Challenges and Limitations of Mycetoma Grains Culture Technique: A Critical Review

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Background

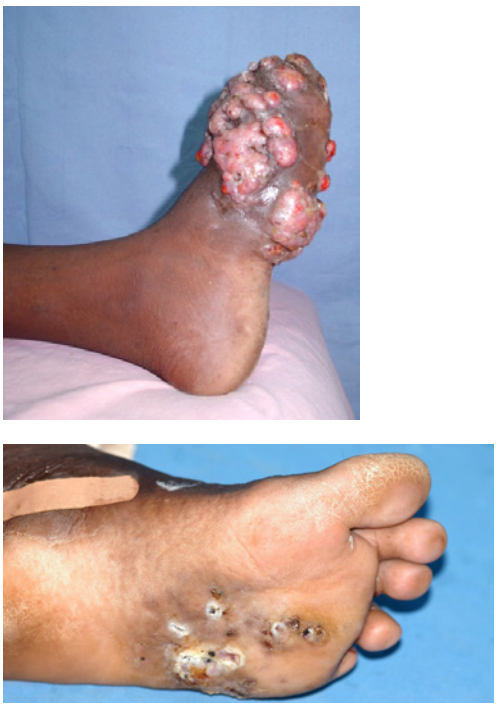
Mycetoma is a chronic, progressive granulomatous infection caused primarily by filamentous bacteria causing actinomycetoma or fungi leading to eumycetoma [1]. This neglected tropical disease (NTD) is endemic in several regions across Africa, Latin America, and parts of Asia, particularly affecting populations in tropical and subtropical climates [2,3,4,5]. Mycetoma causative microorganisms thrive in these environments [6].

Mycetoma pathogenesis commences with the inoculation of the causative pathogen into the subcutaneous tissue, where it induces localised infection and chronic granuloma, which slowly progresses over time [7]. Clinically, it starts as a small, painless nodule or swelling which can go unnoticed [8]. Over time, the infection spreads to invade the deep tissues, muscles, and bones. Multiple sinuses develop that discharge grains in purulent or seropurulent discharge [9]. These grains are aggregates of encapsulated microorganisms in cement substances that give mycetoma its distinctive clinical hallmark [10]. If left untreated, mycetoma can lead to severe tissue destruction, deformities and disabilities with many psychosocial impacts, stigma and community exclusion [11]. Thereby exacerbating poverty in already vulnerable communities.

The greatest challenges in managing mycetoma are its painless nature, gradual progression and the non-specific nature of its early symptoms. The initial stages of the disease often mimic other conditions, such as foreign body granuloma, fibroma, cysts, or other skin and subcutaneous chronic infections and neoplasms, making it difficult to diagnose [12]. As a result, patients frequently delay seeking medical attention, either due to a lack of awareness or limited access to healthcare services [13]. By the time they present to healthcare facilities, the disease is often in an advanced stage, requiring complex and costly treatment that many cannot afford [14,15].

Early diagnosis of mycetoma is crucial for effective treatment and preventing disease progression [16,17]. However, diagnosing mycetoma remains a significant challenge, especially in resource-limited settings where access to advanced diagnostic tools, such as imaging and molecular techniques, is scarce [18,19]. Mycetoma traditional diagnostic methods are few, tedious, and invasive, and they have low specificity and sensitivity, leading to delays in identifying the causative organism [18,19]. This, in turn, delays the initiation of appropriate antimicrobial or antifungal treatment, further compounding the severity of the disease [20].

Moreover, the healthcare infrastructure in mycetoma-endemic regions is often inadequate to meet the diagnostic and treatment needs of affected populations [21,22]. Many healthcare workers lack the necessary training and resources to identify and manage the disease effectively. As a result, misdiagnoses and inappropriate treatments are common, contributing to poor patient outcomes and perpetuating the cycle of disability and poverty.



Massive foot mycetoma

Addressing the diagnostic challenges and improving access to effective treatment is critical to mitigating the devastating impact of mycetoma on affected individuals and communities [22,23]. The traditional grain culture technique, once regarded as the "gold standard" for identifying the causative agent of mycetoma, has become increasingly problematic due to several intrinsic limitations [24]. These limitations not only hinder timely and accurate diagnosis but also delay treatment, contributing to unfavourable patient outcomes. This review explores the challenges associated with the grain culture method and highlights the pressing need for alternative diagnostic approaches that can better serve affected communities.

Grain Culture: An Overview

The hallmark of mycetoma diagnosis is the identification of the causative microorganism encapsulated in the grains to advise on the appropriate antimicrobial or antifungal treatment. However, despite its historical importance, grain

culture is far from ideal in current clinical practice, especially in regions lacking adequate laboratory infrastructure.

Grain Culture Technique Challenges

Grain Culture Technique Challenges

Sample Contamination

One of the most significant challenges in diagnosing mycetoma through grain culture is the high probability of sample contamination, which greatly affects the accuracy of results [25]. The grains used in culture are delicate biological materials that contain microorganisms responsible for the infection. They are of various colours, sizes and consistency depending on their causative microorganisms [11]. These grains must be carefully collected, transported, and processed under strict sterile conditions to prevent contamination [26]. However, this is challenging, particularly in resource-limited settings where maintaining ideal laboratory conditions is often difficult.

Environmental microorganisms, such as non-pathogenic bacteria, fungi, or other organisms present as patient flora or in the surrounding environment, can easily infiltrate the sample at any stage of handling. If the specimen becomes contaminated with these extraneous microorganisms, they can overgrow in culture, leading to a failure in isolating the true causative pathogen [25]. This overgrowth not only obscures the identification of the actual pathogen but can also result in the laboratory identifying non-pathogenic or irrelevant organisms as the cause of infection.

The consequences of sample contamination are severe. Inaccurate identification of the pathogen can lead to misdiagnosis, with patients receiving long-term inappropriate or ineffective treatments. For instance, if an eumycetoma patient is misdiagnosed as actinomycetoma due to contamination, it could result in the use of antibiotics. These antibiotics would be ineffective against fungal infections, could cause serious side effects, and would carry a high risk of contributing to antibiotic resistance. Such delays in proper treatment can allow the disease to progress, causing further tissue damage and increasing the likelihood of longstanding disability [27].

Contamination is a particularly problematic issue in resource-limited settings where sterile conditions may be hard to maintain due to several factors. In many endemic regions, laboratories often lack access to adequate equipment and resources necessary to ensure sterility [28]. Autoclaves, sterile culture media, and disposable laboratory supplies are occasionally in short supply, and laboratories may reuse equipment, increasing the risk of cross-contamination between samples. Additionally, poor infrastructure, such as unreliable electricity or limited access to clean water, further complicates the ability to maintain a sterile working environment. In such situations, even if grains are successfully cultured, the risk of contamination remains high, making the results unreliable.

Another challenge is the lack of personal protective equipment (PPE) and sterilisation protocols. In some settings, healthcare workers may not have access to basic protective gloves, masks, or sterile instruments, increasing the risk of contamination during sample collection and handling. Moreover, the poor design of healthcare facilities, such as inadequate separation between different stages of laboratory processing, can lead to accidental cross-contamination [28].

Sample handling procedures are crucial. The collection of grain samples involves extracting them from the patient, where secondary bacterial infection, may coexist with naturally occurring bacteria and fungi flora [29]. Improper collection can lead to contamination by skin flora, potentially obscuring the true causative agent of mycetoma. Furthermore, if the grains are exposed to air during transport or processing, there is an additional risk of contamination by airborne organisms.

In such scenarios, even if laboratories manage to culture the grains successfully, the results can be inconclusive or outright misleading. Pathologists may mistakenly report the presence of environmental contaminants as the primary cause of infection, leading to diagnostic errors and inappropriate treatments. This underscores the importance of maintaining stringent laboratory procedures and sterile conditions, as well as having access to the necessary resources to ensure that results are as accurate as possible.

Addressing the issue of sample contamination requires a multifaceted approach. Laboratories must be equipped with adequate sterile supplies, including disposable tools and culture media, to minimise the risk of contamination. Healthcare workers must be trained to handle samples in a sterile manner, including proper collection techniques, transportation, and laboratory processing. Improving laboratory infrastructure, access to clean water, reliable electricity, and adequate storage facilities will further support accurate mycetoma diagnosis [30].

Specialised Training Requirements

Performing grain culture to diagnose mycetoma is a complex procedure that requires a high level of technical expertise. The process involves several critical steps, each demanding precise skills and a deep understanding of microbiology and mycology [30]. Laboratory personnel must be proficient in handling delicate biological samples, isolating microorganisms from grains, and accurately identifying the specific pathogens responsible for mycetoma, whether bacterial or fungal [30]. However, many laboratories in mycetoma-endemic areas lack the necessary trained professionals, which severely hampers the diagnostic process and affects patient outcomes [28].

A key challenge lies in the fact that mycetoma-causing microorganisms, particularly fungi, are often slow-growing and morphologically resemble other non-pathogenic species [31]. Fungal cultures can take weeks or even months to develop, during which time contamination, overgrowth of non-pathogenic organisms, or misinterpretation can occur. This requires laboratory personnel to be highly trained in distinguishing subtle differences between pathogenic and non-pathogenic species, a skill that goes beyond routine laboratory work. Personnel must also be familiar with the growth characteristics of both bacteria and fungi in culture, including their appearance, growth rate, and typical biochemical reactions [30]. Without this specialised knowledge, it becomes incredibly difficult to accurately identify the causative agent, increasing the risk of diagnostic errors.

Culture technique

Moreover, the pathogens involved in mycetoma exhibit a range of growth behaviours, further complicating the diagnostic process. For example, certain species of fungi that cause eumycetoma may present in culture as small, slow-growing colonies that can easily be mistaken for environmental contaminants or overlooked entirely [24]. Similarly, some bacterial species causing actinomycetoma may closely resemble non-pathogenic bacteria typically found in the environment, making it difficult to determine whether the cultured organism is the actual causative agent. This level of complexity requires a solid foundation in both microbiology and mycology and the ability to use specialised diagnostic techniques such as biochemical testing, microscopic examination, and advanced staining methods [24,30].

Unfortunately, many healthcare facilities in mycetoma-endemic regions suffer from a shortage of laboratory professionals with such specialised training. In these areas, laboratory staff are often generalists, trained to handle a wide range of common infectious diseases but lacking the specific expertise needed to diagnose rare or neglected diseases like mycetoma. The healthcare infrastructure in these regions may also lack the resources to provide continuous professional development for laboratory personnel, leaving staff without access to the latest diagnostic techniques or updates on emerging pathogens. As a result, even if grain cultures are performed, there is a significant risk that the results may be misinterpreted or delayed due to a lack of specialised knowledge.

The lack of trained personnel not only delays proper treatment but also contributes to the broader challenge of disease management in endemic areas. Misdiagnosis can lead to inappropriate treatments, such as administering antibiotics for a fungal infection, which not only fails to treat the underlying

condition but may also worsen the patient's health. Inaccurate diagnoses also perpetuate misinformation about the disease, as healthcare professionals and the community may come to rely on incorrect assumptions about mycetoma's prevalence, causative agents, or treatment methods. This can have far-reaching consequences, complicating public health efforts aimed at controlling the spread of the disease and improving patient outcomes.

In addition to the direct impact on patient care, the lack of specialised training in laboratory settings also limits the ability to conduct critical research on mycetoma. Endemic regions are home to the majority of the world's mycetoma cases [32]. Yet, research initiatives often rely on laboratories in non-endemic, better-resourced countries where access to trained mycologists and microbiologists is more readily available. This limits opportunities for local researchers and healthcare professionals to develop context-specific solutions for their communities. As a result, there is a critical need to invest in building local capacity through specialised training programmes that focus on the unique challenges of diagnosing mycetoma.

Addressing the shortage of specialised training in mycetoma-endemic areas will require a coordinated effort to improve education, training, and healthcare infrastructure [30]. This includes providing laboratory staff with opportunities for advanced training in microbiology and mycology, as well as ongoing professional development to stay current with evolving diagnostic methods. International collaborations, mentorship programmes, and online training courses can also play a vital role in bridging the skills gap. Additionally, local governments and global health organisations must prioritise investment in laboratory infrastructure to ensure that facilities are equipped to handle the complex requirements of mycetoma diagnosis [30].

Eumycetoma cultures isolated

Extended Culture Time

A major limitation of grain culture techniques for diagnosing mycetoma is the extended time required for the causative organisms to grow and be identified. This prolonged incubation period poses significant challenges for effective disease management, particularly in settings where timely treatment is crucial.

Fungal species responsible for eumycetoma, in particular, are known for their slow growth rates [24]. These fungi can take several weeks to months to develop adequately in culture before they can be reliably identified. Unlike faster-growing microorganisms, these fungi exhibit slow and variable growth patterns, making it difficult to achieve a conclusive diagnosis within a reasonable timeframe. The delay in culture results not only impedes the timely initiation of appropriate treatment but also exacerbates the disease's progression. Mycetoma, if left untreated, can lead to severe consequences such as extensive tissue destruction, deformities, and, in advanced cases, amputations or death [33].

The delay in treatment can result in the disease reaching a stage where more invasive and costly interventions are required. For example, patients may need repetitive surgical debridement or even limb amputation to manage the infection and prevent further complications. These interventions not only have physical and psychological impacts on patients but also place an additional burden on healthcare systems [34].

In low-resource settings, the problem of extended culture time is compounded by inadequate laboratory infrastructure. Many healthcare facilities in endemic regions lack the necessary equipment and resources to support efficient and timely grain culture processing. Insufficient laboratory space, unreliable power supplies, and inadequate supplies of sterile media and equipment can further prolong the culture period. In some cases, laboratories may experience delays due to a backlog of samples, limited personnel, or a lack of specialised training in handling mycetoma cultures. These factors contribute to even longer waiting times for diagnostic results, further delaying patient management and increasing the risk of adverse outcomes.

The impact of extended culture times is particularly pronounced in resource-limited settings where healthcare access is already constrained. Patients in these areas may have to wait weeks or even months to receive a definitive diagnosis, during which time their condition may deteriorate significantly. In many cases, by the time a diagnosis is made, the disease may be so advanced that treatment options are limited and less effective. This can result in a reliance on surgical interventions, which, while necessary,

may not fully address the underlying infection or may result in permanent disability [35].

The extended time required for grain culture also poses challenges for disease surveillance and control efforts. Delayed diagnoses can lead to underreporting of mycetoma cases, as healthcare providers may not recognise the disease in its early stages. This can hinder efforts to monitor disease trends, allocate resources effectively, and implement preventive measures [36]. Additionally, the prolonged diagnostic process can contribute to the spread of misinformation about the disease, as affected individuals and communities may not receive accurate information about the nature of mycetoma or the importance of early treatment.

Addressing the issue of extended culture time requires a multifaceted approach. One potential solution is the development and implementation of faster diagnostic methods, such as molecular techniques (e.g., PCR), that can identify pathogens more quickly than traditional culture methods [37]. While promising, these techniques require investment in specialised equipment and training. Additionally, improving laboratory infrastructure and ensuring that healthcare facilities are equipped with the necessary resources can help reduce delays in culture processing. Enhancing laboratory capacity through training programmes and increasing support for healthcare facilities in endemic regions are also crucial steps in mitigating the impact of extended culture times.

Part of a cultured isolates biobank

Limited Access to Skilled Personnel and Well-Equipped Laboratories

The effectiveness of grain culture techniques in diagnosing mycetoma is intrinsically linked to the availability of skilled personnel and well-equipped laboratories. Unfortunately, in many mycetoma-endemic regions, these critical resources are often insufficient, significantly impacting the reliability and efficiency of diagnostic efforts.

Many laboratories in endemic areas face severe shortages of basic supplies necessary for effective grain culture. Essential items such as culture media, sterile equipment, and reagents are frequently unavailable or in limited supply. Culture media are crucial for providing the appropriate environment for microorganisms to grow, while sterile equipment ensures that samples are not contaminated. Reagents are required for various biochemical tests and identification processes. The absence of these basic supplies hampers the ability of laboratories to perform accurate and reliable grain cultures, leading to inconclusive or erroneous results [30].

The lack of advanced diagnostic tools compounds these issues. Techniques such as molecular identification methods and sequencing offer faster and more accurate identification of pathogens but require specialised equipment and expertise that are often lacking in resource-limited settings [37,38]. Microscopy, another critical tool for identifying mycetoma pathogens, also demands well-maintained instruments and trained personnel to interpret results accurately. Without access to these advanced tools, laboratories are constrained to relying on traditional culture methods, which are prone to limitations such as extended culture times and contamination.

The combined effect of inadequate laboratory infrastructure and limited expertise contributes to unreliable grain culture results. Misdiagnoses or delayed diagnoses due to poor sample handling, incorrect identification, or contamination can lead to inappropriate treatments.

In addition to the direct impact on patient care, the lack of skilled personnel and well-equipped laboratories also hampers disease surveillance and research. Accurate data on mycetoma prevalence, trends, and outcomes are crucial for informing public health strategies and allocating resources effectively. However, without reliable diagnostic data, efforts to monitor and control the disease are severely limited. The lack of research further perpetuates the cycle of underfunding and under-resourcing, making it difficult to develop and implement new diagnostic and treatment approaches [39,40].

Good training in crucial

Addressing the Challenges

Given the numerous challenges associated with grain culture techniques, it is clear that alternative diagnostic methods are urgently required. The limitations of grain culture make it impractical for use in low-resource settings, where mycetoma is most prevalent. As a result, there is a growing emphasis on developing new diagnostic approaches that are faster, more reliable, and better suited to environments with limited resources.

Molecular Diagnostic Techniques

One of the most promising alternatives is molecular diagnostics, particularly polymerase chain reaction (PCR). PCR has the advantage of being highly specific and sensitive, capable of detecting the DNA of both bacterial and fungal pathogens directly from clinical samples [37,38]. This significantly reduces the time needed to identify the causative organism, allowing diagnoses to be made in hours rather than the weeks or months required for traditional culture methods. Moreover, PCR minimises the risk of contamination, as it targets the genetic material of the pathogen directly, bypassing the need for prolonged culturing in potentially compromised environments [41,42].

PCR is also versatile, capable of differentiating between various species of fungi and bacteria that cause mycetoma, which is essential for selecting the most appropriate treatment. The speed and precision of PCR could drastically shorten the diagnostic timeline, allowing for more immediate and tailored interventions, which are essential for preventing disease progression and avoiding severe complications such as deformities and amputations.

Despite its promise, however, the widespread adoption of PCR in mycetoma-endemic regions faces its own set of challenges. Implementing PCR in resource-limited settings requires a certain level of infrastructure, such as reliable electricity, specialised equipment, and trained personnel. Nevertheless, these obstacles are surmountable with the right investments in healthcare infrastructure and training programs. Initiatives that provide portable PCR devices and train local healthcare workers in their use could help bring this technology to remote areas where mycetoma is most prevalent [30].

Advanced Imaging Technologies

In addition to molecular diagnostics, imaging technologies offer another avenue for improving the diagnosis of mycetoma. Mycetoma's gradual progression through soft tissue, bone, and skin often leaves characteristic patterns of tissue damage, which can be visualised through imaging [4346-]. Ultrasound, magnetic resonance imaging (MRI), and X-rays have been shown to help identify the extent of infection and distinguish mycetoma from other soft-tissue infections or tumours [4346-].

Ultrasound is particularly valuable in low-resource settings due to its relative affordability and portability. It can detect the formation of cavities, abscesses and the characteristic grains associated with mycetoma, offering a non-invasive and rapid diagnostic option [46]. MRI provides more detailed imaging and is especially useful for assessing the extent of bone involvement, which is common in advanced cases of mycetoma [44]. X-rays, although less precise in identifying soft tissue involvement, can still help detect bone damage in severe cases [43,45].

The combination of molecular diagnostics and imaging could dramatically enhance diagnostic accuracy. For example, imaging can help localise the infection and assess its severity, while molecular techniques can confirm the specific pathogen responsible for the disease. This comprehensive diagnostic approach would enable healthcare providers to quickly and confidently determine the nature of the infection, facilitating prompt and appropriate treatment.

Conclusions

To address the significant challenges posed by grain culture techniques in diagnosing mycetoma, it is essential to invest in improved healthcare infrastructure and specialised training. Ensuring the availability of well-equipped laboratories and enhancing the skills of healthcare workers, particularly in molecular diagnostics and imaging, are critical steps toward overcoming current diagnostic limitations. New approaches such as PCR and advanced imaging offer promising alternatives that can reduce diagnostic delays and improve accuracy, ultimately leading to more timely treatment and better patient outcomes. By adopting these

modern diagnostic methods and strengthening local healthcare capacity, we can significantly enhance the management of mycetoma in endemic regions, mitigating its long-term burden on affected communities.

Key Points

Sample contamination requires a multifaceted approach that includes:

- Laboratories must be equipped with adequate sterile supplies.
- Healthcare workers appropriate training.
- Adequate laboratory infrastructure.
- Suitable storage facilities.

The shortage of specialised training in mycetoma-endemic areas will require:

- Opportunities for advanced training in microbiology and mycology.
- Continuing professional development.
- International collaborations.
- Mentorship programmes.
- Local governments and global health organisations involvement.

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