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Review Article

Microbiology Section

Exploring the Depths of Cryptic Aspergillosis: Species Variability, Clinical Spectrum, Diagnostic Quandaries and Therapeutic Options

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ABSTRACT

Cryptic aspergillosis, characterised by infections caused by non classical *Aspergillus* species, presents unique challenges in clinical management due to species variability, diverse clinical manifestations, diagnostic complexities, and limited treatment options. This comprehensive review explores the emerging understanding of cryptic aspergillosis, highlighting the spectrum of clinical presentations across different species, diagnostic dilemmas in accurate species identification, and evolving treatment paradigms. Cryptic species, including *A. lentulus, A. udagawae, A. calidoustus*, and others, exhibit intrinsic resistance to commonly utilised antifungal agents, complicating therapeutic approaches. The diagnostic landscape is further complicated by phenotypic and genotypic similarities with classical *Aspergillus* species, necessitating advanced molecular techniques for precise identification. Treatment alternatives are limited, with azoles, echinocandins, and polyenes demonstrating variable efficacy against different cryptic species. Emerging antifungal agents, like fosmanogepix, ibrexafungerp, and rezafungin, hold promise in addressing resistance challenges. This review underscores the need for a multidisciplinary approach integrating clinical, microbiological, and molecular expertise to optimise the management of cryptic aspergillosis and improve patient outcomes.

Keywords: Antifungal treatment, Aspergillus species, Cryptic aspergillosis, Clinical spectrum, Diagnostic challenges, Multidisciplinary approach

INTRODUCTION

Aspergillus species are filamentous moulds that act as saprophytes, and asymptomatic endophytes, along with opportunistic phytopathogens. Also, the estimated number of recognised *Aspergillus* species (spp.) ranges from 300 to 400 [1]. Among the Aspergillaceae family, only five sections- *Fumigati, Flavi*, and *Nigri*, *Terrei*, and *Nidulante*- are known to be responsible for causing diseases in humans. Recently, a rise has been observed in the occurrence and severity of infections triggered by filamentous fungus. These infections present significant hazards to individuals with weakened immune systems, potentially endangering their lives [2]. *Aspergillus* spp. is the most commonly identified mould in clinical samples. So, human aspergillosis has a broad range of clinical manifestations, which span from pulmonary infections, such as invasive pulmonary aspergillosis, straight to different types of hypersensitivity reactions, such Allergic Bronchopulmonary Aspergillosis (ABPA). *Aspergillus fumigatus* is the predominant fungal pathogen in humans, with *A. flavus, A. terreus,* and also *A. niger* being the subsequent most prevalent species [3]. Technological advancements have facilitated the identification of novel species belonging to the *Aspergillus* family. These fungus are commonly known as “cryptic” or “sibling” species by scientists due to their indistinguishable characteristics.

Cryptic aspergillosis refers to a subset of *Aspergillus* infections that are challenging to diagnose due to their atypical presentation, involvement of less common species, and non specific symptoms. This condition is particularly problematic because it often evades initial detection and standard antifungal treatments might be less effective [4]. Unlike typical aspergillosis, which is usually caused by *Aspergillus fumigatus* and presents with classic signs such as lung lesions or respiratory symptoms, cryptic aspergillosis often involves species like *Aspergillus terreus, Aspergillus flavus, or Aspergillus nidulans* [3]. These species can present with more subtle or unusual manifestations and may have different susceptibilities to antifungal drugs, complicating treatment. The symptoms of cryptic aspergillosis can be vague and non specific, resembling those of other infections or conditions, such as fever, weight loss, fatigue, or organ-specific symptoms that do not immediately suggest an *Aspergillus* infection. Traditional diagnostic methods like culture and histopathology might not be effective, necessitating the use of molecular diagnostic techniques like Polymerase Chain Reaction (PCR) and advanced imaging techniques for accurate identification [5]. This condition is often seen in immunocompromised individuals, like those enduring chemotherapy, and organ transplant recipients, or patients with chronic granulomatous disease, who are more susceptible to infections. The cryptic nature of the disease makes management more challenging, as the less common *Aspergillus* species involved might exhibit resistance to standard antifungal therapies, requiring the use of alternative or combination antifungal treatments. This necessitates careful selection and monitoring of antifungal therapy grounded in susceptibility testing. The significance of cryptic species in clinical contexts is increasing. Azole-resistance or even pan-antifungal resistance poses a significant risk, particularly in cases of cryptic aspergillosis, and can greatly increase the likelihood of mortality along with morbidity. This risk is especially pronounced in immunocompromised patients, who have a significantly higher susceptibility to developing Invasive Aspergillosis (IA) compared to the general population. Hence, cryptic aspergillosis is difficult to detect and treat due to its atypical characteristics, involvement of less common species, and non specific clinical presentation, requiring advanced diagnostic tools and tailored treatment strategies for effective management. This review elucidate the clinical spectrum, diagnostic challenges, and treatment alternatives for cryptic aspergillosis, including identifying cryptic species, documenting clinical manifestations, analysing diagnostic difficulties, evaluating current antifungal therapies, and investigating emerging therapeutics to improve patient outcomes.

Differentiation of Cryptic Aspergillosis from Common *Aspergillus*

Cryptic aspergillosis presents significant diagnostic challenges and is associated with higher mortality rates compared to regular

aspergillosis. This difficulty in diagnosis stems from its atypical presentation and involvement of less common *Aspergillus* species characterised by well-known symptoms such as respiratory issues, and is typically responsive to standard antifungal therapies like voriconazole [6]. Unlike regular aspergillosis, typically caused by *Aspergillus fumigatus* with well-characterised symptoms and response to standard antifungal treatments, cryptic aspergillosis involves species like *Aspergillus terreus, Aspergillus flavus, or Aspergillus nidulans* [7]. These species may not only display unusual clinical features but also exhibit resistance to conventional antifungal therapies.

While regular aspergillosis can often be diagnosed through conventional methods such as culture and histopathology, cryptic aspergillosis frequently evades these methods and requires advanced molecular diagnostics, such as PCR, and sophisticated imaging techniques for accurate detection [8]. Furthermore, the less common species involved in cryptic aspergillosis often exhibit resistance to standard treatments, necessitating alternative or combination antifungal therapies. These differences underscore the need for heightened clinical awareness and advanced diagnostic approaches to effectively manage cryptic aspergillosis, particularly in immunocompromised patients [9].

Importance of Identifying Cryptic Aspergillosis

Cryptic aspergillosis poses a growing global challenge, with emerging *Aspergillus* species from tropical and subtropical regions indicating their adaptation to climate change, potentially leading to increased ubiquity of cryptic species in the future [10]. These species pose threats to humans and also animals owing to their elevated Minimum Inhibitory Concentrations (MICs) against antifungal agents and diagnostic complexities. Numerous cryptic species are recognised as human pathogens, with implications for animal health as well. For instance, *A. felis* has emerged as a primary cause of aspergillosis inside cats, while human cases were documented. Additionally, species like *A. alabamensis* have been identified in both dogs and humans [11]. Cryptic species, like their non cryptic counterparts, exhibit widespread distribution and can thrive in various environments [12]. For example, *A. novoparasiticus* has been implicated as a contaminant in sugar cane production, as well as in rice and corn fields. Therefore, identifying cryptic *Aspergillus* species is crucial due to their resistance to common antifungal treatments and their potential to cause severe clinical conditions, including IA. Cryptic species can lead to a range of infections like pneumonia, and exacerbation of for Chronic Obstructive Pulmonary Disease (COPD), keratitis, eyelid infections, and nasal polyposis [13]. Morphological identification in clinical laboratories has shown high accuracy, particularly in identifying known species. However, the detection rate of cryptic species varies among *Aspergillus* sections, with a significantly higher prevalence observed in *Aspergillus* section *Nigri*. Some cryptic species exhibit lower susceptibilities to antifungal drugs compared to sensu stricto species, highlighting the importance of accurate identification for appropriate treatment selection [14]. Despite limited epidemiological data on cryptic species, and case reports and existing investigations highlight the interconnectedness of medical, veterinary, and environmental sciences. However, further research is essential to comprehensively understand the bearing of said species on human, and animal, along with environmental health. Therefore, adopting a one health tactic is crucial for advancing our knowledge and control measures related to cryptic species.

Diagnosis and Identification of Cryptic Aspergillosis

Diagnostic methods are crucial in unraveling cryptic aspergillosis. Culture, which is fundamental to the process of microbiological identification, allows for the observation of the phenotypical properties of fungi. *Aspergillus*, a type of filamentous fungus, can grow well in several types of cultural medium. To prevent the growth of bacteria and promote the growth of the fungus, a selective agar called Sabouraud dextrose agar is employed. This agar is treated with chloramphenicol and gentamicin. *Aspergillus* colonies usually have a soft and fuzzy texture and display a variety of colours, ranging from green to brown, appearing within a period of five to seven days of being kept in a controlled environment between 25-30°C [15,16]. Phenotypic identification is based on the examination of both microscopic and macroscopic features, such as colouration, unique arrangement of conidia, and varying levels of sporulation or development at different temperature microscopic analysis, utilising fluorescence (blancophore stain) and traditional microscopy on lactophenol-supplemented media, can aid in the identification of certain hidden species. However, these characteristics may vary substantially depending on the specific conditions of the media, often lacking discernible physical differences within a particular section of *Aspergillus*. Therefore, phenotypic methods alone are insufficient for cryptic species identification [17].

The taxonomy of the genus *Aspergillus* relies on morphological traits, however, the spotting of species necessitates the examination of morphological, and physiological, and molecular properties. Advanced identification methods, like Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) and sequencing, have enabled the detection of previously unidentified 'cryptic species' [18]. These cryptic species, which cannot be diagnosed by morphology alone, are independent evolutionary lineages with poorly differentiated morphology that were previously misinterpreted as single species. Certain features, such as poor sporulation alongside low in vitro susceptibility to antifungals, led to the identification of new fungi like *Aspergillus lentulus* and *Aspergillus thermomutatus* within the Fumigati species. Another cryptic species, *Aspergillus sydowii* (also called *Emericella sydowii*), causes sinusitis, and onychomycosis, alongside keratomycosis, and has emerged as a pathogen affecting eye vision loss or even blindness. Studies from the USA along with Spain have shown that cryptic *Aspergillus* species make up 10% and 15% of clinical cases, respectively [19].

Serological tests, like the serum Beta-1,3-D-Glucan (BDG) along with Galactomannan (GM) enzyme immunoassays, are valuable for diagnosing IA, with GM showing the best performance on broncho-alveolar fluid instead of serum. Nevertheless, the accuracy and precision of these tests may differ. To boost, the accuracy of serological tests, it is important to consider host characteristics that are vital in the susceptibility to fungal infections and incorporate them into the diagnostic algorithm [20].

The advent of molecular methods revolutionised fungal diagnostics. Also, PCR along with DNA sequencing procedures target certain genetic markers like fungal 18S or even 23S ribosomal DNA, the Nuclear Ribosomal Internal Transcribed Spacer region (ITS), beta-tubulin, the calmodulin gene (cmdA), and the Mini-Chromosome Maintenance protein (mcm7). Numerous other genes can target alternative metabolic passageways, like toxin or even pheromone production. Yet, differentiating within the section often requires integrating multiple targets (multiplex assays), which is labour-intensive, time-consuming, and not universally available [21].

Correct identification has significant clinical relevance. Therefore, as shown in [Table/Fig-1] criteria for diagnosing aspergillosis related disease and other forms of aspergillosis vary significantly across different clinical contexts, such as neutropenia, COPD, Intensive Care Unit (ICU) patients, and chronic conditions [22-25]. The European Organisation for Research and Treatment of Cancer Mycoses Study Group (EORTC-MSG) consensus definitions provide a robust framework for IA diagnosis, incorporating histopathologic evidence and PCR with DNA sequencing, although non culture-based fungal biomarkers have limited clinical application due to reduced efficacy with mould-active antifungals [22]. Bulpa criteria are tailored for COPD patients, while the modified AspICU criteria are specific for

Criteria used	Details
European Organisation for Research and Treatment of Cancer-Mycoses Study Group (EORTC-MSG) Consensus definitions for Invasive Aspergillosis (IA) [22]	**Proven IA:** Histopathologic, and cytopathologic, or even direct microscopic demonstration or culture from sterile aspiration or biopsy specimen with associated tissue damage. Positive PCR with DNA sequencing from FFPE tissue. **Probable IA:** Presence of host factors (e.g., neutropenia, transplantation, immunosuppressant use), clinical features (pulmonary, sino-nasal, CNS infection), and mycological evidence (e.g., galactomannan antigen, *Aspergillus* PCR).
Bulpa Criteria for Chronic Obstructive Pulmonary Disease (COPD) Patients (GOLD Stage III or IV) [23]	Suggestive chest imaging and one of the following: positive microscopy/culture for *Aspergillus* from the lower respiratory tract, and positive serum antibody test for *A. fumigatus*, or even two consecutive positive serum Galactomannan (GM) tests.
Modified Asp ICU Criteria for Intensive Care Unit (ICU) patients [24]	One positive blood biomarker (PCR and/or GM) and more than one of the following: endotracheal aspirate repeated culture/PCR positive, compatible clinical signs, abnormal chest radiography, underlying host risk factors, or positive direct microscopy and BAL culture/PCR for *Aspergillus*.
Influenza-Associated Pulmonary Aspergillosis (IAPA) [24]	An airway plaque, pseudomembrane, or ulcer, along with at least one of the following: a serum GM index more than 0.5, a BAL GM index greater than 1.0, a positive BAL culture, a positive endotracheal aspirate culture, a positive sputum culture, or hyphae clearly indicative of *Aspergillus*.
Chronic Pulmonary Aspergillosis (CPA) [25]	Illness for more than three months and all of the following: persistent cough, haemoptysis, and/or weight loss, progressive cavitary infiltrates and/or pericavitary fibrosis, pleural thickening, or fungal ball on chest radiography, and positive *Aspergillus* IgG assay.

[Table/Fig-1]: Diagnostic schemes and criteria for *Aspergillus*-related diseases [22-25].
BAL: Broncho-alveolar lavage; CT: Computed tomography; CNS: Central nervous system; COPD: Chronic obstructive pulmonary disease; DNA: Deoxyribonucleic acid; FFPE: Formalin-fixed paraffin-embedded; FPCRI: Fungal PCR initiative; GM: Galactomannan; GMI: Galactomannan Index; GOLD: Global Initiative for Chronic Obstructive Lung Disease; IA: Invasive aspergillosis; LFA: Lateral flow assay; ISHAM: International Society for Human and Animal Mycology; PCR: Polymerase chain reaction

ICU patients, emphasising the need for context-specific diagnostic criteria. For Influenza-Associated Pulmonary Aspergillosis (IAPA), direct airway observations coupled with multiple positive biomarkers are essential. Chronic Pulmonary Aspergillosis (CPA) diagnosis relies on prolonged symptoms and specific radiographic and serological evidence, underscoring the need for tailored diagnostic approaches based on patient condition and underlying risk factors [23].

Overview of Cryptic Aspergillosis Species

Cryptic aspergillosis encompasses a diverse group of fungal species within the *Aspergillus* genus that are often challenging to identify and diagnose. These species have unique genetic characteristics and often display differences in antifungal susceptibility compared to more commonly recognised *Aspergillus* species. Cryptic *Aspergillus* species, though pathogenic, offer significant medical and biotechnological benefits. They are a source of novel antifungal and antibiotic compounds, which are crucial in combating drug resistance. Their metabolites show potential in cancer treatment and immunomodulation, and their enzymes are valuable in pharmaceutical synthesis. Additionally, these species contribute to microbial ecology and understanding fungal pathogenesis. In agriculture and environmental management, they enhance soil health and act as natural pesticides. Overall, their unique properties make them important in natural product discovery and therapeutic development. An overview of various cryptic aspergillosis species along with individual descriptions and their respective treatments [23] is shown in [Table/Fig-2], also medically important cryptic *Aspergillus* species, organised by their respective sections.

Description of a Few Individual Species

The following section provides a detailed description of select cryptic *Aspergillus* species, categorised by their respective sections. Each species exhibits unique characteristics that contribute to its clinical significance and environmental presence.

Aspergillus section	Cryptic species
Fumigati	*A. lentulus*
	Aspergillus thermomutatus
	A. udagawae
	A. viridinutans
	A. fumigatiaffini
	A. novofumigatus
Flavi	*A. tamarii*
	A. alliaceus
Terrei	*A. carneus*
	A. alabamensis
Nigri	*A. acidus*
	A. tubingensis
	A. awamori
Versicoloures	*A. sydowii*
	A. creber
Circumdatii	*A. persii*
	A. westerdijkiae
Usti	*A. calidoustus*
	A. insuetus
	A. keveii

[Table/Fig-2]: List of cryptic *Aspergillus* species [23].

Fumigati: This section includes species such as *Aspergillus lentulus*, known for its resistance to common antifungal treatments, which complicates infection management. *Aspergillus thermomutatus* thrives in warmer climates due to its thermotolerance. *Aspergillus udagawae* is notably associated with severe cases of IA, particularly in immunocompromised individuals. *Aspergillus viridinutans* is recognised for causing chronic pulmonary infections, while *Aspergillus fumigatiaffini* shares a close relationship with *A. fumigatus*, yet possesses distinct genetic and phenotypic traits. *Aspergillus novofumigatus*, a recently identified species, is distinguished by unique molecular markers.

Flavi: This section features *Aspergillus tamarii*, commonly found inside soil and also decaying vegetation, with the potential to produce mycotoxins. *Aspergillus alliaceus* is known for its potent aflatoxin production, which poses significant health risks.

Terrei: *Aspergillus carneus*, frequently isolated from indoor environments, has been implicated in human infections. *Aspergillus alabamensis* is a recently identified species with limited clinical data but notable potential pathogenicity.

Nigri: Within this section, *Aspergillus acidus* is typically found in acidic environments and is less commonly associated with human disease. *Aspergillus tubingensis* is recognised for its industrial applications and occasional pathogenicity, while *Aspergillus awamori* is used in fermentation processes but can act as an opportunistic pathogen.

Versicoloures: *Aspergillus sydowii* is known to cause human infections and is found in diverse environmental conditions. *Aspergillus creber* is often involved in indoor mould problems and can contribute to respiratory issues.

Circumdatii: *Aspergillus persii* is found in soil and decaying plant matter, with occasional implications for infections. *Aspergillus westerdijkiae* is known for producing ochratoxins, raising concerns about food contamination.

Usti: *Aspergillus calidoustus* is notably thermotolerant and often resistant to multiple antifungals. *Aspergillus insuetus* is rare but can cause invasive infections, chiefly inside immunocompromised hosts. *Aspergillus keveii* is a recently identified species with limited but emerging clinical relevance.

Clinical Manifestation of Cryptic Aspergillosis

Cryptic aspergillosis, caused by various species within the *Aspergillus* genus, presents a spectrum of clinical manifestations that can affect both humans and animals. While these manifestations share similarities with those caused by non cryptic species, there are distinct features and challenges associated with the diagnosis along with treatment of cryptic aspergillosis [24,25].

Pathogenesis of cryptic aspergillosis: Cryptic aspergillosis, caused by lesser-known or newly identified *Aspergillus* species, presents significant challenges due to the unique biological characteristics of these fungi. Cryptic aspergillosis pathogenesis begins with the inhalation of airborne spores (conidia) from sources such as dust, soil, or decaying organic matter. Upon inhalation, these spores reach the respiratory tract and adhere to epithelial surfaces. The adherence process is facilitated by surface proteins and polysaccharides on the fungal cell wall that interact with host receptors. Once attached, the conidia germinate into hyphal forms, which are more invasive and capable of penetrating deeper into host tissues [26]. The ability of cryptic *Aspergillus* species to elude the host immune system is a critical factor in their pathogenicity. These fungi can modify their cell wall composition to avoid detection by immune cells and produce various enzymes that degrade host tissues. This immune evasion is particularly problematic for immunocompromised individuals, like those undergoing chemotherapy or even organ transplantation, who are more susceptible to severe infections [26]. As the hyphae invade host tissues, they cause cellular damage and necrosis. This invasion can manifest in a range of clinical conditions, including pulmonary infections, sinusitis, and systemic disease. The invasive capabilities of these species are often supported by the production of proteolytic enzymes and mycotoxins, which further contribute to tissue destruction and immune suppression. The diagnosis along with management of cryptic aspergillosis is complicated by atypical clinical features of these infections. Standard diagnostic methods may fail to identify these species, necessitating the use of advanced techniques such as molecular diagnostics (e.g., PCR and sequencing) and specialised culture media. Treatment is further complicated by the resistance of some cryptic *Aspergillus* species to common antifungal agents. For instance, *Aspergillus lentulus* has demonstrated resistance to certain azole antifungals, requiring alternative or combination therapies for effective treatment.

Human pathogenesis: Cryptic aspergillosis pathogensis comprises an array of clinical conditions affecting various organ systems. Common manifestations include IPA along with CPA [27]. IPA usually occurs in immunocompromised individuals and can cause severe respiratory symptoms with a great mortality rate. CPA, on the other hand, affects those with underlying lung conditions, leading to chronic cough, fatigue, and weight loss over an extended period. The clinical spectrum of cryptic aspergillosis varies based on the affected organs and patient circumstances, presenting with respiratory, cutaneous, and systemic symptoms [25]. In the respiratory system, cryptic aspergillosis may manifest as CPA, ABPA, or IA. CPA typically affects immunocompetent individuals with conditions like tuberculosis, bronchiectasis, or cavitary lung diseases. ABPA occurs in patients having asthma or even cystic fibrosis, displaying with allergic lung symptoms. IA is a severe infection seen in immunocompromised patients, like those enduring chemotherapy, organ transplantation, or even with advanced HIV/AIDS.

Cutaneous manifestations include superficial infections of the skin, nails, or cornea, as well as fungal otomycosis or keratitis, often resulting from direct traumatic inoculation and more common in immunocompromised individuals. Systemic symptoms arise from haematogenous spread affecting organs like the central nervous system, cardiovascular system, or causing disseminated infections involving multiple organ systems. IA can lead to severe complications, including brain abscesses, endocarditis, or widespread disease, chiefly in patients having debilitated immune systems [26].

Animal pathogenesis: Cryptic species of *Aspergillus* can also affect animals, with implications for veterinary medicine. For instance, *A. felis* was identified as a significant source of aspergillosis inside cats, presenting with respiratory symptoms, ocular infections, and disseminated disease. Additionally, species like *A. alabamensis* have been described in both dogs and humans, highlighting the zoonotic potential of cryptic aspergillosis [26].

Environmental impact: Cryptic species of *Aspergillus* are ubiquitous in various environments, including agricultural settings. These species can contaminate crops such as sugar cane, rice, and corn, posing threats to both human and animal health. Understanding the environmental distribution of cryptic species is essential for mitigating the spread of infection and implementing effective control measures [27].

Hence cryptic aspergillosis encompasses a diverse array of clinical manifestations with implications for human and animal health. Understanding the pathogenesis, diagnostic challenges, and treatment considerations associated with cryptic species is essential for effective management and control of these infections.

Treatment

Cryptic aspergillosis management presents unique challenges, which is due to the intrinsic resistance of these elusive fungal pathogens to commonly used antifungal agents. Polyenes, historically fundamental in anti-mould therapy, have encountered limitations due to significant toxicity and logistical issues associated with intravenous administration. Liposomal Amphotericin B (LAMB) has addressed some safety concerns, particularly against *A. niger* infections, yet challenges persist with intravenous delivery [2].

Echinocandins, renowned for their advantageous safety profiles, have demonstrated restricted effectiveness against invasive mould infections triggered by cryptic *Aspergillus* species. However, research suggests potential benefits in combination with voriconazole or even as monotherapy inside rare cases of azole resistance [28].

Azoles, extensively studied for their activity against *Aspergillus* species, remain a cornerstone of treatment. Voriconazole is internationally commended as primary therapy for all IA, but concerns regarding safety have steered to the hunt for safer substitutes. Isavuconazole, emerging as a preferred first-line therapy, has demonstrated non inferiority to voriconazole in clinical trials and offers a favorable safety profile [28]. Despite the efficacy of azoles, the escalation of azole-resistant strains, counting cryptic species like *A. udagawae* along with *A. lentulus*, presents a formidable challenge. These species often exhibit intrinsic resistance to multiple antifungal classes, limiting treatment alternatives and necessitating substitute or combination therapies. Cryptic *Aspergillus* species further complicate treatment decisions, potentially leading to inappropriate antifungal utilisation and resistance development [28].

In response to these challenges, new antifungal agents having promising efficacy versus resistant strains were developed. Fosmanogepix inhibits the Gwt1 enzyme and shows broad-spectrum action versus *Aspergillus* species, counting azole-resistant strains. Ibrexafungerp inhibits 1,3-β-D glucan synthase and offers oral bioavailability, making it a promising candidate for outpatient treatment. Rezafungin, with a prolonged half-life, permits once-weekly dosing and shows potent action versus various *Aspergillus* species, counting cryptic species. Olorofim targets dihydroorotate dehydrogenase and demonstrates potent actionversus a broad spectrum of *Aspergillus* species, counting resistant strains [28].

Conversely, opelconazole and encochleated amphotericin B may not be effective versus cryptic aspergillosis owing to the ubiquitous resistance within these classes, highlighting the ongoing need for R&D in the fight versus resistant fungal infections. The [Table/Fig-3] outlines key aspects of antifungal drugs, including their indications, dosage, half-life, CNS penetration, and metabolism. This table

Antifungal agent	Approved indication	Dosage and formulation	Half-life (h)	CNS penetration	Metabolism
Voriconazole	Primary therapy: IA Second line: CPA, ABPA	IV: 6 mg/kg Q 12×24 h, 4 mg/kg Q12 starting day 2 PO: 200 mg Q12 h	6	High	CYP2C19, CYP2C9, CYP3A4
Isavuconazole	CPA: in case of intolerance/toxicity, resistance, or clinical failure to first and second line (limited data)	IV: 200 mg Q8 h×48 h, 200 mg daily starting day 3 PO: 200 mg Q8 h for 48 h, 200 mg daily starting day 3	110-115	High (animal model)	CYP3A4/5
Posaconazole	Prolonged neutropenia prophylaxis for IA CPA: in case of intolerance/toxicity, resistance, or clinical failure to first and second line (limited data)	IV: 300 mg Q12×24 h, 300 mg daily starting day 2 Delayed-release: 300 mg Q12×24 h, 300 mg daily starting day 2 Oral suspension: 200 mg TID	27-35	Low	UGT
Itraconazole	Salvage IA therapy ABPA, CPA: primary therapy	IV-70 mg daily on day 1, 50 mg daily starting day 2	7-10	High (animal model)	Unknown
Liposomal amphotericin B (LAMB)	IA primary therapy, empiric febrile neutropenia therapy	IV: 3 mg/kg/day	9-11	Low (animal model)	Unknown
Caspofungin	IA salvage or empiric febrile neutropenia therapy	Administer 200 mg IV/PO two to three times daily for 3 to 4 days, then 200 mg IV/PO once to twice daily.	-	-	-

[Table/Fig-3]: Clinical use and pharmacokinetics of antifungal agents in aspergillosis treatment [30].
ABPA: Allergic broncho-pulmonary aspergillosis; BID: Bis in die; CPA: Chronic pulmonary aspergillosis; CNS: Central nervous system; IA: Invasive aspergillosis; IV: Intravenous; PO: Per oral

summarises the approved indications, dosage and formulation, pharmacokinetic properties, and metabolism of commonly used antifungal agents in aspergillosis treatment. Voriconazole, and isavuconazole, and posaconazole, and itraconazole, LAMB, and caspofungin are evaluated based on their primary therapy indications, dosage regimens, half-life, central nervous system penetration, and metabolism. This information aids clinicians in selecting the most appropriate antifungal agent per the patient's clinical presentation and individual factors [30].

This information is crucial for effectively treating Aspergillosis, including infections caused by cryptic *Aspergillus* species. For cryptic aspergillosis, the same drugs used for common *Aspergillus* infections might be applicable, though their efficacy can vary. Dosage adjustments may be needed based on pharmacokinetics and patient needs. Understanding the half-life helps in planning dosing schedules, while CNS penetration is relevant for potential CNS involvement. Additionally, drug metabolism can influence treatment, especially if there are interactions with other medications. Thus, tailored treatment based on susceptibility testing and clinical response is essential for managing cryptic aspergillosis effectively [29].

CONCLUSION(S)

Cryptic aspergillosis presents a multifaceted clinical scenario characterised by species variability, diagnostic uncertainties, and therapeutic challenges. While the clinical spectrum encompasses a broad array of presentations, from superficial infections to invasive diseases, accurate species identification remains elusive due to phenotypic and genotypic similarities with classical *Aspergillus* species. This diagnostic quandary underscores the importance of molecular techniques for precise species delineation. Treatment options are further complicated by intrinsic resistance among cryptic species to conventional antifungal agents, necessitating the exploration of novel therapeutic approaches. The emergence of promising antifungal agents offers hope for addressing resistance challenges and improving treatment outcomes. However, a concerted effort is required to elucidate the epidemiology, pathogenesis, and optimal management strategies for cryptic aspergillosis. A multidisciplinary approach, integrating clinical, microbiological, and molecular expertise, is indispensable to traverse the complexities of cryptic aspergillosis and improve patient care in this evolving clinical landscape.

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