

Detection for Virulence Factors of Amoebic Dysentery in Bloody Diarrheal Children Under 7 Years

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Abstract

Background: Amoebiasis, or amoebic dysentery, is a term used to describe an infection caused by the protozoan *Entamoebahistolytica*.

Aim: Identify *E.histolytica* virulence factors (amoebapore and cysteine proteinase) that play a critical role in pathogenesis of amoebic dysentery by using PCR results.

Method: Detect the major virulence factors of the intestinal parasite *E.histolytica* on Stool samples were collected from 56 samples by using PCR technique and. The DNA sequencing analysis was performed for confirmative genetic identification of some local *Entamoebahistolytica*.

Result: To detect the major virulence factors (V.F.) (cysteine proteinase and amoebapore) of *E.histolytica*, PCR technique was conducted, by using specific primers for *E.histolytica*, a 56 samples were positive to *E.histolytica* using PCR technique was diagnosed previously, the result showed that 54 stool samples were bloody & positive to virulence factor cysteine proteinase, and 37 stool samples were bloody & 53 samples were positive to virulence factor Amoeba pore.

Conclusion: Cysteine proteinase and Amoebaporesis the most important virulence factors in *E.histolytica* that play a critical role in the mediated intestinal cell lysis.

Keywords: Bloody diarrheal; Child; amoebic dysentery; Health.

Introduction

E.histolytica is protozoan parasite that causes amoebic dysentery in humans. the infection leads to severe diarrheal disease may be causes colitis in children. Worldwide, amebiasis remains a significant cause of morbidity and mortality in Iraq¹.

According to World Health Organization found the amebiasis is the second or third most frequent parasitic

disease, exceeded only by malaria and schistosomiasis; and contributing towards the high global burden of diarrhea, notably in regions with low economic development and settings with poor sanitation^{2,3}.

The invasive intestinal Amoebiasis remains for several weeks of cramping, abdominal pain, bloody diarrhea and weight loss⁴.

The symptoms of the disease depend on multiple virulence factors among *E. histolytica* presence of these factors has been linked to be risky for complicated signs⁵.

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Materials and Method

56 Stool samples were isolated from bloody diarrheal children & were positive for *E. histolytica* using PCR previously (from the beginning of October 2018 till the

end of January 2019) the samples collected from (Al-Hamza general hospital, AL-Sedeer hospital and private clinic) in Al- Diwaniya governorate in Iraq country . PCR was used to detect two proteins (amoebapore C and cysteine proteinase) as a major virulence factors of *E.histolytica* and The DNA sequencing analysis was performed for confirmative genetic identification of some local *Entamoebahistolytica* isolate No.1 - No.6 based on small subunit ribosomal RNA gene and identified the

genetic variation between *Entamoebahistolytica* isolates and NCBI-Genbank *Entamoebahistolytica* isolates.

Molecular Method: The molecular method conventional PCR was also used in the present study to detection the DNA of *Entamoebahistolytica* in stool samples . The Positive result of conventional PCR was 56 sample as shown in Figure (1) .

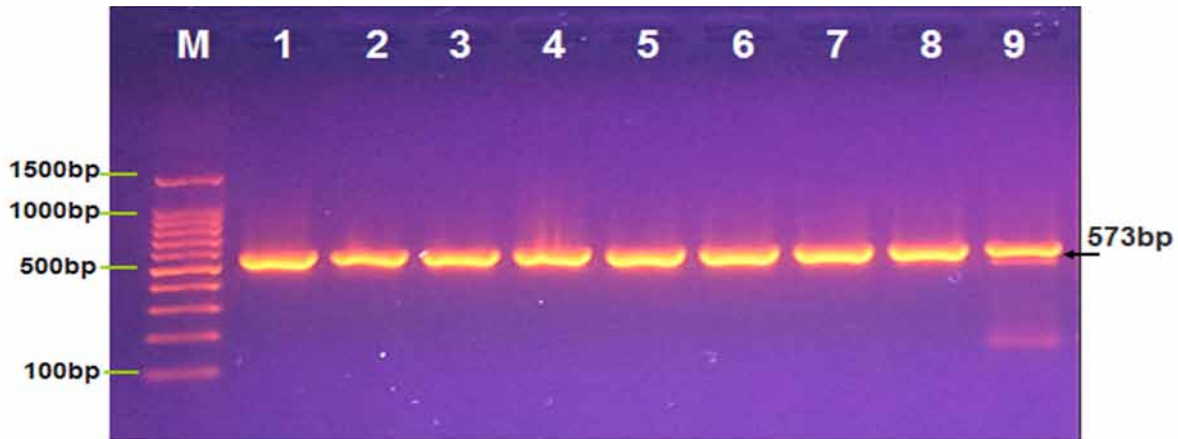


Figure (1): Agarose gel electrophoresis image that showed PCR product analysis for 18S ribosomal RNA gene in *Entamoebahistolytica* isolates. M (Marker ladder 1500-100bp). Lane (1-7) some positive samples at 573bp product size.

The prevalence rate of virulence factors Amoeba pore and Cystein proteinase among children with diarrhea results shows Amoeba pore was seen in 53 (94.6 %), whereas, Cystein proteinase was seen in 54 (96.4 %) of patients.

In the present study the amplified DNA showed that the amoebapore C has (928 bp) in 53 samples, Figure (2).

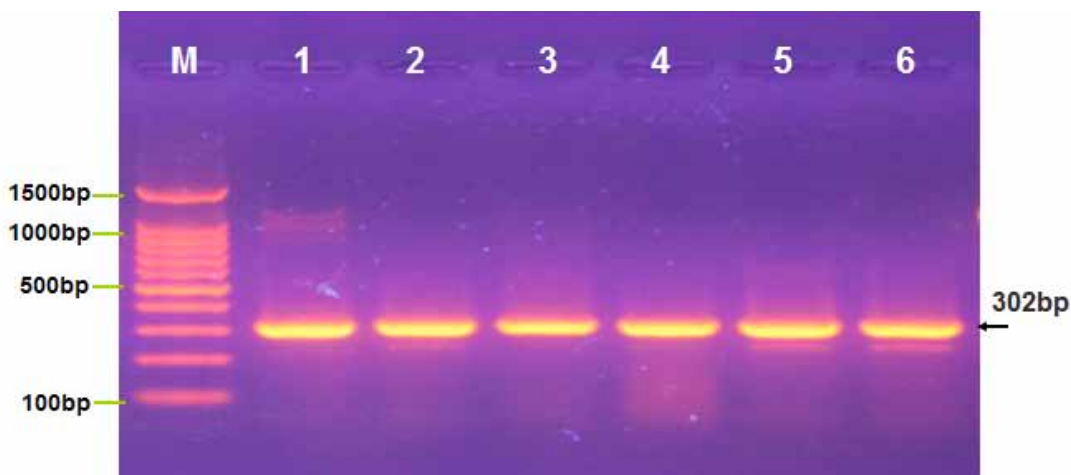


Figure (2): Agarose gel electrophoresis image that showed PCR product analysis for Amoebapore C gene in *Entamoebahistolytica* isolates. M (Marker ladder 1500-100bp). Lane (1-6) some positive samples at 302bp product size.

The results of this estimation revealed that the amplified DNA has (885 bp) for cysteine proteinase in 54 samples, Figure (3).

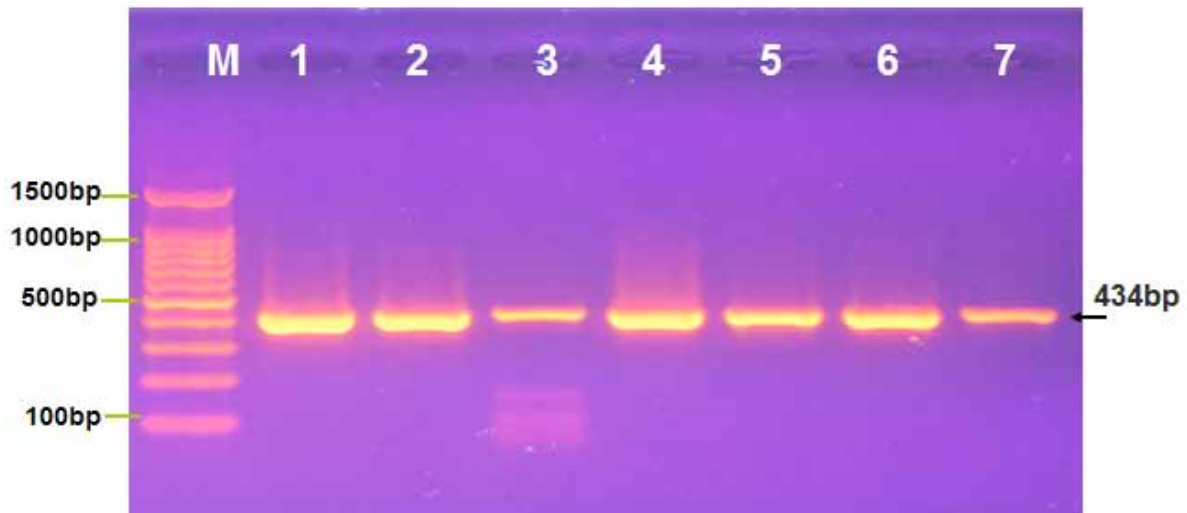


Figure 3: Agarose gel electrophoresis image that showed PCR product analysis for Cysteine protease gene in Entamoebahistolytica isolates. M (Marker ladder 1500-100bp). Lane (1-7) some positive samples at 434bp product size. Amplification Product 900.

The virulence factor Amoeba pore was also more frequently observed in children with bloody diarrhea than children with non-bloody diarrhea, 69.8 % versus 30.2 %, respectively ($P = 0.001$). This result may be due to that amoebapores produced by *E.histolytica* trophozoite play an important role in formation of intestinal ulcer because it cause tissue lysis and flask shape ulcer. *E.histolytica* enzyme insert the trophozoite into epithelial cell of large intestine causes host cell membrane more permeability and cell dying. This agrees with Mirelman et al⁶; Bracha et al⁷; Zhang et al.,⁸.

The virulence factor Cystein proteinase was also more frequently observed in children with bloody diarrhea than children with non bloody diarrhea, 54(100 %) versus 0 (0%), respectively ($P = 0.001$), as shown in table (1). The role of this virulence factors (cysteine proteinase & Amoeba pore) (which is the most important V.F. secreted by *E. histolytica*) the trophozoite of *E.histolytica* can invade the epithelial cell of host cell & lysis of junction between these cell then killed & engulf the debris & erythrocytes, the trophozoite can travel through the portal circulation to the liver & produce liver abscesses by making a breach to the mucosal barrier and this lead to life threatening if not treated^{9,10}.

Table 1: Association between virulence factor Cystein proteinase and Type of diarrhea

Type of diarrhea	Positive n = 54	Negative n = 2	P
Bloody, n (%)	54 (100.0 %)	0 (0.0 %)	< 0.001 ¥ HS
Non bloody, n (%)	0 (0.0 %)	0 (0.0 %)	

DNA Sequence results: The DNA sequencing analysis was performed for confirmative genetic identification of some local Entamoebahistolytica isolate No.1 - No.6 based on small subunit ribosomal RNA gene and identified the genetic variation

between Entamoebahistolytica isolates and NCBI-Genbank Entamoebahistolytica isolates.

The DNA sequencing analysis results were showed that the local Entamoebahistolytica Human

isolates appeared highly genetic confirmation into NCBI-Genbank Entamoebahistolytica isolates with less genetic variation between Entamoebahistolytica isolates according to phylogenetic tree analysis and NCBI-BLAST Homology Sequence identity (%).

The local Entamoebahistolytica isolate No.1 - No.6 were showed closed related to NCBI-BLAST Entamoebahistolytica isolate (AB845672.1) at total genetic changes (0.01-0.004%)

The NCBI-BLAST Homology Sequence identity

(%) between local Entamoebahistolytica Human isolates and NCBI-BLAST submitted Entamoebahistolytica isolates were show (98-99%) as showed in table (2). The genetic identified local Entamoebahistolytica Human isolates (No.1-No.6) were submitted in NCBI-Genbank for accession numbers (MN227232.1-MN227237.1) as showed in table (2).

The local Entamoebahistolytica Human isolates were submitted in Genbank database as (Entamoebahistolytica isolate IQ-D small subunit ribosomal RNA gene, partial sequence) with their nucleotide sequence,

Table (4) the NCBI-BLAST Homology Sequence identity (%) between local Entamoebahistolytica Human isolates and NCBI-BLAST submitted Entamoebahistolytica isolates:

Entamoebahistolytica isolate No.	Genbank Accession number	NCBI-BLAST Homology Sequence identity (%)		
		Identical Entamoebahistolytica	Genbank Accession number	Identity (%)
E.histolytica isolate No.1	MN227232.1	Entamoebahistolytica	AB845672.1	98.52%
E.histolytica isolate No.2	MN227233.1	Entamoebahistolytica	AB845672.1	99.01%
E.histolytica isolate No.3	MN227234.1	Entamoebahistolytica	AB845672.1	98.52%
E.histolytica isolate No.4	MN227235.1	Entamoebahistolytica	AB845672.1	98.52%
E.histolytica isolate No.5	MN22723261	Entamoebahistolytica	AB845672.1	98.52%
E.histolytica isolate No.6	MN227237.1	Entamoebahistolytica	AB845672.1	98.07%

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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