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EMERGING DIAGNOSTIC APPROACHES FOR *Entamoeba histolytica*: IMPLICATIONS FOR RESOURCE-LIMITED SETTINGS

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ABSTRACT

Amoebiasis, a serious public health problem in underdeveloped nations, is caused by the infectious protozoan Entamoeba histolytica. Entamoeba histolytica infection ranges from asymptomatic to symptomatic, and even extraintestinal state such as amoebic liver abscess. Accurate diagnosis is pertinent for effective treatment, prevention of complications, and improved surveillance. Literature for this narrative review was sourced from scientific databases and search engines including PubMed, Google Scholar, and African journal online (AJOL). This review evaluates conventional, immunological, and molecular diagnostic methods for the detection of E. histolytica. It also highlights the advantages, limitations, and applicability of each diagnostic method. This review shows that stool microscopy remains widely used due to its affordability and simplicity. However, stool microscopy has low sensitivity and specificity and cannot differentiate E. histolytica from non-pathogenic species such as E. dispar and E. moshkovskii. Immunological methods, and molecular techniques, demonstrate high accuracy and enable precise species identification. This review also shows that adjunct techniques such as endoscopy and imaging aid diagnosis in extraintestinal cases. In conclusion, combining diagnostic approaches enhances accuracy and clinical decision-making. However, cost, technical expertise, and infrastructure remain limiting factors.

KEYWORDS: *Entamoeba histolytica, Amoebiasis, microscopy, Immunodiagnostics, Molecular diagnostics, Serology*

INTRODUCTION

Entamoeba histolytica is a pathogenic protozoan parasite that causes amoebiasis, a disease that remains an important public health concern worldwide (Guillén, 2022). Most infections are reported to be asymptomatic, however some infected individuals present with symptoms. Accurate and early diagnosis is essential for proper treatment, prevention of complications, and effective disease surveillance (Cooney *et al.,* 2024). Stool microscopy which is the conventional diagnostic method is widely used but often has low sensitivity and specificity. Stool microscopy method also fails to reliably differentiate *E. histolytica* from morphologically identical non-pathogenic species. The low sensitivity and specificity of microscopy in differentiating *E. histolytica* from its non - pathogenic siblings such as *E. dispar* and *E. moshkovskii*, leads to misdiagnosis and

inappropriate management (Datta *et al.,* 2025).

In recent decades, advancements in serological and molecular diagnostic techniques have significantly improved the accurate detection of *E. histolytica*. For instance, serological methods, such as enzyme-linked immunosorbent assays (ELISAs) and rapid immunochromatographic tests, offer improved specificity by targeting the antigens and antibodies of *E. histolytica* (Saidin *et al*., 2019). Furthermore, molecular methods such as nucleic acid amplification tests (NAATs), conventional and real-time polymerase chain reaction (PCR), have proven to be more sensitive and specific in distinguishing pathogenic *E. histolytica* from non-pathogenic amoebas. Despite these advantages, widespread adoption in low-resource settings is made impossible by high costs, the need for stable electricity, and inadequate trained personnel (Garg *et al.,* 2024). This review highlights emerging diagnostic methods for *E. histolytica*, and their implications for resource-limited settings.

LITERATURE SEARCH

Literature for this narrative review was obtained from key scientific databases and search engines such as PubMed, Google Scholar, and African Journal online (AJOL) using *Entamoeba histolytica,* Amoebiasis, microscopy, Immunodiagnostics, Molecular diagnostics, Serology as search terms. The search of the literature was limited to the English Language, to identify relevant peer-reviewed studies on the diagnosis of *Entamoeba histolytica*, as well as technical reports. Both recent and foundational publications were included based on their relevance to the study objectives.

Diagnostic Approaches: A Comparative Perspective

Stool Microscopy

Despite the advent of modern diagnostics, stool microscopy remains the gold standard for detection of *E. histolytica* in resource-limited settings. Stool microscopy remains the most commonly used diagnostic method due to its low cost, minimal equipment requirements, and rapid results (Khan *et al.,* 2019). Stool microscopy procedure involves the microscopic examination of direct wet mounts using saline and iodine preparations. It may be followed with formol-ether concentration techniques to increase the yield of parasitic elements. The detection of *Entamoeba histolytica* trophozoites particularly those containing ingested erythrocytes has been used as a confirmatory marker of invasive intestinal disease (Behravan *et al.,* 2025). Furthermore, microscopy has a low sensitivity and specificity, often ranging from about 60% for intestinal to as low as 30% for extraintestinal amoebiasis. The sensitivity of microscopy is significantly dependent on the expertise of the personnel, quality of samples, workload, the quality of staining, and the parasite density of the stool sample (Tortop *et al*., 2022; Spacek, 2024). This position is in line with Van Den Broucke *et al*. (2018), who reported a 14% sensitivity for stool microscopy in their evaluation of clinical and microscopic predictors of true *E. histolytica* infection among travellers and migrants.

Another limitation of stool microscopy is its inability to differentiate pathogenic *Entamoeba histolytica* from the morphologically identical non-pathogenic commensal *Entamoeba*

species in both cyst and trophozoite forms (Behravan *et al*., 2025). The consequence of this is that a microscopy report cannot distinguish a patient requiring targeted treatment from one with a commensal amoebic infection (Van Den Broucke *et. al.,* 2018). The implication of this in low resource settings is overdiagnosis *E. histolytica* and underdiagnosis of *Entamoeba dispar* and *Entamoeba moshkovskii*.

Culture, Followed by Isoenzyme Analysis

Culture, followed by isoenzyme electrophoresis is one of the earliest laboratory methods developed for the differentiation of *Entamoeba histolytica* from morphologically identical species that are not pathogenic (Çalişkan and Aydin, 2023). These diagnostic methods involve the cultivation of trophozoites in petri dishes, followed by characterization of their isoenzymes using electrophoretic techniques, this procedure is also called zymodeme analysis (Çalişkan and Aydin, 2023). Differences in isoenzyme profiles of some enzymes such as hexokinase and phosphoglucomutase, improve species differentiation of *Entamoeba* species (Sargeaunt *et al.,* 1978; Kumari Yadav *et al.,* 2021). However, despite its diagnostic advantage, culture followed by isoenzyme electrophoresis have several limitations that restrict its routine use in clinical Laboratory (Tokoro *et al.,* 2023). Isoenzyme electrophoresis is highly technical, takes time and is dependent on the results of culture (Parija *et al*., 2014). The long turnaround time limits its routine use in clinical settings, where rapid diagnosis is essential for timely treatment (Parija *et al*., 2014). Additionally, due to more rapid and sensitive diagnostic tools, such as serology and molecular assays, the use of culture, followed by isoenzyme electrophoresis has become unpopular (Tokoro *et al*., 2023). As a result, culture and isoenzyme electrophoresis are now commonly used in research settings rather than in routine diagnostic use (Saidin *et al.,* 2019).

Serological Testing

Serological tests have been reported to play an important role in the diagnosis of extraintestinal amoebiasis, where stool examinations may be negative (Haque *et al.,* 2000; Wuerz *et al.,* 2012). Several studies have developed monoclonal antibody-based tests by using various *E. histolytica* antigens such as the lectin-rich surface antigen, lipophosphoglycan and the 170 kDa amoeba adhesion lectin. The detection of specific antibodies against *E. histolytica* may support diagnosis when clinical suspicion is high, although it may not distinguish between past and current infection (Saidin *et al.,* 2019). Serological tests, such as indirect haemagglutination assay (IHA) and indirect immunofluorescence (IIF) assay, are widely used for the detection of antibodies in patients with suspected *Entamoeba histolytica* infection. Furthermore, IHA and IIF have been successfully used for detection of antibody against *E. histolytica* and are available commercially (Fitri *et al*., 2022). However, it is important to note that IHA and IIF are more useful in non-endemic regions, where a positive antibody test is an indication of active or recent infection due to the low incidence of Amoebiasis (Nath *et al.,* 2018).

Serological tests are less useful for the diagnosis of intestinal amoebiasis, where direct detection of the parasite is important. For this reason, it has been recommended that serology tests should be considered as complementary to the diagnosis of amoebiasis rather than being used alone (Saidin *et al.,* 2019; Morán *et al.,* 2023). It is safe to conclude that, while

serological assays remain valuable particularly for extraintestinal disease, their limitations have brought about the need for other methods that are more sensitive and specific (Haque *et al.,* 2003; Saidin *et al.,* 2019; Morán *et al.,* 2023).

Emerging Immunodiagnostic Techniques for Antigen Detection

Antigen detection assays, particularly enzyme-linked immunosorbent assays (ELISA), has contribute significantly to accurate diagnosis of *Entamoeba histolytica*. Many studies have shown that ELISA-based assays are highly sensitive and specific, when compared with stool microscopy or antibody-based tests (Jing *et al.,* 2025). These assays are designed to detect parasite-specific antigens, such as the Galactose/N-acetyl-D-galactosamine (Gal/GalNAc) lectin, a key virulence factor involved in host cell adherence (Begum *et al*., 2021). ELISA can also be used to detect anti-Entamoeba antibodies during amebic liver abscess (ALA). The ELISA is useful for diagnosis of *E. histolytica* by detecting the antigens during the intestinal phase or antibodies during the extra-intestinal phase (Flores *et al*., 2022). By targeting the antigens, ELISA-based methods provide improved sensitivity and specificity compared with conventional microscopy, and other serology tests (Castellanos-Gonzalez *et al.,* 2018; Saidin *et al.,* 2019).

Some studies have reported that some commercially available antigen test may not successfully differentiate *E. histolytica* and *E. dispar* which is a limitation (Jing *et al*., 2025). However, sandwich ELISA technique, have demonstrated high diagnostic accuracy in its ability to detect parasite antigens in stool samples. These assays are capable of differentiating pathogenic *E. histolytica* from non-pathogenic species, making it valuable for the diagnoses of intestinal histolytica infection (Castellanos-Gonzalez *et al.,* 2018).

Compared with microscopy, ELISA offers several advantages, these include capacity to detect low parasite loads, less dependency on the personnel, and the ability to process multiple samples simultaneously (Morán *et al.,* 2023). These previously listed factors make ELISA suitable for both case diagnosis, and epidemiological studies (Saidin *et al.,* 2019; Morán *et al.,* 2023). However, despite these advantages, ELISA-based antigen detection assays have important limitations. The accuracy of the test depends on proper sample collection, and handling, as antigen degradation may lead to false-negative results (Haque *et al.,* 2000; Castellanos-Gonzalez *et al.,* 2018). Additionally, the requirement for laboratory infrastructure, trained personnel, and cost of test reagents may limit the use of ELISA in resource-limited settings.

Rapid Diagnostic Tests

Rapid diagnostic tests (RDTs), also known as 'rapid lateral flow tests', are immunochromatographic assays designed for the rapid detection of *Entamoeba histolytica* in stool samples. These tests are based on monoclonal antibodies targeted at specific *E. histolytica* antigens that binds to *anti-E. histolytica* antibodies, allowing for the detection of the parasites within 10-20 minutes. The result is visualized by the appearance of a colour on the test cassette. Their ease of use, minimal requirements for expertise and equipment make them suitable in programmatic and resource-limited settings (Momčilović *et al.,* 2019). One of the key advantages of RDTs is their ability to provide results within few a minutes, which makes prompt clinical decision-

making possible (Jing *et al*., 2025). In addition, these tests are portable, require minimal equipment and training, and can be performed at the bedside or in community with poor electricity supply, (Saidin *et al.,* 2019).

Despite these advantages, rapid diagnostic tests generally demonstrate lower sensitivity compared with ELISA and molecular techniques. This limitation may be more pronounced in cases with low parasite burden, which may result in false-negative Laboratory report (Morán *et al.,* 2023). It is advisable that, negative RDT results should be interpreted cautiously, especially in patients with strong clinical suspicion of amoebiasis (Momčilović *et al.,* 2019; Morán *et al.,* 2023).

Molecular Diagnostic Techniques

Molecular diagnostic methods have had remarkable impact in the detection of *Entamoeba histolytica*. They are more sensitive and specific than other previously mentioned diagnostic methods in the detection of *Entamoeba histolytica* (Morán *et al.,* 2023). Molecular methods also have the added advantage of accurately differentiating pathogenic from morphologically identical species Entamoeba that are believed to be non-pathogenic (Shirley *et al.,* 2018; Saidin *et al.,* 2019; Morán *et al.,* 2023).

Conventional Polymerase Chain Reaction (PCR)

Conventional PCR is one of the earliest molecular tools used for the detection of *E. histolytica*. It amplifies parasite 18S ribosomal DNA in stool and tissue aspirates, enabling accurate detection and speciation of amoebic species (Shirley *et al*., 2018; Saidin *et al*., 2019). The main advantage of this method is its ability to differentiate *E. histolytica* from identical non-pathogenic species such as *E. dispar* and *Bangladeshi* (Morán *et al.,* 2023). However, conventional PCR requires identification of amplification products using gel electrophoresis, which increases turnaround time and may easily become contaminated during sample processing (Calle-Pacheco *et al*., 2022). Furthermore, the requirement for trained personnel, constant electricity supply and expensive equipment and reagents limits its use routinely in resource-limited settings (Das *et al.,* 2021; Tokoro *et al.,* 2023).

Nested PCR

Nested PCR is a molecular technique designed to improve on the less sensitive and specific conventional PCR. Here, the amplification product from the first round of PCR is used as a template in the second round of PCR to increase its sensitivity. As a result, nested PCR is useful when parasite density is suspected to be very low, where conventional methods may fail (Ali *et al.,* 2003). Despite its high diagnostic performance, nested PCR also has some limitations such as cost and increase in processing time due to additional amplification step, which may increase the risk of cross-contamination, lead to false-positive results. The long turn-around time in addition to cost, has limits its use for routine diagnostic purpose in health care settings (Das *et al.,* 2021).

Real-Time PCR (qPCR)

Real-time PCR, also known as quantitative PCR, (qPCR) an improvement over conventional and nested PCR techniques. In addition to detection of parasites, this method can also be used to determine the parasite burden. Due to the use of fluorescent probes, qPCR allows the monitoring of amplification process and quantification of parasite DNA real-time. The technique

removes the need for gel-electrophoresis and reduce the risk of contamination (Blessmann *et al.,* 2002). Furthermore, qPCR is more sensitive and has a shorter turnaround time compared to conventional PCR. These attributes make it very suitable for both routine use in clinical settings and as well as for large epidemiological studies (Lotz *et al*., 2025). However, this method is expensive, due to high cost of equipment, maintenance, and reagents. It also requires technical expertise, put together, limits its implementation in many endemic regions (Lotz *et al.,* 2025).

Multiplex PCR

Multiplex PCR has the advantage of being able to amplifying multiple target DNA simultaneously within a single reaction. In a clinical setting, the multiplex PCR can be used to detect multiple intestinal parasites by incorporating a mixture of primers that are specific for parasites of interest (Argy *et al*., 2022). The reducing reagent consumption and turnaround time improves detection of different species of intestinal protozoa in settings where co-infections are common (Robert-Gangneux *et al*., 2025). The limitation of this technique requires careful optimization to avoid primer interactions and ensure balanced amplification of all targets. These technical challenges, along with the need for specialized equipment, may limit its routine use in low-resource laboratories (Mizan *et al.,* 2025).

Loop-Mediated Isothermal Amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is new technique that emerged within this decade. It is a PCR method that is highly sensitive and specific, user friendly and has a short turn-around time. (Parkinson *et al*., 2019). Unlike PCR, LAMP is rapid, and can make results available within an hour, and visualize through a colour change (Notomi *et al.,* 2015). Additionally, LAMP is a portable technology and can serve as a point-of-care testing and field research. These attributes make LAMP one of the most reliable diagnostic methods for amoebiasis in low-resource settings (Yang *et al.,* 2024).

Adjunct Diagnostic methods for detection of E. histolytica

For intestinal amoebiasis, stool-based tests such microscopy, antigen detection assays or molecular techniques provide direct evidence of active parasitic infection and are routinely used in healthcare and research settings (Saidin *et al.,* 2019; Morán *et al.,* 2023). However, there are conditions where these methods may not produce expected results in cases of low parasite burden or intermittent shedding (Saidin *et al.,* 2019). At such times, adjunctive diagnostic methods have been advocated for.

Endoscopy

Endoscopy procedure a process whereby a long, flexible tube with camera at the end is inserted down the throat into a patient's gastrointestinal tract (GIT). The camera allows views of the GIT and may show features of invasive amoebiasis (Sharma and Ahuja, 2021). However, endoscopy cannot be used as a confirmatory test on its own but must be interpreted in addition to evidence provided by Laboratory tests such as microscopy, serology and PCR (Yue *et al.,* 2021).

Imaging Techniques

Another adjunct diagnostic technique is the imaging technology. Imaging entails radiological methods such as ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI). They

have become very useful in the diagnosis of extraintestinal amoebiasis, especially amoebic liver abscess (Priyadarshi *et al*., 2022). These imaging methods can be used to show amoebic lesions when patients are suspected of having extraintestinal amoebiasis. These imaging techniques are useful when assessing the extent of extraintestinal amoebic abscesses, and for guiding therapeutic interventions (Khan *et al.,* 2019; Priyadarshi *et al.,* 2022). The limitation of imaging techniques is that, their findings are not specific to amoebiasis and may overlap with other conditions such as cancers and other pathologies (Priyadarshi *et al.,* 2022). Therefore, like other adjunct diagnostic methods, imaging techniques should not be used in isolation as diagnostic tools for *E. histolytica* infections.

Implications for Resource-Limited Settings

Stool microscopy is insufficient for the accurate diagnosis of *Entamoeba histolytica* in endemic regions that are often poor resource countries due to non-pathogenic morphologically identical species. Therefore, emerging diagnostic techniques are very necessary for endemic and resource-limited regions. The new diagnostic techniques such as antigen-based tests and molecular techniques are more sensitive and specific, and have faster turn-around time compared to stool microscopy (Saidin *et al.,* 2019; Morán *et al.,* 2023). Despite their advantage over stool microcopy, the cost implication in using them, and the need for electricity in the case of molecular techniques, have made them inaccessible for routine use in poor resource settings, especially in public health facilities (Calle-Pacheco *et al.*, 2022).

CONCLUSION

In conclusion, depending only on stool microscopy is insufficient to accurately identify *Entamoeba histolytica*. Consequently, when there is strong suspicion of *E. histolytica* infection, other more sensitive and specific diagnostic techniques should be conducted to enhance diagnostic accuracy and clinical decision-making. While stool microscopy remains the most commonly used method in poor endemic countries such as Nigeria, due to its affordability, antigen-based tests and molecular methods offer superior sensitivity and specificity and can be used for speciation. Policymakers in poor resource countries must be encouraged to prioritize funding for emerging diagnostic techniques in clinical laboratories. As doing so will reduce over-diagnosis of diseases such as amoebiasis and this will in turn prevent over-treatment and drug resistance.

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