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Interpretation of Fungal Culture Results

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Abstract Classically, diagnosis of fungal infections is based on microscopic examination coupled with attempts to culture the responsible fungus from a clinical sample. For some fungal infections (such as dermatophyte infections, infections with dimorphic fungi, and blood stream infections with *Fusarium*, *Acremonium* and allied genera) recovery of the fungus in culture from a patient with clinical symptoms is sufficient for diagnosis. However, in many cases, obtaining a yeast or filamentous fungus in culture is not easily interpreted in isolation. In such circumstances, decisions regarding the clinical significance of an isolate must consider the nature of the organism and the quantity isolated, the likelihood of it accidentally contaminating the specimen, whether fungal elements were seen upon microscopic examination of the sample, the clinical status of the patient, and whether there are other clinical or biological markers suggesting infection. This review discusses these considerations for the different types of clinical samples encountered in a microbiology laboratory.

Keywords Dermatophytosis · Candida · Invasive aspergillosis · Dimorphic fungal pathogens · Microscopy · Culture · Subcutaneous fungal infections · Clinical significance

Introduction

As with all microbiological tests, the ability to accurately interpret fungal culture results and identify fungi suspected of causing infection starts with the careful selection and procurement of the specimen, followed by appropriate transportation to the laboratory. Transportation at ambient temperature is normally recommended, as some fungi are sensitive to temperatures below 10 °C or above 30 °C. However, as fungi and the accompanying microbial biota may continue to replicate in transit, the samples should be processed as soon as possible in order to obtain an accurate result. An important first step is to perform direct microscopic examination to establish the presence of yeast cells, hyphae or other fungal elements. Such a finding will increase the likely significance of an organism with compatible morphology subsequently isolated in culture.

Candida albicans remains the most prevalent yeast species causing both superficial and deep infections, and *Aspergillus fumigatus* is responsible for the majority of invasive mould infections. However, in recent years, the number of other yeast and mould species shown to have pathogenic potential has been increasing in line with the increase in the number of compromised patients susceptible to fungal infection due to medical conditions or their treatment [1–3]. Two terms have been adopted to stem the proliferation of new disease names each time a mould with pathogenic potential is identified. Hyalohyphomycosis describes infection with filamentous fungi that have hyaline (colourless) hyphae, whilst phaeohyphomycosis describes infection with phaeoid (darkly pigmented) moulds. More than 40 species from 20 different genera have been isolated from cases of hyalohyphomycosis and more than 100 species from 60 genera are implicated in phaeohyphomycosis [4]. In total, more than 600 species of yeast and mould have been implicated in human infection, albeit sometimes only as a single case history. Thus, it is becoming increasingly apparent that no fungus isolated from a susceptible patient should be discounted without careful evaluation. However, as

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many of the pathogenic yeasts are commensal organisms and many of the mould pathogens are ubiquitous in the environment and thus likely to contaminate samples or culture plates, care should be taken during procurement of the specimen and processing to minimise the chances of contamination.

Direct Microscopic Examination of Samples

Direct microscopic examination of skin, hair, and nails allows a very rapid diagnosis of dermatophyte infections, and nail infections caused by non-dermatophyte moulds. The clinical sample should be chopped into small pieces and a proportion examined after digestion in 10 % potassium hydroxide (KOH) [or in the cheaper sodium hydroxide (NaOH)] [5, 6]. The ease of detection of fungal elements is greatly facilitated by employing fluorescent brighteners such as Calcofluor white (Sigma-Aldrich) or Blankophor (Blankophor GmbH) in conjunction with a fluorescent microscope [7, 8].

After liquefaction with a mucolytic agent if necessary, respiratory samples should be concentrated by centrifugation (3,000 g, 10 min) before direct microscopic examination. Following centrifugation, the majority of the supernatant is discarded, and the sediment resuspended in the remaining small volume of supernatant. A proportion of the resulting slurry is then mixed with 10 % KOH and fluorescent brightener. The same procedure should be adopted for other body fluids in order to concentrate any yeast cells or hyphal fragments that may be present. If samples are small, as may be the case for aqueous or vitreous humors for example, an aliquot should be examined directly with a drop of KOH and optical brightener. Swabs should be wiped on the surface of a glass slide; the inoculated area should then be covered with a drop of KOH and optical brightener before immediate direct microscopy. Tissue samples should be chopped into small fragments and softened by incubation on a heat block in an Eppendorf tube containing a few drops of KOH. For direct microscopic examination, a portion of softened tissue should be placed on a glass slide, covered by a drop of optical brightener and squashed under a coverslip.

Following microscopic examination, samples should be cultured. There are a variety of media that can be employed, but for the majority of samples, a malt or glucose-peptone agar, (Sabouraud's agar) supplemented with an antibiotic, such as chloramphenicol, to suppress attendant bacterial flora will be suitable. Brain heart infusion agar may encourage the yeast phase growth of the dimorphic fungi [9]. Ideally, plates inoculated with samples from deep sites should be incubated at 30 °C and 37 °C in air, whilst those from superficial sites should be incubated at temperatures of ≤30 °C. Long periods of incubation of up to six weeks may be necessary to allow the growth of slow-growing organisms. However, most fungi causing deep infection, with the exception of some dimorphic pathogens, will grow within a few days, whilst dermatophytes from superficial infections will usually be evident after one week. In a study of 2,173 consecutive clinical cultures, in which plates were incubated at 28 °C, 98 % of yeast isolates and 81 % of mould isolates were detected in the first week, and more than 96 % of moulds were detected by day 14 [10].

Careful examination of the plates should reveal whether any fungal isolates are associated with the area that has been inoculated. Colonies obviously away from the inoculation streak or tissue portion should be discounted as plate contaminants. If there is any doubt, an assessment of maximum temperature of growth may be useful, as many environmental fungi will not be able to grow at 37 °C and so would be unlikely to cause deep infections. Isolates that are considered significant can be identified by a variety of techniques, including biochemical and phenotypic analysis, as well as molecular and proteomic methods [11]. Yeasts or filamentous fungi from blood culture or other deep sites should be identified to species level to allow selection of the most appropriate antifungal therapy [12, 13, 14••, 15••, 16••, 17••]. Specific susceptibility testing of the isolates may also be useful. Table 1 lists the organisms most likely to be implicated in infections from a variety of clinical specimens.

Fungi Recovered from Superficial Sites—Skin, Nail and Hair Samples

Isolation of an anthropophilic or zoophilic "true" dermatophyte or the relatively rare non-dermatophyte cause of skin infection (*Neoscytalidium dimidiatum*) in culture from skin, hair or nails is diagnostic of infection, regardless of whether fungal elements were seen by direct microscopy examination. The exception to this is with *Trichophyton tonsurans*, an anthropophilic agent of tinea capitis, which is known to have a carrier state [18]. Conversely, for the geophilic dermatophytes (*Microsporum cookei*, *Trichophyton terrestre*, *M. fulvum* and *M. gypseum*), isolation in culture is less easy to interpret, as these are potential contaminants of dermatology samples, and clinical significance depends upon their visualisation by direct microscopy. Although a large number of non-dermatophyte moulds have been implicated in fungal nail infections [5, 19, 20], such infections are still relatively rare and usually result following nail trauma. The presence of a non-dermatophyte mould may be indicated by direct microscopic examination, which demonstrates intact hyphae [as compared to arthrospores, which are a key feature of dermatophyte infections (authors' observation)] that are frequently distorted by terminal fronding or hyphal swellings. The decision that a non-dermatophyte mould cultured from nail

Table 1 Likely significance of isolates from a range of clinical samples

Sample	Likely organisms isolated	Comments on significance
Skin	Dermatophyte fungi	Significant if isolated
	Neoscytalidium dimidiatum/hyalinum	Significant if isolated
	Other moulds	Likely contaminants, unless as a result of disseminated infection, e.g., *Fusarium* spp.
	Candida spp.	Significant if seen on direct microscopy
	Other yeast	Unlikely significance, unless as a result of disseminated infection, e.g., *Trichosporon* spp. NB: *Malassezia furfur* is a cause of pityriasis versicolor, but is unlikely to grow in culture
Hair	Dermatophyte fungi	Significant if isolated, some scalp carriage of *Trichophyton tonsurans* reported
Nails	Dermatophyte fungi	Significant if isolated
	Neoscytalidium dimidiatum/ hyalinum	Significant if isolated
	Scopulariopsis spp., *Fusarium* spp., *Aspergillus* spp., *Acremonium* spp.*, some rarer moulds	Significant if seen on direct microscopy and isolated in pure culture from several pieces of nail tissue in the absence of a dermatophyte
	Candida spp.	Significant if isolated from paronychia and seen on direct microscopy
	Rhodotorula spp.	Often isolated, rarely significant even if seen on microscopy
Mucous membrane swab	Yeast species	May be significant if symptomatic and seen in large amounts by direct microscopy. Also common commensals.
	Dimorphic fungi	Significant
Nasal swab/secretions	*Aspergillus* spp. mucoraceous moulds	*Aspergillus flavus* is the most common cause of fungal sinusitis, mucoraceous moulds cause rhinocerebral mucormycosis in appropriate patient groups
	Candida spp.	Likely commensals
Ear swab	*Aspergillus niger*, *other Aspergillus* spp., *Scedosporium* spp., *Scopulariopsis* spp.	Direct microscopy usually positive
	Candida spp.	Common commensals
Sub-cutaneous samples	*Madurella* spp., *Pyrenochaeta romeroi*, *Leptosphaeria* spp.	Cause of dark grain eumycetoma
	Scedosporium spp., *Acremonium* spp.*	Common cause of pale grain eumycetoma and subcutaneous hyalohyphomycosis
	Sporothrix schenckii	Cause of sporotrichosis, tracks up lymphatic system
	Cryptococcus spp.	May result from disseminated infection
	Alternaria spp., *Exophiala* spp.	Cause of subcutaneous phaeohyphomycosis
Corneal tissue	*Fusarium* spp., *Aspergillus* spp., *Acremonium* spp.*, *Candida* spp.	Common causes of fungal keratitis, must be on inoculum site, ideally with positive direct microscopy
	Lasiodiplodia, *Curvularia*, other yeast and mould	Rarer causes but significant if compliant with above
Vitreous / aqueous fluid	*Candida* spp., *Aspergillus* spp., *Fusarium* spp., *Acremonium* spp.*	Usually significant, ensure on inoculum streak, confirmed by positive direct microscopy
Sputum	*Candida* spp.	Very rarely significant, commonly colonises airways, possible role in deteriorating lung function in CF and ABPM patients. Pulmonary candidosis is rare and usually a result of haematogenous dissemination.
	Cryptococcus spp.	Significant
	Aspergillus fumigatus, other *Aspergillus* spp.,*Scedosporium* spp.	May be significant in the appropriate host, common colonisers in CF patients and may contribute to deteriorating lung function
	Exophiala spp.	Common colonisers in CF patients and may contribute to deteriorating lung function
Bronchoalveolar lavage	*Aspergillus fumigatus*, other *Aspergillus* spp.,*Scedosporium* spp. *Cryptococcus* spp.	Likely to be significant in appropriate host Significant
	Dimorphic fungi	Significant
	Candida spp.	Commonly colonise airways—very rarely significant

Table 1 (continued)

Sample	Likely organisms isolated	Comments on significance
Tissue biopsy	*Aspergillus* spp., *Candida* spp., *Cryptococcus* spp., mucoraceous moulds, agents of hyalohyphomycosis and phaeohyphomycosis	Positive direct microscopy enhances significance and is proof of invasive fungal infection. Isolation in the absence of positive microscopy should not be dismissed without careful consideration
	Dimorphic fungi	Significant
Bone marrow	*Histoplasma* spp. and other dimorphic fungi, other yeast, *Aspergillus* spp.	Dimorphic fungi always significant, other yeast and *Aspergillus* usually significant
Blood	*Candida* spp., *Cryptococcus* spp. *Trichosporon* spp. *Rhodotorula* spp. *Malassezia pachydermatis*	Candidaemia is the most common deep fungal infection. Other yeast genera are seen causing fungaemia in immunocompromised patients
	Fusarium spp., *Acremonium* spp. *, *Paecilomyces* spp., *Purpureocillium* spp., *Scedosporium* spp.	*Fusarium* and *Acremonium* spp.* are the most common mould genera isolated from blood during disseminated infection
		Aspergillus spp. and other mould genera are usually contaminants
	Dimorphic fungi	Dimorphic fungi are always significant
CSF	*Cryptococcus* spp., *Candida* spp., other yeast species *Aspergillus* spp.	Usually significant, antigen tests can help to confirm
	Dimorphic fungi	Significant
Prostate fluid	Dimorphic fungi, *Cryptococcus* spp.	Significant
Peritoneal fluid	Yeast isolates, *Aspergillus*, *Paecilomyces*	Probably significant
Pleural fluid	*Cryptococcus* spp., *Coccidioides* spp., *Aspergillus* spp.	*Cryptococcus* spp. *and Coccidiodes* spp. may be found in pleural fluid following pulmonary infection, other dimorphic fungi are rare but significant if isolated. *Aspergillus* spp. are a rare but significant cause of pleural effusion
Joint fluids	*Candida* spp., dimorphic fungi, *Aspergillus fumigatus*	Significant
Pus from abscess	*Candida* spp., *Cryptococcus* spp., *Sporothrix schenckii*	Significant
Urine (non-catheter)	*Candida* spp., dimorphic fungi, *Aspergillus* spp., *Talaromyces marneffei*, *Trichosporon* spp.	*Candida* spp. are significant in symptomatic patients, other organisms are usually found in urine as a result of deep or disseminated infection

*The taxonomy of the genus *Acremonium* has recently been revised, with medically important species being re-assigned to several distinct genera including *Sarocladium* and *Gliomastix*

specimens is clinically significant depends upon positive direct microscopy, failure to culture a dermatophyte, isolation of the non-dermatophyte mould in pure culture from a large proportion of the clinical sample, and preferably its repeat isolation.

Superficial candidosis is more difficult to diagnose by microscopy and culture. *Candida* spp. can cause nail infections, usually associated with paronychia, or superficial skin infections, usually intertrigo of the skin folds associated with obesity or diabetes. The diagnosis of both nail fold infections and *Candida* intertrigo depends to a large degree on clinical presentation. Since *Candida* spp. are normal commensals of the skin, recovery of these organisms in culture from skin swabs or nail specimens is not usually significant unless direct microscopy reveals large amounts of yeast cells/hyphae. *Malassezia* spp. may also be seen on direct microscopic examination; this can be diagnostic for the skin infection known as pityriasis versicolor if yeast cells and short non-branching hyphae are seen. This is especially important to note, as the organisms will fail to grow in culture unless a lipid source is present; for this infection, direct microscopic examination of skin flakes is diagnostic and culture is rarely indicated or attempted [21].

Fungi Recovered from Superficial Sites—Yeast Species Isolated from Mucosal Sites

A variety of yeast species (principally *Candida* spp.) are capable of causing mucosal candidosis (oropharyngeal or vaginal). The clinical presentation of mucosal yeast infections is usually quite characteristic, and is often accompanied by typical white opaque patches of fungal growth macroscopically visible on the mucosal surface. For vaginal candidosis, diagnosis relies upon the presence of typical symptoms, mucosal appearance and the observation of the fungus in smears or its isolation in culture in large numbers. For oropharyngeal candidosis, isolation of the organism in culture alone is insufficient to diagnose the infection (as yeasts are normal commensals of the oral cavity), which should instead be based upon observation of large numbers of the organism in swabs or smears from the affected area.

Otomycosis

If fungal infection of the ear canal is suspected, direct microscopy of waxy material or swabs from the area may reveal the pathogen. As there is an air interface, mould isolates may be able to spore within the ear canal, allowing species identification from direct microscopic examination. The most common isolates from this area are *Aspergillus niger* and *Scedosporium apiospermum* [19].

Subcutaneous Infections—Mycetomas

Subcutaneous infection with certain mould species may follow direct accidental inoculation via thorns or splinters. Eumycetoma formation is slow but relentlessly progressive, and in time may produce discharging sinuses. Pus from these sinuses may yield hard granules that when crushed on a microscope slide can be seen to have been formed from compacted fungal mycelium. Granules may be described as pale grain or dark grain, which are associated with different fungal pathogens. Before culture, grains should be washed to remove contaminating bacteria. Pale grain mycetomas are most often associated with *Fusarium* and *Scedosporium* species, whilst in dark grain mycetomas, *Madurella* species and *Pyrenochaeta romeroi* are often implicated [22].

Chorioretinitis, Endophthalmitis and Keratitis

There are a number of yeasts and moulds regularly implicated in eye infection. Chorioretinitis and endophthalmitis usually arise following haematogenous dissemination (endogenous endophthalmitis), although they can also follow penetrating ocular trauma, either accidental or surgical (exogenous endophthalmitis). Yeast infections, especially due to *Candida*, are far more common in this setting than those caused by moulds, mainly *Aspergillus* [23]. *Candida albicans* was responsible for 92 % of cases in a recent review [24]. Indeed, it is advised that all patients diagnosed with candidaemia undergo fundoscopy to detect such infection at an early stage [25]. In contrast, keratitis usually occurs following trauma, and moulds are more often implicated than yeasts [26]. In particular, there have been serious outbreaks of keratitis with *Fusarium* species in individuals who wear contact lens following the use of contact lens cleaning solution that failed to inhibit mould growth [27, 28]. The majority of cases of postoperative and post-traumatic endophthalmitis due to *Aspergillus* spp. occur in tropical areas such as India [23]. There are also more cases of non-contact-lens-associated keratitis due mainly to *Fusarium* and *Aspergillus* species in Asia and Africa; these cases often follow penetrating injuries with vegetative material in farm workers [26, 29].

Due to the site of infection, samples of tissue, or in the case of endophthalmitis, aqueous or vitreous fluids, are necessarily small. Direct microscopic examination is desirable and can be diagnostic for either yeast or mould infection, but is unlikely to determine the infecting species. Direct species-specific or panfungal PCR tests on such samples can provide definitive identification [30]. However, culture is also useful, and if successful, yields an isolate for definitive identification as well as specific susceptibility testing, which can help to direct appropriate therapy. Isolation in culture in the absence of other confirmatory tests is not proof of infection. but there should be careful consideration of the organism isolated and the likelihood of its role as a pathogen. The degree of significance of an isolate can be improved by marking the area of the plate where the sample has been placed. A cross or a ‘C’ streak in which a needle or scalpel with a small piece of tissue on the end is inoculated onto the agar in the shape of a ‘C’ is most often employed [31]. Growth of a colony of either yeast or mould away from this area should be viewed with suspicion as a probable contaminant.

Respiratory Specimens—Suspected Invasive Pulmonary Infections

Respiratory samples from cases of suspected invasive pulmonary infection should first be concentrated by centrifugation prior to culture of the sediment and incubation at 30 ^{0}C and 37 ^{0}C. It is relatively common to recover a variety of saprophytic moulds (predominantly *Aspergillus* and *Penicillium* species) from such samples, and in the absence of neutropenia, their significance is often questionable [32]. The significance of a positive culture result should be judged on the basis of the results of direct microscopic examination, and should be taken with some scepticism if branching fungal hyphal fragments was not seen microscopically. This is especially important since scant inhaled, non-germinated fungal spores are often present in sputum samples. Such spores will frequently be missed upon microscopic examination, and the recovery of the organism in culture does not necessarily indicate an invasive fungal infection. A caveat to this scenario is that respiratory specimens that have not been processed immediately may sometimes contain germinated spores present as individual fungal micro-colonies, with intact branching hyphae radiating from a central point. Extra vigilance is required to distinguish these microscopically from the fragments of branching mycelium (with broken hyphal ends) typically seen with either invasive fungal infection or fungal colonisation of the airways. However, the recovery of a potentially pathogenic mould from samples from high-risk patients should always be treated seriously, even in the absence of direct positive

microscopy, and it is in these cases that surrogate markers of infection can be useful adjunctive tests. The use of high-resolution computed tomography (HRCT), galactomannan on cerebrospinal fluid (CSF), bronchoalveolar lavage (BAL) fluid or blood, *Aspergillus*-specific and pan-fungal PCR, detection of beta-glucan, and more recently, the lateral flow device for the detection of *Aspergillus* antigen, all have a role to play (see [33] for a review, [34]). Isolation of large numbers of colonies from any specimen could be indicative of recent exposure to large numbers of spores, or a failure in laboratory sterile techniques.

Respiratory Specimens—Cystic Fibrosis Patients

Despite many individual studies, there is still no single consensus approach to the culture of respiratory samples from patients with cystic fibrosis (CF) [35]. Since many of the filamentous fungi associated with airway colonisation in CF patients are ubiquitous in nature, and due to the excessively viscous bronchial mucus in CF, it is again important to distinguish microscopically between recently inhaled spores and growing fungal fragments in respiratory secretions from such patients, and we would advise concentration of specimens by centrifugation prior to microscopic examination. However, culture of concentrated samples results in the recovery of *Aspergillus fumigatus* species complex from up to 50 % of specimens, and due to the rapid growth of this organism on most media and at most incubation temperatures, the recovery of other more slowly growing filamentous fungi known to be important in colonisation and exacerbation of lung function in CF patients (*Scedosporium* spp., *Exophiala dermatitidis*; [35]) is likely to be compromised. Indeed, serial dilution of sputum samples from CF patients known to be persistently co-colonised with *A. fumigatus, S. apiospermum* and *E. dermatitidis* in our laboratory has indicated that the latter two organisms might be present in greater quantities (in terms of colony forming units) of 100-fold to 1,000-fold, but are not detected when undiluted sputa are cultured (unpublished data). Thus, we would advocate culturing CF respiratory specimens that are both undiluted and diluted by at least 100-fold, and incubating cultures at 30 °C and 37 °C. The most common, clinically significant organisms recovered from CF respiratory samples include *Aspergillus* spp. (predominantly *A. fumigatus* species complex), *Scedosporium* spp. (predominantly *S. apiospermum* species complex), *E. dermatitidis*, and *Candida* spp. (predominantly *C. albicans*), all of which are likely to be clinically significant if the appropriate corresponding fungal elements (mould or yeast hyphae) have been seen on direct microscopy [35].

Respiratory Specimens—ABPA/ABPM

Allergic bronchopulmonary aspergillosis (ABPA) is a respiratory disorder provoked by persistent colonisation of the airways by *A. fumigatus*, and characterised by exacerbation of asthma or CF symptoms, pulmonary infiltrates, expectoration of thickened mucus plugs and immunological responses to the fungus (pulmonary eosinophilia and elevated fungus-specific IgE) (see [36] for review). However, it is now accepted that a whole variety of fungi can cause this condition, and the term allergic bronchopulmonary mycosis (ABPM) is more suitable. The principal non-*Aspergillus* species implicated worldwide include *Candida* spp., basidiomycetes (especially *Schizophyllum commune*, and *Trichosporon* spp.), *Scedosporium* spp., *Fusarium* spp, *Cladosporium* spp., *Penicillium* spp., *Alternaria* spp., *Bipolaris* spp., and *Curvularia* spp. (reviewed in [36–38]). Culture of any of these fungi from respiratory specimens from ABPM patients should be considered significant in the presence of asthma or deteriorating CF lung function, eosinophilia, elevated IgE, and transient or permanent lung x-ray abnormalities. As with respiratory specimens from CF patients (see above), fungal elements should normally be apparent upon direct microscopic examination of centrifuged samples.

Respiratory Specimens—Dimorphic Fungal Pathogens

The dimorphic fungal pathogens *Talaromyces* (previously *Penicillium*) *marneffei*, *Histoplasma capsulatum* var. *capsulatum*, *H. capsulatum* var. *duboisii*, *Blastomyces dermatitidis*, *Coccidioides immitis* and *C. posadasii*, *Paracoccidioides brasiliensis* and *P. lutzii* can cause infections in both healthy and immunocompromised patients in defined, endemic regions of the Americas, Africa, Asia and Europe (depending on the particular pathogen, reviewed in [39, 40]). For *Histoplasma* spp., direct microscopic examination of wet preparations of respiratory samples is very rarely diagnostic, since the budding yeast "tissue" form of the organism is predominantly macrophage-associated (although stained smears are more useful). For *T. marneffei*, *B. dermatitidis*, *Paracoccidiodes* spp. and *Coccidioides* spp., microscopic examination of concentrated respiratory samples can permit diagnosis if the appropriate tissue forms are observed: small oval fission yeasts; large, round to oval yeast cells with broad-based unipolar buds; large oval to round yeast cells with multipolar, peripheral budding ("ships wheel" appearance); and thick walled spherules containing endospores, respectively. However, the diagnostic sensitivity using KOH-fluorescent enhancers rarely exceeds 50 % (see for example [41–44]). The recovery of the mould form of these dimorphic fungal pathogens requires the protracted culture of specimens at 30 °C. In non-endemic regions, isolation of these organisms

in culture is always clinically significant. However, even in endemic regions, these organisms are rarely laboratory plate contaminants, and their isolation from respiratory samples (other than in routine surveillance studies) is likely to be indicative of infection, especially if the patients are symptomatic.

Blood Culture

It is usual practice to take more than one blood sample for incubation to maximise the chance of isolation [12, 13, 45–47]. At one time, a yeast isolated from only one sample might have been suspected of being a contaminant; however, it has become apparent that even isolation from one bottle can be significant and is an indication for treatment of candidaemia [13]. For suspected catheter-related blood stream infection, paired blood samples should be taken from the catheter and a peripheral vein, or if this is not possible, from different catheter lumens [45]. Isolation of a yeast species from a line tip removed from a patient without systemic signs of infection most often represents contamination with skin organisms during removal and is not an indication for treatment. It should be remembered that there are many *Candida* species and also a number of other genera of yeast that are regularly implicated in infection, and if isolated, should be regarded as significant pathogens [3, 17••]. Isolation from blood culture can be maximised by terminal sub-culture of blood culture bottles from patients suspected of having a fungal infection, irrespective of whether or not it has flagged as positive [48]. Direct microscopic examination of a spun deposit may also reveal the presence of a slow-growing or nutritionally demanding isolate, such as a *Malassezia* species, which often requires the presence of lipid to grow in culture [49, 50]. Recent proteomic methods have focussed on identifying pathogens directly in blood culture samples without the need for sub-culture, which would overcome these problems and abolish another potentially time-consuming step, thus allowing earlier diagnosis and facilitating prompt institution of appropriate antifungal therapy [11].

It is unusual to isolate filamentous fungi from blood culture even in patients with disseminated infection. Invasive aspergillosis yields positive blood culture in fewer than 5 % of patients, whereas isolation of *Aspergillus fumigatus* from a single blood culture is likely to represent contamination [51]. This is because most deep mould infections are acquired by inhalation of spores; thus, the primary site of infection is the respiratory tract, most often the lungs. During infection, most moulds grow as branching mycelium through the tissues and along the blood vessels, thereby causing infection by contiguous spread. Occasionally, if viable fragments break off, there may be haematogenous dissemination to remote organs, but isolation of viable fragments from blood will be a rare occurrence. The majority of filamentous fungi causing infection do not sporulate in vivo, so there is not production of large numbers of infectious propagules to disseminate around the body. Exceptions to this are with *Fusarium*, and less commonly *Acremonium* and related genera, which have spores evolved for water dissemination and do have the capacity to form spores in vivo. *Aspergillus terreus* may also form chlamydoconidia in vivo, which may account for the propensity of this species to cause disseminated infection. When causing infection of deep tissues, these genera frequently become disseminated by haematogenous spread, and about 70 % of patients with *Fusarium* infection will yield a positive blood culture. A similar number will present with cutaneous lesions due to entrapment of spores in the small capillaries of the skin [15••, 52].

Dimorphic fungi such as *Histoplasma* spp., *Blastomyces* spp., *Paracoccidioides* spp. and *Talaromyces marneffei* display thermal dimorphism and will be yeast forms under the influence of body temperature in vivo. Thus, the yeast forms will have the capacity to disseminate in the blood and may be isolated from blood cultures, usually on prolonged incubation. They will maintain a yeast morphology if sub-culture plates are maintained at 37 °C, but will convert to their respective mould forms when cultured at 30 °C. Outside endemic areas these will always be significant, and even in endemic areas, would very rarely be encountered as plate contaminants. *Coccidioides* spp. are also dimorphic, but are unique in forming spherules in vivo; these are large thick-walled structures that contain multiple endospores. When mature, spherules, which usually originate in the lungs as infection by inhalation of arthroconidia, will rupture, releasing multiple endospores that disseminate. They may be isolated in blood culture, although results are variable and not as reliable as isolation from tissue [53]. Isolation of moulds other than those detailed above are rarely significant, and most often represent contamination either during taking of the specimen or at some point during the processing. Contamination of blood culture with species such as *Aureobasidium pullulans*, *Scedosporium* spp., *Paecilomyces* and *Alternaria* is quite common [54]. However, there have been reported fungaemias in immunocompromised patients with a variety of environmental saprobes, so there should be careful consideration of the clinical condition of the patient and the likely risk factors before discounting unusual isolates.

Tissue

Direct microscopic examination of biopsy or autopsy samples stained with optical brighteners or histological stains can enhance the significance of a subsequent isolate with compatible morphology. Direct microscopy can be diagnostic for

yeast infection, including the yeast forms of dimorphic pathogens, coccidioidomycosis in which spherules may be visualised, mucormycosis, hyalohyphomycosis or phaeohyphomycosis. Visualization of fungal elements is sufficient for proof of invasive fungal infection [55]; however, culture is important, as it can yield an isolate for species identification and possible susceptibility testing, both of which can help to direct appropriate antifungal therapy. Tissues for fungal culture should not be homogenised, as this will disrupt hyphae and may lead to the formation of nonviable mycelial fragments. This is especially likely with mucoraceous moulds, which have few septa to wall off hyphal damage, which means that even quite large fragments may have lost their cytoplasmic contents and become nonviable. This is one of the reasons why culture and even PCR methods may be unsuccessful even when fungal hyphae have been seen on direct microscopic examination. Therefore, if fungal infection is suspected, tissue samples should be chopped into 1 mm pieces, placed on the surface of an appropriate agar, and incubated at 30 °C and 37 °C. Cultures should be examined for the presence of fungal growth after 24 hours and then at regular intervals for up to 6 weeks, depending on the pathogen that is suspected. Hyphae can often be seen emerging from the tissue sample by direct microscopy of the plate or slope even before growth of the colony becomes visible to the naked eye.

Subcutaneous infection may follow accidental inoculation of moulds with either hyaline (hyalohyphomycosis) or darkly pigmented (phaeohyphomycosis) hyphae. These infections, which are most common in individuals with immunological defects, such as renal transplant recipients on tacrolimus, do not present as mycetomas, but on microscopic examination sparse fungal elements may be seen in the tissues. Organisms implicated in these infections include: *Scedosporium*, *Exophiala*, *Cladophialophora*, and *Alternaria* spp. [56–59]. Other deep mould infections are most common in patients who are immunocompromised. *Aspergillus fumigatus* is by far the most common mould causing invasive infection and cerebral aspergillosis is a well-described complication; however, it is also a common contaminant, so careful evaluation is required. Infection with the organisms *Cladophialophora bantiana*, and to a much lesser extent *Cladophialophora arxii* and *Cladophialophora devresii*, which are classified in the European scheme as Hazard Group 3, most often present as cerebral infection, usually in young Asian males, whereas *Rhinocladiella mackenzei* has a similar presentation in patients from the Middle East [60]. Darkly pigmented hyphae may be seen in pus from these lesions or penetrating the brain tissue in biopsy samples. *Aspergillus flavus* is the most common cause of fungal sinus infection in immunocompetent individuals [61]; in immunocompromised or diabetic individuals, mucoraceous moulds are more common [14••].

Conclusions

Whilst isolation in culture alone may be diagnostic for some fungal infections, direct microscopic observation with optical brighteners or histological stains greatly enhances the significance of subsequent isolation of a fungus with compatible morphology. Radiological methods, such as CT scans, can identify abnormal areas and help to direct appropriate sampling. In the absence of confirmatory direct microscopy, consideration should be made of the amount and species of any fungus isolated and its ability to grow at 37 °C, as well as the nature of underlying predisposing factors. Examination of other biomarkers of infection, such as antibody and antigen detection and PCR tests, can help to confirm infection. In a high-risk patient, no fungal isolate obtained in culture should be discounted without careful evaluation.

Compliance with Ethics Guidelines

Conflict of Interest AM Borman and EM Johnson both declare no conflicts of interest.

Human and Animal Rights and Informed Consent All studies by the authors involving animal and/or human subjects were performed after approval by the appropriate institutional review boards. When required, written informed consent was obtained from all participants.

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