Archives of Razi Institute, Vol. 78, No. 1 (2023) 337-343

DOI: 10.22092/ARI.2022.358947.2336

Original Article

Molecular-Genotyping Detection of *Entamoeba histolytica* in Diarrheic Patients

Kareem Kadhim, D[1 *], Abdulsalam Hraija, B[2], Aqeele, G[2]

1. Department of Anatomy and Biology, College of Medicine, University of Wasit, Wasit, Iraq
2. Department of Microbiology, College of Medicine, University of Wasit, Wasit, Iraq

Received 7 June 2022; Accepted 7 July 2022
Corresponding Author: thalsarrai@uowasit.edu.iq

Abstract

Amebiasis is caused by *Entamoeba histolytica*, a protozoan that is found worldwide. The degree of pathogenesis of clinical isolates varies greatly. This study was aimed to molecular identification of *E. histolytica* in children using the nested polymerase chain reaction (nPCR), and then, a genotyping of positive *E. histolytica* isolates using the quantitative PCR (qPCR) assay through targeting serine-rich *E. histolytica* protein (SREHP) gene. For this purpose, a total of 50 bloody diarrheic stool samples were collected from the children attended to the Al-Zahraa' Teaching Hospital and Alkut Hospital for Gynecology, Obstetric and Pediatrics (Alkut, Wasit, Iraq) were subjected to the present study from September to December 2021. Firstly, the extracted DNAs that amplified using specific primers through targeting *18S rRNA* gene and tested using nPCR assay were revealed an overall 48% (24/50) positive samples for *E. histolytica*. For genotyping, our results were detected an existence of four different genotypes (I, II, III and IV) with a significant prevalence of Genotype-II (54.17%) when compared to Genotype-I (20.83%), Genotype-III (12.5%) and Genotype- IV (12.5%). In addition, results of melting temperature of targeted genotypes were 84ºC, 83 - 83.5ºC, 82.5ºC and 81ºC for Genotype-I, II, III and IV, respectively. In conclusion, molecular amplification of *18S rRNA* gene was revealed the large prevalence of *E. histolytica* among bloody diarrheic children of study areas; while, amplification of SREHP gene was reflected the widespread phenotypic variation of the Genotype-II suggesting the high ability of this genotype to spread infection in children. In different endemic areas as Iraq, the utilization of high-resolution genotyping methods showed the extremely polymorphic genetic structure of this parasite.

Keywords: *Entamoeba histolytica*, genotyping, *18S rRNA*, SREHP gene, Iraq

1. Introduction

Entamoeba histolytica is an anaerobic, pseudopod-forming nonflagellated protozoan pathogenic parasite classified in the Entamoebidae family that belongs to Amoebozoa phylum of Eukaryota (1). This parasite distributed worldwide, but the estimation of global prevalence is complicated due to the latent disease as well as limited capacity for detection and surveying among many endemic regions (2). Several reports estimated that 90% of amebiasis are asymptomatic (3). However, there is a high prevalence of infection occurs most commonly in poor socioeconomic population with lowered public health due to increasing the risk factors of infection such as the fecal – oral transmission, poor hand hygiene, and defecation into water sources that being in close proximity with animals (4). Post excretion of trophozoite in terminal part of ileum, colonization occurs in large bowl, but in responding to unknown stimuli, trophozoite moves and initiates pathogenesis (5). The parasite can induce tissue damage by three main events including direct host cell death, inflammation and parasite invasion to

cause an intestinal amebiasis that characterized by diarrhea, amebic dysentery and amebic colitis (6). In rare cases, trophozoites enter the bloodstream resulting in an extra-intestinal amebiasis typically in liver causing amebic liver abscesses, or in brain causing amebic brain abscesses (7). Hence, the National Institute of Allergy and Infectious Diseases (NIAID) classified *E. histolytica* as a category B priority biodefense pathogen due to lowered dose of infection, resistance to chlorine, stability at different environments and properties that having a threat of simple spreading by contaminating of water and feed resources (8).

In laboratory, microscopic examination of stained (wet mounts) or concentrated fresh stool is the commonest method for morphological identification of cysts and trophozoites of *E. histolytica*; however, nonpathogenic *Entamoeba* species make this method is not always easy and possible (9). In the last decades, advancing in molecular detection techniques have supported our knowledge which leading to reorganization and isolation of pathogenic from nonpathogenic *Entamoeba* (10). As well as, apparent variation in lipophosphopeptidoglycan and lipophosphoglycan-like molecules was confirmed throughout avirulent and virulent *E. histolytica* strains (11). DNA typing of polymorphic genetic loci can aid in closely examination the polymorphic structure of field isolates (12). Serine-rich *E. histolytica* protein (SREHP) is abundant immunogenic surface protein, which strongly implicated in *E. histolytica* phagocytosis (13) Great genetic polymorphism in both coding and encoding loci was detected in different *E. histolytica* isolates (14). To demonstrate if there is any association between *E. histolytica* and clinical outcomes of infections, reliable diagnostic assays are needed to genotype the parasite. For this reason, different diagnostic techniques have been developed to investigate variation in *E. histolytica* strains among many years, but a polymerase chain reaction (PCR)-based approaches have revealed a high sensitivity and discriminatory (15). This study was aimed to molecular identification of *E. histolytica* in children using the qualitative nested (nPCR) through targeting *18S rRNA* gene, and then, a genotyping of positive *E. histolytica* isolates using the quantitative PCR (qPCR) assay through targeting SREHP gene in Iraq.

2. Materials and Methods

2.1. Sample Collection

A total of 50 bloody diarrheic patients who referred to the Al-Zahraa' Teaching Hospital and Alkut Hospital for Gynecology, Obstetric and Pediatrics (Alkut, Wasit, Iraq) were subjected to the present study from September to December 2021. Stool samples were collected from all patients into disposable plastic containers that transported under cold temperature conditions to be subjected for molecular analysis.

2.2. Molecular Examination by nPCR

Following the manufacturer instructions, DNAs were extracted from the collected stool samples using the Presto^{TM} Stool DNA Extraction Kit (Geneaid, Korea). After extraction, DNA samples were tested by the Nanodrop spectrophotometer (Thermo-scientific, UK) to estimate their concentrations and purities, and then kept frozen at -20ºC. Targeting *18S rRNA* gene, two sets of primers were designed according to Haque, Ali (16) and provided by the Bioneer Company (Korea) to detect *Entamoeba* spp. [(F:5´-TTT GTA TTA GTA CAA A-3´) and (R:5´-GTA AGT ATT GAT ATA CT-3´)] and *E. histolytica* [(F:5´-AAT GGC CCA TTC ATT CAA TG-3´) and (R:5´-TTT AGA AAC AAT GCT TCT TCT-3´)] at an amplicon size of 900bp and 550bap, respectively. According to the manufacturer instructions of the AccuPower® PCR PreMix Kit (Bioneer, Korea), PCR MasterMix tubes of each set of primers were prepared at a final volume 20µl (5µl DNA template, 1.5µl F primer, 1.5µl R primer and 12µl free-nuclease water). The two reactions were performed in thermocycler system (BioRad, USA) at the following conditions: 1 cycle initial denaturation (5 min at 95°C), 30 cycles of denaturation (30 sec at 95°C), annealing (30 sec at 58°C) and extension (1 min at 72°C) and 1 cycle final extension (5 min at 72°C).

Stained 1% agarose gel with Ethidium bromide (1µg / ml) was used to analysis the PCR products by electrophoresis at 100 volt and 80 Am for 1 hour. DNA fragments were visualized under an ultraviolet transilluminator (Wised, Korea).

2.3. Genotyping by qPCR

This assay was performed for genotyping of positive *E. histolytica* using the specific primers polymorphic targeting the SREHP gene which responsible for distinguishing of *E. histolytica* strains. The reaction was performed in two steps; the first conventional nPCR that amplified a 549-bp and the second phase that performed as qPCR using of the SYBR Green I. The 2 sets of primers were designed in according to Haque, Ali (16) and provided by Bioneer Company (Korea) as following: [(F:5´-GCT AGT CCT GAA AAG CTT GAA GAA GCT G-3´) and (R:5´- GGA CTT GAT GCA GCA TCA AGG T-3´)] and [(F:5´-TAT TAT TAT CGT TAT CTG AAC TAC TTC CTG- 3´) and (R:5´-TGA AGA TAA TGA AGA TGA TGA AGA TG-3´)]. The qPCR MasterMix tubes were prepared using the AccuPower® 2X GreenStarTM qPCR Master Mix (Bioneer, Korea) at a final volume of 50µl (25µl 2X GreenStar MasterMix, 5µl DNA template 2.5µl F primer, 2.5µl R primer and 15µl DEPC water). The qPCR reaction was performed in MiniOpticon Real-Time PCR system (BioRad, USA) at following conditions: 1 cycle initial denaturation (3 min at 95°C), 45 cycles of denaturation (10 sec at 95°C), annealing/extension detection (scan) (30 sec at 60°C) and 1 cycle melting (30 sec at 80-95°C). The genotyping analysis depend on the SYBR Green I melting curve analysis.

2.4. Statistical analysis

All obtained data were reported and analyzed statistically using the GraphPad Prism Software version 6.0.1. One Way ANOVA was applied to detect significant differences between results of molecular genotyping at $P<0.05$.

3. Results

Overall, 48% (24/50) positive samples for *E. histolytica* were detected using nPCR assay (Figure 1). For genotyping, the findings of qPCR assay among 24 positive samples by nPCR were detected an existence of four different genotypes (I, II, III and IV) based on melting curve among with a significant ($P<0.05$) prevalence of Genotype-II [54.17% (13/24)] when compared to Genotype-I [20.83% (5/24)], Genotype-III [12.5% (3/24)] and Genotype- IV [12.5% (3/24)]. The appearance of different genotypes reflecting different values of melting temperature; 84ºC for Genotype-I, 83 - 83.5ºC for Genotype-II, 82.5ºC for Genotype-III and 81ºC for Genotype- IV (Figure 2, Table 1).

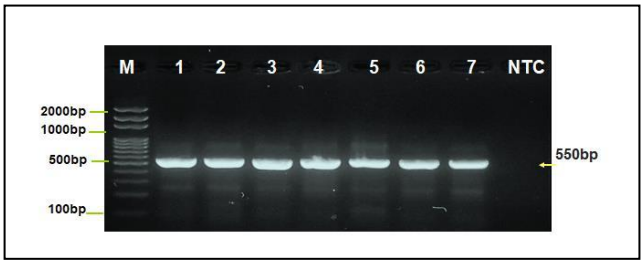

Figure 1. Agarose gel electrophoresis image that shown the Nested PCR product of *18S rRNA* gene that using in detection *E. histolytica*. Where M: Marker (2000-100bp), Lanes 1-10 represented some positive *E. histolytica* stool samples at a product size of 550bp

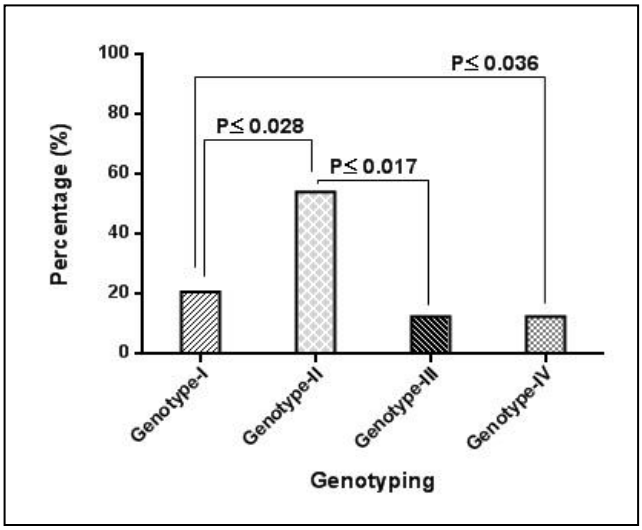

Figure 2. Prevalence of targeted genotypes among 24 positive samples by nPCR

Table 1. Values of melting curve among different *E. histolytica* genotypes

Sample No.	Fluor	Target	Content	Melting Temperature (°C)	Genotype
1	SYBR	*E. histolytica*	Unknown	83.50	Genotype-II
2	SYBR	*E. histolytica*	Unknown	83.50	Genotype-II
3	SYBR	*E. histolytica*	Unknown	81.00	Genotype-IV
4	SYBR	*E. histolytica*	Unknown	83.00	Genotype-II
5	SYBR	*E. histolytica*	Unknown	84.00	Genotype-I
6	SYBR	*E. histolytica*	Unknown	83.00	Genotype-II
7	SYBR	*E. histolytica*	Unknown	84.00	Genotype-I
8	SYBR	*E. histolytica*	Unknown	83.00	Genotype-II
9	SYBR	*E. histolytica*	Unknown	82.50	Genotype-III
10	SYBR	*E. histolytica*	Unknown	83.50	Genotype-II
11	SYBR	*E. histolytica*	Unknown	84.00	Genotype-I
12	SYBR	*E. histolytica*	Unknown	83.00	Genotype-II
13	SYBR	*E. histolytica*	Unknown	81.00	Genotype-IV
14	SYBR	*E. histolytica*	Unknown	83.50	Genotype-II
15	SYBR	*E. histolytica*	Unknown	82.50	Genotype-III
16	SYBR	*E. histolytica*	Unknown	83.50	Genotype-II
17	SYBR	*E. histolytica*	Unknown	84.00	Genotype-I
18	SYBR	*E. histolytica*	Unknown	83.00	Genotype-II
19	SYBR	*E. histolytica*	Unknown	83.50	Genotype-II
20	SYBR	*E. histolytica*	Unknown	82.50	Genotype-III
21	SYBR	*E. histolytica*	Unknown	83.00	Genotype-II
22	SYBR	*E. histolytica*	Unknown	81.00	Genotype-IV
23	SYBR	*E. histolytica*	Unknown	83.00	Genotype-II
24	SYBR	*E. histolytica*	Unknown	84.00	Genotype-I

4. Discussion

Epidemiological investigations regarding an existence of parasites among many regions are commonly aim for identifying a community at risks, as well as description of a disease that having negative impacts on population Shirley, Farr (4). Worldwide, the clinical symptoms of *E. histolytica* could be ranged from asymptomatic illness to amebic dysentery or invasive extra-intestinal infection (17). In comparison to our findings of nPCR assay, *E. histolytica* was identified molecularly in 6% in Iraq (18), 3.4% in Australia (19), 2.6% in Saudi Arabia (20), 10-16.4% in Colombia (21), 9.15% in Malaysia (22), 10% in United Arab Emirates (23), 44.2% in Yemen (24), 1.7% in Ethiopia (25), 0.14% in Iran (26), 14.7% in Egypt (27) and 6.38% in China (28). Difference in incidence of *E. histolytica* infections could be attributed to variation in socio-economic and environmental characteristics, host risk factors such as age, gender, location, education level, occupational status (working), and existence of other family members affected with the parasite or other *Entamoeba* species. In developed countries, exact burdens of infection is challenged to be quantified due to sensitivity of diagnostic modality used, incubation period and symptom severity, sample size, study design and geographic area (4). In Iraq, the rate of morbidities and mortalities correlated to diarrhea is high in particular in children aged < 5 years (29). This elevation in morbidities and mortalities might be due to the multiple challenges of environmental sanitation and basic public health service across Iraq, post many years of war and political instabilities (30).

Mahmood and Bakr (18) noted that the disease outcome in endemic areas could reach 30% in the first year of age and elevated up to 90% at 4 years of age. In recently studies, the results showed that the origins of infection remain extremely higher than expected in children of <2 years of age, and monthly application of PCR assays for testing of diarrheal or normal stool samples can reveal positive results for *E. histolytica* in

children of 2 years of age (6, 31). *E. histolytica* infections might be appeared with variable patterns as following; infection with diarrhea, diarrhea with prior or subsequent asymptomatic colonization, and diarrhea without obvious evidences of infection (11). Several researchers showed that the rate of morbidity was obviously elevated when the testing of children was limited to severe diarrhea (32). The re-emergence of infections was mentioned in Saudi Arabia in children aged <16 years who referred to the hospital with acutely phase of gastroenteritis, in which, this parasite was the almost enteropathogen related to diarrhea (30). Laude, Valot (9) showed that this parasite is one of seven organisms causes dysentery with almost recurrent causes of dysentery due to combination of bacterial and protozoal pathogens. This fact confirmed through utilization of molecular tools and attributed to that, children could exposure for many serious organisms at kindergarten, playground and home (31). Nonetheless, an existence of multiple infections can complicate diagnosing of a particular organism causes the illness and might be resulted in additional impacts that lead to more severely clinical illness (33).

Molecular genotyping had proven to help in close testing the structure of *E. histolytica* field isolates. In targeting of SREHP gene, we detected four *E. histolytica* genotypes (I, II, III and IV) among 24 positive isolates with a significant prevalence of Genotype-II. The first detection of intra-specific difference in *E. histolytica* isolates was reported by Gilchrist, Petri (31) study that confirmed an existence of extensive polymorphisms in size and restriction site of repetitive *E. histolytica* SREHP gene, with identification of 10 distinct DNA patterns in 18 *E. histolytica* isolates from different geographic areas. Ayeh-Kumi, Ali (34) reported that there are 34 distinct patterns of *E. histolytica* in 54 clinical isolates from endemic areas confirming that polymorphisms are found extensively within the single geographic area. Although, highly degrees of polymorphisms existed in *E. histolytica* that is pathogenic, Ali, Mondal (15) found that there is a statistical important variation in genotypes of sample population, proposing the influences of parasitic genomes on the outcome of infections and the existence of the novel mechanism that creating genetic difference. In Turkey, Araz, KORU (35) identified 3 different genotypes in 16 isolates but without significant differences in diarrheic patient, proposing that association could be observed between clinical outcome and parasite load.

This molecular and genotyping investigation revealed the health burden and uncontrolled widespread prevalence of *E. histolytica* among children, calling that the public health authorities for informing diarrhea control program among infants and children in Iraq in particular in low-hygienic and poor regions. Application of both qualitative and quantitative PCR assays could have great benefits in detection the causative pathogen(s). Effective hands hygiene could have a significant role in reducing the severity of contamination or even prevention the spreading of infection. Furthermore, extensive studies are required to demonstrate the potential role of *E. histolytica* or other species of *Entamoeba* in intestinal and extra-intestinal infections and to detect the effect of multiple enteropathogens in severity of clinical illness.

Authors' Contribution

D. K. K. was collected the stool samples from bloody diarrheic children and extraction of DNAs, both B. A. H. and G. A. were responsible for the work of nPCR and qPCR. All authors participated in genotyping of positive isolates, as well in writing of this manuscript.

Ethics

The study carried out under the license of the Scientific Committee of the College of Medicine, University of Wasit (Wasit, Iraq).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Rawat A, Singh P, Jyoti A, Kaushik S, Srivastava VK, Design D. Averting transmission: A pivotal target to manage amoebiasis. Chem Biol Drug Des. 2020;96(2):731-44.
2. Cimino RO, Jeun R, Juarez M, Cajal PS, Vargas P, Echazú A, et al. Identification of human intestinal parasites affecting an asymptomatic peri-urban Argentinian population using multi-parallel quantitative real-time polymerase chain reaction. Parasit Vectors. 2015;8(1):1-7.
3. Begum S, Gorman H, Chadha A, Chadee K. Role of inflammasomes in innate host defense against Entamoeba histolytica. J Leukoc Biol. 2020;108(3):801-12.
4. Shirley D-AT, Farr L, Watanabe K, Moonah S, editors. A review of the global burden, new diagnostics, and current therapeutics for amebiasis. Open forum infectious diseases; 2018: Oxford University Press US.
5. Kantor M, Abrantes A, Estevez A, Schiller A, Torrent J, Gascon J, et al. Entamoeba histolytica: updates in clinical manifestation, pathogenesis, and vaccine development. Can J Gastroenterol Hepatol. 2018;2018.
6. Bercu TE, Petri WA, Behm BW. Amebic colitis: new insights into pathogenesis and treatment. Curr Gastroenterol Rep. 2007;9(5):429-33.
7. Aguilar-Rojas A, Olivo-Marin J-C, Guillen N. The motility of Entamoeba histolytica: finding ways to understand intestinal amoebiasis. Curr Opin Microbiol. 2016;34:24-30.
8. Greene JM, Collins F, Lefkowitz EJ, Roos D, Scheuermann RH, Sobral B, et al. National Institute of Allergy and Infectious Diseases bioinformatics resource centers: new assets for pathogen informatics. Infect. Immun. 2007;75(7):3212-9.
9. Laude A, Valot S, Desoubeaux G, Argy N, Nourrisson C, Pomares C, et al. Is real-time PCR-based diagnosis similar in performance to routine parasitological examination for the identification of Giardia intestinalis, Cryptosporidium parvum/Cryptosporidium hominis and Entamoeba histolytica from stool samples? Evaluation of a new commercial multiplex PCR assay and literature review. 2016;22(2):190. e1-. e8.
10. Foo PC, Nurul Najian A, Muhamad NA, Ahamad M, Mohamed M, Yean Yean C, et al. Loop-mediated isothermal amplification (LAMP) reaction as viable PCR substitute for diagnostic applications: a comparative analysis study of LAMP, conventional PCR, nested PCR (nPCR) and real-time PCR (qPCR) based on Entamoeba histolytica DNA derived from faecal sample. BMC Biotechnol. 2020;20(1):1-15.
11. Gupta S, Smith L, Diakiw AJPC. Amebiasis and Amebic Liver Abscess in Children. 2022;69(1):79-97.
12. Das K, kumar Sardar S, Ghosal A, Saito-Nakano Y, Dutta S, Nozaki T, et al. Multilocus sequence typing (MLST) of Entamoeba histolytica identifies kerp2 as a genetic marker associated with disease outcomes. Parasitol Int. 2021;83:102370.
13. Uddin MJ, Leslie JL, Petri Jr WA. Host protective mechanisms to intestinal amebiasis. Trends Parasitol. 2021;37(2):165-75.
14. Al-Ardi M. Illumination on the structure and characteristics of Entamoeba histolytica genome. Al-Qadisiyah J Pure Sci. 2021;26(4):19-26.
15. Ali IKM, Mondal U, Roy S, Haque R, Petri Jr WA, Clark CG. Evidence for a link between parasite genotype and outcome of infection with Entamoeba histolytica. J Clin Microbiol. 2007;45(2):285-9.
16. Haque R, Ali I, Akther S, Petri Jr WA. Comparison of PCR, isoenzyme analysis, and antigen detection for diagnosis of Entamoeba histolytica infection. J Clin Microbiol. 1998;36(2):449-52.
17. Paul J, Srivastava S, Bhattacharya S. Molecular methods for diagnosis of Entamoeba histolytica in a clinical setting: an overview. Exp Parasitol. 2007;116(1):35-43.
18. Mahmood SAF, Bakr HM. Molecular identification and prevalence of Entamoeba histolytica, Entamoeba dispar and Entamoeba moshkovskii in Erbil City, northern Iraq. Pol J Microbiol.2020;69(3):263.
19. Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, Harkness J. PCR detection of Entamoeba histolytica, Entamoeba dispar, and Entamoeba moshkovskii in stool samples from Sydney, Australia. J Clin Microbiol. 2007;45(3):1035-7.
20. Al-Braiken FA, Salem HS. Diagnosis of Entamoeba histolytica in symptomatic children, Jeddah City, Saudi Arabia. Egypt J Immunol. 2008;15(1):85-92.
21. Peralta ML, Ayala J. Algunas consideraciones sobre la prevalencia actual de Entamoeba histolytica, Giardia duodenalis, coccidios, microsporidios y mixosporidios en Colombia. Revista Salud Uninorte. 2008;24(2):294-302.
22. Ngui R, Angal L, Fakhrurrazi SA, Lian YLA, Ling LY, Ibrahim J, et al. Differentiating Entamoeba histolytica, Entamoeba dispar and Entamoeba moshkovskii using nested polymerase chain reaction (PCR) in rural communities in Malaysia. Parasit Vectors. 2012;5(1):1-7.

23. ElBakri A, Samie A, Ezzedine S, Odeh RA. Differential detection of Entamoeba histolytica, Entamoeba dispar and Entamoeba moshkovskii in fecal samples by nested PCR in the United Arab Emirates (UAE). Acta Parasitologica. 2013;58(2):185-90.
24. Al-Areeqi MA, Sady H, Al-Mekhlafi HM, Anuar TS, Al-Adhroey AH, Atroosh WM, et al. First molecular epidemiology of Entamoeba histolytica, E. dispar and E. moshkovskii infections in Yemen: different species-specific associated risk factors. Trop Med Int Health. 2017;22(4):493-504.
25. Yimer M, Zenebe Y, Mulu W, Abera B, Saugar JM. Molecular prevalence of Entamoeba histolytica/dispar infection among patients attending four health centres in north-west Ethiopia. Trop Doct. 2017;47(1):11-5.
26. Bahrami F, Haghighi A, Zamini G, Khademerfan M. Differential detection of Entamoeba histolytica, Entamoeba dispar and Entamoeba moshkovskii in faecal samples using nested multiplex PCR in west of Iran. Epidemiol Infect. 2019;147.
27. Abozahra R, Mokhles M, Baraka K. Prevalence and Molecular Differentiation of Entamoeba histolytica, Entamoeba dispar, Entamoeba moshkovskii, and Entamoeba hartmanni in Egypt. Acta Parasitologica. 2020;65(4):929-35.
28. Pu L-H, Li Z, Wu J, Zhang Y-L, Chen J-Q, Yang J-F, et al. Prevalence, molecular epidemiology and zoonotic risk of Entamoeba spp. from experimental macaques in Yunnan Province, southwestern China. Epidemiol Infect. 2020;119(8):2733-40.
29. Ahmed S, Klena J, Albana A, Alhamdani F, Oskoff J, Soliman M, et al. Characterization of human rotaviruses circulating in Iraq in 2008: atypical G8 and high prevalence of P [6] strains. Infect Genet Evol. 2013;16:212-7.
30. Harb A, Abraham S, Rusdi B, Laird T, O'Dea M, Habib I. Molecular detection and epidemiological features of selected bacterial, viral, and parasitic enteropathogens in stool specimens from children with acute diarrhea in Thi-Qar Governorate, Iraq. Int J Environ Res Public Health. 2019;16(9):1573.
31. Gilchrist CA, Petri SE, Schneider BN, Reichman DJ, Jiang N, Begum S, et al. Role of the gut microbiota of children in diarrhea due to the protozoan parasite Entamoeba histolytica. J Infect Dis. 2016;213(10):1579-85.
32. Mero S, Kirveskari J, Antikainen J, Ursing J, Rombo L, Kofoed P-E, et al. Multiplex PCR detection of Cryptosporidium sp, Giardia lamblia and Entamoeba histolytica directly from dried stool samples from Guinea-Bissauan children with diarrhoea. Infect Dis. 2017;49(9):655-63.
33. Connor BA, Martin GJ, Riddle MS. Use of the multiplex diagnostic PCR panel in diarrheal disease: expert guidance on the interpretation of results with a focus on Travelers' diarrhea. Official J American College Gastroenterol. 2020;115(10):1553-5.
34. Ayeh-Kumi PF, Ali IM, Lockhart LA, Gilchrist CA, Petri Jr WA, Haque R. Entamoeba histolytica: genetic diversity of clinical isolates from Bangladesh as demonstrated by polymorphisms in the serine-rich gene. Exp Parasitol. 2001;99(2):80-8.
35. Araz RE, Koru Ö, Tanyüksel M, Özekinci T, Ceylan A, Kilbaş HZG, et al. An investigation of the relationship between clinical features of amoebiasis and Entamoeba histolytica genotypes. Turk J Med Sci. 2012;42(1):1147-56.