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RAPD analysis of genetic diversity in *Entamoeba spp.* isolates from clinical samples

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Abstract

The current study investigated the prevalence and molecular characteristics of Entamoeba spp. from 300 stool samples (200 from patients and 100 from healthy controls) collected between October 2023 and February 2025. Direct microscopic examination revealed *Entamoeba spp.* in 110 patient samples (55%), while culture confirmed positivity in 65 cases (32.5%); all control samples were negative. Infection was more prevalent among males (64.5%) than females (35.4%), with participants' ages ranging from 1 to 70 years. The highest prevalence was recorded in the <10 years age group (38.1%), while the lowest was in the 30–40 years group (9%). Infections were frequently associated with mucus and bloody stools. Seasonal variation showed the highest prevalence in December (18.1%) and the lowest in February (1.8%). Among culture media, Entamoeba medium showed the longest survival period of *Entamoeba* (10 days), followed by TYI-S-33 (8 days), PEHPS (7), CLUPS (5) respectively. while the shortest survival was recorded in Blood.A (3 days). PCR analysis revealed *E. moshkovskii* as the most prevalent species (18%), followed by E. histolytica (8%). RAPD technique demonstrates variations in banding patterns among genetically distinct individuals. The present study identified four distinct *E. histolytica* genotypes, mainly in rural areas, and eight *E. moshkovskii* genotypes with wide geographic distribution across Basrah province.

Keywords: *Entamoeba,* Cultivation, PCR, RAPD strain, Genotype

Introduction

Entamoeba, a protozoan parasite, infects various animals, including reptiles, birds, and amphibians. Seven species have been found in human guts, including *E. histolytica, E. dispar, E. moshkovskii, E. coli, E. polecki,* and *E. hartmanni,* with *E. gingivalis* found in the buccal cavity. (El-Dib and Khater, 2022) .*Entamoeba* infections occur globally, especially in low-income countries with inadequate public health infrastructure, making accurate differential diagnosis crucial to distinguish it from similar species. (Servián *et al.,* 2023). *E moshkovskii*, previously considered a free-living environmental organism, recent evidence confirms its role as a human pathogen with significant prevalence in some populations (Heredia *et al.,* 2012). *E. histolytica* is the most common intestinal protozoan parasite causing amoebiasis, causing diarrhea and dysentery. It can invade the intestinal mucosa, form liver and lungs abscesses, and infect humans, with other species also posing risks. (Roure *et al* .,2019).Laboratory diagnosis for *Entamoeba* spp. in humans primarily involves microscopically examining stool samples, but this method is insufficient for distinguishing between species (Argy *et al.,* 2022). The use of polymerase chain reaction (PCR) for molecular diagnosis is crucial due to potential mistreatment of patients, requiring accurate and global implementation. (Bahrami *et al.,* 2019) The study analyzed genetic variability in *Entamoeba histolytica* using random amplified polymorphic DNA (RAPD) using arbitrary primers due to its intrinsic characteristics (Gomes *et al.,* 2000). The Random Amplified Polymorphic DNA (RAPD) technique, also known as Arbitrarily Primed PCR (AP-PCR), is a cost-effective and efficient method for epidemiological studies, enabling rapid genetic fingerprinting of microbial isolates, facilitating strain differentiation and infection source tracking. (Williams *et al.*,1990;Welsh & McClelland, 1991a) . The prevalence of *E. histolytica, E. dispar,* and *E. moshkovskii* was determined using three diagnostic methods: microscopic examination, cultivation, and PCR and Real-Time PCR technique. (Al-Hilfi,2020) . *E. moshkovskii*, previously thought to be a free-living environmental amoeba, was found in 1961 in a symptomatic patient in Laredo, Texas, causing diarrhea, weight loss, and epigastric pain. (Dreyer, 1961). The aim of this study was to identify *Entamoeba* spp. in human stool samples and assess

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