



Aspergillus and Aspergillosis in the Post-Pandemic Era

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Abstract: Aspergillosis is a group of diseases caused by *Aspergillus* infection or allergic reactions to the fungus. The severity ranges from mild allergic reactions to life-threatening invasive infections, depending on the patient's immune status. The COVID-19 pandemic has significantly altered the landscape of fungal infections, particularly those caused by *Aspergillus* species. In the post-pandemic era, aspergillosis has emerged as a major complication, driven by factors such as widespread corticosteroid use, immune dysregulation from SARS-CoV-2 infection, and disruptions in healthcare systems. Thus, the COVID-19 pandemic has reshaped the landscape of aspergillosis. This review summarizes post-pandemic situations and advances in clinically relevant *Aspergillus* taxonomy and species complexes, emphasizing the diagnostic importance of cryptic species and robust molecular identification of functional genes during the biological development in the genus of *Aspergillus*. Moreover, challenges in the prevention against *Aspergillus* infections and perspectives for the discovery and development of novel antifungal strategies have been summarized and discussed.

Key words: *Aspergillus*; Aspergillosis; COVID-19-associated pulmonary aspergillosis (CAPA); Diagnosis; Antifungal resistance

1 Introduction

Aspergillus is a genus of filamentous fungi commonly found in the environment, including soil, decaying vegetation, and indoor air (e.g., in dust, air conditioning systems, and construction sites) [1–4]. The name of *Aspergillus* originates from Latin religious instrument terminology—aspergillum—since the conidial head (spore-bearing structure) of the fungus resembled the aspergillum's shape: a radial “sprinkling” arrangement of chains of spores around a central vesicle, mimicking droplets dispersed from the religious tool [1]. There are over 300 species, but only a few cause infections in humans, including common pathogenic species such as *Aspergillus fumigatus* (the most common cause of infection), *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus terreus* [2, 3, 5, 6]. Accordingly, aspergillosis is a group of diseases caused by *Aspergillus* infection or allergic reactions to the fungus [2, 6, 7]. The severity ranges from mild allergic reactions to life-threatening invasive infections, depending on the patient's immune status [2, 3, 6, 7]. The COVID-19 pandemic has significantly altered the landscape of fungal infections, particularly those caused by *Aspergillus* species [8–11]. In the post-pandemic era, aspergillosis has emerged as a major complication, driven by factors such as widespread corticosteroid use, immune dysregulation from SARS-CoV-2 infection, and disruptions in healthcare systems [10–13]. Notably, we use the term “post-pandemic era” to refer to the period after the acute phase of COVID-19. During this period, the “new” features of aspergillosis mainly reflect new situations in host susceptibility profiles, healthcare exposures, and diagnostic/therapeutic pathways. Clinically, this has been accompanied by the recognition of ICU-associated syndromes such as COVID-19-associated pulmonary aspergillosis (CAPA) [8, 10], more frequent co-/super-in-

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fections and complex airway microbiomes [14, 15], based on rapid, noninvasive, or high-throughput diagnostics when bronchoscopy is limited [8, 10, 16], and heightened concern for azole resistance and cryptic species that challenge routine identification [17–19]. Accordingly, this review links *Aspergillus* taxonomy, biology, and genomics to post-pandemic clinical problems—diagnostic uncertainty, shifting risk factors, and evolving treatment landscapes—and provides an integrated overview of post-pandemic changes in epidemiology, diagnosis, and management of aspergillosis. Specifically, the review is organized as follows: 1) Category or taxonomy of *Aspergillus*, 2) Biological characteristics of *Aspergillus*, 3) Genomics of *Aspergillus*, 4) COVID-19-Associated Pulmonary Aspergillosis (CAPA) and dynamic changes in at-risk populations, 5) Therapeutic and preventive challenges against *Aspergillus* infections, 6) Drug resistance, tolerance, and persistence in *Aspergillus*, 7) Future perspectives.

2 Category or taxonomy of *Aspergillus*

The genus *Aspergillus* belongs to the phylum Ascomycota (class Eurotiomycetes, order Eurotiales, family Aspergillaceae) and represents one of the most important fungal groups, comprising a large number of species with remarkable diversity in morphology, ecology, and both economic and medical significance. Traditional taxonomic classification of *Aspergillus* was primarily based on morphological traits, such as the structure of conidial heads, pigmentation, and spore-bearing structures. In comparison, modern molecular systematics relies on multilocus sequence data, including β -tubulin (*BenA*), calmodulin (*CaM*), and RNA polymerase II subunit B2 (*RPB2*) genes, along with whole-genome information. Based on phylogenetic analyses of multiple genetic markers, the genus is currently divided into subgenera and sections, with major clinically relevant sections including *Fumigati*, *Flavi*, *Nigri*, *Terrei*, *Nidulantes*, *Circumdati*, and *Versicolores* [20, 21].

2.1 The *Fumigati* section

The *Fumigati* section represents the clinically most important pathogenic lineage within the genus *Aspergillus*, with *Aspergillus fumigatus* serving as its core representative species. It is the primary etiological agent of invasive pulmonary aspergillosis (IPA), with mortality often exceeding 50% and approaching 80% in some high-risk im-

munocompromised settings [22]. Species within this section exhibit strong airborne dispersal capacity and remarkable thermotolerance, maintaining efficient growth at human body temperature (37 °C) and tolerating elevated temperatures, a trait considered an important prerequisite for pathogenicity [23]. As summarized in Table 1, *A. fumigatus* is the core pathogen in section *Fumigati* and is typically triazole-susceptible, whereas several morphologically similar cryptic species within the *A. fumigatus/viridinutans* complexes are prone to misidentification and may exhibit reduced susceptibility (elevated minimum inhibitory concentrations, MICs) to triazoles and/or amphotericin B, which can complicate therapeutic decision-making. Given the presence of multiple closely related lineages in section *Fumigati*, species delimitation and clinical identification cannot reliably rely on internal transcribed spacer (ITS) alone; instead, protein-coding phylogenetic markers such as *BenA*, *CaM*, and *RPB2* are commonly used for robust species-level resolution [21, 24].

In addition to *A. fumigatus*, section *Fumigati* includes multiple cryptic species (e.g., *A. lentulus*, *A. udagawae*, and *A. thermomutatus*) that can be phenotypically indistinguishable from *A. fumigatus* in routine culture and microscopy; however, their antifungal susceptibility profiles may differ substantially, often showing elevated MICs to triazoles and/or amphotericin B, underscoring the need for accurate discrimination [24, 25]. Molecular typing markers have therefore become the principal method for reliable species differentiation. Among commonly used markers, ITS sequences show limited discriminatory power, whereas *BenA*, *CaM*, and *RPB2* have been established as core markers for cryptic species identification [21]. In recent years, rapid protein fingerprinting based on MALDI-TOF MS (database-dependent) and genome-based approaches have been increasingly evaluated for precise clinical identification of cryptic species [26, 27]. Therefore, although morphology may allow assignment to the *A. fumigatus* complex, the frequent phenotypic overlap and the potential for distinct MIC patterns among cryptic species make accurate species identification clinically consequential, influencing susceptibility interpretation and, ultimately, antifungal selection and patient management [24, 25].

2.2 The *Flavi* section and other *Aspergillus* groups

The *Flavi* section is an important group of opportunistic pathogens, represented by *A. flavus*. This species not only

Table 1 Clinical and susceptibility features of major *Fumigati* species

Species	Phylogenetic position	Major infection types	Drug susceptibility features	Clinical characteristics and challenges
<i>A. fumigatus</i>	Core species of the <i>Fumigati</i> section	Invasive pulmonary aspergillosis; chronic pulmonary aspergillosis	Usually susceptible to triazoles; acquired azole resistance increasingly reported	Most common pathogenic species; morphology typically supports identification to the <i>A. fumigatus</i> complex, but cryptic species require molecular resolution
<i>A. lentulus</i>	Cryptic species within the <i>Fumigati</i> section (<i>A. fumigatus</i> complex)	Invasive pulmonary aspergillosis; disseminated infections	Reduced susceptibility/elevated MICs to triazoles (often voriconazole/itraconazole); amphotericin B MIC may also be elevated	Frequently misidentified as <i>A. fumigatus</i> by routine morphology; reduced susceptibility may complicate therapy
<i>A. udagawae</i>	Cryptic species within the <i>Fumigati</i> section (<i>A. fumigatus</i> complex)	Invasive pulmonary aspergillosis; disseminated infections; bone/CNS involvement reported	Reduced susceptibility to triazoles (incl. voriconazole) reported; amphotericin B MIC often elevated	Slow growth/low sporulation reported; infections may present as a chronic or refractory disease and can extend beyond the lungs
<i>A. felis</i>	Cryptic species within the <i>Fumigati</i> section (<i>viridinutans</i> complex)	Pulmonary aspergillosis (often chronic); ocular/CNS infections reported	Reduced susceptibility to triazoles reported in the <i>viridinutans</i> complex; MICs may be variable across isolates	Morphologically similar to <i>A. fumigatus</i> ; clinically relevant cryptic species requiring accurate identification and susceptibility testing
<i>A. viridinutans</i>	Cryptic species within the <i>Fumigati</i> section (<i>viridinutans</i> complex)	Chronic pulmonary infections; disseminated infections	Reduced susceptibility to triazoles and amphotericin B reported	Often causes chronic, refractory infections; routine morphology may be insufficient for species-level identification
<i>A. pseudoviridinutans</i>	Cryptic species within the <i>Fumigati</i> section (<i>viridinutans</i> complex)	Opportunistic pulmonary infections reported (limited human data)	Reduced susceptibility to triazoles reported within the <i>viridinutans</i> complex; data remain limited	Rarely reported; risk of misidentification without molecular tools; clinical spectrum still being defined

MIC, minimum inhibitory concentration; CNS, central nervous system; incl., including.

infects humans and animals but also contaminates food and feed commodities (e.g., maize, peanuts, and tree nuts), producing aflatoxins that pose a dual threat to public health and food safety [20]. At the genomic and phylogenetic levels, species differences within the *Flavi* section are partly reflected in the diversity of aflatoxin biosynthetic potential and its regulatory architecture. Several closely related species within this section (e.g., *A. parasiticus* and *A. nomius*) show substantial differences in toxin production capacity, ecological niche preference, and antifungal susceptibility; therefore, polyphasic approaches and multilocus sequence analysis (e.g., *ITS* with limited resolution, together with *BenA* and *CaM*) are commonly required for reliable identification [21, 28, 29].

Clinically, *A. flavus* is an important cause of invasive aspergillosis in some tropical and subtropical regions, and it has been frequently implicated in sino-orbital, cutaneous, and pulmonary infections. Reduced susceptibility or higher MIC distributions to amphotericin B compared with *A. fumigatus* have been reported in multiple settings, which may complicate management in selected cases [30]. Moreover, under selective pressure from environmental azole exposure, decreased azole susceptibility has been reported in non-*fumigatus* *Aspergillus* species, highlighting

a “One Health” implication—that environmentally selected resistance can affect human and animal health through cross-compartment transmission and dissemination [17, 31].

In addition to the *Fumigati* and *Flavi* sections, several other groups within the genus *Aspergillus* exhibit comparatively lower clinical prevalence, including the *Nigri*, *Terrei*, *Versicolores*, and *Circumdati* sections. These groups are widely present in natural environments and are generally considered opportunistic pathogens; nevertheless, under host conditions such as immunosuppression, transplantation, or invasive procedures, they may still cause human infections [21]. The *Nigri* section (represented by *A. niger*) is commonly associated with otomycosis and keratitis, and occasional invasive infections have been reported. The *Terrei* section (e.g., the *A. terreus* species complex) is intrinsically resistant to amphotericin B and has a propensity for dissemination, making it a notable cause of treatment failure in clinical settings [32]. In addition, the subgenus *Nidulantes*, particularly section *Usti* (e.g., *A. calidoustus*), has been increasingly reported in immunocompromised hosts; multidrug resistance and diagnostic difficulties add to the clinical challenges [33].

Overall, although these groups are not the primary

pathogens of aspergillosis, their recognition in routine pathogen identification and resistance surveillance should not be overlooked.

2.3 Other newly defined groups within the genus *Aspergillus*

With advances in genome sequencing and continued phylogenetic re-evaluations, the taxonomic framework of the genus *Aspergillus* has been continuously refined and expanded, and several previously ambiguous lineages have been re-circumscribed under updated subgeneric, sectional, and series-level classifications. For example, within section *Usti*, recent multi-gene phylogenetic studies have supported a clearer internal structure by assigning species into multiple series (e.g., *Calidousti*, *Deflecti*, *Monodiorum*, and *Usti*), and related lineages have been re-evaluated accordingly [20, 34]. In parallel, the broader classification framework has also improved the resolution of sectional boundaries and series-level groupings across subgenera (including *Circumdati*), as supported by multilocus and integrative approaches [20, 35]. Although these refined groups are reported far less frequently in clinical contexts than the major pathogenic sections, their recognition is important for accurate species identification and data interpretation, because correct naming links isolates to validated information on ecology, extrolites, and species-level biology [20, 21]. Clinically, such taxonomic resolution has facilitated high-resolution molecular diagnostics and continual updating/curation of fungal reference databases, and it also underscores the need to include rare *Aspergillus* species in surveillance systems to minimize misidentification and avoid overlooking opportunistic pathogens [20, 36]. As ICU-associated aspergillosis has become more prominent, the clinical value of resolving isolates to the species/complex level—especially cryptic species within major complexes (e.g., *Fumigati*, *Terrei*, *Nigri*)—has further increased in the post-pandemic era, because interspecies differences in intrinsic susceptibility and resistance propensity can directly affect susceptibility interpretation, antifungal selection, and resistance/outbreak surveillance [10, 32, 37, 38].

3 Biological characteristics of *Aspergillus*

3.1 Hyphal polarized growth and its molecular regulatory mechanisms

Hyphal polar growth is a core biological process in *As-*

pergillus and is essential for vegetative growth, tissue invasion, and the expression of pathogenicity. It plays a decisive role in growth rate, morphogenesis, and virulence [39]. Hyphae mainly achieve asymmetric growth through apical extension, which depends on highly coordinated mechanisms for polarity establishment and maintenance, involving polarity axis formation, regulation by small GTPases, vesicle trafficking, dynamic cytoskeletal remodeling, and cell wall biosynthesis networks [39–41]. The establishment of the polarity axis is typically initiated during conidial germination and branch emergence, with key small GTPases such as Cdc42 accumulating at the apical membrane, where they activate downstream signaling pathways and promote the formation of polarity sites. The activity of Cdc42 is regulated by the spatial localization of its upstream modulators, including guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) [39]. In addition, in *A. nidulans*, the cell-end marker TeaA, together with polarity complexes such as the polarisome, contributes to maintaining the orientation of the polarity axis and straight tip growth [41, 42]. Mutations in polarity-associated proteins can result in abnormal branching, apical swelling, or disorganized growth direction, thereby impairing fitness and, in pathogenic species such as *A. fumigatus*, potentially compromising virulence [40, 43]. Once polarity is established, vesicle trafficking systems are rapidly activated. Vesicles are transported along microtubule and actin networks toward the hyphal tip, where they accumulate to form the Spitzenkörper, a central vesicle supply and transit station [40, 42]. This system continuously delivers cell wall synthases, membrane proteins, and polysaccharide substrates to the growing apex, ensuring the supply of essential components for polarized extension. Molecular machineries, including the exocyst tethering complex, the polarisome, and class V myosin (e.g., MyoE), coordinate vesicle fusion and targeted delivery, thereby constituting key molecular mechanisms for maintaining polarized growth [40, 41]. Genetic studies have shown that deletion of the APSES (Asm1p, Phd1p, StuAp, Efg1p, and Sok2p) family transcription factor RgdA causes impaired hyphal growth and morphogenesis and significantly reduced virulence [44].

Polarized growth also requires targeted synthesis and dynamic remodeling of cell wall components at the hyphal apex. Cell wall biosynthetic enzymes, such as chitin synthases (e.g., ChsA) and β -1,3-glucan synthase Fks1,

are enriched at the growth pole and are coordinated with the cell wall integrity (CWI) signaling pathway, which maintains a dynamic balance between hyphal extension and cell wall stability under growth and stress conditions [40, 45]. In *A. fumigatus*, core components of the CWI cascade (e.g., AfMkk2-MpkA and associated regulators) contribute to cell wall homeostasis and morphogenesis, and disruption of this pathway is linked to attenuated stress adaptation and virulence-related phenotypes [45–47].

Defects in polarized growth weaken tissue penetration capacity and compromise colonization efficiency and virulence within the host. The localized secretion of several virulence-associated factors, such as proteases and other secreted enzymes, depends on directional vesicle transport and precise apical targeting. When polarity is disrupted, secretion and delivery become mislocalized or reduced, which can severely impair pathogenicity [40, 45]. Consequently, key nodes associated with polarized growth and its coupling to cell wall synthesis and secretion, including Cdc42, the Rho1-PkcA/MpkA (CWI) module, and cell wall synthases such as Fks1 and chitin synthases, are considered potential targets for antifungal drug development [45]. Table 2 summarizes representative molecules involved in hyphal polar growth and their functional characteristics.

In summary, hyphal polar growth forms the basis of pathogenicity in *Aspergillus*. Its regulatory network spans signal transduction, cytoskeletal remodeling, vesicle trafficking, and cell wall construction. In recent years, the application of multi-omics approaches has substantially deepened our understanding of these processes, providing a theoretical foundation for antifungal strategies and of-

fering key insights into the pathogenic mechanisms of *Aspergillus*.

3.2 Asexual reproduction and its key regulatory factors in *Aspergillus*

Conidia are the principal structures of asexual reproduction in *Aspergillus* and represent the primary means of dissemination and initial infection [48]. Asexual conidiation involves the sequential development of conidiophore stalks (stipes), vesicles, metulae (in biserial species), phialides, and chains of conidia. The formation and differentiation of these structures require precisely regulated morphogenetic stages, orchestrated by multiple molecular regulatory modules to ensure coordinated morphology and function. Among them, the central regulatory cascade is the BrlA-AbaA-WetA pathway, which exerts hierarchical and stage-specific control over conidiation [48–50]. Upon activation, BrlA initiates the transition to conidiogenous cell differentiation (metulae/phialides, depending on species) and subsequently induces AbaA expression, which drives the differentiation and function of phialides [48, 50]. WetA mainly functions during late conidiogenesis, contributing to spore maturation and cell wall construction, and maintaining conidial dormancy and stress resistance [48, 51]. As shown in Figure 1, an overview of the BrlA-AbaA-WetA regulatory network and its associated upstream and accessory regulators in *Aspergillus* conidiation. This tripartite regulatory system is considered the central axis of programmed regulation in *Aspergillus* asexual development, and loss or misregulation of its expression results in severe structural abnormalities or complete failure of conidiation. Upstream of this central cascade, several regulators are involved in Br-

Table 2 Key functional proteins involved in hyphal polarized growth

Protein name	Protein type	Function	Phenotype upon deletion (Mutation)
Cdc42	Small GTPase	Polarity establishment and maintenance at the hyphal tip	Defective polarity establishment/maintenance; abnormal hyphal morphogenesis (e.g., swelling/branching defects)
Exocyst (e.g., Sec3/SecC and other subunits)	Vesicle-tethering complex	Targeted vesicle tethering/docking for polarized exocytosis at the apex	Severe defects in polarized secretion and tip growth (often strong growth impairment; morphology defects such as tip swelling/branching abnormalities)
ChsA	Chitin synthase (cell wall biosynthesis enzyme)	Chitin synthesis contributing to cell wall construction during growth/morphogenesis	Cell wall defects and morphogenetic abnormalities (severity may be isoenzyme-/species-dependent)
Fks1 (FksA)	β -1,3-glucan synthase catalytic subunit	β -1,3-glucan synthesis and deposition (major cell wall component; echinocandin target)	Severe growth and cell wall integrity defects (often not viable as a null; conditional impairment causes strong growth defects)
RgdA	Rho GTPase regulator (e.g., RhoGAP-related regulator)	Regulation of polarized growth/morphogenesis via Rho-family signaling	Abnormal apical growth/morphogenesis; reduced virulence (reported in <i>A. fumigatus</i>)

GTPase, guanosine triphosphatase; Cdc42, cell division cycle 42; Sec, secretion (Sec3/SecC denote exocyst subunits); ChsA, chitin synthase A; Fks1/FksA, β -1,3-glucan synthase catalytic subunit; Rho, Ras homolog (Rho-family GTPase); RhoGAP, Rho GTPase-activating protein.

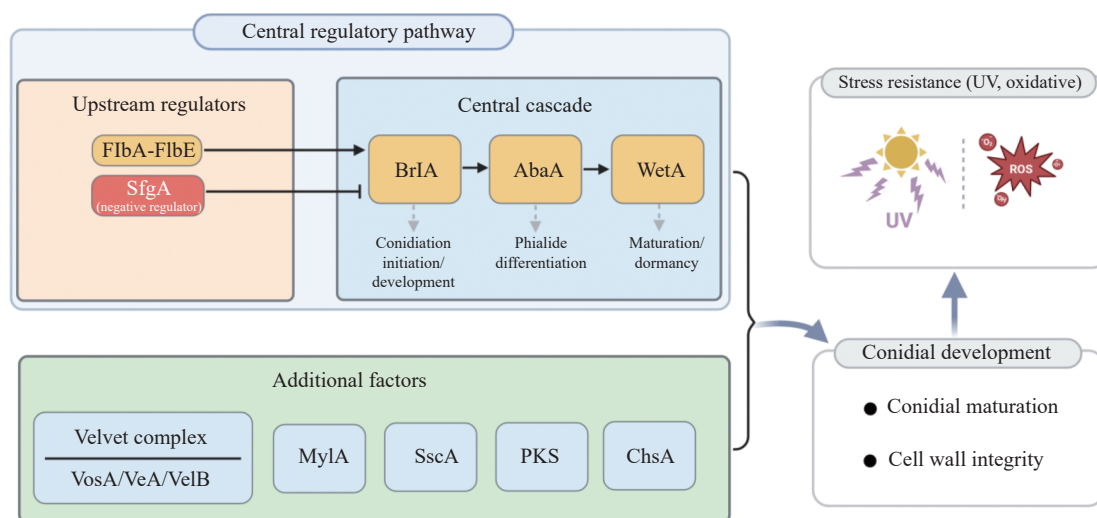


Figure 1 Central regulatory pathway and additional factors governing conidiation in *Aspergillus*. Schematic overview of the core regulatory framework for asexual development in *Aspergillus*. Upstream regulators (FlbA–FlbE) promote activation of the central BrlA–AbaA–WetA cascade, whereas SfgA acts as a negative regulator. The cascade drives stage-specific programs, including conidiation initiation/development (BrlA), phialide differentiation (AbaA), and conidial maturation/dormancy (WetA). Additional factors, including the Velvet complex (VosA/VeA/VelB) and regulators such as MylA and SscA, modulate conidial maturation, cell-wall integrity, and stress-related traits; PKS and ChsA contribute to structural/cell-wall biosynthesis. Collectively, these modules support conidial development and enhance resistance to environmental stresses (e.g., UV and oxidative stress).

IA activation and its precise temporal control. The early asexual developmental regulators FlbA–FlbE play critical roles in integrating upstream signals and initiating *brlA* transcription. Among them, FlbB is a bZIP-type transcription factor, whereas FlbD is a cMyb-type (Myb-like) transcription factor that cooperatively promotes *brlA* expression and the onset of asexual development [52, 53]. In addition, SfgA functions as a negative regulator by repressing BrlA expression, thereby blocking downstream AbaA activation and delaying the onset of asexual development [54, 55].

Beyond these classical regulatory modules, recent studies have revealed novel regulators and epigenetic mechanisms that also participate in asexual development. For example, the MYB-like protein MylA, the spore-specific regulator SscA, and members of the Velvet complex (VosA/VeA/VelB) modulate conidial formation, germination capacity, and stress resistance [56–62]. Deletion of these regulators often leads to disorganized conidial wall structure and reduced germination capacity or stress tolerance, and in pathogenic *Aspergillus* species may also attenuate virulence-associated traits.

The final formation of conidia also depends on polysaccharide biosynthesis and pigment deposition in the cell wall. Studies have shown that chitin synthases (e.g., ChsA in *A. niger*) and their associated regulatory programs criti-

cally influence conidiation and conidial cell wall integrity and architecture [63]. In addition, pigments such as DHN-melanin (e.g., PksP/Alb1-dependent in *A. fumigatus*) and other polyketide-derived conidial pigments (e.g., a gene *wA*-dependent in *A. nidulans*) are synthesized through polyketide synthase (PKS)-mediated pathways [64, 65]. These pigments not only determine spore coloration but also protect conidia by enhancing resistance to UV irradiation and oxidative stress [65–67].

In summary, asexual development in *Aspergillus* is driven by a hierarchically organized regulatory network with well-defined functions. The classical BrlA–AbaA–WetA cascade constitutes the central framework, while newly identified regulators such as the Velvet complex, SscA, and MylA have further expanded our understanding of the molecular mechanisms underlying developmental processes and related traits. Collectively, these studies not only deepen systematic knowledge of fungal developmental regulation but also provide critical molecular insights into stress tolerance and pathogenic potential. Table 3 summarizes the key genes reported to be involved in the regulation of asexual conidiation in *Aspergillus*.

3.3 Virulence-related factors in *Aspergillus*

The virulence of *Aspergillus* species (i.e., the degree of pathogenicity) depends not only on their capacity for en-

vironmental adaptation but also on their developmental programs. Processes such as hyphal polarized growth, conidial formation, and the expression or mutation of regulatory factors can directly or indirectly affect fungal invasiveness, efficiency of tissue colonization, and immune evasion, ultimately shaping overall virulence [68]. Multiple factors influence the pathogenicity of *Aspergillus*, particularly those related to its developmental and structural features: 1) Conidial surface structures are closely associated with virulence. For instance, in *A. fumigatus*, the surface protein RodA forms a hydrophobic rodlet layer that masks underlying pathogen-associated molecular patterns (PAMPs), such as β -1,3-glucan and other immunogenic cell-wall carbohydrate epitopes (e.g., α -mannose-related ligands), thereby evading host immune recognition [69, 70]. RodA-deficient mutants expose these immune-reactive molecules, leading to heightened host inflammatory responses and altered fungal survival *in vivo*, underscoring the crucial role of the immune-shielding conidial surface in successful infection [70]. In addition, DHN-melanin, a major conidial pigment, facilitates immune evasion by modulating host immune responses and interfering with phagocyte processing, and it may also contribute to stress protection (with context-dependent effects on oxidative stress tolerance) [71, 72]. 2) Perturba-

tion of Velvet complex proteins and developmental regulators can affect virulence-associated traits. VelB, VosA, and VeA regulate conidial formation/maturation and secondary metabolite biosynthesis; in *A. fumigatus*, VeA contributes to conidiation control and gliotoxin-associated pathogenic potential in animal models [73]. The conidial-specific regulator SscA has also been shown to influence conidial physiology and early infection-relevant traits; however, its impact on virulence appears to be model- and/or strain-dependent rather than uniformly attenuating [60]. 3) Crosstalk between developmental programs and stress responses contributes to pathogenic potential. Transcription factors such as RgdA and AtfA regulate development-linked processes while simultaneously participating in stress-response pathways, including oxidative stress responses and cell wall homeostasis, thereby supporting adaptation to hostile host environments [44, 74].

Notably, clinical and environmental isolates of *A. fumigatus* can show substantial phenotypic heterogeneity (including conidiation, biofilm-related traits, and stress responses), and these differences may correlate with virulence in a context-dependent manner [75–77]. This suggests that the relationship between development-associated traits and virulence is not strictly linear but can be

Table 3 Major key genes involved in conidiation

Names of genes	Developmental stages	Main functions	Mutant phenotypes
<i>brlA</i>	Initiation stage of asexual development	Activates the asexual developmental program; required for transition to conidiophore development	Bristle-like stalk; failure to form vesicle/metulae/phialides/conidia (conidiation blocked)
<i>abaA</i>	Conidiation stage	Drives phialide differentiation; regulates conidial differentiation	Abacus-like structures; defective phialides, and blocked conidiation
<i>wetA</i>	Spore maturation stage	Regulates conidial maturation and cell wall/viability programs	Reduced conidial viability; cell wall/maturation defects; decreased stress tolerance
<i>vosA</i>	Spore maturation stage	Maintains conidial dormancy; prevents premature germination	Premature germination; reduced conidial viability/stress tolerance
<i>vadA</i>	Spore maturation stage	VosA/VelB-activated regulator of conidial maturation, viability and stress response	Decreased trehalose; reduced viability and oxidative-stress tolerance; altered cell wall traits
<i>velB</i>	Spore maturation stage	Velvet-component; cooperates with VosA in conidial maturation and stress programs	Defects in conidial maturation and stress tolerance (often with reduced viability)
<i>mylA</i>	Conidiation & germination	MYB-like regulator contributing to conidiogenesis and conidial germination	Defects in conidiation and/or conidial germination capacity
<i>sscA</i>	Spore maturation/dormancy	Spore-specific regulator coordinating with VosA in maturation/dormancy/germination	Impaired maturation/dormancy programs; compromised germination control
<i>flbB</i>	Initiation stage of asexual development	bZIP transcription factor upstream of <i>brlA</i> activation	Delayed/strongly reduced conidiation (“fluffy/low <i>brlA</i> ” phenotype)
<i>fluG</i>	Initiation stage of asexual development	Produces/mediates upstream developmental signal required for <i>brlA</i> activation	“Fluffy” colony; severely reduced conidiation (often rescueable by external signal/neighbor WT)

WT, wild type; bZIP, basic leucine zipper (transcription factor); MYB, myeloblastosis (MYB-like DNA-binding domain); VosA/VelB, velvet-family regulators (VosA and VelB); “fluffy”, aconidial or severely reduced conidiation colony morphology; metulae and phialides, conidiophore cell types (metulae present in biserial species).

modulated by strain-specific genetic backgrounds and the host microenvironment.

In summary, the developmental machinery of *Aspergillus* not only governs hyphal morphogenesis and asexual reproduction but also plays critical roles in pathogenesis. Conidial surface architecture, regulatory networks, and stress adaptation together constitute a complex and dynamic system that governs fungal virulence. Elucidating the interconnections among these processes provides new opportunities for antifungal strategies targeting developmental pathways and offers fresh perspectives for clinical pathogen identification and therapeutic intervention. Although core programs of polarized growth and conidiation are conserved, host- and microenvironmental conditions characteristic of severe viral pneumonia and critical illness (e.g., hypoxia, inflammatory cytokines, tissue damage, and bacterial/viral co-pathogens) can reshape fungal growth states (including biofilm-like growth) and secondary metabolism [78, 79]. These context-dependent shifts may influence immune interactions and drug tolerance, underscoring the need to interpret “classical” *Aspergillus* biology through a post-pandemic clinical lens [43, 80–82].

4 Genomics of *Aspergillus*

4.1 Genomic information of representative *Aspergillus* species

Aspergillus is one of the earliest filamentous fungal genera to undergo genome sequencing, and multiple species now have high-quality reference genomes, providing a solid foundation for genetic and functional studies. For example, the widely used clinical reference strain *A. fumigatus* Af293 possesses a genome size of 29.4 Mb, comprising eight chromosomes and 9,926 predicted protein-coding genes, with a GC content of 49.9% [83]. Using integrative multi-platform sequencing approaches, Bowyer *et al.* achieved a telomere-to-telomere (T2T) assembly of reference strain *A. fumigatus* CEA10 and a near-complete assembly of its descendant isolate A1160, laying the groundwork for investigating chromosomal structural variation and other repetitive regions [84]. Reference genomes of other representative species, including *A. nidulans*, *A. niger*, and *A. flavus*, are approximately 30, 33.9, and ~36–37 Mb in size, respectively, with predicted gene numbers ranging from about ~9,500 to ~14,200 [85–

87].

Species also display notable variation in genome structural features. For instance, in *A. fumigatus*, lineage-variable regions (“genomic islands”) and subtelomeric segments are enriched for transposon-related sequences and genes involved in secondary metabolism and host interaction, highlighting chromosomal termini as hotspots for functional evolution and structural rearrangements [88]. With sequencing technology progressing from first-generation Sanger sequencing (relatively long reads but low throughput) and second-generation short-read, high-throughput platforms such as Illumina, to third-generation long-read sequencing platforms including PacBio and Oxford Nanopore, genome assembly quality has improved substantially, facilitating detailed analyses of complex regions such as centromeres, telomeres, and structural variants [84, 89, 90]. In addition, public resources such as the Fungal Pathogen Genomics Resource (FungiDB) have provided critical support for genome annotation and functional analyses [91, 92].

4.2 Core and accessory genes in *Aspergillus*

With the advancement of multi-strain pan-genome sequencing, the concepts of “core genes” and “accessory genes” in *Aspergillus* have become increasingly well defined. Core genes are those conserved across all (or the vast majority of) strains, typically encoding functions essential for basic cellular metabolism and developmental regulation. In comparison, accessory genes are variably present among strains and often encode functions related to environmental adaptation, secondary metabolism, or host interactions [93, 94]. For instance, Barber *et al.* defined the pan-genome of *A. fumigatus* using 300 genomes and identified 12,798 gene clusters that condensed into 10,907 non-redundant orthogroups, of which 7,563 orthogroups (69%) were core and 3,344 showed presence/absence variation [95]. Accessory genes exhibit a higher degree of sequence variability, and many are functionally enriched in categories related to environmental responsiveness and metabolic flexibility, including transmembrane transporters and iron-binding-associated functions, among others [95]. These genes also tend to show non-random genomic distributions; for example, variable genes are frequently enriched toward subtelomeric regions and regions associated with elevated genome dynamism [94, 95]. Accessory genes not only display uneven distribution across lineages but also show substan-

tial polymorphism within species, manifesting as population-structured or clade-biased patterns of gene presence and absence. Lofgren *et al.* integrated population genomics and pan-genomics across 260 *A. fumigatus* genomes, revealing three primary populations with extraordinarily high levels of recombination, and demonstrated that each clade carries distinct suites of accessory genes. Notably, accessory genes were functionally enriched for nitrogen and carbohydrate metabolism, suggesting niche-linked metabolic specialization, and the distribution of putative antifungal resistance determinants was often phylogenetically structured [94].

In *A. flavus*, the AflaPan dataset constructed by Ganurde and colleagues revealed a highly dynamic pan-genome: among 12,316 gene families, only 4,289 (41%) were core, whereas 6,227 (59%) were accessory, accompanied by substantial singleton diversity. These accessory components were implicated in adaptive traits, including stress-associated functions and secondary metabolism-related features, providing a genomic basis for lineage-specific ecological performance and toxigenic potential [96].

Collectively, these results highlight that core genes are more often involved in cellular maintenance and growth, whereas accessory genes tend to encode functions that expand ecological adaptability and condition-dependent pathogenicity. This functional divergence provides the genetic basis for the genomic diversity of *Aspergillus* in different evolutionary contexts and offers new perspectives on the mechanisms underlying ecological differentiation and adaptive evolution [93-96].

4.3 Genomic analysis tools and gene editing technologies in *Aspergillus*

In recent years, advances in high-throughput omics technologies and genetic manipulation approaches have markedly accelerated functional genomics research in *Aspergillus*. In particular, genome annotation, transcriptional regulation analysis, and functional validation of target genes have been widely supported by the application of diverse bioinformatics tools and database platforms. For genome annotation, traditional automated pipelines (e.g., MAKER, AUGUSTUS) have been increasingly integrated with transcriptomic and, where available, proteomic datasets, thereby improving the accuracy of gene structure prediction [97, 98]. The Aspergillus Genome Database (AspGD) provided curated *Aspergillus*-focused annotations and incorporated RNA-seq evidence to refine gene models [99]; its information has been transferred and

is now accessible through broader platforms such as FungiDB, which provides unified annotation resources and experimental support for gene function studies across fungi, including *Aspergillus* [92, 100]. More recently, cross-species orthology inference tools such as OrthoFinder and PANTHER have been widely applied to investigate gene conservation and functional evolutionary trajectories among different *Aspergillus* species or strains [101, 102]. For pan-genome-scale comparisons in *Aspergillus*, orthogroup-based workflows and presence/absence frameworks have been increasingly used to quantify core versus accessory components across large isolate collections [95].

In terms of gene editing, the introduction of the CRISPR/Cas system represents one of the most significant breakthroughs in recent years. In *A. fumigatus*, early CRISPR/Cas9 systems enabled targeted gene disruption [103], and Zhang *et al.* further established a highly efficient CRISPR mutagenesis strategy using very short homology arms via microhomology-mediated end joining (MMEJ), enabling both gene knockout and fusion tagging [104]. Compared with traditional homologous recombination workflows that often require longer homology arms and/or NHEJ-deficient backgrounds, CRISPR-based approaches offer improved programmability and can substantially streamline knockout, knock-in, and promoter/regulatory manipulations in multiple *Aspergillus* species [105]. Moreover, dCas9-based CRISPRi/CRISPRa systems and Cas12a (Cpf1) variants have been adapted in filamentous fungi (including *Aspergillus*) to enable transcriptional modulation and expanded editing capabilities, and CRISPR-based strategies have also been explored for targeted regulation of chromatin and epigenetic states [106-108]. In *A. flavus*, CRISPR/Cas9 has been applied to functionally dissect aflatoxin-related pathways through targeted gene manipulation [109].

Of particular note, in addition to CRISPR-based tools, insertional mutagenesis approaches such as transposon mutagenesis and Agrobacterium-mediated T-DNA insertion have been used to support mutant discovery and library-style functional screening in *Aspergillus*, and recent work on large mobile genetic elements highlights how mobile DNA can mobilize gene cargo and may inspire next-generation high-throughput screening strategies [110-112]. To facilitate comparison, commonly used genome annotation tools and editing systems in *Aspergillus* research are summarized in Table 4.

Table 4 Genomic and genes editing systems or tools used in *Aspergillus*

Names of tool/system	Types	Main functions	Representative <i>Aspergillus</i> applications	Features/advantages
MAKER/MAKER2	Genome annotation pipeline	Evidence-based gene model construction	Used broadly in fungal/ <i>Aspergillus</i> genome projects	Integrates transcript evidence (EST/RNA-seq assemblies) and protein homology evidence; convenient annotation management
AUGUSTUS	Ab initio gene predictor	Gene structure prediction	Commonly used as an ab initio component in fungal annotations	Requires (or benefits from) species-specific training; supports alternative transcripts
CRISPR/Cas9	Genome editing system	Knockout/knock-in/tagging	<i>A. fumigatus</i> (targeted disruption; MMEJ-assisted editing) and other <i>Aspergilli</i>	High efficiency and programmability; widely adopted in <i>Aspergillus</i> toolkits
dCas9-based regulation (CRISPRi/CRISPRa)	Transcriptional regulation	Repression (CRISPRi)/activation (CRISPRa) without DSB	Demonstrated dCas9 regulatory platforms in <i>Aspergillus</i> (activation; repression/epigenetic targeting reported)	No DNA cleavage; useful for essential genes and tunable expression control
Cas12a (Cpf1)	Genome editing system	DNA editing; multiplex editing	Multiplex Cas12a/Cpf1 strategies reported for <i>Aspergillus</i> species; also shown in <i>A. niger</i> editing systems	Alternative PAM (often TTTV) and crRNA processing facilitate multiplexing; a useful complement to Cas9
Insertional mutagenesis (Transposon/ATMT)	Forward genetics/library generation	Random insertion mutant libraries; gene discovery	Transposon mutagenesis and ATMT-based insertional mutagenesis reported in <i>A. fumigatus</i>	Good for high-throughput mutant discovery; insertion bias/position effects should be considered

EST, expressed sequence tag; CRISPR, clustered regularly interspaced short palindromic repeats; DSB, double-strand break; dCas9, catalytically inactive (“dead”) Cas9; CRISPRi/CRISPRa, CRISPR interference/activation; PAM, protospacer adjacent motif; crRNA, CRISPR RNA; Cpf1, CRISPR from *Prevotella* and *Francisella* 1 (Cas12a); TTTV, Cas12a PAM motif (V=A/C/G); ATMT, *Agrobacterium tumefaciens*-mediated transformation.

Beyond functional genomics, sequencing technologies are increasingly integrated into clinical mycology and antifungal-resistance surveillance in the post-pandemic era [113, 114]. Multilocus sequence typing (MLST) offers a standardized and cost-effective approach for lineage tracking [115], whereas whole-genome sequencing (WGS) provides higher resolution for outbreak investigation, resistance mechanism discovery, and within-host microevolution [116–119]. Metagenomic next-generation sequencing (mNGS), in contrast, enables culture-independent detection of *Aspergillus* DNA directly from clinical specimens and can be valuable when bronchoscopy or repeated sampling is constrained in ICU settings [10, 120, 121]. These approaches are complementary: mNGS supports rapid etiologic diagnosis, while targeted sequencing or WGS of cultured isolates enables definitive species identification, resistance genotyping, and genomic epidemiology for surveillance and infection control [116, 122].

5 CAPA and dynamic changes in at-risk populations

5.1 CAPA and its diagnostic criteria

COVID-19-associated pulmonary aspergillosis (CAPA) refers to secondary invasive pulmonary aspergillosis occurring in patients with COVID-19. It was first proposed

as an independent clinical diagnostic entity in 2020 [8]. Traditional diagnostic criteria for IPA, such as those established by the European Organization for Research and Treatment of Cancer/Mycoses Study Group Education and Research Consortium (EORTC/MSGERC), were primarily developed for immunocompromised hosts and require compatible host factors (i.e., classical immunosuppression) for “possible/probable” classification. As a result, many critically ill COVID-19 patients with suspected fungal infection—who often lack these host factors and in whom tissue biopsy is rarely feasible—cannot be classified as “possible/probable” and seldom meet “proven” criteria, leading to under-recognition of CAPA when applying EORTC/MSGERC alone.

To address this gap, the European Confederation of Medical Mycology/International Society for Human and Animal Mycology (ECMM/ISHAM) jointly issued diagnostic criteria for CAPA, introducing a three-tier classification of “possible”, “probable”, and “proven” disease. These criteria incorporate multiple diagnostic indicators, including pulmonary imaging, new pulmonary lesions, bronchoalveolar lavage fluid (BALF) galactomannan (GM) testing, PCR assays, and fungal culture [10]. By moving beyond the requirement of classical immunosuppression/host factors, this framework has made CAPA recognition more consistent with the clinical reality of critically ill COVID-19 patients. As shown in Figure 2,

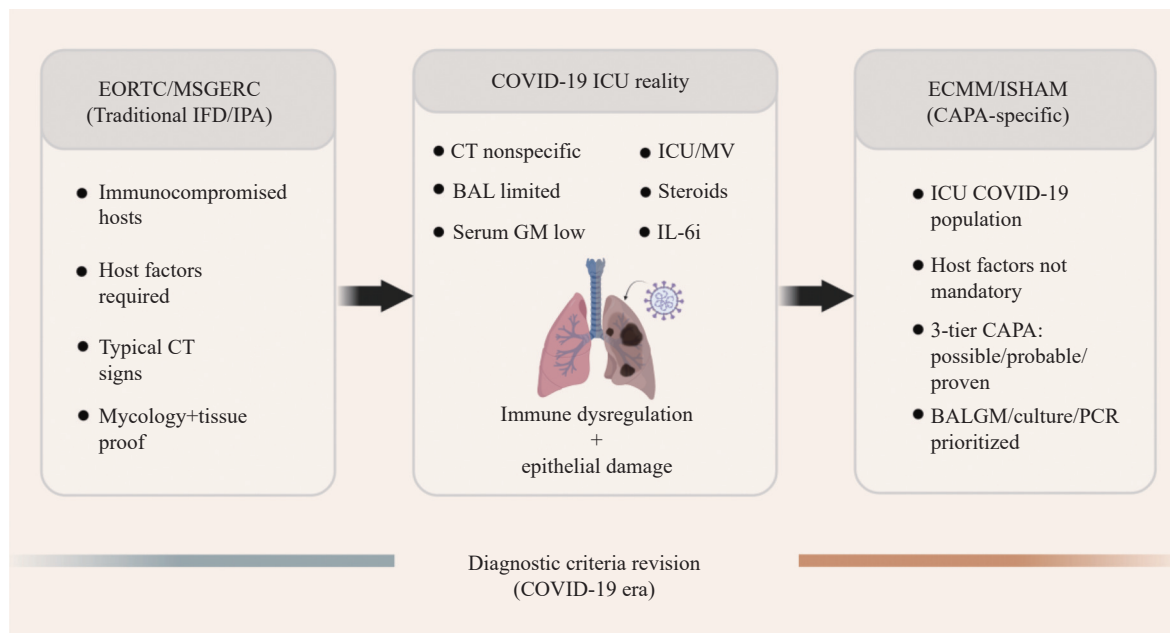


Figure 2 Diagnostic criteria update for CAPA. Schematic comparison of the traditional EORTC/MSGERC framework for IFD/IPA and the clinical constraints in COVID-19 ICU patients that prompted the ECMM/ISHAM CAPA-specific definitions, including non-mandatory host factors, a three-tier classification (possible/probable/proven), and prioritization of BAL-based mycology (GM/culture/PCR). Abbreviations: EORTC/MSGERC, European Organization for Research and Treatment of Cancer/Mycoses Study Group Education and Research Consortium; IFD, invasive fungal disease; IPA, invasive pulmonary aspergillosis; CAPA, COVID-19-associated pulmonary aspergillosis; ICU, intensive care unit; MV, mechanical ventilation; CT, computed tomography; BAL, bronchoalveolar lavage; GM, galactomannan; PCR, polymerase chain reaction; IL-6i, interleukin-6 inhibitor; ECMM/ISHAM, European Confederation of Medical Mycology/International Society for Human and Animal Mycology.

the COVID-19-driven diagnostic criteria change for CAPA was summarized from EORTC/MSGERC to ECMM/ISHAM.

Nevertheless, significant challenges remain in clinical practice. For instance, the risk of aerosol generation restricts bronchoscopy and BALF sampling; serum GM has low sensitivity in this setting; and radiological manifestations are often nonspecific, all of which contribute to underdiagnosis or delayed diagnosis of CAPA [8, 123]. In addition, different studies have adopted heterogeneous diagnostic standards for CAPA: some continue to use the EORTC/MSGERC criteria, others apply the AspICU criteria, while still others follow the CAPA-specific definitions recommended by ECMM/ISHAM. Such discrepancies in diagnostic criteria have led to substantial variation in the reported incidence of CAPA across studies [11, 124]. To facilitate post-pandemic clinical decision-making, Table 5 summarizes the key advantages and limitations of commonly used diagnostic approaches (imaging, conventional mycology, antigen/nucleic acid amplification tests (NAATs; e.g., PCR), and sequencing-based methods).

In parallel with pathogen-focused tests, recent studies in non-neutropenic or ICU populations have evaluated host-response biomarkers and data-driven models to improve risk stratification and prognostication. For example, interferon- $\lambda 1/\lambda 3$ levels and pentraxin-3 have been proposed as diagnostic/prognostic markers, and multicenter machine-learning models that integrate clinical, imaging, and laboratory features have shown promise for supporting early recognition of non-neutropenic IPA [125–127]. Such approaches remain adjunctive and require external validation across diverse settings, but they align with the post-pandemic need for earlier, less invasive, and more interpretable diagnostic pathways.

The introduction of CAPA and updates to its diagnostic framework not only reflect a paradigm shift in clinical recognition of fungal infections in the context of viral infection but also contribute to redefining the risk of invasive fungal diseases in non-classical immunocompromised populations.

5.2 Epidemiological trends of CAPA

With the global spread of CAPA, it has increasingly been

Table 5 Diagnostic approaches for aspergillosis in the post-pandemic era

Method	Specimen(s)	Advantages	Limitations
CT	N/A	Rapid, noninvasive triage in ICU	Low specificity in viral pneumonia/ARDS; transport/instability may limit access
Mic/Cx	Sputum; TA; BALF±NBL; tissue	Provides isolate for species ID + AFST (supports resistance/outbreak surveillance)	Low sensitivity; slow turnaround; sampling is often constrained in the ICU
Histo	Tissue	Definitive evidence of invasion	Invasive; rarely feasible in critically ill patients
GM (EIA)	BALF; serum; TA/NBL*	Standardized; BALF GM supports early diagnosis (incl. CAPA workup when LRT sample is obtainable)	Serum GM is often less sensitive in ICU/non-neutropenic; false positives; cutoffs/specimen type vary by setting
BDG	Serum	Adjunct marker when <i>Aspergillus</i> -directed tests are limited	Non-specific for <i>Aspergillus</i> ; false positives; variable performance across populations
PCR/NAAT (±R-PCR)	BALF; serum/plasma; TA/NBL*	Rapid; improves sensitivity; some assays detect azole-R markers (useful when culture is delayed/negative)	Assay heterogeneity/standardization; contamination; colonization not excluded; resistance coverage incomplete
LFA/LFD	BALF; serum†; TA/NBL*	Fast, simple workflow; supports time-critical ICU screening	Performance varies by platform/cutoff; limited species-level and resistance information
mNGS	BALF; plasma; respiratory samples	Culture-independent; detects co-pathogens/mixed infections (relevant in polymicrobial ICU pneumonia)	Cost; contamination/background reads; thresholds/interpretation not standardized; DNA detection ≠ invasive disease
Host biomarkers/ML	Plasma/BALF + clinical data	Risk stratification/prognostication; may support earlier decisions in non-classical hosts	Not pathogen-specific; requires external validation and standardized cutoffs
MLST/WGS	Isolates	High-resolution typing (WGS > MLST); resistance mechanism/outbreak investigation	Requires isolate and sequencing capacity; not a stand-alone diagnostic; turnaround/infrastructure constraints

* TA/NBL use depends on local protocols/validation. † Platform-dependent. CT, computed tomography; ARDS, acute respiratory distress syndrome; ICU, intensive care unit; CAPA, COVID-19-associated pulmonary aspergillosis; Mic/Cx, direct microscopy/culture; BALF, bronchoalveolar lavage fluid; TA, tracheal aspirate; NBL, non-bronchoscopic lavage; ID, identification; AFST, antifungal susceptibility testing; GM, galactomannan; EIA, enzyme immunoassay; BDG, β -(1,3)-D-glucan; PCR, polymerase chain reaction; NAAT, nucleic acid amplification test; R-PCR, resistance PCR; LFA/LFD, lateral flow assay/device; mNGS, metagenomic next-generation sequencing; ML, machine-learning; MLST, multilocus sequence typing; WGS, whole-genome sequencing; azole-R, azole resistance.

recognized as a frequent secondary fungal infection, particularly in critically ill patients with COVID-19 admitted to the intensive care unit (ICU) [8, 10]. Reported ICU incidence has varied widely across cohorts and case definitions (e.g., 1.0%-39.1% in early reports), and is generally higher than that described in non-COVID-19 ICU populations (approximately 0.3%-5.8% in unselected ICU cohorts) [9, 128, 129]. In an early multicenter ICU study conducted in Europe during 2020, CAPA incidence showed substantial between-study variation (e.g., 19.4% in a Dutch ICU cohort and ~33% in a French ICU report), underscoring the impact of diagnostic definitions and sampling intensity [130, 131]. This pattern supports the concept that severe SARS-CoV-2 pneumonia/ARDS—together with airway epithelial damage, immune dysregulation, and iatrogenic immunomodulation—may increase susceptibility to IPA, although causality remains difficult to establish in observational studies [8, 10].

The rapid increase in CAPA cases is not confined to specific regions. Subsequent retrospective and prospective ICU studies from different countries have continued to report CAPA, with incidence estimates remaining het-

erogeneous [11]. For instance, a German ICU cohort reported CAPA in 34% of patients with severe COVID-19 compared with 8% in contemporaneous non-COVID-19 ICU controls [132]. In an Iranian ICU cohort defined by European Confederation of Medical Mycology/International Society for Human and Animal Mycology (ECMM/ISHAM) criteria, CAPA was confirmed in 14.3% of 119 patients with severe COVID-19 pneumonia [133].

Overall, although significant heterogeneity exists across regions, current epidemiological evidence consistently indicates that CAPA is prevalent and clinically consequential in critically ill COVID-19 patients [10]. Reported risk factors commonly include systemic corticosteroid exposure, prolonged ICU stay, and mechanical ventilation [13, 134]. It is noteworthy, however, that current CAPA incidence data still lack cross-study comparability, owing to differences in case inclusion criteria, diagnostic methodologies, and definitions of CAPA applied across studies [11, 123]. Therefore, future epidemiological studies are urgently needed to establish more representative and comparable international databases based on standardized CAPA diagnostic criteria, thereby enabling more reliable risk

assessment and informing the development of prevention and control strategies [10].

5.3 High-risk populations and their susceptible mechanisms in the post-pandemic era

Before the COVID-19 pandemic, the primary high-risk groups for IPA included patients undergoing organ transplantation, those with hematologic malignancies, or individuals receiving long-term immunosuppressive therapy [10]. However, during the COVID-19 pandemic, numerous cases of CAPA have revealed that severe COVID-19 patients without classical systemic immunocompromised backgrounds can also develop invasive fungal infections [8, 135, 136].

SARS-CoV-2 infection is thought to disrupt the integrity of the pulmonary epithelial barrier, cause local immune dysregulation, and impair leukocyte function, thereby predisposing patients to *Aspergillus* invasion [10, 137, 138]. In addition, the extensive use of corticosteroids and immunomodulatory agents is also considered an important predisposing factor for CAPA [13, 123, 139]. Moreover, ICU interventions such as tracheal intubation and mechanical ventilation may further compromise the respiratory epithelial barrier, while broad-spectrum antibiotic therapy can contribute to respiratory microbial dysbiosis; together, these factors may facilitate *Aspergillus* colonization and infection [13, 140–142]. Notably, some patients, although lacking systemic immunosuppression, may still exhibit susceptibility to CAPA due to localized immune dysfunction and a pro-inflammatory/oxidative milieu; meanwhile, pathogen fitness *in vivo* is closely linked to oxidative stress defenses and metal (e.g., iron/copper) homeostasis pathways [136, 143–147].

In summary, the high-risk population for CAPA has expanded from traditionally immunocompromised individuals to include critically ill COVID-19 patients and those with localized immune impairment. This shift underscores the need to update fungal infection risk assessment strategies and to focus on susceptibility mechanisms driven by viral infection and localized immune dysregulation [10, 136, 148].

5.4 Pathogenic *Aspergillus* species and other unexplored cryptic species in CAPA cases

Alongside the rising number of CAPA cases, a broader spectrum of pathogenic *Aspergillus* species recovered from clinical samples has been reported. CAPA is still attributed primarily to *A. fumigatus*, which is regarded as

the predominant pathogen due to its strong pathogenic potential and ubiquitous environmental distribution [3]. However, CAPA cohorts and case compilations have also documented non-*fumigatus* species, including *A. flavus*, *A. niger*, and *A. terreus*, and occasionally other less common species [9, 149, 150].

Although phylogenetic analyses have clarified the taxonomic positions of cryptic species, their accurate clinical identification remains challenging in routine workflows. Many cryptic species cannot be reliably distinguished using conventional culture- and morphology-based diagnostics, leading to potential pathogen misidentification, inappropriate antifungal therapy, and delayed treatment [19, 151]. Notably, in the post-COVID-19 era, an increasing number of CAPA reports have drawn attention to cryptic and non-*fumigatus* species, yet their contribution may still be underestimated when isolates are reported only at the “*A. fumigatus* complex” level [19, 151]. For instance, *A. lentulus* (a member of the *A. fumigatus* complex) often shows reduced susceptibility to triazoles, including voriconazole; thus, misidentifying it as *A. fumigatus* and treating it with first-line azoles may compromise therapeutic efficacy [152, 153].

The clinical relevance of cryptic species in aspergillosis, including CAPA, is increasingly recognized, yet their epidemiology, virulence, and antifungal susceptibility profiles remain insufficiently characterized. Future efforts should focus on enhancing species-level identification capacity in clinical laboratories and strengthening awareness of the clinical implications of cryptic species to minimize diagnostic and therapeutic biases arising from species misidentification [19, 151].

6 Therapeutic and preventive challenges against *Aspergillus* infections

6.1 Major classes and mechanistic features of anti-fungal agents

Currently, antifungal agents used to treat *Aspergillus* infections are mainly classified into three major categories—azoles, echinocandins, and polyenes—along with several investigational compounds in late-stage clinical development. These drug classes act through distinct molecular mechanisms, which determine their differences in clinical indications, tissue distribution, pharmacokinetics, and resistance profiles.

Azoles are the cornerstone of therapy for invasive aspergillosis (IA). Their antifungal activity is primarily mediated through inhibition of cytochrome P450 14 α -sterol demethylase (CYP51), a key enzyme involved in fungal cell membrane biosynthesis. This inhibition disrupts ergosterol synthesis, leading to impaired membrane integrity and cellular function. Commonly used mold-active triazoles include voriconazole, isavuconazole, and posaconazole (with itraconazole used mainly in selected clinical contexts). Among these, voriconazole has long been a standard-of-care option for IA, supported by randomized clinical evidence, while isavuconazole has been shown to be non-inferior to voriconazole and is widely used as an alternative first-line agent [6, 154, 155]. Notably, posaconazole has also been supported by phase 3 non-inferiority evidence as a primary treatment option for IA, representing an important recent advance [156]. However, clinically relevant differences exist among triazoles in terms of tissue penetration and drug-drug interactions, thereby necessitating individualized dosing strategies and, for several agents, therapeutic drug monitoring [6].

Echinocandins exert their antifungal activity by inhibiting β -1,3-glucan synthase, thereby blocking fungal cell wall synthesis. This class of drugs represents a distinct mechanism targeting the fungal cell wall rather than the cell membrane. Representative drugs include caspofungin, micafungin, and anidulafungin. Echinocandins generally exhibit fungistatic rather than fungicidal effects against *Aspergillus* spp.; therefore, they are not recommended as monotherapy for primary treatment of IA, but may be used as salvage therapy or in combination regimens [6]. When combined with other antifungal agents, echinocandins can show additive or synergistic effects, and randomized trial evidence has evaluated triazole-echinocandin combinations [157].

Polyenes, represented by amphotericin B, exert their antifungal activity by binding to ergosterol in the fungal cell membrane, forming transmembrane pores that cause leakage of intracellular components and ultimately cell death. Although amphotericin B displays broad-spectrum antifungal efficacy, its clinical use is limited by nephrotoxicity with conventional formulations. Consequently, lipid-based formulations such as liposomal amphotericin B (L-AmB) are commonly used to reduce toxicity and may serve as an important alternative option when triazoles cannot be used or when resistance is suspected, in addition to their role in salvage therapy [6].

Beyond these classical antifungal classes, several investigational agents have provided new therapeutic prospects for infections caused by drug-resistant *Aspergillus* strains. Olorofim (F901318), a first-in-class orotomide targeting dihydroorotate dehydrogenase (DHODH) in pyrimidine biosynthesis, has shown activity against *Aspergillus* species, including azole-resistant isolates, and remains under clinical evaluation for refractory invasive mold infections [158]. Fosmanogepix (a prodrug of manogepix) targets Gwt1, a key enzyme in glycosylphosphatidylinositol (GPI) anchor biosynthesis, and is also under clinical development with reported anti-*Aspergillus* activity [159]. In addition, newer glucan synthase inhibitors include the triterpenoid ibrexafungerp and the long-acting echinocandin rezafungin; however, their current regulatory approvals are for *Candida*-related indications rather than aspergillosis, and their clinical roles in *Aspergillus* infections remain to be established [160].

In summary, currently available antifungal agents against *Aspergillus* target multiple cellular pathways, including membrane integrity, cell wall synthesis, and (for emerging agents) pyrimidine and GPI-anchor biosynthesis. However, their clinical application remains challenged by complex drug-drug interactions, limited tissue penetration at certain sites, and emerging resistance. Future efforts should prioritize the development of agents with diverse mechanisms of action, and the optimization of individualized combination therapies to effectively address the evolving spectrum and epidemiology of *Aspergillus* infections.

6.2 Combination therapy and personalized treatment

In the management of IA, combination therapy strategies have gained increasing attention, particularly among high-risk or critically ill patients and in settings with potential antifungal resistance; however, routine upfront combination therapy is not universally recommended, and current guidelines suggest it may be considered only in selected patients with documented IPA [6, 161]. Traditional monotherapy, such as voriconazole or amphotericin B, can control infection to some extent; however, its efficacy is often constrained by factors such as pathogen resistance, pharmacokinetic variability (including drug-drug interactions), and host immune status [162, 163]. Therefore, to enhance antifungal efficacy and potentially improve outcomes in difficult-to-treat disease, increasing clinical research has focused on the combined use of tria-

zoles with echinocandins and other adjunctive approaches [6, 157].

The rationale for combination therapy lies primarily in synergistic interactions and complementary molecular targets. For example, a randomized trial evaluating voriconazole plus anidulafungin versus voriconazole monotherapy did not demonstrate a definitive overall mortality benefit, but suggested potential benefit in selected subgroups (e.g., galactomannan-positive patients) [157]. Earlier observational studies have also explored triazole-echinocandin combinations (e.g., voriconazole plus caspofungin), but the evidence base remains limited compared with randomized data [164]. In addition, preclinical studies have reported enhanced anti-*Aspergillus* activity when antifungal drugs are combined with certain adjunctive agents or host-directed immunomodulatory strategies, providing further insights for combination therapy design [165, 166]. Representative combination regimens, their target populations, and mechanistic features are summarized in Table 6.

Meanwhile, personalized treatment approaches are also advancing. Given the variability in pharmacokinetics, organ function, drug-drug interactions, and immune background among patients, precise selection of the optimal drug regimen and dosing strategy is critical for improving therapeutic outcomes, and therapeutic drug monitoring (TDM) is recommended/commonly used for selected triazoles such as voriconazole in clinical practice [6, 167]. Some clinical centers have begun to implement individualized antifungal management guided by TDM and infection-type classification. In critically ill patients (including CAPA/viral-associated IPA), combination regimens may be used empirically in selected severe cases, but high-quality evidence for routine use remains insufficient [6,

162]. Furthermore, with the ongoing development of multi-target antifungal agents, future combination regimens will place greater emphasis on target complementarity and alignment with the patient's immune status [160].

Overall, combination and personalized therapeutic strategies represent key directions in post-pandemic antifungal management, aiming to maximize efficacy, minimize resistance risk, and enable more precise clinical interventions.

6.3 Clinical challenges and possible reasons for treatment failure

Despite continuous advances in antifungal therapeutics, IA still exhibits high rates of treatment failure and mortality. Major clinical trials and observational cohorts indicate that approximately one-third to nearly one-half of IA patients may experience treatment response failure and/or death despite standard antifungal therapy, with even higher rates observed among profoundly immunocompromised populations [6, 154]. The underlying causes are multifactorial, encompassing delays or inaccuracies in both diagnostic and therapeutic processes, increasing antifungal resistance, misidentification of cryptic species, and host immune dysfunction.

Diagnostic delay remains one of the primary determinants of poor outcomes in IA. The early clinical manifestations of IA are often nonspecific, and radiological findings frequently overlap with other pulmonary infections. Moreover, the diagnostic performance of commonly used biomarkers, such as galactomannan (GM) and β -D-glucan, is variable across host populations and clinical contexts, and false positivity/negativity may occur, leading to misdiagnosis or delayed confirmation and, consequently, loss of the optimal therapeutic window [6, 168]. In critically ill patients, particularly those with CAPA, addition-

Table 6 Combination regimens for *Aspergillus* infections

Combination regimen	Drug class combination	Clinical context/target population	Mechanistic features
Voriconazole + Echinocandin (e.g., caspofungin/anidulafungin)	Azole + Echinocandin	Severe IA; selected high-risk patients; salvage/poor response	Dual targeting (membrane + cell wall); potential add-on benefit
Isavuconazole + Echinocandin	Triazole + Echinocandin	Severe IA/CAPA in practice; sub-optimal response or intolerance	Dual targeting; interactions range from synergy to indifference; clinical data limited
Liposomal amphotericin B + Azole	Polyene + Azole	Salvage use in selected cases; not routine	Possible synergy or antagonism depending on exposure; use cautiously
Azole + Adjunctive immunotherapy (e.g., IFN- γ /GM-CSF; selected)	Antifungal + Host-directed adjunct	Profound immune dysfunction; refractory IA (highly selected)	Host immune augmentation to support antifungal activity
Fosmanogepix + Liposomal amphotericin B	Novel-target agent + Polyene	Experimental/early clinical context; refractory invasive mold disease	Dual targeting; combination benefit supported mainly by animal models

IA, invasive aspergillosis; CAPA, COVID-19-associated pulmonary aspergillosis; IFN- γ , interferon gamma; GM-CSF, granulocyte-macrophage colony-stimulating factor.

al obstacles include the difficulty of obtaining high-quality lower-respiratory samples and the presence of complex co-infections, both of which complicate early detection [10].

Antifungal resistance and cryptic species misidentification represent additional key contributors to therapeutic failure. In recent years, azole-resistant *A. fumigatus* isolates have been increasingly reported in clinical settings across multiple regions, substantially compromising first-line drug efficacy [169, 170]. Furthermore, cryptic species such as *A. lentulus* and *A. udagawae* can show reduced susceptibility to commonly used antifungals; if identification relies solely on morphology or conventional culture, these organisms are easily misidentified as *A. fumigatus*, resulting in inappropriate antifungal treatment and poor clinical outcomes [171, 172].

Host immune status and underlying conditions are also decisive factors influencing treatment outcomes in IA. Persistent immunosuppression, corticosteroid exposure, and post-transplant immunomodulation can markedly impair the overall efficacy of antifungal therapy, even when *in vitro* susceptibility appears favorable [6, 173]. In addition, severe hepatic or renal dysfunction limits the safe use and dosing of many antifungal agents, further compounding the therapeutic challenge.

Finally, drug-drug interactions and insufficient drug exposure warrant significant attention. Azoles exhibit extensive metabolic interactions with immunosuppressants and other commonly used ICU medications, which can result in subtherapeutic plasma concentrations or increased toxicity [6, 174]. In the absence of TDM, clinicians face difficulty in achieving optimal dosing precision, thereby constraining therapeutic efficacy and increasing the risk of treatment failure [174].

7 Drug resistance, tolerance, and persistence in *Aspergillus*

7.1 Global trends and challenges in *Aspergillus* resistance

The global spread of COVID-19 has not only altered the clinical spectrum of invasive fungal infections but also intensified concern about the emergence and dissemination of antifungal-resistant *Aspergillus* strains. In the post-pandemic era, the expanded recognition of CAPA and other ICU-associated aspergillosis has increased reliance on

mold-active triazoles in critically ill populations, thereby heightening the clinical relevance of pre-existing resistance reservoirs and potentially adding selection pressure in healthcare settings [175, 176]. In parallel, genomic and epidemiological evidence indicates that a substantial fraction of drug-resistant infections can be acquired from environmental sources, emphasizing the importance of a One Health perspective [113].

In recent years, azole resistance rates have continued to increase across multiple countries and regions. In a genomic epidemiology study of European CAPA isolates, 14.3% (3/21) showed phenotypic azole resistance [177]. In regions such as India and Iran, both environmental and clinical isolates of *A. fumigatus* increasingly harbor canonical *cyp51A* resistance mechanisms (e.g., TR34/L98H and TR46/Y121F/T289A), which are strongly associated with azole resistance [178–181]. In addition, non-*fumigatus* or cryptic species with reduced susceptibility to multiple antifungal classes (e.g., *A. terreus* and *A. lentulus*) are being detected with growing frequency, further complicating empiric azole-based therapy [32, 152, 182].

The widespread agricultural use of azole-based fungicides is considered a major selective pressure for the emergence of environmental azole-resistant strains. Environmental “hot spots” have been identified in settings such as composting and plant-waste material, which can contain azole residues and release high burdens of resistant spores [183, 184]. Together with heterogeneity in laboratory capacity and surveillance frameworks, such environmental reservoirs and monitoring gaps complicate resistance tracking and containment across regions [175, 185, 186].

In summary, antifungal resistance in *Aspergillus* during the post-pandemic era presents multidimensional challenges: the broadened ICU-associated aspergillosis burden increases the clinical impact of resistance; the rising prevalence of resistant isolates limits the efficacy of first-line triazoles; and environmental reservoirs together with uneven surveillance complicate resistance management and containment. Under this evolving landscape, effective aspergillosis control must transcend individual clinical management and be addressed as a coordinated global public-health priority.

7.2 Molecular mechanisms of antifungal resistance in *Aspergillus*

In recent years, extensive research on the molecular

mechanisms of antifungal resistance in *Aspergillus* species, particularly resistance to azole antifungals, has uncovered multiple key mutations and regulatory pathways. In *A. fumigatus*, azole resistance is predominantly associated with alterations in the *cyp51A* gene, which encodes 14 α -sterol demethylase, the primary target of azoles. Common resistance-associated alterations include tandem repeats in the promoter region (e.g., TR34, TR46), which are linked to increased *cyp51A* expression, and point mutations within the coding sequence (e.g., L98H, Y121F, T289A), which can reduce azole susceptibility by affecting the target enzyme. These alterations may occur individually or in combination, such as TR34/L98H and TR46/Y121F/T289A, and together they are frequently associated with high-level azole resistance phenotypes [187, 188].

Beyond *cyp51A*, azole resistance in *A. fumigatus* may also involve altered regulation of *cyp51B* (less commonly reported), as well as activation of transmembrane efflux systems. Multiple transporters have been implicated, including ATP-binding cassette (ABC) transporters such as Cdr1B and other ABC/MFS transporters (e.g., AfuMDR1/AfuMDR4 in some studies), which can actively export azole compounds and lower intracellular drug concentrations [189–191]. Moreover, transcription factors coordinating sterol biosynthesis and efflux regulation have been demonstrated to play critical roles in azole resistance. AtrR directly regulates both *cyp51A* and the ABC efflux pump Cdr1B, whereas SrbA (SREBP) primarily governs sterol biosynthesis and hypoxia adaptation and contributes to azole tolerance, including via regulation of *cyp51A*; therefore, increased transcriptional activity and/or overexpression of these regulators (rather than generic “mutations”) can enhance azole resistance levels in *Aspergillus* [43, 188, 192, 193].

Echinocandins, which inhibit β -1,3-glucan synthase and thereby impair fungal cell wall biosynthesis, were long considered to exhibit reduced efficacy against *Aspergillus* mainly due to intrinsic tolerance rather than frequent acquired resistance. However, recent findings indicate that acquired echinocandin resistance, although rare, can occur and has been linked to *fks1* hot spot mutations, while the caspofungin paradoxical effect is generally viewed as an adaptive cell-wall stress response phenotype rather than stable target-based resistance [194, 195].

Resistance to polyenes such as amphotericin B remains relatively uncommon; when reduced susceptibility is re-

ported, it may involve remodeling of membrane sterol composition and broader stress-response pathways [196, 197].

Taken together, antifungal resistance in *Aspergillus* results from multiple synergistic factors, including target-gene alterations and transcriptional regulation, activation of efflux systems, and metabolic reprogramming. The interplay among these mechanisms not only increases the difficulty of clinical treatment but also places higher demands on the development of next-generation antifungal agents targeting novel molecular pathways.

7.3 Biofilms in persistent and drug-tolerant *Aspergillus* infection

Biofilm formation represents one of the major strategies employed by *Aspergillus* species to evade host immune clearance and withstand antifungal therapy. Within the host, particularly in the airway lumen and chronic pulmonary lesions, *A. fumigatus* adheres to surfaces and secretes extracellular matrix (ECM) components, progressively forming dense and highly organized three-dimensional biofilm-like structures [81, 198, 199]. These structures not only provide physical barriers that limit antifungal penetration, but also enable fungal cells embedded within the biofilm to reprogram metabolic pathways and decrease their susceptibility to antifungal drugs [80, 81]. Under biofilm-forming conditions, biofilm assays frequently show increased sessile MICs (SMICs) or reduced susceptibility to voriconazole and amphotericin B compared with planktonic growth; this phenotype is generally interpreted as biofilm-associated drug tolerance and is not directly comparable with clinical resistance breakpoints [81, 200, 201].

The biofilm-associated drug-tolerant phenotype of *Aspergillus* arises from multiple interconnected mechanisms. These include sequestration of antifungal agents by ECM components, such as polysaccharides and extracellular DNA, as well as reduced metabolic activity, enhanced reactive oxygen species (ROS) detoxification, and alterations in membrane composition, all of which contribute to fungal stress adaptation [81, 198, 202]. In addition, transcription factors and regulators such as SrbA (hypoxia/sterol homeostasis) and MedA (adhesion and host interaction) have been shown to influence biofilm development and related gene networks; disruption of these regulators can impair biofilm maturation or adherence, while SrbA also contributes to azole tolerance under hypoxic conditions [43, 80, 203].

Another major biofilm-associated clinical challenge is persistent infection, in which fungal lesions fail to resolve completely despite antifungal therapy, allowing *Aspergillus* to persist and to reactivate when host immunity fluctuates. Studies and reviews have suggested that biofilms can harbor subpopulations with quiescent or low-metabolic (persister-like) phenotypes, which show reduced susceptibility to fungicidal activity and may contribute to treatment refractoriness and clinical relapse [81, 204, 205]. Persistent biofilm-associated infection is particularly problematic in patients with chronic pulmonary diseases such as chronic obstructive pulmonary disease (COPD) and chronic pulmonary aspergillosis (CPA), where *Aspergillus* may colonize airway surfaces long-term, establishing chronic infectious foci and increasing the risk of treatment failure, recurrence, and poor outcomes [200, 206, 207].

8 Future perspectives

8.1 Discovery and development of novel antifungal agents

Over recent years, the rising global incidence of *Aspergillus* infections and the continued escalation of antifungal resistance have increasingly limited the effectiveness of conventional therapies, driving accelerated efforts toward the development of new anti-*Aspergillus* agents. Several leading candidates in the current pipeline reflect two major innovation directions: 1) first-in-class agents with novel molecular targets, such as the orotomide olorofim (a DHODH inhibitor) and the Gwt1-targeting pro-drug fosmanogepix; and 2) innovations in drug delivery and exposure optimization, exemplified by inhaled triazoles such as opelconazole (designed to achieve high pulmonary exposure with limited systemic exposure), as well as orally available glucan synthase inhibitors such as ibrexafungerp. These agents collectively highlight advances in target novelty and route-of-administration design, and many have progressed into various stages of clinical evaluation, underscoring their translational potential [158, 208].

Among them, olorofim has progressed through phase 2b clinical evaluation for invasive fungal diseases with limited therapeutic options, supporting its potential role in refractory aspergillosis and other difficult-to-treat mould infections [158, 209]. Fosmanogepix, which targets GPI-anchor biosynthesis via Gwt1, has shown broad activity in

preclinical and early clinical development programs and remains a promising future candidate for mould infections [208]. Opelconazole, a novel inhaled triazole formulation, is under clinical evaluation as an adjunct to systemic therapy for pulmonary aspergillosis, aiming to increase local drug exposure in the lung while minimizing systemic toxicity and drug-drug interactions [210].

Overall, next-generation antifungal agents have achieved advances across multiple dimensions, including target innovation, clinical development progress, and optimization of drug-delivery strategies. To facilitate a systematic comparison of their mechanisms, indications, antifungal spectra, and developmental status, key features of representative agents are summarized in Table 7. Although several compounds remain in clinical-trial phases and require further assessment of long-term efficacy and safety, they collectively offer renewed therapeutic potential for managing azole-resistant *Aspergillus* infections.

8.2 Drug repurposing for aspergillosis

In recent years, therapeutic strategies for aspergillosis have expanded beyond de novo antifungal drug development to include drug repurposing and repositioning approaches, particularly in response to the escalating challenge of antifungal resistance. In this context, clinically approved drugs with well-characterized safety profiles, as well as clinical-stage compounds originally developed for non-fungal indications, have emerged as attractive candidates for alternative anti-*Aspergillus* applications [211]. Several non-antifungal agents have been reported to exert inhibitory or adjuvant effects against *A. fumigatus* in experimental settings. For example, brilacidin, a synthetic host defense peptide mimetic originally developed as an antibacterial agent, has been shown to potentiate echinocandin activity (e.g., caspofungin) against *A. fumigatus* [212]. In parallel, a range of natural compounds and commonly used drugs (e.g., quercetin and tetracycline-class antibiotics as adjuvants) have exhibited anti-*Aspergillus* potential *in vitro* and/or in infection models, with reported effects including inhibition of hyphal growth, interference with virulence-associated traits, and modulation of host responses [213–215].

Beyond the agents discussed above, drug repurposing studies have also explored a broader range of non-antifungal drugs, including anti-inflammatory agents, antiparasitic drugs, oncology therapeutics, and even antibacterial antibiotics used as antifungal adjuvants [211]. For example, the polymyxin antibiotic colistin can potentiate caspo-

Table 7 Preclinical-stage antifungal drugs against *Aspergillus* infections

Drug	Mechanism of action	Antifungal spectrum	Clinical indications	Clinical development stage	Key features advantages
Olorofim	DHODH inhibitor	Strong activity against <i>Aspergillus</i> spp. (incl. difficult-to-treat cases); limited activity against many yeasts	Invasive aspergillosis with limited options (e.g., refractory or unsuitable for azoles)	Phase III (OASIS)	Novel target oral dosing limited cross-resistance potential
Fosmanogepix	Gwt1 inhibitor	Broad spectrum, including <i>Aspergillus</i> and other molds	Invasive mold infections; candidemia invasive candidiasis	Phase III (candidemia invasive candidiasis); Phase III initiated for invasive mold infections	IV and oral formulations favorable tissue distribution novel target
Opelconazole	Triazole CYP51 inhibitor	Predominantly <i>Aspergillus</i> spp.	Pulmonary aspergillosis settings (e.g., chronic or adjunct approaches)	Phase II/III (ongoing evaluation)	Inhaled delivery, high pulmonary exposure, low systemic toxicity
Ibrexafungerp	β -1,3-glucan synthase inhibitor (distinct binding vs echinocandins)	Primarily <i>Candida</i> ; limited <i>Aspergillus</i> activity	<i>Candida</i> -focused indications; <i>Aspergillus</i> role investigational	Approved for selected <i>Candida</i> indications; additional studies ongoing	Oral option cell wall target alternative scaffold
Rezafungin	Long-acting echinocandin β -1,3-glucan synthase inhibitor	<i>Candida</i> strong; <i>Aspergillus</i> activity similar to the echinocandin class	<i>Candida</i> -focused invasive infections; <i>Aspergillus</i> mainly adjunct combination context	Late stage/approved in some regions for <i>Candida</i> indications	Once-weekly dosing improved PK

DHODH, dihydroorotate dehydrogenase; OASIS, Olorofim *Aspergillus* Infection Study (Phase III trial; NCT05101187); CYP51, cytochrome P450 51 (sterol 14 α -demethylase); incl., including; IV, intravenous; vs, versus; PK, pharmacokinetics.

fungin activity against *A. fumigatus* by perturbing membrane permeability and modulating calcium homeostasis/stress responses, with efficacy supported across *in vitro* and *in vivo* infection models [216]. Notably, miltefosine (an antiparasitic drug) has demonstrated activity against *A. fumigatus* *in vitro* and *in vivo*, supporting its potential as a repurposing lead for difficult-to-treat aspergillosis [217]. Such agents may exert antifungal effects through diverse mechanisms, including interference with fungal signaling pathways, disruption of membrane integrity, or modulation of metal ion homeostasis, thereby showing potential activity against drug-resistant strains [211]. Collectively, these findings suggest that drug repurposing represents a viable approach to broadening clinical antifungal treatment options, offering opportunities to reduce the time and cost associated with conventional drug development. Representative repurposed agents and their proposed mechanisms of action are summarized in Table 8.

8.3 Multiple targetted strategies against *Aspergillus* infections

The clinical efficacy of single-agent antifungal therapies has been increasingly constrained by the rising prevalence of resistant strains, the high conservation of antifungal drug targets, and the protective effects conferred by fungal biofilms. These limitations have driven growing interest in multi-target combination strategies for the development of novel anti-*Aspergillus* therapeutics. Such

strategies encompass not only the combined use of agents with distinct mechanisms of action, but also the integration of multiple active moieties within a single molecular scaffold to simultaneously disrupt several essential fungal physiological processes. For example, combining cell-wall-active agents with inhibitors of fungal stress-response signaling (e.g., the calcineurin-Hsp90 network) can concurrently weaken cell-wall integrity and blunt adaptive stress tolerance, leading to enhanced antifungal activity against *A. fumigatus* in experimental settings [218, 219]. In addition, epigenetic modulators such as histone deacetylase inhibitors have been reported to potentiate azole activity against *Aspergillus*, including biofilm-associated growth, supporting their potential as adjuvant components in multi-target regimens [220].

Notably, combinations of antifungal agents with immunomodulatory factors have emerged as an important research direction in the post-pandemic era. Approaches that pair antifungal drugs with immune checkpoint modulators, vaccine adjuvants, or host defense peptides aim to simultaneously enhance host immune responses and antifungal efficacy [221, 222]. Such strategies may reduce the required dosage of individual drugs, mitigate toxicity, and potentially delay the emergence of antifungal resistance. Looking ahead, with continued advances in high-throughput drug screening, computer-aided drug design, and pharmacodynamic modeling, multi-target combination strategies are expected to play an increasingly important

Table 8 Repurposed drug candidates for therapy against *Aspergillus* infections

Drug	Original indication/class	Proposed anti- <i>Aspergillus</i> mechanism	Validation approach
Brilacidin	HDP mimetic was originally developed as antibacterial	Potentiates echinocandin activity; membrane-active stress sensitization	<i>In vitro</i> assays; infection models reported
Ebselen	Organoselenium compound (repurposed agent)	Redox-active antifungal effect; reduced fungal burden <i>in vivo</i>	Murine invasive aspergillosis model
Quercetin	Natural flavonoid	Reduces fungal load and suppresses TLR4-driven inflammation	<i>In vitro</i> assays and animal models (keratitis)
Minocycline	Tetracycline-class antibiotic	Potentiates amphotericin B activity against <i>Aspergillus</i> spp.; the mechanism is not fully defined	<i>In vitro</i> assays
Glabridin	Licorice-derived natural compound	Immunomodulation (Dectin-2/NLRP3-related) with reduced disease severity in keratitis models	Animal models (keratitis)

HDP, host defense peptide; TLR4, Toll-like receptor 4; Dectin-2, dendritic cell-associated C-type lectin 2 (CLEC6A); NLRP3, NOD-, LRR- and pyrin domain-containing protein 3.

role in the individualized treatment of invasive aspergillosis and to emerge as a key direction in antifungal drug development.

Beyond cytokine adjuvants, host-directed adoptive immunotherapies are being explored to compensate for impaired antifungal immunity in high-risk patients, including the adoptive transfer of *Aspergillus*-specific T cells and genetically engineered chimeric antigen receptor (CAR) T cells [223–225]. Dectin-1 (CLEC7A)-based CAR T cells (D-CAR), which leverage the β -glucan-binding extracellular domain of Dectin-1, have shown direct anti-hyphal activity against *A. fumigatus* *in vitro* and *in vivo* [224, 225]. More recently, human T cells were engineered to express a CAR that targets a conserved *A. fumigatus* hyphal cell-wall protein antigen (Af-CAR), and adoptive transfer of these cells reduced fungal burden and improved survival in preclinical invasive pulmonary aspergillosis (IPA) models [226]. Although key translational challenges remain (e.g., antigen specificity, manufacturing time, and the risk of cytokine-associated toxicities, as well as optimal integration with antifungal agents), these studies provide a forward-looking yet plausible adjunctive strategy for profoundly immunocompromised hosts [225, 227]. In parallel, immune checkpoint modulation—most notably programmed cell death protein 1 (PD-1) blockade—has shown therapeutic benefit and synergy with caspofungin in murine IPA models, supporting further evaluation in carefully selected contexts [221, 228].

8.4 Frontiers and challenges in fungal vaccine development

In the post-pandemic era, the development of fungal vaccines has increasingly been recognized as a promising strategy to address the rising incidence of aspergillosis and the ongoing spread of antifungal resistance. Current

vaccine research targeting *A. fumigatus* has primarily focused on four major categories: protein subunit vaccines, polysaccharide- or glycopeptide-conjugate vaccines, live-attenuated or inactivated whole-cell vaccines, and vector-based or other genetically engineered vaccines [229, 230]. Among these, protein subunit vaccines based on immunodominant antigens such as AspF3 (and other candidate antigens including Crf1/AspF9 in some formulations) have been shown to induce protective cellular immune responses in animal models, leading to a significant reduction in pulmonary fungal burden [230–232]. In addition, β -glucan (laminarin)-protein conjugate vaccines can elicit protective anti- β -glucan antibodies and have shown cross-fungal protection in experimental models [233, 234].

In recent years, host-directed immunomodulatory strategies have been incorporated into fungal vaccine design, including the use of optimized immune adjuvants and dendritic cell-based vaccine approaches to enhance vaccine immunogenicity [222, 230]. Moreover, delivery platforms based on nanoparticles, liposomes, or viral-vector/DNA strategies have shown advantages in improving antigen stability and targeting efficiency, thereby enhancing mucosal immune responses [229, 230].

Despite these advances, fungal vaccine development continues to face multiple challenges. The populations most susceptible to aspergillosis are predominantly immunocompromised patients, whose capacity to mount effective immune responses is inherently limited. In addition, the complex interactions between fungi and the host immune system remain incompletely understood. Other major obstacles include the lack of broadly applicable preclinical models and reliable immune correlates of protection, the translational gap between experimental efficacy and clinical feasibility (e.g., immunogenicity and safety in immunocompromised hosts, delivery practicality, and

manufacturing scalability), as well as the high cost and prolonged timelines associated with clinical trials [222, 230].

Overall, future efforts in *Aspergillus* vaccine development will require continued breakthroughs in antigen selection, optimization of delivery systems, and translation-al strategies to achieve safe, effective, and broadly appli-cable immunoprophylactic approaches for high-risk popu-lations.

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Author's contributions

Author 1: Can Yuan: Data curation, validation, writing manuscript.

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Author 3: Ling Lu: Data curation, Project administration, writing manuscript.

Data availability

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