Review

Recent Advances and Future Perspectives in Mitigating Invasive Antifungal-Resistant Pathogen *Aspergillus fumigatus* in Africa

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Abstract

Purpose of Review Azole resistance in *Aspergillus fumigatus* is an emerging public health issue with global distribution and has been linked to use in agricultural and horticultural settings. In 2022, the World Health Organization (WHO) created a fungal pathogen priority list, and *A. fumigatus* was listed as a critical pathogen. Currently, Africa lacks effective surveillance systems for this emerging threat, mostly due to lack of capacity and diagnostics to determine azole resistance in routine clinical settings. This review aims to address and improve on the current diagnostic tools and future perspective strategies in tackling clinical and environmental antifungal-resistant (AFR) *A. fumigatus* in Africa. We emphasized on the importance of early diagnosis and misdiagnosis associated with aspergillosis

caused by *Aspergillus* sp., cross talk between clinical and environmental, mode of action and resistance mechanism, collaborative one health approach, and future perspectives for AFR *A. fumigatus* management strategies.

Recent Findings Early diagnosis and effective management of invasive aspergillosis are critical. On the continent, very few laboratories routinely conduct antifungal susceptibility testing on *Aspergillus* species. Where this occurs, it is culture-based in vitro antifungal susceptibility testing. Drug repurposing and the need for a non-culture-based molecular method (PCR) are critical.

Summary Enhancing promising future perspectives of non-cultured approaches such as whole-genome sequencing, CRISPR/Cas9, and RNAi-mediated technologies to complement the culture-based approach as important strategies to mitigate and overcome emerging issues of AFR *A. fumigatus* in Africa.

Introduction

The prevalence of fungal infections is increasing and poses a global threat to humans [1]. Over 1.6 million deaths annually are caused by fungal diseases globally, and more than 1 billion people are at extremely high risk from severe fungal infections [2, 3]. There are several reasons for this increase, and the major risk factor for invasive fungal infections is the prolonged survival in immunocompromised patients with diseases such as HIV/AIDS, chronic obstructive pulmonary disease (COPD), tuberculosis (TB), and severe acute respiratory syndrome coronavirus (SARS-CoV-2) [4–6]. Fungal infections occur across a range of medical conditions, usually as co-infections or opportunistic infections [7, 8]. Therefore, invasive fungal infections are complicated, which hinders diagnosis and management of immunocompromised patients [9].

The World Health Organization (WHO) developed the first global fungal priority pathogens list (FPPL), which aligns with public health requirements [10]. Recently, the WHO updated the list of fungal pathogens into three priority groups (critical, high, and medium) using a multi-criteria decision analysis (MCDA) approach for use in public health systems in response to fungal infections and antifungal resistance [11•]. The critical group of priority pathogens are *Aspergillus fumigatus*, *Candida albicans*, *Candida auris*, and *Cryptococcus neoformans*. Therefore, the focus is on invasive acute and subacute systemic causative fungal pathogens with drug resistance or management challenges.

Africa accounts for one fifth of the world's population that are at risk for contracting fungal infections with limited access to healthcare, harmful environmental factors, and generally poor livelihoods [12]. The high incidence of fungal infections in Africa is a major concern, and not all healthcare systems are able to address this challenge. Data compiled by the Global Fund Action for Fungal Infections (GAFFI) suggests that 47.6 million Africans suffer from fungal diseases, of which 1.7 million suffer from a severe fungal infection [13]. However, these assessments are based on data from only a handful of African countries and likely underestimate actual prevalence. Fungal infections such as bloodstream infections, wound infections, and urinary tract infections are caused by antifungal-resistant (AFR) *A. fumigatus* pathogen, which have been reported in Nigeria and West Africa [14].

To date, the treatment of invasive fungal infections caused by *Aspergillus* spp. relies primarily on these antifungals: amphotericin B (polyenes), fluconazole, voriconazole, itraconazole (azoles), and caspofungin (echinocandins) [15–17]. However, resistant fungi react quickly to chemical attacks [18], and failed treatment is a common consequence of resistance. This is attributed to an interplay between underlying host immunodeficiencies, antifungal drug properties (drug-target interactions, pharmacokinetics, and pharmacodynamics) [19], biofilm formation [20], and fungal properties (varied cell morphologies, antifungal tolerance, antifungal resistance, genetic mutations,

and induced protective mechanisms) [21, 22]. Rapid plasmid-mediated spread of resistance has not been demonstrated in fungi relative to bacteria [23], and such mechanisms need to be investigated.

Unfortunately, studies report global resistance to azole, echinocandins, and polyene antifungal drugs in both clinical and environmental fungal strains, which were previously effective against *A. fumigatus* [15, 24] and *C. auris* [25, 26••]. Azole drug resistance has severe clinical consequences, with retrospective studies showing a 25% increase in mortality at day 90 in patients with drug-resistant aspergillosis relative to patients with wild-type (WT) infections [27]. In general, the prevalence of azole resistance in *A. fumigatus* is characterized by the expression-upregulating tandem repeat (TR) mechanisms in the promoter region of the *Cyp51A* gene through point mutations, which decrease the affinity of azoles for the target protein [28, 29]. Hence, the most common alleles, namely TR34/ L98H and TR46/Y121F/T289A, are associated with high levels of itraconazole and voriconazole resistance [30], both inside and outside clinics [27].

In Africa, emerging high drug-resistant pathogens are gaining clinical importance [26••]. Modern genomic epidemiological methods have been extensively explored and revealed possible eco-evolutionary associations between the environment and increasing clinical resistance of the azole-resistant genotypes *A. fumigatus* [24] in patients with no history of antifungal treatments. However, ecological "hotspots" have been postulated [18], where both biotic and abiotic conditions allow fungal growth when exposed to azole concentrations below the minimum inhibitory concentration (MIC), creating suitable conditions for adaptation to drug pressure. Therefore, these pathogens need to be screened and ascertained for the burden of outbreak infections if they are to be prioritized for health interventions. In this review, we focused on these priority areas with the aim of outlining current advances and future perspectives regarding key research strategies needed to mitigate the invasive antifungal-resistant pathogen *A. fumigatus* in Africa.

Diagnosis and misdiagnosis associated with aspergillosis

Aspergillosis is undoubtedly the most common human disease, ranging from superficial to deep-seated and potentially fatal infections [31, 32], for example, otomycosis, onychomycosis, keratitis, chronic pulmonary aspergillosis (CPA), allergic bronchopulmonary aspergillosis (ABPA), saprophytic pulmonary, or sinusoidal aspergillomas, which develop rapidly and are often fatal, especially if diagnosis is delayed or missed [33, 34]. Some of these fungal infections, particularly skin diseases that affect individuals without any preexisting conditions who live and work in close proximity to certain environmental niches [35, 36].

The morphological diagnosis of aspergillosis is usually obtained by the observation of thin, septate, acute-angled, or dichotomously branched hyphae [37]. *Aspergillus* is a large genus of about 250 species that has a number of important pathogens. Some pathogenic species are present in culture contaminants, with varying pathogenicity and susceptibility to antifungal agents. The number of these pathogenic species is increasing rapidly due to adaptation to temperature, and their numbers may be currently underestimated [36]. Nonetheless, the rapid and accurate identification of clinical isolates is very important in selecting appropriate antifungal agents.

Diagnostic challenges also occur at the morphological level, as there are some hyaline septate molds that appear to be indistinguishable. For example, *Fusarium* spp*.*, *Scedosporium* sp*.*, and *Pseudallescheria* sp. were diagnosed

in patients between histology and culture ranges from 17 to 22% [38–40]. The therapeutic importance of this is significant, as there are significant differences between the treatment of aspergillosis and that of mucormycosis (mucormycetes). Molecular methods are more time-consuming than direct microscopy and response to treatment depends on early diagnosis and the use of appropriate antifungal agents and, therefore, considered a diagnostic challenge [41].

Clinical diagnosis of aspergillosis is often challenging due to nonspecific clinical features, and the significance of this is that they are being treated with wrong medicines [42, 43]. Therefore, most lesions do not respond well to therapy and may become chronic or kill the patient. The prevalence of onychomycosis due to *Aspergillus* sp. varies in different parts of the world. The incidence of onychomycosis by *Aspergillus* has shown an increase in recent years, representing 34–60% of onychomycosis due to non-dermatophyte molds [44, 45]. Several *Aspergillus* spp. have been isolated from human nails such as *A. fumigatus*, *A. flavus*, *A. versicolor*, *A. niger*, *A. terreus*, *A. sclerotiorum*, and *A. nidulans* [44]. *Aspergillus* onychomycosis infections are often misdiagnosed and targeted with fluconazole leading to treatment failure and chronicity of the disease [46].

Over 3 million cases of chronic pulmonary aspergillosis have been estimated to occur globally each year, and 10 million cases of fungal asthma occur yearly, yet a significant proportion remain undiagnosed in resource-poor communities [3, 47•]. In addition, fungal asthma, caused by airborne fungi such as *Aspergillus* spp., exacerbates asthma in millions of adults and children [48, 49]. Limited diagnosis or misdiagnosis and poor estimates of disease morbidity result in the true burden of disease not being fully known [50]. In pulmonary aspergillosis, the rate of misdiagnosis due to nonspecific clinical findings and atypical radiological manifestations is up to 73% [51]. Previous study showed that chronic pulmonary aspergillosis (CPA) can be diagnosed as bacteriologically negative pulmonary tuberculosis (TB) [52]. This has been attributed to lack of awareness and limited access to *Aspergillus*-specific IgG testing and CT imaging in India. In 29 to 45% of aspergillosis cases, ABPA are often confused with pulmonary TB, and patients receive many doses of anti-tuberculosis drugs before diagnosis are performed [53].

Patients with severe COVID-19 pneumonia are susceptible to secondary viral, bacterial, and fungal infections due to diffuse alveolar lung damage and dysregulated immune response [54–56]. Early diagnosis of these co-infections is important in order to initiate appropriate antimicrobial therapy [57]. Coronavirus disease (SARS-CoV-2, COVID-19)–associated pulmonary aspergillosis (CAPA) is a syndrome affecting COVID-19 patients with acute respiratory distress syndrome (ARDS) requiring intensive care in intensive care units (ICUs), with incidence rates ranging from 3.8 to 33.3% [58], and thus, these diagnostic variations are attributed to differences in the patient populations and CAPA. The diagnosis of CAPA is complex due to the commonly observed atypical radiological features, the lack of established host factors, and the difficulty in obtaining mycological evidence due to the low sensitivity of serum galactomannan in CAPA [59, 60]. Early cases of CAPA were diagnosed postmortem [61], and most patients with severe COVID-19 pneumonia are often too severely sick and hemodynamically unstable to

undergo invasive diagnostic procedures such as bronchoscopy and lavage or lung biopsy. Furthermore, such procedures are contraindicated in COVID-19 patients due to the high risk of generating aerosols that are harmful to both patients and healthcare workers [62, 63].

Invasive pulmonary aspergillosis was diagnosed in 23% of critically ill patients with H1N1 viral infection, a median of 3 days after ICU admission, and corticosteroid use was found to be an independent risk factor for this superinfection [64]. Influenza was identified as an independent risk factor for patients diagnosed with invasive pulmonary aspergillosis and, thus, was associated with high mortality [65]. Previously, patients were often misdiagnosed with rhinocerebral aspergillosis, and invasive aspergillosis was also misdiagnosed, which was confused with a malignant disease [66]. Furthermore, image features of sino-orbital aspergillosis are nonspecific and may be confused with various orbital pathologies, and most common is an idiopathic orbital inflammatory disease [67, 68]. The involvement of the paranasal sinuses is usually a helpful clue in diagnosis, although it may not be present in certain cases; for example, concomitant sinus disease has been reported in 60–90% of cases in previous literature [69]. Orbital aspergillosis has also been misdiagnosed several times as malignancy [70, 71], optic neuritis [72], orbital apex syndrome [73], and typical bacterial cellulitis with orbital abscess [74].

Cross talk between clinical and environmental antifungal resistance *Aspergillus fumigatus*

The extensive application of fungicides containing active ingredients such as azoles and their interminable distribution in the environment are the link between clinical and environmental AFR strains of *A. fumigatus* [73, 74, 75••] (Fig. 1). In addition, recent studies have shown that AFR *A. fumigatus* strains in patients with invasive aspergillosis arise from environmental sources rather than through *de novo* mutation and selection in patients during antifungal drug treatment [24, 76, 77]. Several ecological hotspot niches contain AFR *A. fumigatus* strains, which includes flowerbeds, compost, leaves, seeds, soil, paddy fields, hospital environments, and hospital air samples [75, 78].

Clinically isolated strains have shown cross-resistance to voriconazole, posaconazole, itraconazole, and six triazole fungicides extensively used in agriculture, contributing to the widespread dissemination of multidrug-resistant pathogenic fungi in patients and hospitals [79]. While horizontal gene transfer is an important mechanism for the spread of antibiotic-resistant genes among fungal pathogens, conducting such a study is imperative because it has only been studied in bacteria. However, once multidrug-resistant genotypes merge in fungal pathogens, such genotypes can spread very quickly to other geographical regions and ecological niches through vegetative cells and airborne spores [80, 81]. Previous studies from several countries have shown that clinical strains of AFR *A. fumigatus* are associated with the use of the mentioned fungicides in agriculture and that these resistant strains have identical mutations of TR34/
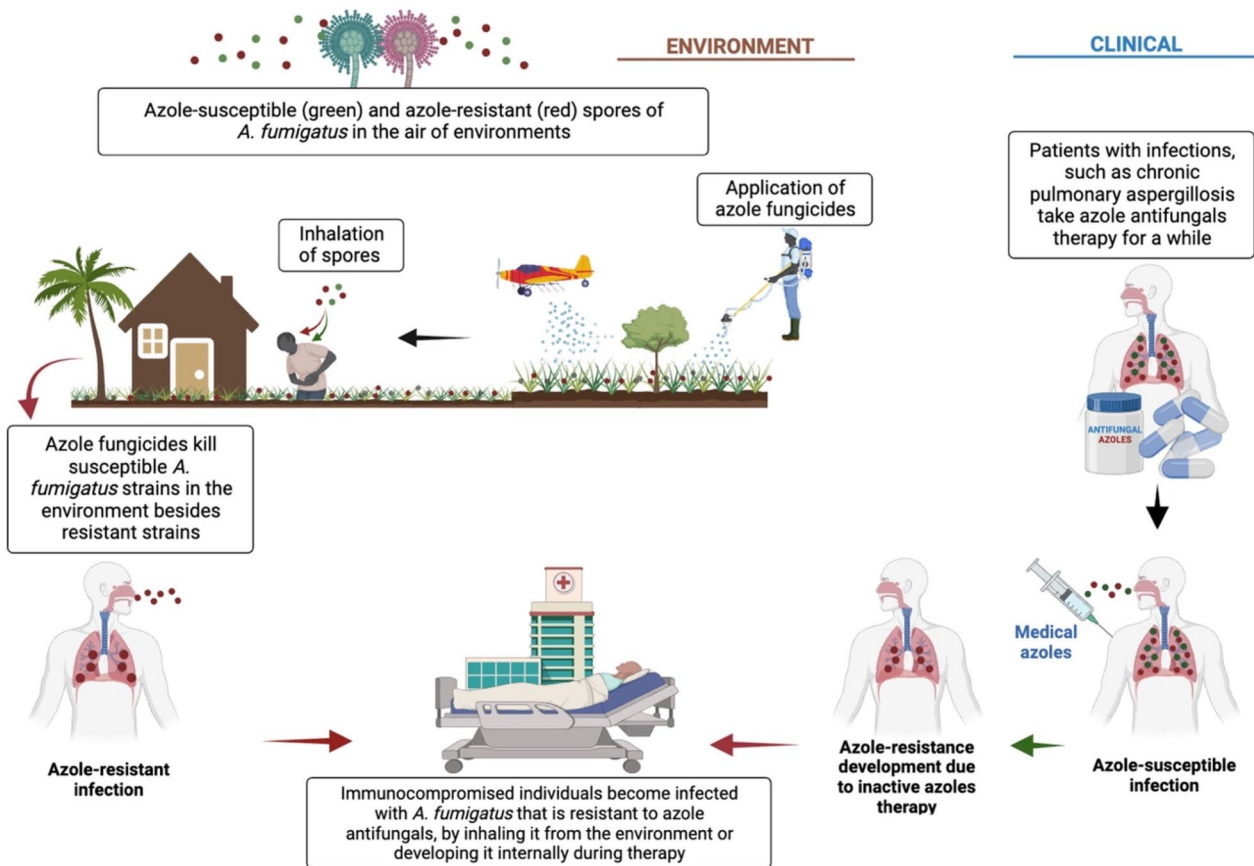
Fig. 1 Possible routes of prevalence of antifungal-resistant *Aspergillus fumigatus* from the environment to the clinical health system through human inhalation. Application of chemical azole fungicides similar to medical azoles in an environment may result in the selection of azole-resistant *A. fumigatus* strains in the environment. Therefore, azole-susceptible individual may develop azole-resistant infections after inhaling these already azole-resistant strains. In the clinical healthcare system, azole-resistant *A. fumigatus* develops in immunocompromised individuals receiving long-term azole therapy for chronic aspergillosis. Figure created with http://biorender.com.

L98H and TR46/Y121F/T289A clones have been found worldwide from both environmental and clinical sources [81, 82]. AFR *A. fumigatus* with the TR34/L98H mutations was identified in the environment of Columbia [83], Portugal [84], Thailand [85], Netherlands [86], and the USA [77]. Clinically isolated strains have showed cross-resistance to voriconazole, posaconazole, itraconazole, and six triazole fungicides extensively used in agriculture, contributing to the widespread dissemination of multidrug-resistant pathogenic fungi in patients and hospitals [79].

Mode of action and mechanisms of resistance

The mode of action (MoA) of all major groups of antifungal agents such as azoles, echinocandins, and polyenes is similar, as they regularly influence cell structure and rigidity by interacting with cell wall or cell membrane components (Fig. 2A). Azoles are classified as either imidazoles or triazoles,
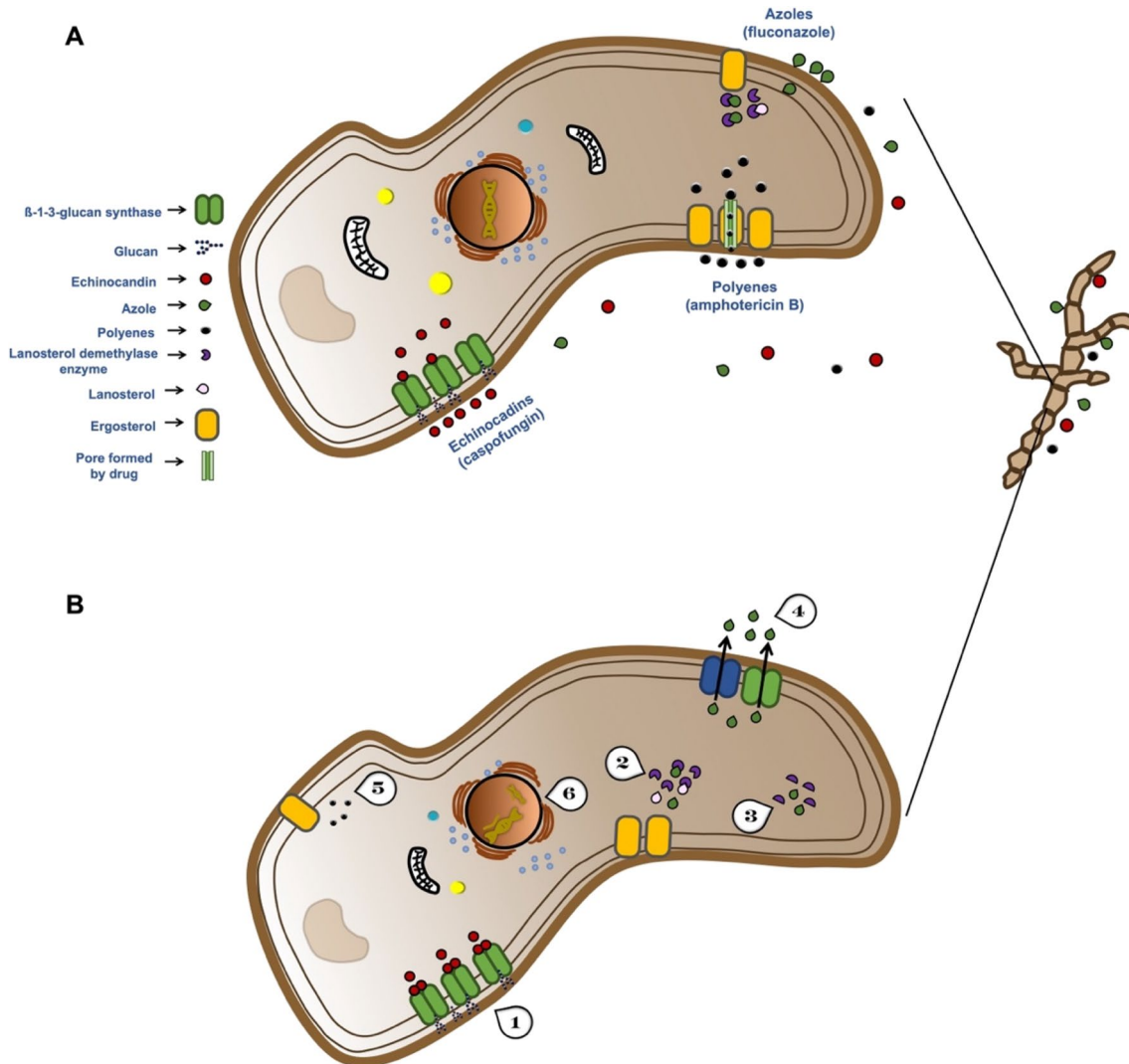

Fig. 2 Schematic representation showing the mode of actions of the primary antifungal drug classes in a fungal cell (**A**) and the known mechanisms of antifungal drug resistance in a fungal cell (**B**)—number (1) refers to mutations in *Fks1/2* that inhibit echinocandins from binding and blocking the drug target 1,3-β-d-glucan synthase, (2) mutations in *Erg11* that leads to overexpression of drug target lanosterol demethylase (LD) protein, (3) mutations in *Cyp51A* that inhibit azoles from binding and blocking LD, (4) increased efflux activity or transporter expression, e.g., ATP-binding cassette (ABC) or major facilitator superfamily (MFS) transporters, (5) ergosterol reduction, and (6) changes in ploidy. Figure created with PowerPoint in http://microsoftoffice.com.

which targets ergosterol biosynthetic pathway in fungal cells by inhibiting the cytochrome P450-dependent enzyme and lanosterol demethylase (LD), which is encoded by the *Erg11* and *Cyp51* genes [87, 88]. The LD enzyme plays an essential role in the synthesis of ergosterol; inhibition of the enzyme results in the accumulation of sterol precursors and 14α-methylated sterols in the fungal cell membranes [89, 90]. This means that azole exposure in the fungal cells leads to a reduction in ergosterol, altered plasma membrane structure, leakage of cell contents, increased cell permeability, and impaired growth [91, 92]. Therefore, it is important that azoles effectively bind to fungal lanosterol 14α-sterol-demethylase without affecting drug metabolism in the host. Echinocandins have a distinctive mode of action by inhibiting β-1-3-glucan synthase, which leads the breakdown of glucans that are important for cell wall components of several fungi [93]. Finally, polyenes have an unusual mode of action compared to other antifungal drug classes, as they do not bind to a specific enzyme, but instead interact with an important molecule—ergosterol, creating channels in the fungal cell membrane and killing cells by allowing ions and other cellular components to escape [94••].

Resistance mechanisms of AFR can be either intrinsic or acquired [95, 96]. Intrinsic resistance includes inherent resistance to antimicrobial drugs; for example, *A. fumigatus* [97] and other *Aspergillus* sp. [98] are intrinsically resistant to fluconazole. In contrast, acquired resistance is usually a result of exposure to antimicrobial selective pressures and can be caused by mutations, overexpression of resistance gene products, and/or genome rearrangements [99, 100••] (Fig. 2B). Furthermore, several resistance mechanisms of AFR in fungal strains may be because of upregulation of drug efflux and resistance genes or mutation [101], as the efflux pumps are transmembrane proteins that can efficiently transport drugs outside of the cell, thereby decreasing intracellular drug concentrations [102, 103]. In addition, overexpression due to upregulation of *Erg11*/*Cyp51A* genes that encode efflux transporters results in overexpression of these proteins [104]. Finally, mutations in the amino acid sequence of drug targets can also lead to resistance [105]. The most distinctive resistance mechanisms to AFR include the alterations in aneuploids and ergosterol synthesis. For instance, aneuploids are cells that have more or fewer chromosomes than the usual number [106, 107] and have recently been linked to azole AFR [108, 109]. The reduction of ergosterol synthesis through changes in expression or mutation has been shown to result in resistance to polyenes and amphotericin B [94].

Collaborative One Health approach for antifungal resistance *Aspergillus fumigatus*

A collaborative One Health approach that includes contributions at local, regional, national, and global levels could help manage AFR in Africa. Human, animal, and environmental health are closely linked, and responding to the threat of AFR requires the concerted efforts of all stakeholders

such as mycologists (clinical, veterinary, and plant), farmers, and fungicide manufacturers as well as policymakers [110•].

Engaging employees from different sectors will change institutional and sociocultural beliefs to address this threat. Raising awareness through stakeholder networking will significantly reduce the risk and burden of AFR, thereby preserving the efficacy of AFR drugs like azole for clinical use [81]. The primary prevention and control measures for AFR include community engagement through media advertising (radio, television, or social media) and farmer meetings within the farm settlements. Therefore, education and dissemination of information on the effects of excessive and excessive use of azole fungicides on human, animal, and environmental health will be of significant benefit.

By bridging knowledge gaps about the impacts of fungicides and agricultural practices (composting), indiscriminate practices are harnessed. Information such as the effectiveness of applying low fungicide doses, aerating composting, rotating, or mixing azole fungicides with another fungicide with different modes of action limits resistance, reduces the development of azole resistance, and prolongs the benefits of azole [111, 112]. Education about the use of fungicides to prevent disease rather than promote plant growth is an alternative community orientation strategy.

Establishing antifungal stewardship in public health and the environment will improve and promote the appropriate use of azole and limit the risk of cross-resistance [113]. This is achieved by regulating the production and sale of fungicides and obtaining approval before bringing new fungicides to market. In addition, azole fungicides could be withdrawn from the market or replaced by another fungicide with a different mechanism of action or structure and fungicides with the same mode of action as medicinal azole will not be approved. Likewise, avoiding the use of fungicides in gardening or ornamental plant cultivation minimizes harmful effects and reduces the risk of resistance development. In addition, guidelines on dosage and frequency of fungicide application and breeding of resistant plants will limit the risk of cross-resistance and prevent dependence on fungicides [81].

In Africa, surveillance data on AFR is limited, and the prevalence of AFR is still underestimated. Therefore, collaboration between different sectors is urgently needed to enable informed decision-making, advocacy, and policymaking in rationalizing the available medical azole against azole resistance. Coordinated data sharing provides high-quality data to fill surveillance gaps. Creating an efficient standardized data collection registry requires the commitment of all stakeholders such as mycologists (clinical, animal, and plant), environmentalists, and government authorities. The data will increase knowledge and help determine the incidence of AFR across countries and regions. Therefore, addressing the extent of azole resistance in Africa cannot be emphasized due to the unavailability and inaccessibility of alternative therapies.

Interdisciplinary surveillance between researchers and stakeholders should be promoted. Multinational studies on AFR and discovering other potential resistance hotspots in Africa will generate data and solutions useful for public health interventions. In addition, the interlaboratory collection of *Aspergillus* strains will contribute to the knowledge of AFR incidence through

collaboration with equipped clinical, local, regional, and reference laboratories with the capacity for susceptibility testing within a country or outside the African region. Regional data collection provides local data for informed planning and data-driven decisions within a community or place that are of value to a country. Finally, the lack of laboratory capacity to perform antifungal susceptibility testing (AFST) in most of our clinical laboratories requires collaboration with international organizations in capacity building to bridge this disparity.

Future perspective

The review studies described here have provided valuable insights into many aspects surrounding AFR *A. fumigatus* and collectively reported current approaches that could potentially mitigate the pathogen. However, the upsurge needs for improved treatment, and management of aspergillosis fungal pathogens is an ongoing challenge [6, 30, 114]. Special attention should be considered to applying new approaches such as drug repurposing, whole-genome sequencing, and RNA-based technology. Therefore, it is paramount to advance our understanding by deciphering the AFR *A. fumigatus*.

Drug repurposing

Development of repurposed drugs was essentially based on conventional drug delivery, consisting of de novo identification and new molecular units [115]. It is a time-consuming and an expensive process with a high risk of error [116, 117]. However, modern omics technology coupled with systems biology and high-throughput drug screenings enables a novel approach to drug repositioning in mitigating human diseases [118, 119]. Considering the repurposing of drugs to treat emerging diseases, this is a tremendous advantage when certain drugs are not yet available or developed. One of the major challenges in the global fight against infectious diseases is the inconsistent occurrence of diseases and the lack of treatment options for rare diseases, like the novel coronavirus disease (COVID-19) [120, 121] and currently aspergillosis in COVID-19 patients and individuals who have recovered from COVID-19 [122, 123].

The pathway to patient-specific personalization of drugs is important in the advancement and treatment of aspergillosis since the efficacy of drugs can be very dependent on different gene profiles because of the heterogeneity of human diseases [124, 125]. Human diseases are expressed through complex mechanisms that can be ascertained from several sources such as genetic aberrations, infectious diseases, and degenerative diseases [126]. In general, diseases often involve many intricate signaling cascades that vary greatly in specific individuals [127]. Individuals in different human populations have specific sets of inherited or non-inherited genetic abnormalities that can make

certain individuals less responsive or unresponsive to common treatments or drugs [128]. In addition, registered drugs may be worthless to a particular individual if a specific drug target is missing and may not elicit the general response to a particular drug [129]. Therefore, it is crucial for drug personalization to reduce the lack of drug efficacy and drug repurposing.

Whole-genome sequencing

Establishing important analytical approaches to understand the acquisition of AFR *A. fumigatus* in Africa is fundamental to mitigating and controlling invasive fungal infections in patients and clinical environments. Regarding the advent of whole-genome sequencing (WGS) and its accessibility to the scientific domain, new diagnostic assessments using high-throughput DNA sequencing of next-generation sequencing (NGS) approach can provide a significant alternative for diagnostics and establish knowledge of fungal biodiversity for epidemiological purposes [114, 130]. This technique has been revolutionized and is currently being used to study the association between various fungal strains isolated from immunocompromised patients when a cluster of cases occurs in order to comprehend disease outbreaks. It provides large sample sizes, allows evaluation of mutation frequencies that correlate with resistance, and identifies the most dominant community. For example, the successful studies on hospital outbreaks of aspergillosis in immunocompromised patients [131] and in transplant patients were attained using NGS analysis [132, 133•]. In addition, studying the correlation of the sequence of genes associated with resistance in fungal and clinical environmental isolates showing resistance requires understanding how the mutation may affect cell composition linked to drug susceptibility [100]. Another complementary approach is to examine the mechanisms of antifungal resistance with less susceptible strains and higher minimal inhibitory concentration (MIC) to a drug, compared to the control [134].

RNA-based technology

The last decade has produced led to an increase in the number of molecular techniques available to understand the function of genes involved in pathogenesis. However, RNA interference (RNAi) and clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein (Cas-9) (CRISPR-Cas9)-based technology are the leading approaches that can provide valuable insights into aspergillosis [135, 136], its associated pathogens and, thus, have the potential to uncover new and interesting biological and clinical information.

RNA interference pathways are highly conserved in eukaryotes to negatively regulate gene expression by short RNAs or small non-coding RNAs (sRNAs) [137]. The established RNAi pathway produces double-stranded

RNA (dsRNA) by RNA-dependent RNA polymerases, which are eventually processed by Dicer enzymes to produce the sRNAs (Fig. 3). Thus, these endogenous RNAs are used to suppress different target sequences [138, 139•]. RNAi is initiated by introducing a dsRNA, which is homologous to the target sequence. The dsRNA is processed to 21–25 nucleotides by an endonuclease known as DICER. One strand of the resulting small interfering RNAs (siRNA) associates with the effector protein, Argonaute, which then binds and degrades target mRNA in a homology-dependent manner, silencing gene transcript levels.

CRISPR/Cas-9 mechanism generates specific double-stranded pieces at the target locus that trigger DNA repairs [140•] (Fig. 4). The designed single guide RNA (sgRNA) identifies the target sequence in the gene of interest through a complementary base pair. While the Cas-9 nuclease creates double-stranded pieces at a location, three base pairs upstream of the protospacer adjacent motif, then the double-stranded piece becomes cellular repaired either by knockouts (KO) through non-homologous end joining or knock-ins (KI) by homologous recombination. Until date, the *Aspergillus* genus of the *A. fumigatus* offers the largest range of molecular genetic tools for studying gene function [141, 142]. Other related aspergillosis-causing genera lack similar genetic toolkits, and only the RNAi technology was accessible in *A. oryzae* [143]. Fortunately, this grim situation is changing owing to the extension of CRISPR-Cas9 technological approach against relevant medical fungi.
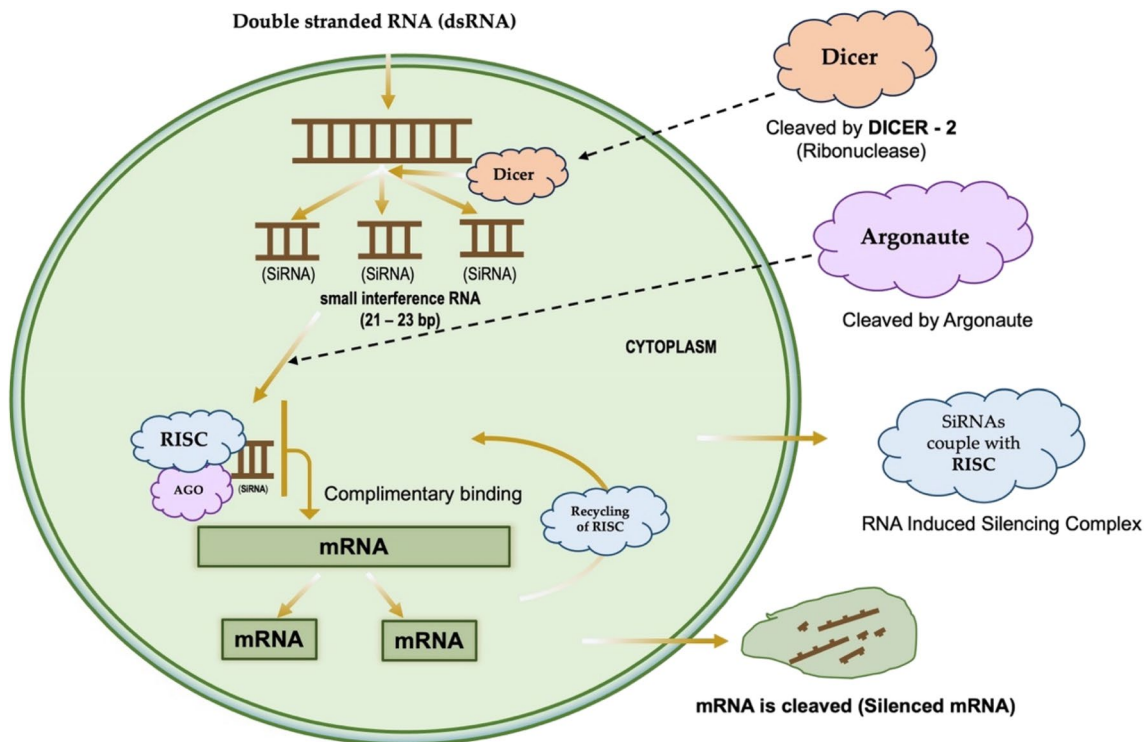

Fig. 3 Schematic representation of RNAi-mediated silencing mechanism in fungi. Figure created with PowerPoint in Microsoft http://office.com.
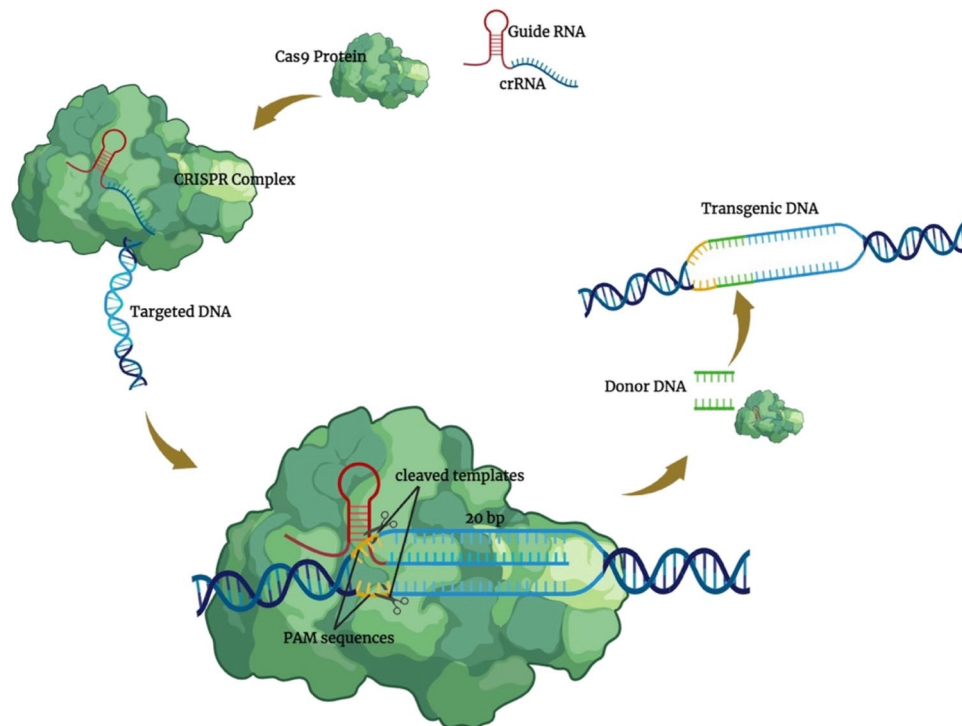
Fig. 4 Schematic representation of CRISPR/Cas9-mediated gene editing mechanism in fungi. Figure created with http://biorender.com.

However, the methods should be optimized as they only work in genes that generate a selectable phenotype upon mutation, making their widespread use impossible. In light of this, a promising plasmid-free CRISPR-Cas9-based method was established that produces stable transformants in *Candida* [144], *Cryptococcus* [145], and *Aspergillus* sp. [146] which can facilitate a rapid process by allowing the targeted mutation of any gene and the use of microhomology repair templates [147].

Special attention should also be given to deciphering the mechanisms that confer intrinsic and acquired resistance to antifungal drugs, in particular which genes of *Aspergillus* fungi are expressed during aspergillosis. It is worth noting that the existing circumstances hamper the progress of treatments to tackle the disease, as advances made in one species are not confirmed in detail in other species or genera. This vital question has been very challenging to answer for technical reasons. Precisely, when isolating total RNA from a host that has been infected with any microbe, thus, the signal from host transcripts usually overcomes the signal from the infecting microbe, and the pathogen RNA consists of only a tiny fraction, about 0.1% of the total amount of RNA extracted [148, 149]. The successful improvement approaches to screen for an enrich fungal transcript from total RNA samples obtained from infected mouse tissues have been applied *in vivo* in mouse infection models of *A. fumigatus* [150, 151]. Therefore, applying careful enrichment RNA transcript methods to investigate the expression of AFR genes on variable isolates of *A. fumigatus* in mouse models of aspergillosis could certainly

elucidate important virulence genes that could potentially be useful as diagnostic biomarkers.

The current innovative approaches to understanding and mitigating the function of genes involved in pathogenesis are RNAi and CRISPR-Cas9-based technology [136]. Thus, these approaches have the potential to uncover new and interesting biological and clinical information of invasive fungal pathogens. Nonetheless, it is crucial that we use comprehensive research tools to combat antifungal pathogens. Therefore, it is of crucial importance to use such techniques in the mitigation and control of AFR *A. fumigatus* in Africa, thereby improving the treatment of aspergillosis disease.

Conclusions

The AFR *A. fumigatus* is widely recognized as a threat to humans throughout the world and the risk posed by this emerging pathogen across Africa, as well as the level of antifungal exposure in the environment and its impact on AFR in humans are understudied. Given the incomplete removal or inactivation of antifungal agents during agricultural practices and the direct application of effective antifungal concentrations to agricultural products, the environmental dimension of AFR requires greater attention since commercial and subsistence agriculture is generally practiced in African countries. The potential and expectation of insight into the recent advances and future perspective strategies can improve the effectiveness of emerging antifungal-resistant pathogen in Africa, and thus, it is critical to invest in reducing the burden and enhancing the clinical outcome of diseases.

To combat AFR *A. fumigatus* in Africa, it is important to improve current diagnostic tools (e.g., antifungal drug susceptibility) and the availability of these tests in poor and disadvantaged settings. In addition, the use of next-generation and metagenomic sequencing has the potential to enable screening of this pathogen. Community engagement and advocacy to improve access to safe use of azole fungicides and effective therapy with triazoles in clinical settings are needed. However, a better understanding of the resistance mechanisms of this fungal pathogen is promising for the development of new strategies to target this pathogen while strengthening the host immune response. Comprehending this resistance mechanism will facilitate the high-throughput screening applications, with the CRISPR/Cas9 system and RNAi silencing–mediated approach are at the forefront of this development on inhibiting known genes associated with AFR resistance. Ultimately, if these novel high-throughput approaches are used to combat the invasive AFR pathogen *A. fumigatus* in Africa, it can be expected that further improvements in this technology will be achieved, and some of the challenges such as efficiency will be overcome in the future.

Author contribution

C.C.A. contributed to the study conception and design. C.C.A, A.D., and O.O.K. wrote the main manuscript. C.C.A. prepared figures 1, 2, 3, 4. C.B.N. and R.O. contributed to the editing of the manuscript. All authors reviewed the manuscript and approved the manuscript for publishing.

Compliance with Ethical Standards

Conflict of Interest
Conrad Chibunna Achilonu declares that he has no conflict of interest. Adeyinka Davies declares that she has no conflict of interest. Okezie O. Kanu declares that he has no conflict of interest. Colin B. Noel declares that he has no conflict of interest. Rita Oladele declares that she has no conflict of interest.

Human and Animal Rights and Informed Consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

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•• Of major importance

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