Fungal infections in the intensive care unit

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LIST OF ABBREVIATIONS

ABPA	Allergic Broncho-Pulmonary Aspergillosis
APACHEII	Acute Physiology and Chronic Health Evaluation II
BAL	Bronchoalveolar Lavage
CI	Candida Colonization Index
CVC	Central Venous Catheter
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
EORTC-MSG	European Organization for Research and Treatment of Cancer-Mycoses Study Group
IA	Invasive Aspergillosis
IC	Invasive Candidiasis
ICU	Intensive Care Unit
IDSA	Infectious Disease Society of America
PCR	Polymerase Chain Reaction
PNA-FISH	Peptide Nucleic Acid-Fluorescence in Situ Hybridization
TPN	Total Parenteral Nutrition

SUMMARY

Title: Fungal Infections in the Intensive Care Unit
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Background and Aims: Treating infections in patients at the intensive care unit is a complex matter which is nevertheless complicated by the critical state that the patients are usually in. The mortality rate due to fungal infections haven't changed much in the last few years and so; dealing with fungal infections in the setting of critically ill patients can be challenging and there is an ongoing need for more research and for the development of new approaches. While previous discoveries of antifungal drugs were deemed successful in treatment, they created room for emergence of new fungal species and the widespread use of antibiotics and antifungal drugs has led to a growing resistance. Difficulties with early detection of fungal infections are currently still problematic while it has been shown to be crucial for the success of the treatment. With a shift in species and emerging resistance, while diagnostics and mortality rates aren't getting much better; new approaches to fungal infections in the ICU (Intensive Care Unit) are being sought for.

Methods: PubMed and Google Scholar were used to find relevant material. Search terms included but were not limited to: Invasive Candidiasis, ICU, treatment, Aspergillosis, risk factors etc. The primary criteria for study selection were evidence-based research and quality of design.

Findings: Predictive rules to select high-risk patients who might benefit from prophylactic antifungal therapy have been found to be successful when used in combination with a clinical suspicion of fungal infection; but still need to be incorporated into clinical praxis. New diagnostic techniques (β-D-glucan, PCR) are under development and would lead to faster recognition of patients with fungal infections and hence faster administration of treatment; which has been shown to positively affect the survival rate. The recommended treatment has changed toward newer antifungal drugs but also depend on the age of the patient, immune status, localization of infection and isolated fungal species.

Key words: Invasive fungal infection, Candidemia, Invasive aspergillosis, ICU.

SAŽETAK

Naslov: Gljivične infekcije u jedinici intenzivnog liječenja
Autor: Dajana Džeko

Pozadina i ciljevi: Liječenje infekcija kod pacijenata u jedinici intenzivnog liječenja je složeno, a dodatno se komplicira životno ugrožavajućim stanjem u kojem su pacijenti. Stopa smrtnosti zbog gljivičnih infekcija nije se znatno promijenila u posljednjih nekoliko godina. Stoga u okruženju kritično bolesnih pacijenata bavljenje gljivičnim infekcijama još uvijek je izazovno i postoji potreba za daljnjim istraživanjem i razvojem novih pristupa. Prethodna otkrića antifungalnih lijekova su bila uspješna u liječenju, ali su stvorila prostor za pojavu novih gljivičnih vrsta, a široko korištenje antibiotika i antifungalnih lijekova dovelo je do sve veće rezistencije. Još uvijek postoje poteškoće u ranom otkrivanju gljivičnih infekcija iako znamo da je upravo to presudno za uspjeh liječenja. Uz današnju pojavu novih vrsta gljiva i porast rezistencije traže se novi pristupi dijagnostici i liječenju gljivičnim infekcijama u jedinici intenzivnog liječenja (JIL).

Metode: PubMed i Google znalac upotrijebljeni su za pronalaženje relevantnog materijala. Izrazi za pretraživanje su uključivali, ali nisu bili ograničeni na: invazivnu kandidijazu, JIL, liječenje, aspergiloza, čimbenike rizika itd. Primarni kriteriji za odabir studija bili su istraživanja temeljena na dokazima i kvaliteta dizajna studija.

Nalazi: Postoje pravila za odabir visokorizičnih bolesnika koji bi mogli imati koristi od profilaktičke antifungalne terapije i pokazala su se uspješnima kada se koriste u kombinaciji s kliničkom sumnjom na gljivičnu infekciju, ali još uvijek treba koristiti dobru kliničku praksu. Nove dijagnostičke tehnike (β-D-glukan, PCR) su u razvoju i dovode do bržeg prepoznavanja bolesnika s gljivičnim infekcijama i time bržem početku liječenja; što se pokazalo da pozitivno utječe na stopu preživljavanja. Preporučeno liječenje se promijenilo prema novijim antifungalnim lijekovima, ali također ovisi o dobi pacijenta, imunosnom stanju, lokalizaciji infekcije i izoliranim vrstama gljiva.

Ključne riječi: Invazivna gljivična infekcija, Kandidemija, Invazivna aspergiloza, JIL.

1. INTRODUCTION

Fungal infections are a major burden of mortality and morbidity in the critical care setting and are one of the leading causes of nosocomial infections despite recognition of risk factors and improvement in infection prevention. As one of the leading causes of infections in the ICU (Intensive Care Unit), fungi cause opportunistic infections (infection develops when the hosts' immune system is impaired) (8). There are many different fungal species that can be isolated in critically ill patients but the most common species are *Candida*, *Aspergillus* and *Mucorales* (1) where *Candida* is by far the most prominent cause of nosocomial fungal infections (8). *Candida* exists as part of the normal flora of the skin, the gastrointestinal and the genitourinary tract of healthy humans and colonizes the mucus membranes of 30-60% of humans (2, 9). Candidemia is the fourth most common cause of nosocomial bloodstream infections in the USA (3, 4) and the 6^{th}-10^{th} in Europe (2). Studies show that 80% of cases of severe invasive fungal infections are due to *Candida spp* and 0, 3-19% are due to *Aspergillus spp*. Since the critically ill patients who are treated for invasive fungal infections are a very heterogeneous group in combination with the finding of *Candida spp.* being responsible for 17% of all infections in a worldwide ICU prevalence study; the approach to these infections is complex but of high importance. Selecting which patients would benefit the most from antifungal therapy, which drug to use, the right dose and duration of treatment is a challenge. Since the occurrence of infections is changing (species) and the emergence of resistance to antifungal agents (3, 9) it is of great importance to be cautious when treating these infections. The recognition of risk factors for fungal infections has been somewhat established, where multiple studies have agreed on which the major risk factors are. There have been several attempts of creating prediction rules using these risks, to single out the high-risk patients who would benefit the most from the treatment. The search for prediction rules is indeed valid, considering the fact that 10-40% of cases of candidemia are associated with sepsis or septic shock (2). Other fungal species are far less common with unique risk factors and will be mentioned only briefly.

Treating fungal infections, especially in patients in the ICU (Intensive Care Unit), is a delicate and important matter since the performance of different antifungal agents affects patients' mortality. Studies have shown that episodes of invasive candidiasis occur 5-10 times more often in the ICU compared to other wards and that two thirds of candidemia

occur in the ICU or on surgical wards (10). The mortality remains high (11) and there are still difficulties considering the diagnosis and treatment, while it has been established that early treatment increases survivability (5, 12). New insights into diagnostics and therapeutic strategies have changed the approach towards fungal infections. This paper is presenting the latest consensus, to this date, considering epidemiology, risk factors, diagnostics and treatment of fungal infections in the ICU.

2. EPIDEMIOLOGY OF FUNGAL INFECTIONS IN THE ICU

2.1 Candida Infections

Candida spp. represent a great proportion of nosocomial fungal infections and is the 4th most common blood stream infection in USA (3, 4) while being the 6th-10th in population-based European studies (2). The absolute majority of invasive fungal disease due to *Candida* species (over 90%) is due to the five most common pathogens *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis* and *C. krusei (7)*. A shift from albicans to non-albicans species and to former non-pathogenic species has been observed. This might be explained with the fact that we can now detect new species in the laboratories and also due to an increase in the number of vulnerable people (1). Around 50% of the isolated species detected are *C. albicans* but the rate of identified nonalbican species are increasing (2, 4). Non-albican species that are becoming important pathogens are *C. glabrata* in northern Europe, Canada and the United States and *C. parapsilosis* in southern Europe, Asia and South America (3). The incidence is age-specific, with the maximal rates at the extremes of age (3). The incidence in reality might be higher than noted because of the low rate of positive blood cultures and the difficulty in making a diagnosis of candidiasis without candidemia (1). Candidiasis is the leading cause of fungal infections in the ICU (Intensive Care Unit), ranging from 1 to 10/1000 ICU admissions (2) and is an important cause of morbidity and mortality in ICU patients (1). The mortality rate differs significantly between patients. The mortality rate for a patient who has developed candidiasis or has multifocal colonization is 30-50% (4, 6) versus a patient without this diagnosis, 8-14%, or with unifocal colonization (13).

Virulence and antibiotic susceptibility vary between different candida species and of note is that antifungal agents (especially azoles) have been associated with a consecutive

infection with *C. glabrata* and *C. krusei* (2, 3). *C. parapsilosis* and *C. krusei* are less virulent than *C. albicans, C. tropicalis* and *C. glabrata*. *C. dubliniensis*, *C. lusitaniae*, *C. kefyr*, *C. guilliermondii* are less frequent and are associated with specific hosts or with susceptibility patterns. *C. dubliniensis* is common in immunosuppressed individuals, especially in HIV-infected patients (3). A newly discovered, multidrug-resistant species is *C. auris* which causes invasive nosocomial infections (2).

Invasive candidiasis can be divided into candidemia and deep-seated tissue candidiasis, where candidemia is more common. The deep-seated infection arises from direct inoculation to a sterile site, or due to earlier or unrecognized hematogenous dissemination. It can lead to secondary candidemia or stay localized (3). Other studies divide the *Candida* related diseases into three categories; candidemia with or without endophthalmitis, disseminated hematogenous infection with deep organ involvement and chronic disseminated candidiasis (1).

2.2 Non-Candida Infections

Aspergillus is a genus with ≥180 species but only a few cause infections in humans. This group of fungi has a wide environmental distribution and can be found in soil, water, air, decomposing organic matter and ventilation systems. The species that are most clinically important are; *A. fumigatus*, *A. flavus*, *A. niger, A. terreus*, and *A. nidulans* (1, 8). *Nesartorya udagawae* has emerged with increasing clinical impact (1). The clinical characteristics of an infection (degree of infection, response to treatment and outcome) depend on the patient's immune status. *Aspergillus* species can cause allergic hypersensitivity reactions (rhinitis, asthma, pneumonitis, ABPA (Allergic Broncho-Pulmonary Aspergillosis)) or more serious invasive (pulmonary) disease. The manifestations of the invasive disease once again depend on the immune status of the host. In neutropenic patients it tends to lead to disseminated extrapulmonary disease through hematogenous or continuous spread, while it tends to lead to localized disease in non-neutropenic patients (8). The incidence in patients depends on the immune status and is in a decreasing manner; patients with hematopoietic stem cell transplantation, stem cell transplantation, solid organ transplantation, HIV and hematologic malignancy.

When *Aspergillus* species have been isolated in a patient, it is difficult to discriminate

between colonization and infection (1) but what is known is that it is a bad prognostic marker (8). Overall mortality ranges between 17-90 % depending on the type of infection and immune status of the patient, with the highest rate in patients who are liver transplant recipients (1, 8).

Mucormycosis is a rare but serious infection with emerging importance and the most common genera are *Rhizopus*, *Mucor* and *Rhizomucor* (1). Other genera are *Lichtheimia*, *Cunninghamella*, *Apophysomyces, Saksenae* and other species. They cause an invasive fungal infection and are considered a fungal emergency. Mortality depends on the underlying conditions and ranges from 19-35 % without any underlying condition and up to 66 % for malignancy (1, 14, 15). The most common sites of infection are the sinuses, lung and the skin (in a decreasing order) but the central nervous system and gastrointestinal tract have also been described (15, 16).

Infections with rare yeast species have been associated with specific conditions and exist as opportunists, causing invasive infections in selected patients. *Cryptococcus*, *Histoplasmosis* and *Pneumocystis* usually cause invasive disease in patients with severe T-lymphocyte dysfunction. Other fungal species that are even more rare but can be seen in the ICU (Intensive Care Unit) setting are; *Fusarium, Scedosporium, Trichosporon, Hansenula, Rhodotorula* and *Malassezia* (8, 17). According to the latest ESCMID guidelines (to date) one should keep in mind that almost all of these rare invasive fungal infections are considered intrinsically resistant to echinocandins when deciding for treatment in the ICU (17).

3. RISK FACTORS FOR FUNGAL INFECTIONS IN THE ICU

There is an overlap of the risk factors for different fungal species and with the characteristics of critically ill patients. Generally speaking, the risk increases when several areas of a host's defense mechanisms are impaired (8). There are numerous known risk factors for invasive candidiasis in the ICU but the ones that have been supported most frequently by different studies are central venous catheter, recent surgery and broad-spectrum antibiotics (3, 4), see table 1. Patients from the surgical ICU and especially

patients who underwent abdominal surgery, experienced anastomotic leakages and had more than one surgery are considered to be at a higher risk (3, 6). Regarding the increasing age of patients treated in the ICU and the growing number of comorbidities, more and more patients have several risk factors. These are therapy with immunosuppressive agents, neutropenia, TPN (Total Parenteral Nutrition), diabetes mellitus, pancreatitis, renal insufficiency, high disease severity score (APACHEII>20), major trauma, solid organ transplants and hematologic malignancy (1-3). Considering colonization with *Candida spp.*, it can be considered an independent risk factor for development of invasive candidiasis (1, 3, 5) and more so if the colonization is multifocal (6) and is assessed using the colonization index (2). Studies have had differing conclusions though. Some studies have concluded that it is the disruption in the hosts' defenses that creates the association with developing IC (Invasive Candidiasis) and not the degree of colonization (8). In a prospective multicenter study at surgical ICUs, the colonization of urinary tract/rectum and a higher APACHEII score wasn't found to increase the risk of developing invasive candidiasis, while receiving antifungal agents was found to decrease the risk. A retrospective study didn't find any significant association between receiving corticosteroids, immunosuppressive drugs, abdominal surgery and chronic hemodialysis and being at an increased risk of developing candidiasis. They suggest receiving broad spectrum antibiotics, diabetes mellitus and new onset hemodialysis to be significantly associated with the risk (13). Worth noting is that the sample was small and the circumstances described were rare events. Studies have shown that mortality might be independently affected by the severity of the disease while not being greatly affected by the chosen antifungal drug or presence of a CVC (Central Venous Catheter) (18). Receiving prophylactic treatment (fluconazole) has shown emergence of *Candida krusei* in bone marrow transplanted patients (4).

Table 1. Risk factors associated with *Candida spp.* infections in the ICU
Use of broad spectrum antibiotics
Presence of central venous catheter
Use of immunosuppressive drugs, glucocorticoids, chemotherapy
Major trauma
Recent surgery (particularly abdominal surgery with anastomotic leakage & ≥1 surgery)
Neutropenia
Solid organ transplantation
Hematologic malignant disease
Hemodialysis
Pancreatitis
Total parenteral nutrition
Diabetes mellitus
Multifocal colonization (colonization index ≥0,4)
Bold indicates major risk factors. For further information see references (1-6)

CVCs (Central Venous Catheters) have been recognized as a major risk factor for candidemia due to the discovery that *Candida* species produce biofilms and adhere to catheters and the observation that the fungal infection may persist until removal of the catheters (3). Biofilms have been associated with creating a higher resistance to antifungal drugs and to the hosts' immune response (9). The effect of removal of central venous catheters hasn't yet to be agreed upon with studies showing different results; making it into a controversial debate (11). A cohort of 842 adults from two randomized clinical trials didn't find any significant effect of early CVC removal. Early removal was defined at two points in time; within 24 hours or 48 hours after initiation of antifungal therapy. They concluded that APACHEII score (disease severity score), older age and neutropenia were the most beneficial variables and that CVC removal should be individualized (19, 20). A patient-level review of 1915 patients from seven trials concluded that the impact of CVC removal depended on the APACHEII score; with significant impact for the three lower quartiles but no association with improvement in the highest APACHEII quartile (score >24) (18). This would again point to an individualized approach towards CVC removal. Studies have also shown that removing catheters during candidemia or abscess drainage in invasive candidiasis is an independent factor of mortality reduction (2, 21). When interpreting the results of some studies that found that the removal of CVCs is associated with better

mortality it should be noted that there is a bias toward better outcome since a patient has to be alive to have the CVC removed (11, 18). In conclusion, it is recommended by the latest IDSA guidelines (2016) that the CVC should be removed as soon as possible in non-neutropenic patients with IC (Invasive Candidiasis), if the CVC is considered to be the source of the infection (7).

Since there are many different risk factors associated with the development of a fungal infection and taking all of these into account would include a great scope of patients, there have been attempts to develop systems for guiding the clinical doctors in identifying the patients at the highest risk of developing such an infection. This is important because of the development of resistance but also from a cost-efficient perspective. The Candida Colonization Index and the candida score are two examples of such attempts. *Candida* Colonization Index (CI) is the ratio of the culture positive sites over the total number of sites cultured for candida. Studies have concluded that a CI ≥ 0,4 could be used as the cutoff-value for introducing preemptive treatment (1, 3). Using a corrected *Candida* Colonization Index [(number of sites with heavy colonization/number of sites colonized) x colonization index] ≥0, 4 has had an efficiency of 100% of predicting IC (Invasive Candidiasis) (5). Candida Score (CS) is based on specified risk factors (surgery upon admission, TPN (Total Parenteral Nutrition), severe sepsis and multifocal colonization) and a value of CS > 2,5 puts the patient in the category of a high-risk patient, who might benefit from prophylactic treatment (1). A prospective cohort study compared the usage of CI, CS, β-D-glucan and anti-*Candida* antibodies for differentiating between colonized and infected non-neutropenic patients. CS was found to be better than CI considering both sensitivity, specificity and number of patients needed to be included to predict one infection (8,7 vs 20,8 respectively). They suggest to use a cutoff-value for the CS >3, since the rate of patients having invasive candidiasis and a CS score <3 was 5 %. Considering the serological biomarkers, this study found that β-D-glucan had a sensitivity of 77,8 % and a specificity of 52,7 % while the anti-*Candida* antibodies wasn't found to be useful. (6).

New studies have concluded that genetic variations, polymorphisms, might play a role in the susceptibility to candidemia. Studies have shown that the risk increases by 19 times in ICU (Intensive Care Unit) patients in the presence of newly discovered single-nucleotide polymorphisms. Disease progression has been associated with cytokine polymorphisms despite antifungal treatment. Polymorphism in the TLR/interferon pathway has also been

shown to increase the susceptibility in ICU patients vs. controls matched for underlying diseases (2, 3). These findings have opened up for further interest in genetic risk factors where one might speculate that the possibility of screening for specific polymorphisms could be a future method of selecting patients who would benefit from prophylactic antifungal therapy.

Regarding *Aspergillus spp.*, there are often multiple risk factors present in the patients who develop IA (invasive aspergillosis) at the ICU. The first risk factor to be recognized, since several decades ago, is neutropenia. Today it is believed that it's not only the number of neutrophils that matters, but also the overall neutrophil functional status. Very often though, compromised immune functional status and neutropenia coincide. The duration and degree of neutropenia plays an important role as well and this concerns recipients of solid organs, especially lung (1). The recognized risk factors for developing IA are immunocompromised status with hematologic malignancy, neutropenia, stem cell and solid organ transplant recipients (especially lung) and steroid treatment (1, 8). Corticosteroids have been recognized as a major risk factor (8) and a category of patients who have been noted to have an increasing incidence are patients with chronic obstructive pulmonary disease who are treated with corticosteroids (8). Other risk factors are prior antibiotic treatment, AIDS, H1N1 infection, chronic granulomatous disease, acute renal failure and receiving treatment with TNF-α inhibitors (1).

The risk factors for mucormycosis in critical care setting are neutropenia, diabetes, hematopoietic stem cell transplantation and penetrating trauma (14) but also include malignancy, deferoxamine treatment and renal failure (1, 15). Iron overload may be a risk factor and so an adjunctive treatment for patients at risk might be to induce iron depletion through chelators and preferably the new agent deferasirox (1, 22). Infections of rare invasive fungal species have also been noted due to using contaminated medical devices such as tongue depressors and bandages (8, 23).

Considering the importance of the immune system and host defenses that are involved in fungal infections, it has been stipulated that this knowledge might be used in future treatment options. Different parts of the immune defense (cells, cytokines) have been recognized to be of value in different phases of a fungal infection (24). Research on mice have shown that the IL-1R/TLR system plays a role in developing disease due to *C.*

albicans and *A. fumigatus* and is one of the strategies that might be developed for further targeting the development of invasive fungal infections in humans (8).

4. DIAGNOSIS OF FUNGAL INFECTIONS

4.1 Traditional Methods

4.1.1 Candida Infections

The diagnostic methods can be divided into direct and indirect methods (3). Direct methods are cultures of samples of blood or tissue from sites that are normally sterile. Cultures have a sensitivity ranging from 21-71%, have a long incubation time and are usually negative in deep-seated candidiasis (3). Cultures are also often negative when prophylaxis with fluconazole has been used (1) and they are not considered optimal for the diagnosis of candidemia or deep-seated candidiasis (2). After identifying *Candida* on blood cultures or with direct microscopy, the PNA-FISH method can be used to differentiate between different *Candida* species (8) or to identify the ones that are likely to be fluconazole-resistant (16).

Indirect diagnostic methods are for example biomarkers and PCR assays. The biomarker tests that have been shown the most interest are the ones detecting fungal cell wall components such as galactomannan antigens, antimannan antibodies and β-D-glucan. Biomarkers are specific but lack in sensitivity (1, 3). The negative predictive value in the β-D-glucan is a major diagnostic benefit (3) and studies have shown that it could also be used to predict when to stop empirical treatment (2), but further investigation is always needed after obtaining a positive test result (16). A retrospective case-control study found that the best sensitivity/specificity ratio for testing β-D-glucan was 0, 65/0, 74 respectively and that the detection of mannan was more specific while lacking in sensitivity. Finding a high correlation between invasive candidiasis and these two tests being positive; they suggest that β-D-glucan testing could be used as a first-line screening and depending on the value of the result; mannan detection could be indicated in a second step. Furthermore, they suggest that patients receiving antifungals, with negative blood cultures, could be monitored for glucanaemia and mannanaemia as to predict relapses (25). The

sensitivity of biomarker tests has been shown to be higher for *C. albicans* than for other *Candida* species. It is also of importance, that it has been concluded that a negative serum β-D-glucan doesn't rule out invasive fungal infection, as long as the clinical picture is suggestive of it (26). The performance of PCR assays has had different results in different studies. Studies have shown that PCR-tests have a sensitivity of 60% for detecting candidemia (1). The sensitivity for detecting deep-seated candidiasis with negative blood cultures has been concluded to range from invaluable to as high as 89% in different studies (1, 3) while some studies have shown that it has a higher sensitivity than β-D-glucan in patients with IC or deep-seated candidiasis (2). It has been shown that PCR is positive at an earlier point versus using direct methods for detection (cultures). There needs to be a standardization of the PCR-tests (3) and it is to date not commercially available (2). According to the ESCMID guidelines the recommended test for invasive candidiasis is galactomannan antigen and antibody (3). In conclusion, there are many different tests for detecting candida infections but none is completely satisfactory or superior to the other methods, and so it would be necessary to perform several diagnostic tests, and preferably to combine them, for a greater accuracy.

4.1.2 Non-Candida Infections

Direct microscopy is very valuable because results are achieved fast but also because culture isn't a good method for identifying some species (eg. *Mucorales*), certain species have a unique appearance (eg. capsule of *Cryptococcus* species) and direct visualization confirms that the organism is in the specimen and excludes contamination (16).

The gold standard for diagnosing aspergillosis is to prove its presence histologically but this can be problematic to achieve in critically ill patients and so; combining clinical and radiological findings with microbiological evidence might be more appropriate (8, 16). Cultures of respiratory specimens have been shown to be useful only in high-risk patients. Galactomannan and β-D-glucan are both cell wall components and are found in body fluids and indicate a probability of infection. Galactomannan antigens in serum haven't been proven useful in the ICU but measuring it in BAL fluid (Bronchoalveolar Lavage) has shown a sensitivity of 50-88 % and specificity of 87 % and the galactomannan index can be calculated based on the concentration found in the sample (1, 8, 16). If it is not appropriate to obtain BAL, three sputum samples can be sent for microscopy and culture

(16). PCR testing has a high specificity and sensitivity but can't be used to differentiate colonization from infection and is to this date not recommended to be used as a diagnostic technique by itself (1, 16). Combining galactomannan tests with PCR increases the sensitivity and has led to earlier recognition and administration of treatment but of note, these tests are affected by the use of prophylactic or empirical antifungal drugs (16). Depending on which *Aspergillus* species that is isolated, there is an increased risk (*A. flavus*) or decreased risk (*A. niger*) of IA (invasive aspergillosis) (16). According to the EORTC/MSG consensus group, the diagnosis of aspergillosis can be based on three criteria; 1) risk and host factors, 2) clinical and radiological signs and symptoms, and 3) laboratory testing that indirectly or directly proves the existence of *Aspergillus*. Using these criteria, the diagnosis can then be divided into proven, probable and possible infection (27, 28). A proven infection is a positive culture of a sterile site or a positive histopathological or cytopathologic examination. For a probable infection, all the three criteria mentioned need to be positive but the sites used for laboratory testing need not be otherwise sterile. Sputum, bronchoalveolar lavage etc. may be used. For a possible infection, the first and the second criteria need to be present but the evidence of *Aspergillus* on laboratory testing isn't necessary (1). The radiological tests that may be used are chest x-rays and CT scans, where the presence of the “halo sign” on the latter would be pathognomonic for the disease. The “halo sign” is a nodule with a dense center, surrounded by ground glass opacity (1). It is usually absent in patients at the ICU though (8) and the radiological findings might seem normal or non-specific in neutropenic patients with IA (pulmonary) who would more commonly have signs such as infiltrates, consolidations and nodules (16).

There are different conclusions about the diagnostic methods to be used when suspecting mucormycosis. Diagnostic methods are overall deemed limited and histopathology is considered the only method that can confirm the presence of *Mucorales*, while PCR isn't routinely used but has been described and on radiological testing there are usually no specific findings (1). If an invasive fungal infection is suspected in a patient with suggestive lesions on a CT-scan together with a negative *Aspergillus* galactomannan serum test and negative BAL; an invasive mucormycosis infection should be suspected. In selected patients (hematologic malignancy, recipients of stem cell transplants) imaging with CT can show the “reverse halo sign” which can help differentiate mucormycosis from aspergilloma. The “reverse halo sign” is a ring of consolidation with a center of ground glass opacity, but the diagnostic value depends on the pre-test probability (14).

The latest ESCMID guidelines, to date, conclude that the three methods that are strongly recommended for diagnosis are direct microscopy, histopathology and culture and that histopathology may help differentiate *Mucorales spp*. from *Aspergillus spp*. Identifying the species and testing susceptibility is also strongly recommended for gaining epidemiological knowledge but not so much for guiding treatment since a significant association between minimal fungicidal concentration and clinical outcome hasn't been found. (14)

Considering the *Cryptococcus spp*, the capsule antigen of *C. neoformans* and *C. gattii* can be tested for in serum and CSF. Testing for β-D-glucan is not appropriate for the *Cryptococcus* and *Mucorales spp.* since they don’t produce it (16, 17). It is also important to mention that when one is evaluating possible invasive fungal sinusitis (which could lead to serious complications such as spread to the CNS due to hematogenous or direct sinus extension) it is crucial to perform a cross-sectional CT or MRI imaging (16).

4.2 New Methods

Since *Candida* species are becoming increasingly resistant to antifungal drugs, it is becoming important to rapidly identify species, so that effective treatment can be started early. Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF) is currently a promising method for rapid identification of bacterial and fungal organism once they have been isolated (1) and works by automatically analyzing the mass distribution of proteins (29). Turnaround time is 10-15 minutes and the diagnostic accuracy is at least 95 % (2) and 84-99 % when compared to conventional methods (30). MALDI-TOF is gaining popularity as an accurate, fast and cost-effective new method of diagnosing infectious diseases (29). It is also considered to possibly become a future diagnostic tool for rare invasive fungal infections (17) (30).

Peptide nucleic acid-fluorescence in situ hybridization (PNA-FISH) is a method that uses artificial PNA polymers. PNA is different from DNA or RNA with the phosphate ribose ring being replaced by a polyamide, making the backbone of PNA acyclic and neutral. This method uses hybridization of PNA probes to species specific regions of ribosomal RNA and has a high sensitivity and specificity. It has been shown to be able to differentiate

between *Candida* species after identified growth in blood cultures and does so within hours (1, 2, 8) and can be used with direct microscopy to identify *Candida* species that might be fluconazole-resistant (16). This could be of use when selecting antifungal agents. Studies concerning these new interesting technologies have been promising but they still need to be established into the diagnostic work-up and further studies need to be performed to evaluate the clinical benefits.

5. PROPHYLACTIC, PREEMPTIVE AND EMPIRIC STRATEGIES

5.1 Prophylactic Strategies

Clinical studies have given little support for prophylactic treatment, unless for specific high-risk groups (incidence of at least 10%) (12). Prophylaxis with fluconazole have been effective for patients who recently underwent abdominal surgery, have recurrent gastrointestinal perforations, anastomotic leakage (1, 3) or are recipients of bone marrow transplants (13). A study which compared prophylactic fluconazole with placebo in ICU patients didn't find any significant difference in the incidence of candida infections, mortality or length of stay (10). Antifungal prophylaxis have been shown to reduce the incidence of candidemia by 50%, but there is to this date no evidence that has shown that the survival is increased (3) outside the group of bone marrow transplanted patients (13). It has been shown though, that survivability increases if preventative antifungal agents are administered early (5) and more specifically within 24-48 hours of the first positive blood culture; which is problematic since the culturing methods that are used to this date require 48-72 hours to be able to test positive for yeast (12).

A recent multicenter, randomized, placebo-controlled study used the echinocandin caspofungin as antifungal prophylaxis in ICU patients who were found to be at high risk of developing IC (Invasive Candidiasis). A clinical prediction rule was used to select patients. The study didn't find any benefit in the high-risk ICU patients who were treated prophylactically with caspofungin. Of note is that the patients undergoing abdominal surgery were put in the same group as patients undergoing other types of surgeries. Since different types of surgeries carry different rates of risk of fungal infection, this could've

affected the outcome of the study (31).

It is suggested that one should be careful with prophylactic recommendations because of the rising incidence of resistant *Candida spp.* and also due to the cost of the drugs. Hence, prophylaxis is considered useful only for the groups of patients that it has been shown to be effective for: patients with gastrointestinal anastomotic leakage, patients that had pancreatic or small bowel transplants, specific patients undergoing liver transplants and extremely low-birth-weight neonates in specific settings (3, 32). In a prospective study evaluating this concept, studying high risk surgical patients (recurrent gastrointestinal perforations/anastomotic leakage or acute necrotizing pancreatitis) patients were treated prophylactically with caspofungin and the study concluded that intra-abdominal IC (Invasive Candidiasis) was prevented in 95% of the patients. They administered a loading dose of 70 mg, followed by 50mg/day until the patient's surgical condition was resolved (median duration of treatment was 16 days) (5).

Administering echinocandins to prevent IC in patients at the ICU (Intensive Care Unit) is still being debated [reviewed in (33)]. Based on moderate quality evidence, the latest IDSA guidelines (2016), recommend administering fluconazole with a loading dose of 800mg and then 400 mg/day in high-risk patients at the ICU. With low quality evidence, they recommend echinocandins as an alternative. Caspofungin (70mg loading dose, then 50mg daily), anidulafungin (200mg loading dose, then 100mg daily) or micafungin (100mg daily) (7).

Developing prediction rules for patients at high risk of developing IC and using these as guidance for prophylactic, preemptive and empirical therapy is complex and the results have been controversial. Most often, these studies have been retrospective and prospective studies are needed to validate the prediction rules before they can be used in clinical practice.

A retrospective study aiming to find predictive rules for identifying patients at the surgical ICU who are at increased risk of developing candidiasis, found a set of criteria that identified 78% of the patients who would develop candidiasis. By using their rules, the patients needed to treat before preventing one case of candidiasis ranged from six to 19 and the estimated cost was found to be lower than the estimated cost of treating a case of

candidiasis (5). Even though the sample was small and there needs to be prospective validation of using the suggested rule, it shows that antifungal prophylaxis in the ICU based on risk evaluation might be possible.

Another retrospective multicenter study aimed to develop and evaluate clinical prediction rules in the ICU setting. They concluded that the best performing rule was any systemic antibiotic or central venous catheter with at least two of the following; TPN (Total Parenteral Nutrition), dialysis, major surgery, pancreatitis, steroid treatment or immunosuppression. They also specified on which number of days, since ICU admission, that these criteria should've been fulfilled to be marked as a factor in the prediction rules. In this study, by using these rules one might have been able to predict 34% out of all the cases of patients that developed IC (Invasive Candidiasis) (34).

Three published clinical risk predictive models for invasive candidiasis were evaluated in a prospective multicenter study. These models took into account clinical risk factors only or they also incorporated *Candida* colonization parameters. The predictive models were applied prospectively on ICU patients who were admitted for at least 72 hours, to see if these models could be included in management algorithms. Cases were found by evaluating for EORTC-MSG criteria. 15 out of 615 patients developed invasive candidiasis and there were 11 infections due to *C. albicans*. When comparing the performance of the models that only used clinical risk factors, the results were different than in the previously published studies with better sensitivity but worse specificity and positive predictive value. When adding the candida colonization parameters post-hoc, the results improved, except for the sensitivity which decreased. The results of the performance of the colonization index were similar to the models that used clinical risk factors. It was concluded that risk predictive models should include both clinical risk factors and colonization parameters to achieve a higher performance (12).

The presence of *Aspergillus* species in an immunosuppressed patient, whether it is colonization or infection, is a poor prognostic marker (1). Prophylactic treatment of aspergillosis has not been validated for use in the ICU, but has been administered in neutropenic patients (1).

Considering prophylactic treatment of *Mucorales spp.* it is recommended to be provided to

immunosuppressed individuals with a previous diagnosis of mucormycosis, to prevent recurrence. The choice of drug should be the antifungal agent that was effective last time in combination with surgical resection. Neutropenic patients or individuals with a graft-versus-host-disease outbreak should also be given prophylaxis with posaconazole (14). In complex situations such as preventing relapse in patients who were successfully treated for mucormycosis but need further immunosuppressive treatment for their underlying conditions, there are no clear guidelines and sufficient evidence based data is lacking. It is important to educate patients about the signs of relapse of a *Mucorales spp.* infection (such as facial swelling and black nasal discharge) and encourage them to seek medical attention if these signs appear.

5.2 Preemptive Strategies

It is of great importance that early presumptive treatment lowers mortality and increases survival of patients with invasive candidiasis. Retrospective observational studies have shown that early presumptive treatment lowers the mortality in patients with invasive candidiasis but this still has to be validated by prospective studies (3). When deciding which patients are best suitable for pre-emptive treatment, clinical prediction rules such as CS (Candida Score) can be used to select patients at high-risk of developing invasive candidiasis and these individuals' serum biomarkers (β-D-glucan or PCR) would then be followed. Administering pre-emptive treatment based on elevated serum β-D-glucan has been shown to reduce the incidence of proven invasive candidiasis (2).

A retrospective case-control study suggesting that β-D-glucan testing can be used as screening, with mannan test as a second step if indicated, propose an algorithm for preemptive treatment of patients at the ICU (Intensive Care Unit). They consider this being a very useful approach since the excess use of antifungal agents is expensive (25). The test that was used for detecting mannan epitopes poorly detects the mannan epitopes on *C. parapsilosis*, which is a species that was present frequently in the sample groups in this study. This could have affected the conclusions that were drawn from the study and might be considered significant since *C. parapsilosis* is currently becoming more common in southern Europe, Asia and South America (3). The CI (Candida Colonization Index) was suggested to be used only as a last step. Other studies have concluded though, that a CI > 0, 4 is the cutoff-value for preemptive treatment (1, 3). Preemptive treatment is not

indicated for patients with multifocal colonization of candida and risk factors for infection but without an established infection (1). It is instead suggested in patients with risk factors and positive serology (1). There is a need for more studies to establish which patients are suitable for pre-emptive therapy and since pre-emptive treatment would lead to more broad use of antifungal agents, there needs to be an evaluation of how this affects the epidemiology of fungal species (2).

Preemptive treatment of aspergillosis is usually defined as the one administered when there's mycological evidence but no developed infection. The benefit with this, versus empirical therapy, is controversial (1).

5.3 Empiric Strategies

A majority of the patients (two thirds) at the ICU receive empirical antifungal treatment while it is still a controversial approach without completely conclusive results (35). Early empirical treatment has been recommended in selected patients (those with risk factors for IC (Invasive Candidiasis), sepsis of unknown cause and positive serum biomarkers) but its effect on outcome needs to be further investigated (2). Empirical therapy may also be administered to patients with refractory fever being treated with broad-spectrum antibiotics (1). Combining the CS (Candida Score) and serum β-D-glucan test results has been suggested to be used to guide empiric antifungal treatment (6).

In studies that were powered for noninferiority, it has been shown that fluconazole, voriconazole and caspofungin are as effective as amphotericin B deoxycholate with less toxic effects and lower rates of discontinuation of treatment (3). As a result of studies like these, amphotericin B deoxycholate isn’t today the preferred first option for treatment of invasive candidiasis (2). Noninferiority studies have also shown that anidulafungin should be preferred over fluconazole, especially in treatment of *C. albicans*, despite the severity of the disease (3). Echinocandins have been shown to be a better choice over azoles and amphotericin B when considering survival rates and clinical outcome, especially considering infections with *C. albicans* and *C. glabrata*. (3)

Concerning the removal of intravascular catheters in patients with candidemia and the resulting effect on survival and outcome, studies have had somewhat different

conclusions. There is a need for randomized, blinded studies of the effect that catheter removal might have on mortality and outcome and many retrospective studies that have been done to date have shown different outcomes (3). In a pooled patient-level analysis of seven randomized treatment trials, where the patients were treated with an echinocandin in combination with catheter removal, the conclusion was that two factors were concluded to be associated with improved survival (18).

Studies have shown that, regardless of the type of invasive candidiasis and APACHE II score (disease severity score), an echinocandin should be considered the drug of choice. The latest ESCMID and IDSA guidelines state that documented invasive candidiasis (positive blood culture) should be treated with echinocandins as a first line antifungal drug and in cases of unstable patients or patients with recent use of fluconazole (1). There are exceptions in some specific circumstances, where treatment with triazoles would be preferred. These scenarios are; treating for *C. parapsilosis* infection (since the minimal inhibitory concentration for echinocandins is high (18)), if the patient used echinocandins previously for a prolonged period or if the patient has meningitis, endophthalmitis or a urinary tract infection (11). Echinocandins is not suitable for urinary tract infections because of their pharmacokinetics; the active drug isn't excreted in urine (1). Exposure to echinocandins have been shown to induce resistance to the drug but also multidrug resistance (36) and the choice of drug would hence be a triazole (fluconazole), because empirical studies have shown that the patient in this case, after using an echinocandin, would most likely have developed a *C. parapsilosis* infection (3). Studies have also shown that in a specific subgroup of patients (stable, low-risk, likely to be fluconazole susceptible) (11) the drug of choice should be fluconazole instead of an echinocandin. The first line treatment in these patients should be a loading dose of 500 mg fluconazole and then 400 mg (1, 3, 7).

It has been suggested that clinical prediction rules might be used for starting empirical treatment and that the negative predictive value of β-D-glucan could be used to guide the discontinuation of the treatment (2). Echinocandins should be used until the patient reaches clinical stabilization, no matter the species of *Candida* that is in question (except *C. parapsilosis*) , since they have been proven to increase survival (3). Limiting the duration of treatment to a specific number of days has been debated and is highly

controversial. Some studies have concluded that, if the conditions are right, the treatment with intravenous echinocandins can be switched to oral azoles after five days (3). Other studies have concluded that there's a need of consecutive blood cultures and treatment for 14 days after the last positive blood culture, with fundoscopy examination (to exclude endocular infection) and removal of central venous catheters and implanted devices (1).

Based on moderate quality evidence, the latest IDSA guidelines (2016) recommend empirical treatment of non-neutropenic critically ill patients with risk factors for IC (Invasive Candidiasis) and no other known cause of fever, with an echinocandin. Administering caspofungin (70mg loading dose, then 50mg daily), anidulafungin (200mg loading dose, then 100mg daily) or micafungin (100mg daily) is recommended. Also with moderate quality evidence, fluconazole is recommended as an alternative treatment (800mg loading dose, then 400mg) if the patient hasn't recently been exposed to azoles and the identified *Candida* species aren't azole-resistant (7).

Empirical treatment of aspergillosis is suggested to be administered to neutropenic patients at risk, with a prolonged febrile period, while having already received broad spectrum antibiotics (1). An echinocandin in combination with amphotericin B may be used (8).

6. TREATMENT

6.1 Candida Infections

When managing *Candida* infections the treatment varies considerably and depends on many aspects; the specific *Candida* species responsible for the infection, the susceptibility to antifungal drugs, the anatomical location of the infection, the underlying immune status and comorbidities of the patient and the patients' risk factors for infection.

A randomized multicenter study compared voriconazole treatment with amphotericin B followed by fluconazole for invasive candidiasis in non-neutropenic patients. The median duration of treatment was 15 days and the intravenous catheters were removed in the vast majority of patients in both groups. Voriconazole was given intravenously 6mg/kg twice in

the first 24 hours, then 3mg/kg twice daily for three days, then 200mg orally twice daily. The conclusion was that it is as effective but with fewer toxic side effects compared to the regimen with amphotericin B/fluconazole (37).

Treating invasive candidiasis with intravenous anidulafungin (200 mg loading dose, then 100 mg/day) was compared to intravenous fluconazole (800 mg loading dose, then 400 mg/day) in a randomized, double-blind, non-inferiority study. The majority of patients (97%) were non-neutropenic. The treatment lasted for ≥14 days after the last negative blood culture with improvement in symptoms and signs. Oral fluconazole (400mg daily) could also be administered to patients in both groups after ≥10 days of intravenous therapy. The results were successful in 75, 6% of patients treated with anidulafungin versus 60, 2% of the patients receiving fluconazole. The adverse effects were similar in both groups. While finding that anidulafungin was more effective than fluconazole in some circumstances (at the end of the therapy and at 2-week follow-up) but had a lower rate of success for infections with *C. parapsilosis* (echinocandins have a lower efficacy against this agent in general); it was concluded that overall, anidulafungin is non-inferior to fluconazole. Of note, patients with proven infection of *C. krusei* were excluded from the study (38). There are clinical scenarios though, when azoles may be more appropriate than echinocandins such as in patients with meningitis, endophthalmitis and candiduria (39). The emergence of resistance to echinocandins in *C. glabrata* and *C. krusei* has also been noted to be a clinically relevant problem and in these situations one might use a higher dose of fluconazole (800 mg/day) (8).

Table 2. Recommended treatment for candidemia in non-neutropenic patients

1st line	***Alternative regimen***
Caspofungin (70 mg loading dose, then 50 mg daily) Micafungin (100 mg daily) Anidulafungin (200 mg loading, then 100 mg daily)	Fluconazole (800 mg loading, then 400 mg daily) Lipid-formulation Amphotericin B (3-5 mg/kg/day)
Adopted from IDSA (infectious disease society of America) guidelines 2016 (7)	

According to the new clinical guidelines from IDSA (see table 2) the recommended initial treatment for candidemia in non-neutropenic patients is an echinocandin. Caspofungin (70mg loading, then 50mg daily), micafungin (100mg daily) or anidulafungin (200 mg loading, then 100mg daily). Recommended alternative choices are fluconazole (800mg loading, then 400mg daily) in selected patients, unlikely to have fluconazole-resistant *Candida* species, and lipid formulation amphotericin B (3-5mg/kg daily) in case of intolerance, limited availability or resistance to other antifungal drugs. Transition from an echinocandin to fluconazole within 5-7 days is recommended for clinically stable patients, in the case of *Candida* species that are susceptible to the drug, and in whom repeat cultures on antifungal therapy are negative. For infections due to *C. glabrata* the transition to fluconazole or voriconazole is recommended in susceptible species. For infections due to *C. krusei*, voriconazole is recommended as a step-down therapy. For all cases of blood-stream *Candida* infections; susceptibility of azole should be tested, a dilated ophthalmology exam performed within the first week of diagnosis and follow-up cultures at least every other day. Recommended duration of therapy is 2 weeks following negative blood culture results and resolution of candidemia related symptoms (7).

Considering the echinocandin group of antifungal drugs, there are a few limitations that have inspired further research. Among these are the need for daily intravenous dosing, high cost, limited spectrum, emerging resistance and liver and cardiac toxicity. The continuous interest in this group of drugs has led to an exciting new discovery; their ability to prevent and treat *Candida* biofilm formation, and studies on caspofungin-coated medical devices is ongoing. Since *Candida* infections of medical devices are costly, this could be a first step towards a great discovery [reviewed in (33)].

6.2 Invasive Mold Infections

Definitive treatment of aspergillosis is defined as treatment given to a patient with a proven infection. The drug of choice in IA (Invasive Aspergillosis) is voriconazole (1, 8) (6 mg/kg i.v. twice daily loading dose, then 4 mg/kg twice daily). Itraconazole and posaconazole are considered second line treatment options. When a single agent isn't successful, combination therapy may be used. Caspofungin together with amphotericin B can be used when treatment with other agents isn't successful or isn't appropriate because of adverse events. Amphotericin B has mostly lost its predominance because of its adverse effects,

where nephrotoxicity is one of the most common adverse effects. It has been noted that it is paradoxically less toxic at higher doses, though there are no differences in the outcome. Adjunctive treatment may be used, including Granulocyte-Colony Stimulating Factor and Interferon-Gamma and resection of the lesions might be needed in some cases (1). Duration of the treatment depends on the immune status of the patient. For a non-immunosuppressed patient 6-12 weeks are suggested while an immunosuppressed patient is suggested to be treated until the immune status changes and the clinical and radiological signs and symptoms resolve. There is no need to achieve a negative laboratory test to certify the eradication of the fungus. Relapses may occur due to incomplete eradication or lack of sterilization of foci (1).

The treatment options for mucormycosis are limited and the degree of difficulty to treat infections with this organism is higher than for other invasive fungal infections (40). The general consensus is that immediate treatment increases survival and a combination of pharmacological treatment with surgical debridement whenever possible and correction of the underlying risk factors is suggested (1, 16). Reversal of the predisposing risk factors has been concluded as an important step in the treatment of mucormycosis and examples are administering Granulocyte-Colony Stimulating Factor, preventing hyperglycemia, ketoacidosis and limiting to a minimal dose/ceasing treatment with glucocorticosteroids (14, 40). In the ESCMID guidelines, the drug of choice is amphotericin B in the liposomal or lipid-complex form (at least 5 mg/kg/day) and the use of amphotericin B deoxycholate is discouraged because of its severe adverse effects (14). Other studies conclude that amphotericin B in deoxycholate, lipid complex or liposomal form may be used in the doses 1-1, 5 mg/kg/day, 5-7, 5 mg/kg/day and 5-10 mg/kg/day respectively (1).

Combination therapy with amphotericin B and an echinocandin or deferasirox (an iron chelator) has also been suggested for mucormycosis but is controversial (16). A phase II trial showed that patients with mucormycosis had a higher mortality at 90 days if treated with deferasirox and was therefore not supportive of it as adjunctive therapy, while other studies claim that it reduces the risk of mucormycosis by inducing iron starvation (1, 41, 42). The ESCMID guidelines conclude that there is only a marginal recommendation to use deferasirox outside clinical trials of hematological patients, for diabetic patients (14). Salvage treatment with posaconazole 200 mg orally four times daily has been described and is strongly recommended in cases of refractoriness of disease, intolerance of other

antifungals or a combination of both (14).

The duration of treatment of invasive mold infections should be individualized and continued until the clinical signs, immunosuppression and radiological signs, if present, resolves (1, 14). Testing for resistance should be guided by local epidemiology but it is suggested to test *Aspergillus fumigatus* for itraconazole and voriconazole susceptibility (16). In general, for rare invasive fungal infections, the latest ESCMID guidelines to date suggest administering amphotericin B (lipid formulation) in combination with flucytosine for better drug penetration, depending on susceptibility of the fungal species (14).

7. CONCLUSION

Fungal infections in the ICU (Intensive Care Unit) are of great importance because they are associated with high mortality and morbidity. Despite growing knowledge about the epidemiology and risk factors for developing invasive fungal infections, it still remains a challenge to diagnose and treat these infections in the critical care setting. Combinations of different diagnostic tests seem to be the most prudent approach. Early intervention strategies are crucial and the use of personalized risk profiling based on immunogenetics might be a useful approach in the future, even though more studies are needed. The management of invasive fungal infections has changed in the last few years partly due to growing resistance to antifungal drugs and timely use of drugs is necessary to avoid further changing the ecology of the fungal species.

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10. BIOGRAPHY

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