostic method. The method has been used for the detection of mutations in human genetic diseases (Pena et al., 1994), for the study of sequence variation in human mitochondrial DNA (Barreto et al., 1996) and for genetic typing of infectious agents such as Papilloma virus (Villa et al., 1995), *Trypanosoma cruzi* (Vago et al., 1996) and *E. histolytica* (Gomes et al., 1997)

## **5. Future of the mutation detection field**

The whole mutation detection field is undergoing a major revolution with the recent development of techniques based on hybridization to oligonucleotides immobilized in silicon microchips called "DNA chips" or "Biochips" (Editorial, 1996). On the surface of a microchip of only 1.28 x 1.28 cm it is possible to synthesize as many as 409,600 different oligonucleotides. The target DNA is fluorescently labeled and hybridized to the array, thus binding to sequence-specific constellations of oligonucleotides. The binding is quantified in a robotic system by a confocal fluorescent microscopy and the results are analyzed automatically by computer. With one specific biochip containing only 66,276 oligonucleotides it is possible to determine all the nucleotide sequences of the mitochondrial DNA of an individual in less than 10 minutes (Chee et al., 1996). Biochips were initially developed to determine mutations in breast cancer 1 gene (BRCA1) (Hacia et al., 1996) and to unravel gene expression patterns in human normal and tumor tissues (De Risi et al., 1996). It is not too far in the future when we will be able to scan whole human or parasite genome for mutations with the help of just few biochips in less time.

## **TREATMENT OF AMOEBIASIS**

### **1. Intestinal amoebiasis**

Luminal amoebicides are poorly absorbed and reach a high concentration in bowel, but their activity is limited to amoeba lying close to mucosa. Iodoquinol and paramomycine are used as luminal drugs. Luminal agents are indicated for asymptomatic carriers, patients with colitis and ALA to eradicate cysts. The majority of individuals who pass cysts are colonized with non pathogenic *E. dispar* or probably *E. moshkovskii*, which does not warrant a therapy. However, it is prudent to treat asymptomatic individuals who pass cyst unless *E. dispar* or *E. moshkovskii* colonization can be definitely demonstrated by specific test like PCR.

After an oral or parenteral administration the tissue amoebicides reach high concentrations in blood and tissue. Metronidazole is a nitroimidazole compound; it is very effective in treating invasive amoebiasis. Patients with amoebic colitis should be treated with intravenous or oral metronidazole (750mg three times daily for 5 to 10 days) (Kasper et al., 2005). The metronidazole therapy has side effects like nausea, vomiting, abdominal discomfort, and a disulfiram - like reaction. Other imidazole compounds like tinidazole and ornidazole are also effective. All patients should also receive a full course of luminal agents, since metronidazole is not effective against the cyst stage of *E. histolytica* (Kasper et al., 2005).

## **2. Extra-intestinal amoebiasis**

The drug of choice for treatment of ALA is metronidazole. In developing countries the longer acting nitroimidazole (tinidazole and ornidazole) have been shown to be effective as single dose therapy. With early diagnosis and treatment the mortality

due to ALA is less than 1%. If possible, treatment with second line therapeutic agents like emetine and chloroquine should be avoided because of potential cardiovascular and gastrointestinal side effects of the former and the higher relapse rate with the latter. Studies conducted on South African population reveals that 72% of liver abscess patients were asymptotically infected with *E. histolytica* in the intestine (Gathiram and Jackson, 1985); thus, the treatment regimen should include a luminal agent to eradicate cyst stage of *E. histolytica* and prevent further transmission.

Majority (>90%) of ALA patients respond dramatically to metronidazole therapy with decrease in both pain and fever within 72 hrs (Kasper et al., 2005). There is no evidence that aspiration, even of large abscess (up to 10cm), enhance healing. Surgical intervention should be reserved only for instances of bowel perforation and rupture into the pericardium (Haque et al., 2003).

## **CONCLUSION**

The thrust area in the field of diagnostic amoebiasis is to break the deadlock of accurate diagnosis of amoebiasis and to know the true epidemiology of intestinal amoebiasis which would save the patients from unnecessary treatment with anti-amoebic drugs which have side effects and the expenditure on these drugs can be avoided.

As per the WHO, patients infected with even a single *E. histolytica* cyst / trophozoite should be treated with anti-amoebic therapy even if the patients are asymptomatic (not showing symptoms of amoebiasis) because such patients are potential carriers and may spread the infection in community (WHO, 1997), whereas those infected with *E. moshkovskii* or *E. dispar* do not warrant anti-amoebic therapy.

To make the diagnosis of amoebiasis simpler, cost effective and time saving, many laboratories world wide are evaluating different strategies which could detect and differentiate *E. histolytica* from *E. histolytica*-like amoeba in stool samples. We believe that as the trend of evidence based medicine is heralding these newer techniques will soon be available for accurate diagnosis of amoebiasis for better patient care.