Changing epidemiology of systemic fungal infections

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ABSTRACT

Species of *Candida* and *Aspergillus* remain the most common causes of invasive fungal infections, but other yeasts and filamentous fungi are emerging as significant pathogens. Opportunistic yeast-like fungi and moulds such as Zygomycetes, *Fusarium* spp. and *Scedosporium* spp. are increasingly being recognised in patient groups such as those with leukaemia and in bone marrow transplant recipients. Recognition of these epidemiological changes is critical to patient care. The key elements in selecting an appropriate antifungal agent are the type of patient (solid-organ or stem-cell transplant), severity of immunosuppression, history of prolonged exposure to antifungal drugs, and knowledge of the genera and species of the infecting pathogen and its typical susceptibility pattern.

Keywords Epidemiology, fungal infections, review, risk-factors

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INTRODUCTION

Invasive fungal infections are increasingly common in the nosocomial setting. [1–5]. Furthermore, because risk-factors for these infections continue to increase in frequency, it is likely that the incidence of nosocomial fungal infections will continue to increase in the coming decades. This expansion is based on an increase in the number of immunocompromised patients, including cancer patients with chemotherapy-induced neutropenia, transplant recipients receiving immunosuppressive therapy, and human immunodeficiency virus (HIV)-infected patients [6–9]. Better control of underlying diseases and improved antimicrobial therapies result in prolonged survival, thus putting these patients at higher risk of acquiring an opportunistic fungal infection. The predominant nosocomial fungal pathogens include *Candida* spp., *Aspergillus* spp., Mucorales, *Fusarium* spp., and other moulds, including *Scedosporium* spp. [1,10–12]. These infections are difficult to diagnose and cause high rates of morbidity and mortality, despite antifungal therapy. Early initiation of effective antifungal therapy and reversal of underlying host defects remain the cornerstones of treatment for nosocomial fungal infections. In recent years, new antifungal agents have become available, resulting in a change in the standard of care for many of these infections. Nevertheless, the mortality rate of nosocomial fungal infections remains high, and new therapeutic and preventive strategies are needed. In the USA, the estimated annual incidences of invasive *Candida* and *Aspergillus* infections are 72–228 and 12–34 infections per million population, respectively [10,13,14]. At this time, we face a marked shift in the epidemiological profile of fungal infections: new and emerging pathogens such as Zygomycetes (e.g., *Rhizopus* and *Mucor* spp.), hyaline moulds (e.g., *Fusarium* spp.), yeast-like fungi (e.g., *Trichosporon* spp.) and some dematiaceous fungi are increasingly being reported (Table 1). This article reviews selected aspects of the epidemiological profiles of invasive mycoses, risk-factors for infections and the susceptibility to antifungal agents.

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Table 1. Spectrum of opportunistic fungal pathogens[a]

Candida spp.	*C. albicans*	Other yeasts	*Blastoschizomyces* spp.
	C. glabrata		*Cryptococcus neoformans*
	C. gulliermondii		*Malassezia* spp.
	C. kefyr		*Rhodotorula* spp.
	C. krusei		*Saccharomyces* spp.
	C. lusitaniae		*Trichosporon* spp.
	C. rugosa	Zygomycetes	*Absidia* spp.
	C. parapsilosis		*Cunninghamella* spp.
	C. tropicalis		*Mucor* spp.
Aspergillus spp.	*A. fumigtus*		*Rhizomucor* spp.
	A. niger		*Rhizopus* spp.
	A. flavus	Dematiaceous moulds	*Alternaria* spp.
	A. terreus		*Bipolaris* spp.
Other hyaline moulds	*Acremonium* spp.		*Curvularia* spp.
	Fusarium spp.		*Cladophialophora* spp.
	Paecilomyces spp.		*Exophiala* spp.
	Scedosporium spp.		*Phialophora* spp.
	Scopulariopsis spp.		
	Trichoderma spp.		

[a]Adapted from reference [109].

MEDICALLY IMPORTANT YEASTS AND YEAST-LIKE FUNGI

Candida

Bloodstream infections (BSIs) due to *Candida* spp. have become common in both adult and paediatric intensive care units (ICUs), accounting for 10–15% of hospital-acquired BSIs. This rising incidence has been attributed to several risk-factors that are prevalent in critically ill patients, such as debility, underlying malignancy, blood and bone marrow transplantation, AIDS, prolonged ICU and hospital stay, neutropenia, use of antibiotics and corticosteroids, and administration of parenteral alimentation. In most cases, the yeast's portal of entry is the gut, but in other patients, especially those with central venous catheters (CVCs), the skin is the most likely culprit. Candidaemia may occur in both immunocompromised and non-immunocompromised patients. The development of candidaemia, however, strongly implies immunodeficiency, since it often occurs in immunocompromised patients, and more specifically, in 10–20% of those with myeloproliferative disorders or leukaemia and in up to 74% of patients with AIDS.

An increasing incidence has been observed for all clinical manifestations, including oropharyngeal infections, post-operative site infections, and urinary tract infections, but is especially dramatic for candidaemia. This rising incidence of *Candida* infections and candidaemia can be attributed to a variety of mostly endogenous and exogenous factors. Granulocytopenia is the most important risk-factor for the development of systemic candidosis in patients with cancer. The improved and intensified treatment of patients with cancer induces granulocytopenia of significantly long duration. Damage to the oropharygeal mucosa through the use of aggressive cytostatic drugs facilitates *Candida* colonisation and subsequent invasion.

One of the most important groups of opportunistic fungal pathogens comprises *Candida* spp. [2,10,11,15–19], which account for 8–10% of all nosocomial BSIs and remain important pathogens in ICUs [20]. Invasive infections are associated with an attributable mortality rate of 10–49%, an excess duration of hospital stay and an enormous excess of costs [21]. Although some centres report an increased mortality rate associated with species of *Candida* other than *Candida albicans* as compared with *C. albicans*, no consistent pattern has emerged.

More than 100 species are known, with *C. albicans* being the main representative. The frequency of *Candida* spp. distribution varies according to the geographical setting [3,15,22,23]: 44% and 62% of BSIs due to *C. albicans* were documented in Latin America and in Europe, respectively. Nearly 95% of episodes of candidaemia are caused by *C. albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei* [3,4,10,11]. *C. albicans* infections occur less frequently with increasing patient age [2], after exposure to azole antifungals [22], and in the ICU setting [3]. Since the 1990s, fluconazole has been widely used for prophylaxis and treatment of invasive fungal infections in immunocompromised patients; this resulted in a decreased incidence of BSIs worldwide [16,24,25], but an increased incidence of *C. glabrata* and other non-*albicans Candida* infections. *C. glabrata* is emerging, and accounts for 20% of BSIs in the USA (12–37%), 15% in Europe, 10% in Asia, and 5% in Latin America [26]. The reason for the occurrence of *C. glabrata* appears to be multifactorial [26,27], and includes geographical characteristics [23,26], age [27], patient populations [16,22] and use of fluconazole [16,22]. *C. parapsilosis* is the second most common species recovered from blood cultures in Europe [28] and tends to form biofilms on the surface and lumens of catheters [29,30]; biofilm-producing microbes may be completely resistant to antifungals [29]. *C. parapsilosis* has also been known to colonise the hands of healthcare workers, thus emphasising the importance of hand hygiene and proper catheter care. This species is most common in neonates and children, and is associated with a lower mortality rate. *C. parapsilosis* isolates can be divided into three groups on the basis of molecular studies [31,32]. Two new species, *Candida orthopsilosis* and *Candida metapsilosis*, are proposed to replace the existing designations of *C. parapsilosis* groups II and III, respectively. The species name *C. parapsilosis* is retained for group I isolates. None of the clinical *C. orthopsilosis* isolates were found to produce biofilm *in vitro*.

C. tropicalis is known to be an important cause of infections in patients with cancer [16,22] and appears to be more virulent than *C. albicans* in patients with haematological malignancy. Dissemination is associated with high mortality rates. In a retrospective survey of candidaemia performed at the University Hospital Vienna between 2001 and 2006, the number of non-*C. albicans* infections increased, with *C. tropicalis* causing 7% [33]. Most infections appear to originate from the patients' endogenous microflora. An outbreak of *C. tropicalis* sternal wound infections following cardiac surgery was traced to a colonised scrub nurse [34]. Most *C. tropicalis* strains are susceptible to amphotericin B, flucytosine, and triazoles [35]. Paradoxical growth of *C. tropicalis* strains was noted in RPMI-1640 and antibiotic medium three at caspofungin concentrations of 12.5 mg/L [36].

C. krusei is a fluconazole-resistant species and is an uncommon cause of candidaemia (<3%) [36]. However, the emergence of *C. krusei* may have a profound effect on clinical outcome. A comparative study of fungaemia in immunocompromised patients showed the mortality rate associated with *C. krusei* to be 49%, as compared to a rate of only 28% with *C. albicans*. Antifungal response rates are also lower with *C. krusei*, although amphotericin B achieves a success rate of 51% [37]. Endogenous spread from the gastrointestinal tract is the main mechanism of infection, particularly in patients with haematological malignancy [38]. Several reports have described an increase in *C. krusei* colonisation and infection in granulocytopenic patients that was associated with use of fluconazole as prophylaxis.

Candida lusitaniae is an important cause of nosocomial infection among immunocompromised hosts. It is an endogenous pathogen but nosocomial transmission can occur. Initial resistance or rapid development of resistance to amphotericin B is characteristic of this species. Most strains are susceptible to azoles. Colony morphology switching of *C. lusitaniae* might be associated with the acquisition of multidrug resistance in this species [39].

Candida rugosa is a fungus that appears to be emerging as a cause of infection in some geographical regions. Pfaller *et al.* [40] observed geographical and temporal trends; *C. rugosa* accounted for 0.4% of *Candida* spp. and the frequency of isolation increased from 0.03% to 0.4% between 1997 and 2003. *C. rugosa* was most common in the Latin American region (2.7%), and 40.5% of strains were susceptible to fluconazole. Isolates from Europe and North America were much more susceptible (97–100%) to voriconazole than those from other geographical regions (55.8–58.8%). Bloodstream isolates were the least susceptible to both fluconazole and voriconazole.

Factors associated with an increased risk of invasive candidosis are shown in Table 2. Candidaemia was found to be an independent risk-factor for death during hospitalisation [41], and the outcome depended on early administration of adequate therapy [21].

Antifungal prophylaxis has been proven to decrease invasive candidosis in neutropenic

Table 2. Co-morbid conditions for candidaemia in hospitalised patients

Underlying factors[a]
Broad-spectrum antimicrobial agents (number and duration)
Steroids (therapy and prophylaxis)
Extremes of age (<1 and >70 years)
Chemotherapy
Malignancy
Previous colonisation (≥2 sites)
Gastric acid suppression
Indwelling catheter (CVC, Hickmann catheter)
Total parenteral nutrition
Neutropenia (<500/mm^3)
Surgery (gastrointestinal) or gastrointestinal damage
Mechanical ventilation
Any renal failure (haemodialysis)
Malnutrition
ICU stay (>10 days), >APACHE II score
Severity of disease
Mucositis
GvHD (acute and chronic)
Stem-cell and organ transplant

CVC, central venous catheter; ICU, intensive care unit; GvHD, graft vs. host disease.
[a]Data compiled from references [3,4,9,12,13,19,20,25,110–116].

patients [16]. The data are less compelling for non-neutropenic ICU patients. Initial treatment should be guided by combining the local epidemiology and the most important risk-factors. Usually, *C. albicans*, *C. parapsilosis* and *C. tropicalis* are susceptible to polyenes, the azoles and the echinocandins (Table 3). *C. glabrata* is inherently less susceptible to fluconazole, and *C. krusei* is intrinsically resistant to fluconazole [42].

A particular problem with patients with candidaemia and invasive candidosis is that they are difficult to distinguish clinically from patients with bacterial sepsis, at least early in infection. This often leads to an initial delay in instituting antifungal therapy, and furthermore the choice of initial empirical therapy may be inappropriate. Parkins and colleagues investigated the relationship between the adequacy of initial empirical antifungal therapy and outcome of bloodstream and invasive *Candida* infections by reviewing 207 patients who had an invasive *Candida* infection over a 5-year period [43]. Only one-third of patients received empirical antifungal therapy, and furthermore, the therapy was deemed appropriate in 26% of patients. Crucially, the authors were able to demonstrate that adequate empirical therapy was associated with a significant reduction in mortality from 46% to 27%. Notably, in this study, around half of the infections were demonstrated to be due to species of *Candida* other than *C. albicans*, with 22% being due to *C. glabrata* alone.

Opportunist yeast-like fungi

Trichosporon

Trichosporon spp. are normal residents of human skin and can be isolated from soil and water.

Table 3. In-vitro susceptibility data for *Candida* spp. commonly associated with bloodstream infections[a]

	Susceptibility[b]						
***Candida* spp.**	**AMB**	**5-FC**	**FLU**	**ITR**	**VOR**	**POS**	**CAS**
C. albicans	S	S	S	S	S	S	S
C. glabrata	S-I	S	S-SDD-R	S-SDD-R	S-I	S	S
C. krusei	S	R	R	S-SDD-R	S-I	S	S
C. lusitaniae	S-R	S	S	S	S	S	S
C. parapsilosis	S	S	S	S	S	S	S-I
C. tropicalis	S	S	S	S	S	S	S

S, susceptible; I, intermediate (susceptibility is not certain); SDD, sensitive dose-dependent; AMB, amphotericin B; 5-FL, flucytosine; FLU, fluconazole; ITR, itraconazole; VOR, voriconazole; POS, posaconazole; CAS, cancidas.
[a]Data adapted from references [2,13,23,112,117,118].
[b]The CLSI has established interpretative breakpoints for *Candida* spp. tested against fluconzole, itraconazole and flucytosine using CLSI-recommended guidelines for broth dilution testing. On the basis of these breakpoints, resistance is defined as MIC of >64 mg/L for FLU, >1 mg/L for ITR and >32 mg/L for 5-FU. For the purposes of this table, susceptible is defined as MIC <1 mg/L of AMB, CAS, VOR and POS.

Eight species of *Trichosporon* are known human pathogens, with *Trichosporon asahii* and *Trichosporon mucoides* accounting for the majority of deep-seated and disseminated infections. Risk-factors for infection include immunosuppression, disruption of mucosal integrity, and CVCs. A major risk-factor for trichosporonosis is underlying haematological malignancy. In a review by Girmenia *et al*. [44], 63% of 287 *Trichosporon* cases had an underlying haematological malignancy. Those at greatest risk include neutropenic cancer patients receiving cytotoxic treatment. Less commonly, disseminated infection has been seen in solid-organ transplant recipients, burn patients, low-birth-weight infants, and persons with AIDS. Factors that enhance mucosal colonisation and subsequent invasive infection include broad-spectrum antibiotic treatment and breaks in anatomical barriers. The overall mortality rate is high, ranging from 60% to 80% in earlier reports. Some improvement has been achieved with recent developments in diagnosis, treatment and prevention. Isolates of *T. asahii* are often resistant to amphotericin B *in vitro*.

The optimal antifungal treatment for trichosporonosis is currently unclear, and despite antifungal therapy, the prognosis is poor, with over three-quarters of patients dying. Echinocandins have poor activity against *Trichosporon* spp. and, indeed, breakthrough cases in patients receiving micafungin and caspofungin have been reported [45,46]. Azole antifungals seem to be more potent than amphotericin B, and the newer triazoles, voriconazole, posaconazole, and ravuconazole, appear to be active *in vitro*. Successful treatment of trichosporonosis with triazoles has been reported [47].

Rhodotorula

Rhodotorula spp. have increasingly been recognised as important human pathogens. Immunoconmpromised patients, particularly those with CVSs or other indwelling devices, are at highest risk for infection. While *Rhodotorula* strains appear to be less virulent than the more common yeast pathogens such as *Candida*, *Rhodotorula* infection has been associated with a crude mortality rate of up to 15% and can cause sepsis syndrome and other life-threatening complications. *Rhodotorula* BSIs have been successfully managed with line removal alone, antifungal therapy with line removal, and a combination of these approaches. In a recent review by Tuon *et al*. [48], where all cases of CVC-related fungaemia due to *Rhotorula* spp. reported in the literature were considered, it was found that all patients but one in the 88 cases examined had some form of underlying disease, including 69 (78.4%) who had cancer. *Rhodotorula mucilaginosa* was the species most frequently recovered (75%), followed by *Rhodotorula glutinis* (6%). Amphotericin B deoxycholate was the most common antifungal agent used as treatment, and the overall mortality rate was 9.1%. The authors conclude by stressing that fungaemia caused by *Rhodotorula* is rare but is increasingly seen in immunocompromised and in ICU patients. Additionally, it is noted that *Rhodotorula* spp. are resistant to fluconazole. Recent in-vitro data extend our understanding of antifungal activity against *Rhodotorula* spp. [49]. Here, the activities of eight antifungals against 64 *Rhodotorula* isolates collected in surveillance programmes between 1987 and 2003 were determined. The strains were resistant *in vitro* to fluconazole (MIC_{50}, >128 mg/L) and caspofungin (MIC_{50}, >8 mg/L). Amphotericin B (MIC_{50}, 1 mg/L) and flucytosine (MIC_{50}, 0.12 mg/L) were both active *in vitro*, and the new and investigational triazoles all had some in-vitro activity, with ravuconazole being the most active (MIC_{50}, 0.25 mg/L).

Geotrichum capitatum

G. capitatum, formerly known as *Trichosporon capitatum* or *Blastoschizomyces capitatus*, is an uncommon, but frequently fatal, cause of invasive infections in immunocompromised patients, particularly those with haematological malignancies [44]. In a recent retrospective multicentre study from Italy, the incidence of *G. capitatum* infection among patients with acute leukaemia was 0.5%, with a 55.7% crude mortality rate [44]. *G. capitatum* is susceptible to amphotericin B and azoles, in particular voriconazole, but is intrinsically resistant to echinocandins [44]. This is exemplified in a further series of three patients from the same authors, where it was shown that, in one patient, infection improved when caspofungin was replaced by voriconazole [50]. In a second patient, a breakthrough *G. capitatum* infection developed while the patient was undergoing antifungal prophylaxis with caspofungin; and in the third patient, the choice of voriconazole therapy was based on the observation of multiple maculo-papular skin lesions suggestive of disseminated *Candida* infection.

Pichia (Hansenula)

The main species implicated in human infections are *Pichia anomala* (previously *Hansenula anomala*) and *Pichia angusta* (previously *Hansenula polymorpha*). Recently, there was a report of *P. angusta* and *P. anomala* being responsible for cases of fungaemia in a Brazilian paediatric ICU [51]. The source of the infection was never found. Patients with *P. anomala* fungaemia seem to have risk-factors in common with those who have candidaemia. A number of transient cases of candidaemia caused by *Candida utilis* (*Pichia jadinii*) have been reported.

Debaromyces

Debaromyces hansenii is the anamorph form of *Candida famata*. *C. famata* has been repeatedly associated with catheter-related BSIs, and occasionally with infections of the central nervous system. The reservoir of *C. famata* is not known, but there is a possibility that nosocomial infections can occur via air contamination [52]. No studies on the antifungal susceptibility of *Debaryomyces* are available.

Klyveromyces

Candida kefyr, the anamorph of *Klyveromyces marxianus*, has occasionally been involved in opportunistic infections in immunocompromised persons. *Klyveromyces* is found on certain foodstuffs (mainly dairy products). The collective experience at three particular French hospitals exemplifies the apparent emergence of *C. kefyr* colonisation in patients with onco-haematological diseases [53]. During a 6-year period (2000–2005), 64 417 isolates of *Candida* spp. were collected from three French teaching hospitals with 4150 beds, including 305 onco-haematological unit beds. In total, 12 834 (20%) of 64 417 *Candida* isolates were recovered from patients hospitalised in onco-haematology wards. *C. kefyr* was isolated twice as often from patients in onco-haematology wards as from those hospitalised in other wards (4.8% vs. 1.9%; $p < 0.001$). Among the 1604 *C. kefyr* isolates recovered during this period, 623 (38.8%) were isolated from patients in onco-haematology wards ($p < 0.001$). During the same period, ten patients developed a *C. kefyr* BSI, of whom nine (90%) were hospitalised in onco-haematology wards. Most patients (seven of nine) had myeloid or lymphoblastic leukaemia. The reasons why *C. kefyr* is an emergent yeast in the fungal flora of neutropenic patients are not known, because the gastrointestinal yeast flora of healthy individuals is composed mainly of *C. albicans* and *C. glabrata*. Even though *C. kefyr* is found on certain foodstuffs (mainly dairy products), it is not known why this yeast is isolated more frequently from patients with onco-haematological diseases. It can be hypothesised that: (i) the use of empirical therapeutics as well as antifungal prophylaxis could induce selection of *C. kefyr* in the gastrointestinal flora, because some strains of *C. kefyr* have high MICs against amphotericin B; and (ii) colonisation of neutropenic patients with *C. kefyr* could be favoured by modifications in gastrointestinal homeostasis, in particular by the induction of mucositis by antimycotics (i.e. mucositis could favour colonisation by *C. kefyr*).

Saccharomyces cerevisiae

S. cerevisiae (also known as 'baker's yeast' or 'brewer's yeast') is mostly considered to be an occasional digestive commensal. However, since the 1990s, there have been a growing number of reports about its involvement as an aetiological agent of invasive infection in 'fragile' populations. A particular feature of such infections is their association with a probiotic preparation of *S. cerevisiae* (subtype *Saccharomyces boulardii*) for treatment of various diarrhoeal disorders (see below). The nature of *S. cerevisiae* (subtype *S. boulardii*) and its clinical applications have been recently reviewed [54,55].

In a recent clinical review, 92 cases of *Saccharomyces* invasive infection were presented, only 15 of which were diagnosed before 1990 [56]. All patients had at least one condition facilitating the opportunistic development of *S. cerevisiae* infections. Predisposing factors were similar to those of invasive candidosis, with intravascular and antibiotic therapy being the most frequent. Blood was the most frequent site of isolation (78%, or 72 patients). *S. cerevisiae* (subtype *S. boulardii*) accounted for 51.3% (47 cases) of fungaemias, and was exclusively isolated from blood.

There are several additional reports and reviews regarding the safety of *S. cerevisiae* (subtype *S. boulardii*) probiotic preparations. For example, there have been cases of acquired

S. cerevisiae fungaemia [57,58]. The authors concluded that probiotics should be used cautiously in certain high-risk populations. A review of the current literature reinforces the view that fungaemia and sepsis are rare complications of the administration of *S. cerevisiae* (subtype *S. boulardii*) in immunocompromised patients but confirms that the most important risk-factor for *S. cerevisiae* fungaemia is the use of probiotics [59,60]. This raises the question of the risk/benefit ratio of these agents in critically ill or immunocompromised patients, who are likely to develop an infection after exposure to high amounts of a microorganism with low virulence.

Cryptococcus

Cryptococcal infections occur with a near worldwide distribution in immunosuppressed hosts. Infection is typically caused by *Cryptococcus neoformans*, an encapsulated yeast, and infection is acquired from the environment. The organism lives in soil and organic matter containing high concentrations of pigeon and bird excreta. *C. neoformans* is neurotropic, and most patients with cryptococcal meningitis suffer from a defect in cellular immunity. The infection is seen most frequently in association with lymphoma, AIDS, transplantation, and corticosteroid therapy [61]. In a recent review of cryptococcal infection in HIV-negative patients, splenectomy was reported to be a risk-factor for infection in 3% of cases [62]. Non-*neoformans* cryptococci have been generally regarded as saprophytes and rarely reported as human pathogens [63]. However, the incidence of infection due to these organisms has increased over the past 40 years, with *Cryptococcus laurentii* and *Cryptococcus albidus*, together, being responsible for 80% of reported cases. Conditions associated with impaired cell-mediated immunity are important risk-factors for non-*neoformans* cryptococcal infections, and prior azole prophylaxis has been associated with resistance. *Cryptococcus gattii* causes disease in immunocompetent people in a geographically restricted area in Australia [64]. It is notable that there are invasive *C. gattii* infections in immunocompetent humans on Vancouver Island, western Canada [65,66]. The organism, which is thought to thrive only in tropical regions, was recovered in the temperate climatic zone. It has been postulated that environmental factors may support its propagation.

PATHOGENIC FILAMENTOUS FUNGI

Aspergillus

In general, the clinical significance of filamentous fungi can be inferred from the following data. In Europe, *c.* 18 000 patients will be diagnosed with acute leukaemia alone, and *c.* 13 000 will die of this disease each year. It is estimated that approximately 99 000 patients will be treated for a haematological malignancy, and that *c.* 18 800 will undergo a bone marrow or organ transplant. Significant proportions of the patients undergoing lung or allogenic bone marrow transplantation and of the patients with acute leukaemia will develop invasive mycoses. Roughly estimated, 5000–6000 cases involve filamentous fungi, which mostly become the ultimate cause of death.

Aspergillus spp. are opportunistic moulds that cause both allergic and invasive syndromes. The genus *Aspergillus* contains approximately 175 species, but only a minority of them have been associated with human disease. Infections are caused mostly by *Aspergillus fumigatus*, followed by *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus niger* and *Aspergillus nidulans*. *Aspergillus* is found in soil, water, food, and the air, and grows on a wide variety of organic material, such as decaying vegetation. The conidia (spores) are easily aerosolised. The route of transmission is via the air. Although exposure is universal, invasive infection occurs almost exclusively in immunocompromised individuals. Infections have frequently been described in patients with haematological malignancies and solid-organ transplant recipients, and also in patients undergoing chronic intermittent haemodialysis, in whom these infections were associated with hospital construction and/or ventilation systems contaminated with *Aspergillus* spp. Even hospital water is a frequently overlooked source of nosocomial aspergillosis.

Aspergillus spp. are common throughout the world and are ubiquitous in air, soil, and decaying matter [67]. Invasive aspergillosis (IA) has emerged as a leading cause of morbidity and mortality in immunocompromised patients [68]. *A. fumigatus* is most frequently isolated from cases of IA, followed by *A. flavus*, *A. niger*, and *A. terreus* [69,70]. *A. terreus* has been recognised as a cause of frequently lethal infections [71,72]. In certain hospitals, *A. flavus* is more common than

A. fumigatus, and the reasons for increased numbers of non-*A. fumigatus* infections are not fully elucidated [73]. Outbreaks associated with *A. flavus* appear to be associated with single or closely related strains, in contrast to those associated with *A. fumigatus*. Common clinical syndromes associated with *A. flavus* include chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and osteomyelitis following trauma and inoculation. In addition, *A. flavus* produces aflatoxins, the most toxic and potent hepatocarcinogenic natural compounds ever characterised. The *flavus* complex currently includes 23 species or varieties, including two sexual species, *Petromyces alliaceus* and *Petromyces albertensis*.

Infections caused by a recently recognised new species of *Aspergillus* have been reported [74,75]. The organism, which, according to classic morphological typing methods, is typically identified as *A. fumigatus*, clusters as a unique species with multilocus sequence typing, supporting the proposed designation of *Aspergillus lentulus* (Balajee 2005). The organism may be of particular interest because isolates exhibit low susceptibility to multiple antifungals *in vitro*.

Many factors, such as unique environmental exposure, specific host-related characteristics (e.g., chronic obstructive pulmonary disease (COPD), chronic granulomatous disease (CGD), cystic fibrosis (CF)), and the net state of immunosuppression of the affected patients, may partially account for this trend [5,76–80]. Overall, the outcome of infection appears to depend more on host factors than on the virulence or pathogenicity of the individual *Aspergillus* species. Studies including stem-cell and solid-organ transplant recipients revealed *Aspergillus* spp. to be the predominant moulds, followed by zygomycetes, and fusarium [81]. Solid-organ transplant recipients were more likely to develop candidosis, whereas stem-cell transplant recipients were at higher risk for moulds [63]. More than 60% of patients with IA have underlying haematological diseases or have undergone bone marrow transplantation [45,78]. Recovery of *Aspergillus* spp. from the respiratory tract secretions of ICU patients should be considered as a marker of infection [82]. Similar findings are made in patients with haematological malignancies [80].

Multiple analyses have identified patients at high risk (Table 4). IA is associated with a high mortality rate of 65% [45,79,80]. In-vitro susceptibility testing is not routinely recommended, but may be useful when the infection fails to respond. The vast majority of *Aspergillus* spp. remain susceptible to the various antifungal agents (Table 5).

Table 4. Co-morbid conditions for aspergillosis and mould infections in patients at risk[a]

Haematological malignancies	Organ transplant patients
Leukaemia	Lung, liver, heart, renal
Myelodysplastic syndrome	Acute and chronic rejection
Stem-cell transplant	Steroids
GvHD (acute and chronic)	Haemodialysis
Prolonged neutropenia	Tacrolimus
Induction chemotherapy	OKT 3
Fungal colonisation	Renal failure
Local epidemiology	Cytomegalo virus (CMV)
Steroid prophylaxis	Re-transplantation
Neutrophil dysfunction	Splenectomy
Cytotoxic drugs	Alemtuzumab
Infliximab	Local epidemiology
Alemtuzumab	Diabetic ketoacidosis[b]
T-cell-depleted stem-cell products	Iron overload[b]
CD34-selected stem-cell products	Diabetes mellitus[b]
Diabetic ketoacidosis[b]	Deferoxamine therapy[b]
Iron overload[b]	Skin breakdown[b]
Diabetes mellitus[b]	
Deferoxamine therapy[b]	
Skin breakdown[b]	

GvHD, graft vs. host disease.
[a]Data compiled from references [6,9,20,110,111,113,114, 119–124].
[b]Related to zygomycosis.

Several recent reviews have highlighted the increasing incidence of invasive *Aspergillus* infections that are associated with critical care medicine [82–84]. Critically ill patients undergoing intensive care exhibit a complex change in immune function, characterised by deactivation of macrophages and an altered cellular response due to the severity of illness, which is also termed 'immunoparalysis'. This immunological derangement might explain why *Aspergillus* infections are able to develop in critically ill patients who do not display the predisposing classic risk-factors. Many other factors will negatively influence immune function during critical illness, such as (acute) hyperglycaemia and the use of corticosteroids. Corticosteroids have profound effects on the distribution and function of neutrophils,

Table 5. In-vitro susceptibility of *Aspergillus* spp. and moulds to several antifungal drugs[a]

Species	AMB	ITR	VOR	POS	CAS
A. fumigatus	S	S	S	S	S-R
A. flavus	S	S	S-R	S	S-R
A. terreus	S-R	S	S	S	S
A. niger	S	S	S	S	S
Mucor sp.	S-R	S-R	R	S-R	R
Rhizopus sp.	S	S-R	R	S-R	R

R, resistant; S, susceptible; AMB, amphotericin B; ITR, itraconazole; VOR, voriconazole; POS, posaconazole; CAS, cancidas.
[a]Data adapted from references [90,117,118,125].
No breakpoints are established for *Aspergillus* sp. and moulds. For the purposes of this table, susceptibility is defined as ≤2 mg/L for all drugs tested.

monocytes, and lymphocytes, and they directly stimulate the growth of *A. fumigatus in vitro*, possibly via sterol-binding proteins in the fungus. In particular, intravenous corticosteroid treatment in patients with COPD is associated with a rising incidence of IA [84]. Also, broad-spectrum antibiotics, which affect the distribution of the normal flora, have been described as a risk-factor [84]. However, not every critically ill patient in the ICU is at risk of developing invasive fungal infections. Apparently, other specific—patient-related—predisposing conditions seem to be associated with the development of IA; COPD and other chronic lung diseases, diabetes mellitus, acute liver failure/advanced liver cirrhosis, chronic renal failure and near-drowning have been described. In these, mainly retrospective, studies, a mean in-hospital mortality rate of 80% was found in patients with highly suspected or proven IA in the presence of at least one of these underlying conditions, despite antifungal therapy. Remarkably, patients who were suspected of being colonised only with *Aspergillus* spp. (i.e., no signs of pulmonary infection) demonstrated an even higher in-hospital mortality rate, which might suggest that colonisation should be considered as a potentially important finding [83].

IA appears to be gaining a foothold in the ICU in patients without classic risk-factors. In a large study performed in a medical ICU, the investigators found microbiological or histopathological evidence of *Aspergillus* infection in 6.7% of patients; 64% were patients who did not have an underlying haematological malignancy [83]. This observation has been confirmed in other studies in ICUs [85]. Three 'new' risk-factors stand out for IA: COPD, steroid use, and severe hepatic failure. Even critically ill patients without any risk-factor apart from a prolonged stay may develop complex immunological derangements, which put them at risk for developing IA.

Early identification of patients who require antifungal therapy is very important and requires diagnostic tools validated in the ICU population that show positive results in an early phase of the infection. Critical care physicians need a helpful instrument to guide clinical practice in the ICU. The clinical signs associated with IA are notoriously vague (fever, increased respiratory secretions, high oxygen requirements, haemoptysis) and lack specificity in the ICU. Mechanical ventilation and atypical radiological abnormalities (atelectasis, pleural effusions) preclude the use of chest radiography as a helpful diagnostic tool for the ICU patient. The halo sign on computed tomography plays an important role in the neutropenic patient early in the course of the disease. Unfortunately, computed tomography in the ICU patient is of little benefit, as no lesion specific for IA has been identified in this population. Respiratory cultures of *Aspergillus* take a minimum of 48 h to grow. Culture and direct microscopy of respiratory samples have a sensitivity of approximately 30% [84]. Although a positive culture suggests that the patient has IA, it does not discriminate between invasive forms and colonisation. The positive predictive value varies, depending on the degree of immunosuppression, between 20% and 60% in non-neutropenic patients [84].

Application of non-culture-based methods, including galactomannan, PCR, and β-1–3D-glucan, may improve sensitivity, allowing an earlier diagnosis to be made. The use of β-1–3D-glucan in the ICU is hampered by its low positive predictive value, because of false positivity associated with other bacterial and fungal infections, the use of albumin, and haemodialysis [84]. Thus far, no prospective data on PCR detection are available for ICU patients.

Treatment of IA is difficult and mortality remains high, despite the introduction of new antifungals [85]. A significantly better outcome (response rate 52% vs. 30%) was demonstrated in a randomised study that compared voriconazole with conventional amphotericin B [86]. However, 86% of the patients were treated for a haematological malignancy, and no definite conclusions can be drawn for the ICU population. In addition, many aspects of antifungal therapy that are relevant to the ICU population have not been sufficiently addressed, including the pharmacokinetic profile in patients with underlying renal and hepatic dysfunction, interactions with frequently used 'ICU' drugs, the best route of administration, and the value of plasma level monitoring.

In an era of increased availability of new immunosuppressive drugs and better intensive care, with prolonged survival, we can expect to see a continuing increase in the incidence of IA. Important questions remain. Is the incidence of IA in one medical ICU in the same order of magnitude as in other ICUs (surgical, mixed)? Can the delineation of patients at risk be improved? Is, for instance, a hydrocortisone infusion of 7 days enough for *Aspergillus* conidia to germinate and to cause invasive disease? Do non-culture-based methods applied to respiratory samples lead to an early diagnosis and a potential survival advantage in the ICU? Diagnostic techniques should be interpreted with caution and should preferably be validated against post-mortem findings, because proven cases offer the most valuable information. Finally, the results from trials of combination antifungal therapy are eagerly awaited.

From the existing literature, the key learning point is that ICU physicians should be alerted to the possibility of IA in a mechanically ventilated patient at risk with unexplained pulmonary infiltrates.

Filamentous fungi—beyond *Aspergillus*

The last decade has witnessed the emergence of new opportunistic pathogens, including Zygomycetes, *Fusarium* spp., *Paecilomyces* spp., *Scedosporium* spp., and the dematiaceous fungi (e.g., *Alternaria* spp.) [1,87,88]. Some centres face a tremendous increase in the number of infections due to Zygomycetes. *Mucor* spp., *Rhizopus* spp., *Rhizomucor* spp., *Absidia* spp. and *Cunninghamella* spp. are known to cause diseases [89–92]. These pathogens are resistant to voriconazole and caspofungin *in vitro* and *in vivo*.

Fusarium spp., *Alternaria* spp. and *Scedosporium* spp. also account for mould infections among solid-organ transplant recipients, with a crude mortality rate of 90% for *Scedosporium prolificans* and 55% for *Scedosporium apiospermum* [87,88]. *S. apiospermum* strains are typically susceptible to the azoles, and *S. prolificans* strains tend to be resistant to various antifungal agents. Other agents of hyalohyphomycosis include *Acremonium*, *Paecilomyces*, *Penicillium*, *Scopulariopsis* and *Trichoderma* [87,88].

Acremonium

Fungi of the genus *Acremonium* are environmental saprophytes, found in the soil and decaying plant material, and are rarely human pathogens. In immunocompetent individuals, *Acremonium* spp. mainly cause foot mycetomas or corneal infections after inoculation during penetrating injuries. *Acremonium* spp. are being increasingly recognised as opportunistic pathogens. It appears that the major predisposing factors comprise prolonged use of corticosteroids, splenectomy and bone marrow transplantation, along with subsequent tacrolimus administration.

At least 35 *Acremonium* infections in adults have been described in the literature [87]. Fifteen cases of documented *Acremonium* infection (excluding mycetoma and keratitis) in children have been reported [93]. In the recent reports, in both children and adults, *Acremonium strictum* is the most commonly identified species. The presence of adventitious forms of *A. strictum* provides a mechanism for haematological spread and dissemination of infection. Fungaemias caused by *A. strictum* have been reported mainly in neutropenic patients [87].

Paecilomyces

Paecilomyces is a cosmopolitan filamentous fungus which inhabits the soil, decaying plants, and food products. Some species of *Paecilomyces* are isolated from insects. *Paecilomyces* is usually considered to be a contaminant but may also cause infections in humans and animals. The genus *Paecilomyces* contains several species. The most common are *Paecilomyces lilacinus* and *Paecilomyces variotii*.

P. lilacinus is an emerging pathogen that causes severe human infections, including devastating oculomycosis [94]. Usually, it shows low susceptibility to conventional antifungal drugs *in vitro*, and variable susceptibility to novel triazoles. A review of the published literature identified 119 reported cases of human infection by *P. lilacinus* between 1964 and 2004. Most were cases of oculomycosis (51.3%), followed by cutaneous and subcutaneous infections (35.3%), and a smaller group of miscellaneous infections (13.4%). Direct cutaneous inoculation may lead to these infections, which may involve almost any organ or system of the human body; soft-tissue, pulmonary and cutaneous infections, cellulitis, onychomycosis, otitis media, endocarditis, osteomyelitis and catheter-related fungaemia have all been reported [94]. Peritonitis and sinusitis are the most common infections caused by *P. variotii*.

Lens implantation is the most frequent predisposing factor for oculomycosis. Cutaneous and subcutaneous infections occur mainly in solid-organ and bone marrow transplant recipients, although surgery and primary or acquired immunodeficiency are also relevant predisposing factors. Infections in apparently immunocompetent patients have also been reported.

Surgical debridement combined with antifungal drug therapy, or the correction of predisposing factors, such as neutropenia, is usually required to obtain improvement. Treatment with traditional antifungal drugs often fails. Voriconazole has demonstrated good activity in both cutaneous and ocular infections in the few cases in which this drug has been used. The new triazoles ravuconazole and posaconazole show good in-vitro activity against *P. lilacinus* and could be promising therapeutic alternatives. Caspofungin and terbinafine appear to be active *in vitro* against *P. variotii*. *Paecilomyces* spp. do not appear to be sensitive to fluconazole.

A report of *P. lilacinus* infection in a liver transplant patient serves as an illustrative case of infection caused by this mould [95]. A 56-year-old male who was 12 months post-liver transplant presented with a 2-month history of painful, erythematous nodules over the right knee. Several biopsies yielded a mould that was initially phenotypically identified as a *Penicillium* species, but molecular sequence analysis ultimately determined the identity as *P. lilacinus*. Several courses of oral voriconazole were required for resolution of the infection. Skin and soft-tissue infections were the most common presentation. This case highlights the fact that treatment of *Paecilomyces* infections may require multiple courses of antifungal therapy, often with surgical debridement. On the basis of their experience, the authors suggest that voriconazole may be a useful treatment alternative to the more traditional therapy with amphotericin B-based agents.

Scedosporium

S. apiospermum is a significant opportunist with very high levels of antifungal resistance [96]. Previously, it was mainly known to be involved in traumatic, subcutaneous infections and in asymptomatic pulmonary colonisation, but in recent years, new disease entities have emerged. The fungus has now become recognised as a potent aetiological agent of severe infections in immunocompromised patients. Currently, *Scedosporium* infections are among the most common deep mould infections. With a frequency of about 9%, *S. apiospermum* is among the most common filamentous fungi colonising the lungs of CF patients. The intrinsic clinical potency of *S. apiospermum* can be deduced from its extremely infrequent isolation from outside and indoor air but its high prevalence in the lungs of susceptible patients. The natural environmental habitat of the fungus is unknown; nutrient-rich, brackish waters such as river estuaries have been suggested. The fungus is strongly promoted by agricultural and, particularly, by industrial pollution.

S. apiospermum has been specifically listed as an important cause of death in transplant recipients, with a frequency of one per 1000 patients [97]. A comparable frequency (0.4%) has been observed in patients with haematological malignancies [97]. The role of *S. apiospermum* in fatal infections may be underestimated, due to the lack of accurate diagnosis.

S. apiospermum is common in temperate climates and is less frequently encountered in the tropics. Its natural niche is not known; all environments from which it is currently isolated are strongly influenced by human activity. It is a eutrophic fungus that is commonly found in soil. Its occurrence is promoted in manure-enriched or polluted environments, such as agricultural land, garden soil, sewer or ditch mud and polluted pond bottoms. It is also found in hydrocarbon-contaminated soils, being able to assimilate natural gas and aromatic compounds, and it has therefore been suggested for use in bioremediation.

Diagnosis is achieved with a combination of direct microscopy, histopathology, culture, radiology and serology. Numerous studies have shown that antifungal drugs such as amphotericin B, itraconazole, flucytosine, fluconazole and terbinafine show low in-vitro activity against *S. apiospermum*; however, some studies have demonstrated that itraconazole does have some activity. The new triazoles are promising: voriconazole and ravuconazole appear to be active, but posaconazole has shown variable activity. The in-vitro activity of echinocandins against *S. apiospermum* has generally been considered to be modest. However, it is important to exercise caution in interpreting in-vitro susceptibility results, because clinical improvement of *S. apiospermum* infections with amphotericin B treatment has been reported despite apparent in-vitro resistance to this drug.

A major problem in recognition of *S. apiospermum* infections is the fact that the fungus is a typical opportunist. Therefore, none of the clinical entities is fully characteristic for the species. Three basic clinical syndromes can be distinguished [96]: (i) localised disease after trauma; (ii) largely asymptomatic or symptomatic colonisation of the cavities; and (iii) systemic invasive disease. Traumatic infections are found in otherwise healthy persons. Pulmonary infections are observed in patients with predisposing pulmonary disorders. Systemic disease occurs if the immune status of the patient is severely impaired and in victims of near-drowning. The fungus then shows marked neurotropic behaviour. Secondary cutaneous manifestations are infrequent with severe dissemination. *S. apiospermum* has long been considered to be a coloniser of the lungs of patients with pulmonary disorders, but its consistent occurrence in the lungs of patients with CF has only recently received proper attention [96]. The isolation of *S. apiospermum* from the lungs of patients with CF is remarkable, given its infrequent isolation from indoor air, although there appear to be regional differences.

S. apiospermum is increasingly recognised as an important opportunistic pathogen in transplant recipients [97]. Infection is associated with a high rate of dissemination and poor outcome overall. The authors carried out a retrospective analysis of the Cleveland Clinic lung transplant database and identified five patients with *S. apiospermum* isolated from respiratory tract specimens. Disseminated disease developed in three patients, whereas two appeared to be only colonised. This report and the authors' review of the literature highlight the importance of early diagnosis and differentiation from *Aspergillus*, since *Scedosporium* is inherently resistant to amphotericin B. Effective therapeutic approaches being explored include combinations of antifungals, because even the newer triazoles have a 50% response rate in clinical studies. Surgical debridement and immune recovery are associated with improved prognosis, favouring the use of agents that expedite immune reconstitution in these patients. Close monitoring of clinical improvement and frequent re-evaluation of treatment is essential. In conclusion, *S. apiospermum* appears to be a truly emerging environmental pathogen and to display a remarkable shift in its clinical spectrum. For an in-depth review of all aspects of *S. apiospermum*, see the exhaustive review by Guarro *et al*. [96] and the website of a joint European Confederation of Medical Mycology societies and the International Society for Human and Animal Mycology Working Group on *Pseudallesheria–Scedosporium*: http://www.scedosporium-ecmm.com.

Scopulariopsis

Scopulariopsis brevicaulis is a rare and emerging pathogen that has been increasingly reported in the past two decades as a cause of deep mycosis in hosts presenting with factors that predispose them to infection [87]. *S. brevicaulis* and other *Scopulariopsis* spp. have mainly been associated with onychomycosis, but their spectrum of human infections includes post-traumatic keratitis and endophthalmitis, disseminated skin lesions and meningitis in AIDS patients, endocarditis related to valvuloplasty or prosthetic valves, subcutaneous hyalohyphomycosis in immunocompromised hosts, fungus ball and pneumonia, and disseminated infections in stem-cell transplant patients or hosts with leukaemia.

S. brevicaulis has been reported to be resistant *in vitro* to amphotericin B, flucytosine, terbinafine, and azole compounds. Invasive infections due to *S. brevicaulis* are unlikely to respond to a particular antifungal treatment, and several therapeutic approaches have been considered, such as debridement or excision of necrotic tissue plus chemotherapy, prolonged monotherapy with azole agents or terbinafine, and combinations of antifungal agents. The combined activity *in vitro* of antifungal agents against *S. brevicaulis* has not been assessed until recently. One study describes the activities of ten combinations of antifungal compounds against clinical isolates of this species [98]. An unexceptional effect was observed for all combinations. Synergy was observed for some isolates and combinations, particularly with posaconazole–terbinafine (68% of strains), amphotericin B–caspofungin (60%), and posaconazole–caspofungin (48%).

Trichoderma

Trichoderma spp. are common, soil-borne, filamentous fungi and have long been known as non-harmful microorganisms. They are used in biotechnology as sources of enzymes and antibiotics. Moreover, they are applied to agricultural crops as plant growth promoters and biofungicides. However, as recently emerging fungal pathogens, *Trichoderma* strains have been detected on the skin, in the lung and as causative agents of peritonitis in peritoneal dialysis patients, and have been found to be disseminated in the liver, brain, heart and stomach of immunocompromised patients [2]. The majority of the pathogenic *Trichoderma* isolates are members of the species *Trichoderma longibrachiatum*. Despite systemic antifungal therapy, the prognosis for *Trichoderma* infection is poor, regardless of the type of infection and the therapy used.

Agents of mucormycosis (zygomycosis)

The Mucorales are opportunistic fungi capable of causing acute, rapidly developing and often fulminant infections in the compromised host. Infections are thought to be acquired by inhalation or by progression of previously localised cutaneous lesions, and occur in patients with neutropenia. Several recent reviews and commentaries underline the increasing prevalence and awareness of these infections [19,89,91,92]. The spectrum of zygomycosis is reviewed here.

Zygomycetes may also cause lethal infections in patients with diabetes, patients receiving deferoxamine therapy, injection drug users, and patients with no apparent immune impairment. Invasive zygomycosis is clinically similar to aspergillosis; fungi commonly affect the paranasal sinuses (39%), the lungs (24%), and the skin (19%). Dissemination develops in 23% of cases and the mortality rate is 96%; risk-factors are shown in Table 4.

Major risk-factors include ketoacidosis in untreated type 1 diabetes mellitus, lymphoma, leukaemia, neutropenia, corticosteroid or other long-term immunosuppressive therapies, and deferoxamine therapy of dialysis patients with aluminium or iron overload. To date, there are only a few reported infections associated with AIDS, so AIDS does not appear to be a significant risk-factor. Trauma to the skin, including severe burns, intravenous catheters, intravenous drug abuse, or even an insect bite, may also result in infection in the immunocompetent host.

The pathogenesis of mucormycosis is unclear, and while it is undoubtedly exogenous, possible sources of infection have only occasionally been suggested, e.g., adhesive dressings, air-conditioning filter units, and food. Cutaneous trauma may be an underestimated event in the initiation of many cases of mucormycosis. However, most infections follow inhalation of spores that have been released into the air, and the lungs and nasal sinuses are common sites of infection. The most common predisposing illness for gastrointestinal infection is severe malnutrition or disruption of the gastrointestinal mucosa.

The infectious propagule responsible for the establishment of zygomycosis is unproven. Many studies have suggested that sporangiospores are responsible, while others have indicated that small hyphal fragements initiate disease. Since sporangiospores of mucoraceous fungi are uncommon in air and are readily contained by phagocytic cells normally found in pulmonary tissues, it is possible that in some cases, especially where trauma is the associated factor, hyphal fragments are the infectious propagules.

Mucormycosis is distributed worldwide. Many different organisms have been implicated, but the most common causes of human infection, listed in order of apparent incidence, are *Rhizopus oryzae* and *Rhizopus microsporus* var. *rhizopodiformis*. Other less frequent aetiological agents, but for which a major pathogenic role in humans has been established, include *Absidia corymbifera*, *Apophysomyces elegans*, *Cunninghamella bertholletiae*, *Mucor* spp., *Rhizomucor pusillus* and *Saksenaea vasiformis*. These moulds are ubiquitous, are thermotolerant and can be isolated in large numbers from soil or decomposing organic matter, such as fruit and bread. Their spores can often be found in the outside air. Nosocomial outbreaks of mucormycosis are not as common as hospital-related *Aspergillus* infections, but have been reported in leukaemic patients. Nosocomial cutaneous infections with *R. microsporus* var. *rhizopodiformis* have been traced to contaminated dressings and wooden tongue depressors used as immobilising splints in a neonatal ICU. The major risk-factors predisposing individuals to mucormycosis include uncontrolled diabetes mellitus, other forms of metabolic acidosis, burns and malignant haematological disorders. Treatment is seldom of benefit unless these underlying conditions can be corrected.

In most cases, mucormycoses occur in patients already receiving treatment because of another disease. For precisely this reason, they may not be immediately recognised. The clinical hallmark of mucormycosis is vascular invasion resulting in thrombosis and tissue infarction/necrosis. In most cases, the infection is relentlessly progressive and results in death unless treatment with a combination of surgical debridement and antifungal therapy is initiated promptly.

Mucormycosis is an opportunistic infection and is seldom seen in normal persons. Various forms are recognised, each of which is associated with particular underlying conditions. Like the aetiological agents of aspergillosis, the causal organisms of mucormycosis have a predilection for vascular invasion, causing thrombosis, infarction and necrosis of tissue. The anatomical distribution of lesions appears to correlate to a certain degree with defined predisposing conditions, e.g., craniofacial involvement in individuals with diabetic acidosis, pulmonary and disseminated infection in patients with acute leukaemia, and gastrointestinal and cutaneous lesions following local trauma. Indeed, cutaneous infections have possibly replaced craniofacial and pulmonary disease as the prevalent clinical manifestation of mucormycosis, a change mirrored by the emergence of *R. microsporus* var. *rhizopodiformis* (as *R. rhizopodiformis* in many cases) as a significant pathogen.

Zygomycetes appear to be susceptible to amphotericin B and are generally not susceptible to the triazoles and echinocandins (Table 5) [99]. Among the extended-spectrum triazoles, posaconazole appears to be active against most of the Zygomycetes [100,101].

Rhinocerebral mucormycosis

The terms rhinocerebral and craniofacial mucormycosis are used to describe infection that begins in the paranasal sinuses and then spreads to involve the orbit, face, palate or brain. The term should be used only when there is documented brain involvement in addition to involvement of sinuses alone or to involvement of both sinuses and orbit respectively. It is not clear whether paranasal and rhinorobital mucormycosis are simply rhinocerebral mucormycosis in evolution or whether the extent to which mucormycosis progresses beyond the paranasal sinuses to involve the orbit and brain is dependent on the host response. Several recent case series and reviews describe a clinical variety of rhinocerebral mucormycosis termed rhino–orbital–cerebral mucormycosis. Nasal endoscopy appears to be useful in diagnosis, tissue debridement and follow-up of patients.

Rhinocerebral mucormycosis is most commonly seen in acidotic individuals, particularly those with uncontrolled diabetes mellitus, but it also occurs in leukaemic patients and organ transplant recipients. It is the most common clinical form of mucormycosis and is often fatal within a week of onset if left untreated. It is assumed that the initial event is colonisation of the nasal mucosa, allowing the fungus to spread via the paranasal sinuses into the orbit. Involvement of the brain and cavernous sinus occurs by way of the orbital apex. Multiple approaches to treatment are required. Early clinical and laboratory diagnosis is crucial. If the infection spreads into the palate, a black necrotic lesion is often found. This is an important diagnostic sign, and necrotic lesions may also be found on the nasal mucosa. Nasal septum or palatal perforation is frequent. Drainage of black pus from the eye is an ominous but useful diagnostic sign. From the orbit, infection may spread into the brain, leading to frontal lobe necrosis and abscess formation. These features result from invasion of the fungus through the cribiform plate of the ethmoid bone. The cerebrospinal fluid (CSF) findings are non-specific. CSF cultures are sterile.

The radiological findings are non-specific, but are useful in delineating the extent of the infection.

Pulmonary mucormycosis

Pulmonary mucormycosis is seldom diagnosed during life. Mucormycosis may develop in the lungs as a result of aspiration of infectious material, or following inhalation, or from haematogenous or lymphatic spread during dissemination. Most cases occur in leukaemic patients undergoing remission induction treatment. Pulmonary infiltrates develop with infarction of focal areas of lung. The clinical signs are those of bronchitis, pneumonia, and thrombosis. There is necrosis of the parenchyma, leading to cavitation; the bronchi may be perforated, resulting in haemoptysis. If untreated, haematogenous dissemination to other organs, particularly the brain, will often occur. There are no characteristic symptoms or signs to distinguish mucormycosis from aspergillosis. The infection is fatal within 2–3 weeks.

Clinicinans are aware that pulmonary zygomycosis is being seen more frequently in patients with cancer. However, the clinical manifestation is similar to that of invasive pulmonary aspergillosis. Computed tomography imaging could potentially differentiate the two infections. Most cases of pulmonary zygomycosis in these patients appear to develop as breakthrough infections if treatment with antifungal agents effective against *Aspergillus* spp. is administered.

Cutaneous mucormycosis

Cutaneous mucormycosis is a particular problem in infected patients with burns, in whom spread to underlying tissue is common. The initial signs include fever, swelling and changes in the appearance of the burn wound. The development of severe underlying necrosis and infarction in a burn should suggest the diagnosis.

Cutaneous infections account for 16% of all forms of zygomycosis, with an associated mortality rate of 16%, as compared to 67% for rhinocerebral, 83% for pulmonary and 100% for disseminated infection. The most common causative organisms are *Rhizopus* spp., although others, such as *Mucor* and *Absidia*, are also frequently seen, whereas *S. vasiformis* and *A. elegans* are rare pathogens. Cutaneous zygomycosis is less likely to be associated with severe systemic illness than are other forms, while local predisposing factors such as burn, trauma, surgery, needlesticks and others play a major role. The most common sites involved in cutaneous zygomycosis are the lower and upper extremities, followed by the head and neck, and then the abdomen.

Mucormycotic gangrenous cellulitis can follow other forms of trauma to the skin. In diabetic or immunosuppressed patients, cutaneous lesions may arise at an insulin injection site or a catheter insertion site. More massive trauma, such as open fractures or crash injuries, has been seen in other patients. Necrotising cutaneous mucormycosis has occurred in patients who have had contaminated surgical dressings applied to their skin. Infections have also been described in a variety of individuals after traumatic injury or near-drowning. The most recent examples of zygomycosis in trauma patients are exemplified by survivors of the tsunami in Southeast Asia on 26 December 2004.

Disseminated mucormycosis

Disseminated mucormycosis may follow any of the four forms of mucormycosis described so far, but it is usually seen in neutropenic patients with pulmonary infection. Less commonly, dissemination occurs from the gastrointestinal tract, or from burns or other cutaneous lesions. The most common site of spread is the brain, but metastatic necrotic lesions have also been found in the spleen, heart and other organs. Zygomycetes can complicate peritoneal dialysis.

Disseminated mucormycosis is usually diagnosed after the patient has died of the infection. Occasionally, metastatic cutaneous lesions permit an earlier diagnosis.

Cerebral infection following haematogenous dissemination is distinct from the rhinocerebral form of mucormycosis. It results in abscess formation and infarction. Patients present with sudden onset of focal neurological deficits or coma. Investigation of the CSF is unhelpful: protein, glucose and cell abnormalities are nonspecific, and cultures are sterile. Computed tomography and magnetic resonance scans are useful in locating the lesions.

Mucormycosis in AIDS

Mucormycosis in cases of HIV disease is rare. However, it can be the presenting opportunistic infection in AIDS. Predisposing factors for mucormycosis in HIV disease include low CD4 count, neutropenia, and active intravenous drug use. Mucormycosis can present in the basal ganglia, the skin, the gastrointestinal tract, or the respiratory tract, or may be disseminated.

Mucormycosis in haematological malignancies

The incidence of mucormycosis in patients with haematological malignancies has increased during the last decade, probably due to the more severe and prolonged post-chemotherapy neutropenia. The diagnosis is usually made at autopsy, and its incidence in autopsy studies in patients with haematologic malignancies ranges between 0.4% and 0.9%. Several retrospective studies have been published [102].

Voriconazole is increasingly used as prophylaxis in patients with haematological malignancies. Several reports have linked the prolonged use of this agent to an increase in infections caused by Zygomycetes [103–105]. However, an increase in zygomycosis was reported before the availability of voriconazole [106].

Mucormycosis in solid-organ transplant recipients
Mucormycosis is a rare infection in renal transplant recipients; however, mortality is exceedingly high. Risk-factors predisposing to this disease include prolonged neutropenia, diabetes, and immunosuppression. Mucormycosis is a rare but highly invasive infection following orthotopic liver transplantation.

CHANGING EPIDEMIOLOGY AND IMPLICATIONS FOR THERAPY

The epidemiology of invasive fungal infections in immunocompromised patients is rapidly changing. Factors that influence the current trends in the epidemiology of opportunistic fungal infections are given in Table 6. Recognition of these epidemiological changes is critical to patient care. Key elements in the selection of the appropriate antifungal agent are the type of patient (solid-organ or stem-cell transplant), severity of immunosuppression, history of prolonged exposure to antifungal drugs, and knowledge of the genera and species of the infecting pathogen and its typical susceptibility pattern.

Table 6. Variables that account for the current trends in the epidemiology of opportunistic fungal infections[a]

Increasing number of susceptible hosts: transplant type
Centre-to-centre differences: patient selection
Greater laboratory expertise in detection and identification of fungi
Use of new transplant modalities for haematopoietic stem-cell transplantation
Changing surgical techniques: evolution in organ transplant practices
Use of corticosteroid-sparing regimens and overall conservative approach to immunosuppression
Use of novel immunosuppressive agents
Antimicrobial prophylactic practices
Exposure to azoles
Better control of underlying diseases

[a]Data compiled from reference [110].

More than 17 different species of *Candida* have been identified as aetiological agents of BSIs and other manifestations of systemic candidosis. About 95% of candidaemia cases are caused by four species: *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* [2]. The excellent activities of new and established systemic antifungal agents against *C. albicans*, *C. parapsilosis* and *C. tropicalis* are well-documented. Among these common species, only *C. glabrata* can be said to be truly emerging as a cause of candidaemia, due in part to its intrinsic and acquired resistance to azoles and other commonly used antifungal agents. The remaining 5% of candidaemias are caused by 12–14 different species. Of note is the emergence of *Candida dubliniensis* in some centres [107]. Although these species must be considered as rare causes of BSIs, several have been observed to occur in nosocomial clusters and/or to exhibit innate or acquired resistance to one or more established antifungal agents. Given that these uncommon species may emerge as important opportunistic pathogens in the future, it is imperative that an accurate diagnosis and identification of the fungal pathogen is made. Furthermore, it is useful to keep in mind that broad and injudicious use of any anti-infective agent in severely immunocompromised hosts may result in superinfections due to organisms that are both unusual and drug-resistant.

It is evident that there has been an increase in rare mould infections in recent decades [108]. These infections have been reported primarily in severely immunocompromised patients. The emergence of these organisms is multifactorial and can be related to more intense immunosuppression, the prolonged survival of patients who have what were previously fatal diseases, and the selective pressure of broad-spectrum antifungal agents used for prophylaxis or therapy. Among the organisms causing these rare mould infections, the Zygomycetes are the most commonly encountered, and in some institutions, the increase in the incidence of these organisms appears to be associated with the use of voriconazole. *A. terreus*, a species that is resistant to amphotericin B, and less frequently, *Aspergillus ustus* and *A. lentulus*, have been noted increasingly as causes of invasive aspergillosis in tertiary-care centres in the US. Several species of *Scedosporium* with innate resistance to many antifungal agents have emerged as major causes of disseminated mould infections that are frequently very difficult to treat. Among patients who have haematological malignancies, are neutropenic or have received a haematopoietic stem-cell transplant, infections due to *Fusarium* spp. respond poorly to many antifungal agents. Dematiaceous, or brown–black fungi, most often associated with chronic localised infections, are now increasingly reported as a cause of disseminated infection in immunosuppressed hosts. Concomitant with the increased number of infections due to these rare moulds, several new mould-active antifungal agents have been developed. The new expanded-spectrum azole voriconazole has changed our approach to moulds such as *S. apiospermum*, *Fusarium* spp. and *A. terreus* that are amphotericin B-resistant. Posaconazole, the most recently approved expanded-spectrum azole, is the first drug in the azole class to show activity against the Zygomycetes and has proven extremely useful for step-down therapy after initial treatment with amphotericin B. It is not known whether posaconazole is effective as primary therapy for zygomycosis; the use of this agent for that purpose awaits clinical trials with the recently developed intravenous formulation of posaconazole.

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