



Molecular Diagnosis and Genetic Polymorphism of Entamoeba Histolytica in Iraqi Samples

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Abstract

The aim of this study was to determine the prevalence of the *E. histolytica* between males and females, as well as to determine its spread depending on the type of infection, whether it is associated with bacteria or not, in addition to studying the *E. histolytica* genetically through RNA to determine the genetic sequence of this *E. histolytica*. A total of 100 samples (stool) were collected from patients suffering from diarrhea with inflammatory feature from several different locations in the Adiwaniyah City, Iraq, including: Al-Diwaniya Teaching Hospital, Maternity and Children Hospital, Hamza general Hospital and some many private medical laboratory during the period from October 2021 to April 2022. The current study was done at AL-Qadisiyah University. The stool samples were classified to be healthy, infected with *E. histolytica* according to genetic examinations. The results showed *E. histolytica* and bacteria infection higher in male than female; it were in male 30(60%) while lower results in Entamoeba only 8(16%). There are significant differences among the three groups. In the female high results show mixed infection of *E. histolytica* and bacterial infection which recorded 18(36%) and parasite infection only recorded the same results, while bacterial infection only showed that 4(8%). The current study concluded that the mentioned parasite affects males more than females, as well as infection of the type associated with bacteria is the most prevalent in males and females, and the current study concluded that there are mutations that occurred in the parasite's genome that can increase the virulence of this parasite.

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KeyWords: *E. histolytica*, Intestinal Infections, *E. coli*.

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Introduction

Entamoeba histolytica, a protozoan parasite, causes amoebiasis, which may manifest clinically as anything from asymptomatic colonization to amebic dysentery and invasive extraintestinal amoebiasis, which most often manifests as liver abscesses [1]. *E. histolytica* is thought to impact up to 50 million people worldwide, mostly in underdeveloped nations, and it is the cause of over 100,000 fatalities annually. Because of fecal cyst excretion, transmission often happens via the consumption of contaminated food or water, or even through fecal-oral transfer within families. Seroprevalence investigations have shown antibodies in people living in highly endemic regions of Asia and Latin America ranging from 12 percent to 65 percent of the time, even in asymptomatic people [2]. Numerous

immunological assays may assess the antibodies that arise after invasive infection, but these tests distinguish between previous infection and present amoebiasis. Incidence of intestinal amoebiasis in highly endemic areas ranged from 13 percent to 67 percent among people with diarrhea, according to studies using more sensitive tests that can distinguish pathogenic *E. histolytica* from non-pathogenic species, such as enzyme-linked immunosorbent assay (ELISA) stool antigen detection or polymerase chain reaction (PCR) [3]. Amoebiasis continues to pose a significant threat to public health in many areas, particularly in the "bottom billion" nations where poverty and low income are pervasive and complicated problems are impeding their ability to progress economically.

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Parts of India, Bangladesh, tropical African nations, Brazil and Mexico, China, and South-east Asia are among the regions having high incidence of amoebic infection [4]. Areas with poor sanitation, limited water treatment, and low socioeconomic level are often where human infection is detected. The sole reservoir is human, and infection occurs when hands or food come into contact with feces that contain cysts. Oral-genital and oral-anal contact have been documented to constitute human-to-human transmission routes, particularly among homosexuals and others with poor personal hygiene [5]. For the early diagnosis of several infectious disorders, including amoebiasis, molecular diagnostic techniques have taken precedence. For the identification and detection of Entamoeba species in feces, tissues, and liver lesion aspirates, several DNA amplification methods are available. They include multiplex PCR, loop-mediated isothermal amplification assay, nested PCR, real-time PCR, and standard PCR [3].

covered with cover slip and examined at 40x and 100x (oil lens) to show the trophozoite and cyst according to [6].

3. Genotypic Identification

Multiplex Polymerase Chain Reaction (PCR) technique was performed for confirmative molecular detection and DNA sequencing method was performed for Phylogenetic relationship analysis study of positive sample isolates of local *E. histolytica* with NCBI GenBank Global Entamoeba histolytica isolates. The sequencing of the PCR product of 18S rRNA gene, where the 6bp PCR product was purified from agarose gel by using (EZ EZ-10 Spin Column DNA Gelextraction Kit.

Table 1. PCR detection gene primer with their nucleotide sequence and product size

Primer	Sequence (5'-3')	Product Size
<i>E. histolytica</i> 18s rRNA	ATTGGAGGGCAAGTCTGGTG GCCTTGTGACCATACTCCCC	616bp

Materials and Methods

1. Study Design and Sampling

A total of 100 samples (stool and blood) were collected from patients suffering from diarrhea with inflammatory feature from several different locations in the AlDiwaniyah city, Iraq, including: Al-Diwaniya Teaching Hospital, Maternity and Children Hospital, Hamza General Hospital and some many private medical laboratory during the period from october2021 to April 2022. Before samples are collected, case history has been taken from all patients who were targeted in the study including the name, age, gender, address, clinical symptoms (fever and bloody diarrhea). General stool examination had been done to samples within 30 minutes, after that, according to this test result, the stool samples were classified to be healthy (non infected), infected with *E.histolytica* only, infected with bacteria only and accompanied infection between *E.histolytica* and bacteria. Each infected stool sample was divided into two parts one for morphological examination, while the other part, were stored at -20 °C (deep freezing) for DNA extraction used for PCR.

2. Microscopic Examination

A small drop of fecal sample have been picked up by stick and mixed with a drop of saline then

4. Statistics Analysis

The Statistical Analysis System- SPSS program, the Chi square and one way anova were used to analyze different groups in study parameters. Least significant difference -LSD test was used to compare between means in this study.

Results

1. Distribution of Entamoeba Histolytica and Bacterial Infection and Bacterial Infection According to the Gender

The results of distribution of *E. histolytica* and bacterial infection according to the gender that included 100 stool samples (50 males and 50 females) in male the mixed of *E.histolytica* and bacteria infection results show higher than female it were in male 30(60%) while lower results in entamoeba only it were 8(16%) as in table (2). The results show a highly significant difference amongst three groups.

In the female results from a total 50 samples, high results show mixed infection of *E.histolytica* and bacterial infection which recorded 18(36%) and bacterial infection only recorded the same results, while bacterial infection only showed that 4(8%) as in table (2). The results show significant difference between three groups while there was no



significant difference between male and female.

Table 2. Distribution of infection of Entamoeba histolytica and Mixed infection and bacteria only infection according to gender

Gender	<i>E.histolytica</i>	<i>E.histolytica</i> and <i>bacteria</i>	<i>Bacteria</i> only	X ²	P value
Male	8(16)	30(60)	12(24)	22.86*	0.001
Female	4(8)	18(36)	18(36)	9.46	0.009*
Total	12(13)	48(53.33)	30(33.33)	8.78	0.004
X ²	1.76	5.76	1.71		
P value	0.18	0.016*	0.190		

2. Sequencing and Phylogenetic Construction

Nine of 18s rRNA gene of *E.histolytica* from Human detected from different regions & was sent to GenBnk-Bankit submission for recording these isolates in the NCBI National center for biotechnology information and were obtained the submission IDs for all 9 sequences that were sent. All results of nine isolates analyzed and examined and took the Accession numbers as Fasta options begins ((ON086987.1, ON086988.1, ON086989.1, ON086990.1,

ON086991.1, ON086992.1, ON086993.1, ON086994.1, ON086995.1). These results recorded and published in the International Nucleotide database Sequence Collaboration (INSDC) this location containing National Center for biotechnology Information (NCBI) GenBank, European Nucleotide Archive (ENA) and DNA Data Bank of Japan (DDBJ). The alignment and identity of our results with global strain as in table of table (3).

Table 3. Alignment of homology sequence of our isolates of *E.histolytica* with global strains and percent of identity

Isolate No.	Genebank Accession number	NCBI-Blast isolate	Source	Identity%
1	ON086987.1	<i>Entamoeba histolytica</i>	Iraq	This study
2	ON086988.1	<i>Entamoeba histolytica</i>	Iraq	This study
3	ON086989.1	<i>Entamoeba histolytica</i>	Iraq	This study
4	ON086990.1	<i>Entamoeba histolytica</i>	Iraq	This study
5	ON086991.1	<i>Entamoeba histolytica</i>	Iraq	This study
6	ON086992.1	<i>Entamoeba histolytica</i>	Iraq	This study
7	ON086993.1	<i>Entamoeba histolytica</i>	Iraq	This study
8	ON086994.1	<i>Entamoeba histolytica</i>	Iraq	This study
9	ON086995.1	<i>Entamoeba histolytica</i>	Iraq	This study
10	MT296778.1	<i>Entamoeba histolytica</i>	Iraq	99%
11	MT296775.1	<i>Entamoeba histolytica</i>	Iraq	99%
12	MT296772.1	<i>Entamoeba histolytica</i>	Iraq	99%

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3. Phylogenetic Tree Construction

Phylogenetic tree construction was performed according to BLAST NCBI-GenBank databases by using MEGA-X program, sequence analysis of 18s rRNA gene of *E. histolytica* by using world references to publish strains of the parasite of *E.histolytica*. The result of identity score among our isolates & world references strains of *E.histolytica* was ranging from 99%-100% as compare with other members of *E.histolytica* in Iraq and in the world. Most of our isolates of human fell together with iraqi strain like MT296778.1, MT296775.1, MT296772.1 with identity percentage 99% while the Egypt strain with

accession number MK332025.1 identity 98% with Iraqi strain of our results as in figure(1).



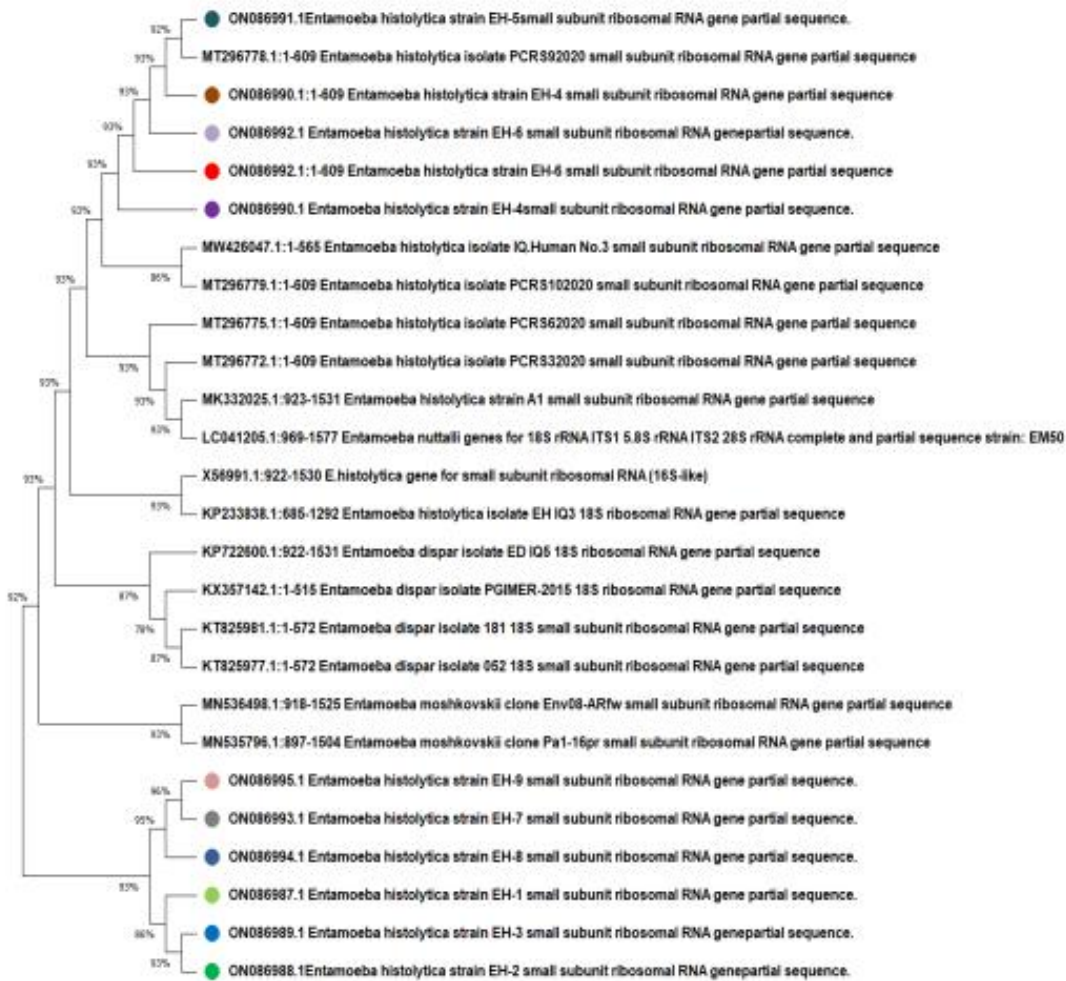


Figure 1. Phylogenetic tree analysis depends on partial sequences in local *Entamoebahistolytica* isolates, which are used for genetic species identification.

Discussion

1. Distribution of Samples According to Gender

The results of microscopic examination rise in male infection than in female. The results show from total 50 for male samples 38 positive samples distributed between *E. histolytica* infection only and *E. histolytica* with bacteria. This results agreed with Al-ardawy (2021). The incidence of male infection rises because the males are more likely to touch the source by the continuous exit from their homes to work and eat from shops and streets that lack hygiene. Therefore, many bad practices such as putting the fingers in the mouth and pitting nails all cause an increase in males than females in infection. The results of the current study agree with the results of several studies such as the study of Al-Malki (2014) [7], when studying the

prevalence of *E. histolytica* in Jaddah Saudi Arabia, reports that a higher infection level in males than females due to manual jobs far from their homes, where they are often inclined to eat and drink water from sources such as roadside kiosks, so their chances of an infection increase. Dhanabal et al. (2014) [8] also found a higher prevalence in males than females and noted that the infections are likely to be associated with individuals' daily activities rather than gender. The present results agree with Al-Hasheme et al. (2020) [9] who found that parasitic intestinal infection in males was higher than in females in the holy city of Karbala, Iraq.

2. Sequencing and Phylogenetic Construction

Sequencing and phylogenetic tree of *E. histolytica*. There were many of researchers are



studied phylogenetic tree analysis of parasite of *Entamoeba spp* in the world, but our study provides new information about this parasite in Iraq, in Babel Malaa et al.,(2019) [10]were recorded five isolate of *E.histolytica* by using 18S rRNA gene to detection and the results showed identity 100% to Japan country,the present study showed 9 isolate of *E.histolytica* were closed related to the MW426046.1,MT296778.1,MT296775.1,MT296772.1, MT296779.which identity with Iraqi isolate from Baghdad with identity 99%.there was difference in other strain of *Entamoeba spp* like *E.dispar.E. moshkovskii* that mean the parasite was endemic and distributed through contaminated water.

Conclusions

The current study concluded that the mentioned parasite affects males more than females, as well as infection of the type associated with bacteria is the most prevalent in males and females, and the current study concluded that there are mutations that occurred in the parasite's genome that can increase the virulence of this parasite.

References

- M. Kantor et al., "Entamoeba Histolytica: Updates in Clinical Manifestation, Pathogenesis, and Vaccine Development," *Can J Gastroenterol Hepatol*, vol. 2018, p. 4601420, Dec. 2018.
- M.L.M. Gonzales, L.F. Dans, and J. Sio-Aguilar, "Antiamoebic drugs for treating amoebic colitis," *Cochrane Database Syst. Rev.*, vol. 1, p. CD006085, Jan. 2019.
- S. Saidin, N. Othman, and R. Noordin, "Update on laboratory diagnosis of amoebiasis," *Eur. J. Clin. Microbiol. Infect. Dis.*, vol. 38, no. 1, pp. 15–38, Jan. 2019.
- M. Tanyuksel and W.A. Petri Jr, "Laboratory diagnosis of amebiasis," *Clin. Microbiol. Rev.*, vol. 16, no. 4, pp. 713–729, Oct. 2003.
- L.R. Rivero, F.A.N. Fernández, and L.J. Robertson, "Cuban parasitology in review: a revolutionary triumph," *Trends Parasitol.*, vol. 24, no. 10, pp. 440–448, Oct. 2008.
- J.J. Verweij, D. Laeijendecker, E.A.T. Brienen, L. Van Lieshout, and A.M. Polderman, "Detection and identification of entamoeba species in stool samples by a reverse line hybridization assay," *J. Clin. Microbiol.*, vol. 41, no. 11, pp. 5041–5045, Nov. 2003.
- S. Jamila, "Factors associated with high prevalence of Entamoeba histolytica/dispar infection among children in Jeddah." *KSA*, 2014.
- J. Dhanabal, P. P. Selvadoss, and K. Muthuswamy, "Comparative study of the prevalence of intestinal parasites in low socioeconomic areas from South chennai, India," *J. Parasitol. Res.*, vol. 2014, p. 630968, Jan. 2014.
- I.H.M. Al-Hasheme, T.A.A. H. Al-Tammime, and K.A.H. Al-Morshidy, "Study of some hematological and immunological parameters associated with the infection of

intestinal parasites in the holy city of Kerbala, Iraq," *Annals of Tropical Medicine and Public Health*, vol. 23, no. 12, 2020, doi: 10.36295/asro.2020.231207.

Malaa, Al Tufaili, and Hamza, "A phylogenetic study of Entamoeba Histolytica isolated from patients in the babylon hospital of Iraq based on 18s ribosomal RNA gene," *Indian J. Public Adm.*, [Online]. Available: https://www.researchgate.net/profile/Saba-Malaa/publication/336064667_A_Phylogenetic_Study_of_Entamoeba_Histolytica_Isolated_from_Patients_in_the_Babylon_Hospital_of_Iraq_Based_on_18S_Ribosomal_RNA_Gene/links/5f18639045851515ef3e60be/A-Phylogenetic-Study-of-Entamoeba-Histolytica-Isolated-from-Patients-in-the-Babylon-Hospital-of-Iraq-Based-on-18S-Ribosomal-RNA-Gene.pdf

