

# Mixed mould species in laboratory cultures of respiratory specimens: how should they be reported, and what are the indications for susceptibility testing?

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## ABSTRACT

**Aims** To investigate how clinical microbiology laboratories should report and interpret mixed mould isolates including *Aspergillus* species from clinical samples and the criteria for susceptibility testing of the isolates.

**Methods** Retrospectively collected data from our laboratory information system of moulds isolated between January 2005 and December 2007. Patient case notes were also reviewed.

**Results** A total of 502 isolates (from 273 patients) were found. 20 patients with clinical diagnosis of a probable fungal infection had mixed *Aspergillus* species.

**Conclusions** In most instances, the isolation of *Aspergillus* species from non-sterile sites does not represent clinical disease, but only colonisation/contamination. However, for high-risk patients including transplant recipients, a positive culture is associated with invasive disease. Our tertiary centre routinely reports single fungal isolates and mixed cultures with appropriate comments, and those considered significant will also have susceptibility testing carried out. The correlation of culture results with clinical features can differentiate between invasive disease and contamination.

## INTRODUCTION

*Aspergillus* species are the most important group of filamentous moulds causing invasive fungal infections (IFI), and they continue to pose a clinical challenge owing to the high mortality associated with these infections.<sup>1</sup>

The culture and isolation of *Aspergillus* species in the microbiology laboratory could be interpreted as contamination, colonisation or invasive disease in a patient. However, the isolation of *Aspergillus* species, especially *Aspergillus fumigatus*, from high-risk patients including bone marrow transplants (BMT), haematology and solid organ transplants (SOT) recipients is associated with IFI. Cultures from sterile sites including biopsies and repeated cultures from non-sterile sites can help to distinguish true positives from colonising species or laboratory contaminants.<sup>2</sup>

The issue of mixed cultures from patient samples is becoming important as the significance of infections caused by species of *Aspergillus* other than *A fumigatus* becomes increasingly recognised.<sup>3–4</sup> The isolation of fungal isolates is not only important for mapping out the epidemiology of IFI but also critical in the choice of appropriate antifungal therapy. The British Society for Medical Mycology has produced guidelines for the laboratory investigation

of IFI<sup>5</sup> but has not given any advice about how to report and how to deal with mixed culture isolates. We are unaware of any guidelines that advise clinical microbiology laboratories about how to interpret mixed cultures, especially from high-risk patients.

The main aim of this study is to establish how to report mixed isolates (more than one mould species) of mould including *Aspergillus* species from clinical samples. Should all *Aspergillus* species be reported, or should laboratories only concentrate on reporting *A fumigatus*? Another important consideration is what criteria are to be used to select mould isolates for susceptibility testing including minimum inhibitory concentration tests (MICs). This is a report of the current laboratory practice in our tertiary centre.

## METHODS

We retrospectively collected information from our laboratory information system about all *Aspergillus* species and other moulds isolated from clinical samples between January 2005 and December 2007. We have excluded mould isolates cultured from the sputa of cystic fibrosis patients, from all ear swabs and other superficial sites. We identified a subsection of patients who had mixed *Aspergillus* species from repeat samples and those with mixed cultures from sterile sites. The clinical history and diagnosis of these patients were reviewed from information gathered from their case notes.

## RESULTS

There were 502 isolates (from 273 patients) over the 3-year period. Twenty patients with suspected clinical diagnosis of fungal infection or allergy had repeated mixed *Aspergillus* species cultured. The clinical features and types of mixed culture isolates from these patients are presented in table 1.

Among these patients, the largest group was those with probable airways disease, including allergic bronchopulmonary aspergillosis and *Aspergillus* bronchitis (patients 1, 2, 8, 9 and 11–20). Three patients had chronic pulmonary aspergillosis (patients 3, 7 and 10), and three possibly had invasive aspergillosis (patients 4, 5 and 6).

In all 20 cases, *A fumigatus* was cultured. The species most commonly isolated in addition were *Aspergillus niger* (six cases) and *Aspergillus flavus* (five cases), with three instances of *Penicillium* spp. Two different morphologies of *A fumigatus* were found in one patient, which may or may not represent different strains on typing.

**Table 1** Patients, diagnosis, specimens and culture results

Patient no (age)	Diagnosis	Specimen	Cultures result
1 (68 years)	Bronchitis	Sputum/BAL	<i>A fumigatus</i> / <i>Penicillium</i> sp
2 (39 years)	Persistent bronchial infection (primary immunodeficiency)	Sputum	<i>A fumigatus</i> / <i>A glaucus</i>
3 (26 years)	Aspergilloma	Bronchoscope biopsy/BAL/sputum	<i>A fumigatus</i> / <i>A niger</i>
4 (28 years)	Small cell carcinoma (finger)	Sputum/lung biopsy	<i>A fumigatus</i> / <i>A nidulans</i>
5 (87 years)	Carcinoma bladder (+ chest infection)	Sputum	<i>A fumigatus</i> / <i>A flavus</i>
6 (81 years)	Lobectomy (+ pneumonia)	BAL/sputa	<i>A fumigatus</i> / <i>A niger</i>
7 (63 years)	Chronic pulmonary aspergillosis (+ <i>Mycobacteria malmogensis</i> infection)	Chest fistula pus/sputum	<i>A fumigatus</i> / <i>A versicolor</i>
8 (63 years)	Heart transplant	BAL/sputa	<i>A fumigatus</i> / <i>Penicillium</i> sp
9 (84 years)	Chronic cough	BAL	<i>A fumigatus</i> / <i>A niger</i>
10 (38 years)	Tuberculosis	BAL/sputa	<i>A fumigatus</i> / <i>A flavus</i>
11 (55 years)	COPD, emphysema	BAL/sputa	<i>A fumigatus</i> / <i>A niger</i>
12 (64 years)	Single lung transplant (cystic fibrosis)	BAL/bronchial aspirate	<i>A fumigatus</i> / <i>Trichoderma harzianum</i>
13 (65 years)	ABPA	Sputa	<i>A fumigatus</i> / <i>Penicillium</i> sp
14 (64 years)	COPD, bronchiectasis	Sputa	<i>A fumigatus</i> / <i>A flavus</i>
15 (39 years)	ABPA	Sputa/BAL	<i>A fumigatus</i> / <i>A flavus</i>
16 (65 years)	Bronchiectasis	Sputa/BAL	<i>A fumigatus</i> / <i>A flavus</i>
17 (56 years)	COPD/emphysema	Sputa/BAL	<i>A fumigatus</i> / <i>A niger</i>
18 (42 years)	ABPA	Sputum/BAL	<i>A fumigatus</i> / <i>A terreus</i>
19 (65 years)	Severe asthma with fungal sensitisation	Sputa	<i>A fumigatus</i> / <i>A niger</i>
20 (34 years)	Double lung transplant	Sputum	<i>A fumigatus</i> (two morphologies)

ABPA, allergic broncho-pulmonary aspergillosis; BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease.

## DISCUSSION

In most instances, the isolation of *Aspergillus* species from non-sterile sites is not associated with clinical disease but only reflects colonisation or laboratory contamination. However, for high-risk patients including allogeneic BMT recipients and SOT patients, a positive culture is often associated with invasive disease.<sup>6</sup> 'Significant' isolates are not restricted to invasive aspergillosis only but may be found in allergic and chronic pulmonary aspergillosis. As a result, IFI is increasingly recognised as an important cause of morbidity and mortality in both immunocompromised and chronic pulmonary disease patients. Our 900-bed university teaching hospital provides tertiary care for patients including heart–lung transplantation and oncology patients who are at high risk for IFIs, as well as thousands of patients with COPD, asthma, bronchiectasis, interstitial lung disease and other chronic pulmonary diseases. The microbiology laboratory is therefore keen to ensure that mycology services are efficient and guided by good evidence-based recommendations for reporting. All single fungal isolates are reported with appropriate comments, and those considered significant (following discussion with the clinical team) are also tested routinely for antifungal susceptibility including MICs using appropriate standards.

With mixed cultures however, it is still unclear as to how best to report these, since no guidelines exist. It has become our standard practice to notify all mixed cultures to clinicians and also to do MICs on all isolates from biopsies and those from sterile sites. Extensive discussion with clinicians is also warranted in order to ascertain the significance of isolates from other samples, especially those from high-risk patients. The need to send repeat samples where possible and to investigate further using other diagnostic methods including radiology, antigen and IgE and IgG antibody testing and other molecular tests where appropriate needs to be discussed.<sup>7,8</sup> The correlation of culture results with the clinical features can help to differentiate between invasive disease and colonisation or contamination.<sup>9</sup> Given that all our patients had *A fumigatus* isolated, it is also possible that this was the most important isolate, given its remarkable pathogenicity and allergenicity, and the other

## Take-home messages

- ▶ It is our experience that mixed fungal species are not uncommon in clinical samples from immunocompromised patients.
- ▶ There is a need to develop a consensus guideline on how best to report mixed fungal cultures especially from sterile samples and biopsies received from immunocompromised patients.
- ▶ The practice of reporting only *A fumigatus* from mixed cultures should be discouraged, since non-*fumigatus* infections are also increasingly reported in clinical practice.

isolates found are not contributing to disease. However, all the other isolates we found are important allergenic species and could be contributing to worse airways disease in those with asthma.<sup>10</sup> If two morphologies of *A fumigatus* are found, as in one patient in this series, both should be susceptibility-tested.

It is important to note, however, that a considerable workload is associated with the isolation and susceptibility testing of *Aspergillus* species. This has resource implications for general microbiology laboratories involved in this type of work, although it will go a long way to improving the quality of their service.

In conclusion, mixed infections with *A fumigatus* and other moulds are seen in clinical practice, especially among immunocompromised patients. A prospective study will be required in order to assess the significance/impact of mixed infections on the clinical outcome of these patients.

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