J Antimicrob Chemother 2017; **72** Suppl 1: i5–i11
doi:10.1093/jac/dkx028

Journal of Antimicrobial Chemotherapy

Changes in the epidemiological landscape of invasive mould infections and disease

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Although a wide variety of pathogens are associated with invasive mould diseases, *Aspergillus* spp. have historically been one of the most common causative organisms. Most invasive mould infections are caused by members of the *Aspergillus fumigatus* species complex and an emerging issue is the occurrence of azole resistance in *A. fumigatus*, with resistance to amphotericin B documented in other *Aspergillus* spp. The epidemiology of invasive fungal disease has shifted in recent years as non-*A. fumigatus Aspergillus* spp. and other moulds have become progressively more important, although there are no consolidated data on the prevalence of less common species of moulds. The incidence of mucormycosis may have been underestimated, which is a potential concern since species belonging to the order Mucorales are more resistant to antifungal agents than *Aspergillus* spp. All species of Mucorales are unaffected by voriconazole and most show moderate resistance *in vitro* to echinocandins. *Fusarium* spp. may be the second most common nosocomial fungal pathogen after *Aspergillus* in some tertiary hospitals, and show a susceptibility profile marked by a higher level of resistance than that of *Aspergillus* spp. Recently, *Scedosporium aurantiacum* has been reported as an emerging opportunistic pathogen, against which voriconazole is the most active antifungal agent. Other mould species can infect humans, although invasive fungal disease occurs less frequently. Since uncommon mould species exhibit individual susceptibility profiles and require tailored clinical management, accurate classification at species level of the aetiological agent in any invasive fungal disease should be regarded as the standard of care.

Aspergillosis

Invasive aspergillosis (IA) currently constitutes the most common cause of infectious pneumonia-related mortality in patients undergoing HSCT and is an important cause of opportunistic respiratory and disseminated infections in other immunocompromised patients.[1] Overall, the genus *Aspergillus* contains about 250 species divided into eight subgenera (*Aspergillus*, *Fumigati*, *Circumdati*, *Candidi*, *Terrei*, *Nidulantes*, *Warcupi* and *Ornati*),[2] which in turn are subdivided into several sections or species complexes. Of these, <40 species are known to cause diseases in humans. Most invasive infections are caused by members of the *A. fumigatus* species complex, followed by *A. flavus*, *A. terreus* and *A. niger* species complexes.[3] These data contrast with epidemiological data from a decade previously, where the majority of cases were due to *A. fumigatus*.[4] Irrespective of this, some institutions may find *A. flavus* or *A. terreus* to be the most frequently recovered species of *Aspergillus*.[5] Consideration should be given to the infecting species of *Aspergillus* since some representatives may display resistance to a broad panel of antifungal therapies.

Both the change in microbial epidemiology and the steady rise in aspergillosis have multiple causes. These include the increasing number of patients with weakened immune systems such as patients with cancer and organ transplant recipients, and advances and modifications to healthcare practices in response to worldwide concerns about hospital-associated aspergillosis. In addition, azole resistance seems to be an emerging problem in some geographical areas, and it has been reported that cryptic species of *Aspergillus* with resistance to antifungal agents can be more common than expected.[2–5]

Patient populations

It is estimated that ~200 000 cases of IA occur worldwide per year.[6] Approximately 50% affect patients with haematological malignancies such as AML and ALL, and recipients of allogeneic HSCT.[7,8] The risk of early infections is influenced by the underlying disease, presence of persistent neutropenia, type of stem cell and conditioning protocols; late infections are associated with the onset of graft-versus-host disease and with cytomegalovirus (CMV) infection and disease.[9] Environmental factors such as nearby construction work or inadequate infection control can play a contributory role. Gene polymorphisms affecting the innate immune response, such as the toll-like receptor 4,[10] interleukin-1β

and β-defensin 1,[11] long pentraxin 3[7,12] and dectin-1,[13,14] are associated with a higher risk of developing IA. Impaired production of reactive oxygen species, and qualitative and quantitative immune cell defects, also increase risk.[15–17] The introduction of mould-active prophylaxis with posaconazole resulted in a reduction of IA incidence in haemato-oncological patients from 15.5% to 7%,[18] but on the other hand led to observations of rare fungal pathogens being involved in breakthrough infections.[19] Mortality rates associated with IA in haematological patients reached 29%.[11,20]

The dominant risk factor for IA in solid organ transplant recipients is not the depletion of phagocytic neutrophils[21,22] but their functional impairment by immunosuppressant drugs. The 1 year incidence of invasive mould infections in this setting is highest for IA (0.65%), with the majority of infections typically occurring more than 3 months after transplantation (late-onset infection).[23] IA is commonly found in lung and heart–lung recipients. Airway colonization in lung transplant recipients with cystic fibrosis is a risk factor for disease following transplantation.[24,25] Increasing intensity of immunosuppression, haemodialysis and CMV infection are risk factors for liver and renal transplant recipients.[26,27] Donor CMV seropositivity is a risk factor for late-onset infection.

ICU patients represent the second largest at-risk population for IA, with incidence rates varying between 6.1 and 57/1000 ICU admissions.[28,29] This applies to patients suffering from chronic obstructive pulmonary disease and receiving high-dose corticosteroids, and those with severe alcoholic liver cirrhosis.[30,31] Mortality rates range from 46% to 80%.[4,21–23,25] The degree, duration and type of immunodeficiency influence pathogenesis and disease outcome.[21,22] It is not uncommon for IA to be associated with concurrent viral respiratory infections due to H1N1 influenza or adenovirus.[32]

The pathogens: from A. fumigatus to non-A. fumigatus

An emerging issue is the occurrence of azole resistance in *A. fumigatus*. The full extent of the problem is still unknown, but the global prevalence of azole resistance in *Aspergillus* is estimated to be ∼3%–6%.[33] Resistant isolates have been identified in Europe, the Middle East, Asia, Africa, South America and, recently, in the USA.[34] Some studies suggest that resistance in *A. fumigatus* may be partially driven by the use of agricultural azoles, which protect crops from fungi. Other species of *Aspergillus* may also be resistant to amphotericin B, including *A. lentulus*, *A. nidulans*, *A. ustus* and *A. versicolor*. Hence, the identification of unknown *Aspergillus* clinical isolates to species level may be important given that different species have variable susceptibilities to multiple antifungal drugs. Thus, knowledge of the species identity may influence the choice of appropriate antifungal therapy. Table 1 provides an overview of susceptibility trends from infections due to a non-*A. fumigatus* species complex.

Other mould infections

Many mould species are able to cause opportunistic invasive fungal diseases since they are saprobes with a cosmopolitan presence and are widely distributed in the environment. These filamentous fungi are usually rare pathogens, but their prevalence appears to have increased steadily over the last two decades. Rare species of filamentous fungi can cause colonization and superficial mycoses, as well as local infections after penetrating trauma and disseminated diseases in patients with predisposing factors. The course of infection can differ between mould species, and some exhibit a decreased susceptibility or are even intrinsically resistant to antifungal agents.[41] It is therefore highly relevant to obtain an accurate species identification to inform clinical management. The epidemiological features of the most common species of the rare moulds implicated in human infections are summarized in Table 2.

Mucormycosis

Mucormycosis is the disease caused by organisms belonging to the order Mucorales. The most common species of Mucorales involved in human infections are the *Rhizopus* spp. although other species belonging to the genus, such as *Mucor*, *Rhizomucor*, *Lichtheimia*, *Mycocladus*, *Apophysomyces*, *Saksenaea*, *Cunninghamella*, *Cokeromyces* and *Syncephalastrum*, have also been isolated in cases of mucormycosis.[48,49] The classical risk factors for mucormycosis are diabetes and penetrating trauma, but immunosuppression and granulocytopenia have become the most common predisposing factors in many tertiary hospitals over recent years.[8,23]

There are no reliable data to assess the incidence of this mycosis in the general population. A number of studies have reported an incidence of 0.5–1.2 cases per million inhabitants/year.[50] Some population-based surveys have isolated Mucorales species in 5%–15% of clinical samples with filamentous fungi. In recent reports, mucormycosis was 7-fold less common than IA in haematological patients, and mortality rates ranged from 25% to 50%.[42]

Some studies have pointed out that the incidence of mucormycosis may be underestimated due to the low performance of diagnostic techniques based on conventional microbiological procedures, such as culture and microscopy. The most useful methods for detecting Mucorales are still microscopic examination of tissues and histopathology, which offer moderate sensitivity and specificity. Recent clinical studies have reported that mucormycosis is the cause of >10% of all invasive fungal infections when techniques based on DNA amplification by quantitative DNA[51] are used to complement conventional methods.[52–54]

Species belonging to the order Mucorales are more resistant to antifungal agents than *Aspergillus* spp. All species of Mucorales are unaffected by voriconazole and most show moderate resistance *in vitro* to echinocandins.[55,56] In fact, the use of voriconazole as first-line treatment for aspergillosis, and use of echinocandins as empirical treatment for febrile neutropenia and disseminated candidiasis, have been blamed for the increased incidence of mucormycosis.[57] Amphotericin B and posaconazole show the most potent activity *in vitro* against the Mucorales.[58–60] Current guidelines recommend the polyenes as first-line treatment, with posaconazole as alternative and salvage therapy.[42] Both itraconazole and isavuconazole exhibit an intermediate activity *in vitro* against these fungal species.[61] *In vitro* activity of azoles against Mucorales is species-dependent with *Mucor* spp. being less susceptible and *Lichtheimia* spp. and *Rhizopus* spp. exhibiting the lowest MICs. Although the MIC values for posaconazole are lower than for isavuconazole, in the clinical setting this may be compensated for by higher isavuconazole exposure at standard dosing.[60]

Table 1. Features of selected non-*A. fumigatus* infections

Species	Diseases	Specific characteristics	References
Emericella nidulans	IA in CGD	• More virulent than *A. fumigatus* • Higher mortality • Propensity to spread from the lung to adjacent structures and to disseminate • Intrinsic resistance to amphotericin B	35
Emericella quadrilineata	IA in CGD and IA	• Resistant to caspofungin?	35
Aspergillus calidoustus	IA	• Propensity to disseminate • Intrinsic resistance to azoles • Intrinsic resistance to caspofungin?	35, 36
Aspergillus terreus	IA	• Propensity to disseminate (63%) • Intrinsic resistance to amphotericin B	5
Aspergillus tubingensis	IA, airway colonization and ear infections	• Acquired resistance to azoles • Lower propensity to disseminate (10%-30%)	37
Aspergillus lentulus	IA	• Resistant to azoles and echinocandins • Resistant to amphotericin B	38
Aspergillus alliaceus	IA	• GM negative • High MICs of amphotericin B and caspofungin	35
Aspergillus carneus	IA	• GM low positive	39
Aspergillus novofumigatus	IA	• Resistant to azoles	35
Aspergillus alabamensis	Mainly airway colonization	• Resistant to amphotericin B	40
Aspergillus ustus	IA	• Resistant to amphotericin B, azoles and echinocandins	36
Aspergillus felis	IA	• High MICs against voriconazole and caspofungin	35

IA, invasive aspergillosis; CGD, chronic granulomatous disease; GM, galactomannan.

Table 2. Epidemiological features of rare mould species

Species	Diseases	Specific characteristics	References
Mucorales	IFD in patients with risk factors	• Increasing prevalence in haematological patients • Higher mortality than aspergillosis • Resistance to voriconazole	42
Fusarium spp.	Local and disseminated mycoses in patients with risk factors	• Leading cause of IFD in haematological patients in some areas (Brazil) • Mortality >75% in IFD cases • Unpredictable resistance to some antifungal agents	43, 44
Scedosporium apiospermum complex	Colonization, local infections and IFDs	• More common in temperate areas • High mortality in IFD cases • Voriconazole is the most potent antifungal agent against them	43, 45
Scedosporium prolificans	Colonization, local infections and IFDs	• More common in southern Europe, Australia and California • Mortality >90% in IFD cases • Multiresistant organism	43, 46
Other rare mould species	Colonization, local infections and IFDs	• Unreliable data on prevalence and mortality • Identification at species level and AST are compulsory for correct management	43, 47

IFD, invasive fungal disease; AST, antifungal susceptibility testing.

Infections by *Fusarium* spp.

The genus *Fusarium* includes several fungal species complexes. These are ubiquitous soil saprophytes and pathogenic for plants. Only a few species cause infections in humans.[43] Among these are the species complexes *F. solani*, *F. oxysporum*, *F. verticillioides* and *F. proliferatum*. *Fusarium* spp. have been involved in superficial and deep mycosis and are the leading cause of fungal keratitis in the world.[43,62] Clinical interest is currently growing since they have been identified as emerging and multiresistant pathogens causing opportunistic disseminated infections.[63,64]

Few clinical studies have described the incidence of *Fusarium* spp. Several surveys have indicated that *Fusarium* could be the second most common nosocomial fungal pathogen after *Aspergillus* in some tertiary hospitals.[41] In some geographical areas, clinical studies have calculated that *Fusarium* spp. cause between 3% and 5% of invasive fungal diseases.[44] A Spanish population-based study found *Fusarium* spp. in only 1.2% of mould clinical isolates recovered from deep human samples, including tissues, sterile fluids and respiratory secretions.[50] However, in Brazil, *Fusarium* is the leading cause of invasive mould infections, followed by *Aspergillus*.[44,63,64] The main risk factor for invasive fusariosis is neutropenia, predominantly in patients with acute myeloid leukaemia and haematopoietic cell transplant recipients.

Fusarium spp. show a susceptibility profile marked by a higher level of resistance than that of *Aspergillus* spp. Some *Fusarium* isolates can be susceptible *in vitro* to amphotericin B, voriconazole and posaconazole, and isavuconazole, although not to itraconazole or micafungin.[65] However, the mortality rate in disseminated fusariosis exceeds 75%.[44,50,66] Most experts consider *Fusarium* spp. to be multiresistant organisms and treatment recommendations include combination antifungal therapy with adjunctive measures and surgery when possible.

Infections by *Scedosporium* spp.

The genus *Scedosporium* has undergone a taxonomic reclassification. According to the new classification, the most common *Scedosporium* spp. involved in human infections are *S. apiospermum* (telemorphic state, *Pseudallescheria apiosperma*), *S. boydii* (*Pseudallescheria boydii*), *S. aurantiacum* and *S. prolificans* (*Lomentospora prolificans*). Other species that are very rarely isolated from human samples are *S. dehoogi*, *Pseudallescheria ellipsoidea*, *S. desertorum* and *S. minutisporum*.[67] Owing to epidemiological reasons, most recent reports divide human infections by these species into mycoses caused by the *S. apiospermum* complex (which includes *S. apiospermum*, *S. boydii* and *S. aurantiacum*) and by *S. prolificans*.[43]

Species belonging to the *S. apiospermum* complex are cosmopolitan, being ubiquitously present in the environment, but predominantly in temperate areas. They are commonly isolated from soil, sewage and polluted waters, composts, and from the manure of horses, dogs, cattle and fowl.[45] *S. prolificans* appears to have a more restricted geographical distribution, being found largely in hot and semiarid soils in southern Europe, Australia and California.[46]

The clinical spectrum of infections by *Scedosporium* includes colonization, allergic bronchopulmonary pneumonia, superficial and subcutaneous infections (onychomycosis, otitis and mycetoma), local deep infections such as arthritis, osteomyelitis, endophthalmitis, endocarditis and pneumonia, and disseminated infections with CNS involvement. Disseminated infections are related to immunosuppression in >95% of cases and in these patients the infections are difficult to treat, with a high rate of therapeutic failures and relapses, and with mortality rates close to 90%.[43,45,46]

The incidence of infections by *Scedosporium* has been analysed in some countries.[50,62,68–71] A Spanish population-based survey found *Scedosporium* to be the second most common mould after *Aspergillus* in human samples isolated from tissues and respiratory secretions.[50] A multicentre Australian study analysed moulds isolated from deep samples during 2004 to 2012. *Scedosporium* spp. was isolated in 33.3% of cases in which infection was due to non-*Aspergillus* species.[65] *Scedosporium* has been reported as the cause of 1% of invasive fungal infections caused by moulds among haematological patients in Italy,[69] and of 5.6% of deep mould mycoses in solid organ transplant recipients in the USA.[62] A 1 year prospective study performed at 11 German laboratories calculated the prevalence of *Scedosporium* spp. in respiratory samples from patients with cystic fibrosis to be 3.1%, with 90% of isolates belonging to the *S. apiospermum* complex.[67] Recently, *S. aurantiacum* has been reported as an emerging opportunistic pathogen colonizing the lungs of patients suffering from chronic diseases.[71]

Regarding their susceptibility profile, voriconazole is the most active antifungal agent for the *S. apiospermum* complex, with more moderate activity for isavuconazole.[72] Some isolates of *S. apiospermum*[73] and *S. boydii* are susceptible to isavuconazole, posaconazole, itraconazole, amphotericin B and echinocandins, but *S. aurantiacum* seems to be susceptible only to voriconazole. *S. prolificans* is a multiresistant organism and all antifungal compounds available exhibit high MIC values against this mould.[74–76] The management of disseminated mycoses caused by *Scedosporium* spp. (mainly *S. prolificans*) is very difficult and current guidelines recommend voriconazole, or amphotericin B as an alternative. In addition, adjunctive therapy and surgery (when possible) should be considered. Salvage therapy with combination antifungal treatment has been successfully prescribed in some cases.[43,45,46]

Infection by other rare moulds

Other mould species can infect humans, although invasive fungal disease occurs less frequently. Some hyaline fungal species such as *Acremonium*, *Paecilomyces* and *Penicillium* have been involved in cases of local and disseminated deep mycoses. Melanized (black) fungi can also be isolated from humans and they have been rarely reported as agents of deep-seated mycoses. Both rare hyaline moulds and melanized fungi are saprobes and ubiquitous organisms in the environment. They can cause cutaneous and respiratory colonization, local infections after accidental inoculation, and invasive fungal disease in patients with predisposing factors, mainly those receiving immunosuppressive therapy. There are no reliable data about their prevalence. The susceptibility profile of these species varies from susceptibility to resistance to all antifungal agents. Identification at species level is compulsory for the correct management of mycoses caused by these rare moulds, and sequencing of DNA targets may be necessary for their classification. Updated guidelines for the diagnosis and the treatment of rare mould infections have been published recently.[43,47]

Conclusions

Aspergillus spp., largely those belonging to the *A. fumigatus* complex, remain the leading cause of invasive fungal disease in most geographical areas and clinical settings. Other species of *Aspergillus* are prevalent in some countries and sibling/cryptic species of *Aspergillus* are an emerging problem since they can be resistant to some antifungal agents. The expanding use of antifungal agents with high activity against *Aspergillus* in prophylactic, empirical and targeted therapy may be promoting the emergence of other mould species that exhibit resistance to certain antifungal agents. There are no consolidated data on the prevalence of these less common species of moulds, but some reports indicate that they can cause between 10% and 15% of all cases of invasive fungal disease. Since these species exhibit individual susceptibility profiles and require tailored clinical management, accurate classification at species level of the aetiological agent in any invasive fungal disease should be regarded as the standard of care.

Funding

This Supplement was funded by Basilea Pharmaceutica International Ltd, Basel, Switzerland. Editorial support was provided by a freelance medical writer (C. Dunstall) with funding from Basilea.

Transparency declarations

C. L.-F. has received research funding, consultancy fees, speaker's fees and travel/accommodation/meeting expenses from Astellas Pharma, Pfizer, Gilead Sciences, Merck Sharp and Dohme, and Basilea. M. C-E. has received grant support from Astellas Pharma, Basilea, BioMerieux, Gilead Sciences, Merck Sharp and Dohme, Pfizer, Schering Plough, Soria Melguizo SA, Ferrer International, the European Union, the ALBAN programme, the Spanish Agency for International Cooperation, the Spanish Ministry of Culture and Education, the Spanish Health Research Fund, the Instituto de Salud Carlos III, the Ramon Areces Foundation and the Mutua Madrileña Foundation. He has been an advisor/consultant to the Pan-American Health Organization, Astellas Pharma, Gilead Sciences, Merck Sharp and Dohme, Pfizer and Schering Plough. He has been paid for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas Pharma and Schering Plough.

This article forms part of a Supplement sponsored by Basilea Pharmaceutica International Ltd, Basel, Switzerland.

Author contributions

The authors co-wrote the article and approved the final version for publication.

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