ORIGINAL ARTICLE

Audit of laboratory mycology services for the management of patients with fungal infections in the northwest of England

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Background: Fungal infection is increasingly recognised as an important cause of morbidity and mortality, especially in immunocompromised patients. Little information exists on laboratory services available and the methods used by general microbiology laboratories to diagnose these important infections.

Aim: To investigate the services microbiology laboratories in northwest England provide towards the diagnosis and management of superficial and deep fungal infections.

Methods: A questionnaire was sent to laboratories to get a holistic view of the support given to clinicians looking after patients with fungal infections. The aim was not to investigate details of each laboratory's standard operating procedures. The completed questionnaires, which formed the basis of this report, were returned by all 21 laboratories which were recruited. This study was conducted between March 2004 and September 2004.

Results: Services were provided to District General Hospitals and to six tertiary centres, including eight teaching hospitals by 16 laboratories. Their bed capacity was 250–1300 beds. Total specimens (including bacterial and viral) processed annually were 42 000–500 000 whereas fungal ones were 560–5400.

Conclusion: In most microbiology laboratories of northwest England, clinicians were aware of the potential of fungal pathogens to cause infections especially in immunocompromised patients. Additional measures such as prolonged incubation of samples were introduced to improve fungal yield from patients at high risk. It is necessary to train and educate laboratory and medical staff about the role of serology and molecular methods in diagnosis and management of patients with fungal infection.

In recent decades, the incidence of fungal infections has increased rapidly.¹ The morbidity and mortality associated with these infections are substantial—for example, in the US, 70% of the estimated 2300 infection-related deaths in 1992 were caused by invasive candidiasis, aspergillosis and cryptococcosis.³ Centres with active surveillance and robust diagnostic programmes have lower mortality.⁴

Most initial tests for diagnosis of fungal infections in northwest England are carried out in general microbiology laboratories. Several mycology regional laboratories (eg, Salford Royal Hospital and Leeds) provide specialist services. The mycology reference laboratory in Bristol complements these services. The questionnaire-based audit was designed to investigate the services, including methods used by general microbiology laboratories to diagnose fungal infections. A similar national study was conducted in 1996.⁵

METHODS

Questionnaires were sent to 21 laboratories in northwest England (see appendix). These were completed by senior biomedical scientists, consultant microbiologists or clinical scientists. The questionnaire contained 14 composite questions requiring various responses (yes/no; tick; numerical answers and brief comments). Responders were asked about workload and services offered, hospitals or specialties served, number of fungal infections diagnosed per year and current or future needs. The responses were collated and analysed using an Excel spreadsheet.

RESULTS

Of the 21 laboratories, 9 supported transplant services (bone marrow and solid organs); 20 supported intensive care units (adults or children); 13 supported dialysis (haemodialysis or peritoneal dialysis); and 13 supported other haematology, oncology or radiotherapy services.

Blood cultures

Annually, 10 laboratories documented >10 cases of candidaemias and eight others had <10 cases. Three laboratories did not indicate their candidaemia rates.

Various automated systems were used to culture fungi from blood including BacT/Alert (Biomérieux, Hazelwood, Missouri; n = 11). Seven laboratories indicated that they used additional special culture methods if candidaemia was clinically suspected—for example, use of BacT/Alert FA (Biomérieux), Myco F lytic bottle (Becton Dickinson Microbiology Systems, Franklin Lakes, New Jersey) or increasing the period of incubation up to 2 weeks.

All laboratories, except two, routinely speciated *Candida* isolates from blood cultures (these two laboratories speciated on the basis of clinical history).

Sterile site

All laboratories except one speciated *Candida* isolates from deep sterile sites: 13 of them did so routinely, whereas the rest would do so depending on history.

Lines

All laboratories except three speciated *Candida* isolates from lines: 7 of them did so routinely whereas 10 would do so depending on history. One laboratory did not respond to this query.

All laboratories except one routinely speciated clinically important mould isolates from patients at high risk, including those in the intensive care unit, haematology or oncology and those who received transplants. Twelve laboratories indicated that they possessed in-house capability for identifying moulds.

Abbreviation: NEQAS, National External Quality Assessment Scheme

Antifungal sensitivity testing

All but one laboratory provided susceptibility testing of *Candida* species (four on site; the rest by referral). Only eight laboratories carried out sensitivity testing of moulds (one laboratory on site; the rest by referral).

Serology

Table 1 shows the responses to questions on serology testing.

Molecular diagnosis

Only 6 of the 21 laboratories provided a molecular detection service (eg, PCR for *Candida* and *Aspergillus*; three on site; three off site).

Dermatology samples

All 21 laboratories received dermatology samples (including skin, nail and hair) and only two laboratories indicated that they would refer all for examination to a specialist or to a reference centre. Nineteen laboratories carried out direct microscopy on skin, nail and hair (all using potassium hydroxide, whereas only five laboratories used a fluorescent dye such as calcofluor white). All laboratories cultured samples for dermatophytes and identified them to genus level, whereas only three laboratories indicated that they speciated them.

National External Quality Assessment Scheme

All laboratories participated in the National External Quality Assessment Scheme (NEQAS) for identification of fungi.

Comments on current or future needs

Table 2 shows the comments on current or future needs for identification of fungi.

DISCUSSION

In northwest England, awareness of the potential of fungi as important causes of infections in patients at high risk is encouraging. This may partly be because most laboratories now provide services to clinicians who are managing immunocompromised patients, including those who have received transplants.

The incidence of fungal infections has increased substantially as a result of a rise in the number of patients at high risk, including those with organ transplants.^{1-6,7} Opportunist infections are important causes of morbidity and mortality, they pose a diagnostic challenge to clinicians and also to microbiology laboratories.⁸ It is therefore important that laboratories take steps to maximise fungal yield.

Several laboratories had indicated that they would use additional special culture or diagnostic methods if candidaemia were suspected. The detection of fungaemia is important in the diagnosis of invasive fungal infection.⁹⁻¹¹ In disseminated mould infections, however, no growth is found in blood cultures. Since the 1990s, there has been improvement in detection of fungaemia as a result of advances in blood

Table 2 Comments on current and future needs for fungal identification

Comments	No of laboratories
Will like to do in-house sensitivity tests on yeasts.	4
Require training	
Access to improved diagnostic tests including PCR	1
Information on antifungal assays (reference ranges and frequency of monitoring)	1
Algorithm for requesting molecular tests (sampling or frequency)	1
Centralisation of dermatophyte identification, antifungal assay and serology	1
No comments	13

culture methods.⁹⁻¹¹ The lysis centrifugation method provides a flexible and sensitive but labour intensive method for detecting fungaemia that is caused by common yeasts and moulds. Sensitivity is also improved when more than one system is used for blood culture,⁹ but this comes at a cost beyond the capability of many general microbiology laboratories in the UK.

Most laboratories did speciate yeasts from blood cultures (90%), sterile sites (95%) and line tips (81%). Also, all laboratories except one routinely speciated clinically important moulds from patients at high risk. This complies with the proposed standard of practice recommended by the British Society for Medical Mycology.² Although the primary reasons for this audit did not include measuring practices against these standards, it is encouraging to see that some aspects are being incorporated into the standard operating procedures of most laboratories.

All laboratories carried out susceptibility testing on yeasts, as compared with only eight laboratories that did so on moulds; 13 (62%) laboratories would not even consider susceptibility testing on moulds. Although the reasons for the lack of interest in carrying out sensitivity testing on moulds, as opposed to testing on yeasts, was not specifically asked for, it may reflect the poor predictive value of these tests and the perceived lack of contribution to the overall patient management. Issues relating to turn-around times are also critical. Despite limitations, education on the potential role of sensitivity tests is necessary.

Antifungal assays are also becoming a routine part of the management of patients receiving antifungals. In all, 16 (76%) laboratories monitored routinely for efficacy or toxicity. This is commendable and ensures optimum management, especially when potentially toxic agents are used. Automation has also made it easier for laboratory personnel to acquire the necessary analytical skills. Although reference ranges of some agents, including itraconazole, are well validated, those for newer agents, such as voriconazole, require further research.¹²

Without further probing, it was impossible to tell precisely why some laboratories did not use some of the serology tests at all. It may be because of a lack of understanding of the exact roles of these tests, although there was a suggestion that laboratories that do not use the cryptococcal antigen tests, for example, do not service hospitals or units that have patients who are at a high risk of cryptococcosis, including HIV.

Molecular detection methods including PCR are yet to find routine application in the management of invasive fungal infections as only six laboratories, of the 19 that responded use them. This may be because of the uncertainty about the role of these relatively expensive tests in the management of patients, as well as issues regarding turn-around time.

Table 1 Result of serology testing—do you do the following tests?

Tests	Yes	Yes	No
	In-house	Referral	
<i>Candida</i> antigen (Mannan)	0	3	16
<i>Candida</i> antibody (precipitins)	0	9	11
<i>Aspergillus</i> antigen (Galactomannan)	3	4	12
<i>Aspergillus</i> antibody (precipitins)	0	14	6
Cryptococcal antigen	3	11	6
Cryptococcal antibody	0	8	12

Take-home messages

- Incidence of fungal infection has increased rapidly in recent years and many laboratories have become more adept at looking for fungi, some also using additional culture methods in suspected clinical samples.
- Most laboratories speciated yeasts from sterile sites and also routinely speciated clinically important moulds, complying with the British Society for Medical Mycology recommendations.
- Future needs around training issues towards in-house sensitivity testing, use of serology and molecular methods in diagnosis remain.
- All laboratories participated in the National External Quality Assessment Scheme, which if reflected nationally, represents an improvement compared with the earlier survey.
- Adequate labelling of samples and forms filled accurately with clinical details are important steps towards effective laboratory diagnosis.

Laboratory investigation of dermatophyte infections is widespread and only two laboratories referred specimens to specialist laboratories for routine investigation. One aspect that may require improvement (via education or training) is microscopic investigation. Evidence suggests that using fluorescent dyes increases sensitivity compared with using potassium hydroxide.^{13 14} The method is simple, rapid and inexpensive, but requires a small capital investment towards the purchase of a fluorescent microscope, which nowadays is commonly found in many microbiology laboratories in the UK.

All laboratories participate in NEQAS, which remains the benchmark for quality improvement. If this finding is reflected nationally, it will seem to be an improvement compared with an earlier survey.⁵

The general remark, as regards future needs, was on training issues towards in-house sensitivity testing and the use of serology and molecular methods. Currently, there is no reason why laboratories that wish to do susceptibility testing of *Candida* against fluconazole cannot do so. The disc method of testing is well validated and requires minimal training and could be incorporated in most laboratories.¹⁵ Other areas of antifungal susceptibility testing, at present, require specialist expertise.

Although cultures and histological examination remain the principal means of laboratory diagnosis of fungal infection, a need for more rapid non-cultural methods of diagnosis still remains.⁹ With few exceptions (eg, cryptococcal antigen test), these tests have not yet made any major clinical impact, largely because of poor sensitivity, specificity and standardisation. The cryptococcal tests are well standardised and detect the capsular polysaccharide antigen of *Cryptococcus neoformans* with sensitivity >95% (meningitis) and 67% (disseminated infections).^{2 16}

Several assays (including in-house) detect *Aspergillus* antigen (free or bound) in serum, bronchoalveolar lavage or urine.^{8 17} *Aspergillus galactomanan* circulates during infection. Enzyme-linked immunosorbent assays with sensitivities of 90% and specificities of 84% exist and are more suitable for detection of *A. galactomanan* in serum than in urine and can also detect it in bronchoalveolar lavage.^{18 19} Repeat testing may be required to confirm results, but false positives are

known to occur. A negative result, however, does not rule out the possibility of invasive aspergillosis.

Mannan (cell wall polysaccharide component of *Candida*) has a short half-life in serum and is easily bound by anti-mannan antibody. The low sensitivity of assays has limited its use.²⁰ Other potential antigenic sites such as enolase,¹¹ mannoprotein²¹ and (1-3)- β -D-glucan²² are being investigated and look promising. Often, antibody tests for both *Candida* and *Aspergillus* cannot distinguish between current and past infections or colonisation, and a negative result does not rule out infection.

The application of molecular biology offers great hope for a rapid diagnosis of fungal infections. Research has focused on diagnosis of invasive candidosis, as well as infection by other fungi, including *Aspergillus*.^{20 22-24} *Candida*-specific DNA had been recovered from blood or body fluids, with a sensitivity of 76-96% in clinical practice.^{20 23} Current PCR diagnosis is cumbersome but promising, and issues of standardisation of techniques and distinction between colonisation and infection need to be resolved.

In conclusion, this audit highlights the growing awareness about fungal infection. A lack of consensus regarding the use of non-cultural tests for assisting the clinical diagnosis of these infections, however, still remains. Training is also required for laboratories wishing to develop in-house disc sensitivity testing for *Candida*.

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APPENDIX

QUESTIONNAIRE USED TO COLLECT DATA

2004

FUNGAL INFECTIONS

AUDIT OF LABORATORY SERVICES IN THE North West

2. TYPE OF HOSPITAL (tick) BED CAPACITY

- District Gen. Hosp. (Teaching Hospital)
- District Gen. Hosp. (not Teaching Hospital)
- TERTIARY HOSPITAL
- OTHERS (SPECIFY)

3. Clinical specialties served by your lab (tick)

- Bone Marrow Transplant/Peripheral stem cell Transpl.?
- Other Specialist Clinical Haematology?
- Oncology?
- Radiotherapy?
- Solid organ Transplant (specify type)?
- Dialysis (specify type)?
- ID unit (including HIV)?
- Burns/Plastics?
- Intensive care unit (specify bed capacity):?
- Adult: _____ Paediatric: _____ Neonatal: _____
- Dermatology?
- Others (specify)

4. LAB WORKLOAD (specimens per year)

- Total number of specimens (bacteriology, virology, serology, etc)
- Dermatology specimens
- Fungal serology
- Others (specify)

5. Blood culture system(s) in use

- Name:
- Any special culture/diagnostic methods in use if fungal sepsis suspected? Yes/No
- If yes, indicate/explain
- No of positive fungal blood cultures per year

6. IS YOUR LAB. POLICY TO FULLY IDENTIFY YEAST SPECIES IN (tick)

Yes—all isolates or Yes—as per local policy or clinical details or No—never or very rarely

- Blood cultures
- Deep sterile sites
- Line tip isolates
- Vaginal isolates
- Urine isolates
- Isolates from patients receiving intravenous antifungal therapy
- Isolates from patients about to receive intravenous antifungal therapy
- Isolates from HIV/AIDS patients
- Isolates from burns patients
- Other specialist units (eg listed in item 2 above)

7. Do you do susceptibility testing on yeasts (tick)

- YES (On site)?
- YES (referral to a specialist lab-give name)?
- NO?
- other comments

8. IS YOUR LAB. POLICY TO FULLY IDENTIFY MOULDS (including referral to reference lab.) TO SPECIES LEVEL IN

Yes or No or Medical decision

- ICU patients:
- Haematology:
- Oncology
- All transplants
- HIV/AIDS patients
- Chest patients
- Others (specify e.g. see item 2 above)
- In-house capability for identifying moulds (comment)

9. Do you do sensitivity testing on moulds (tick)

- YES (On Site)?
- YES (referral to a specialist lab-give name)
- NO?

10. SKIN/NAIL/HAIR (DERMATOPHYTES)

DO YOU ROUTINELY DO: Yes or No

- Microscopy with KOH
- Microscopy with fluorescent dye (specify)
- Culture
- Identification to genus level
- Others (specify)

11. SEROLOGY

No or Yes (in-house or referral)

- Do you do *Candida* antigen (Mannan)
- Do you do *Candida* antibody (precipitins)
- Do you do *Aspergillus* antigen (Galactomannan)
- Do you do *Aspergillus* antibody (precipitins)
- Do you do *Cryptococcal* antigen
- Do you do *Cryptococcal* antibody
- Others (specify)

12. Do you carry out antifungal assay(s)

- YES (On site)? _____
- YES (referral to a specialist lab-give name)?
- NO?
- other (explain)

13. Does your laboratory participate in nequas for mycology?

- YES? _____
- NO?

14. Does your laboratory carry out molecular detection methods?

- YES ON SITE?
- YES BUT OFF SITE. Give details?
- NO?

15. Please comment on current/future needs for mycology services**16. Please indicate the name of your laboratory (optional)**

IMPORTANT: Please Note:

17. This mycology audit data may be presented at microbiology meetings—but your identity will not be disclosed without your approval

Please indicate if you wish to remain anonymous:

- THE NAME OF MY LABORATORY MUST NOT BE RELEASED
- I HAVE NO OBJECTION TO THE REPLIES BEING IDENTIFIED AS FROM MY LABORATORY

ANY QUERIES REGARDING THIS QUESTIONNAIRE SHOULD BE DIRECTED TO DR. IBRAHIM A. HASSAN, CONSULTANT MICROBIOLOGIST AT WYTHENSHAW HOSPITAL MANCHESTER (OR TO COLLEAGUES DRS ISALSKA OR CULLEN). TEL: 0161 291 2885/4756/2884/2892. email: microbiology@smuht.nwest.nhs.uk

Thank you for taking the time to complete this questionnaire

Please return by Friday 10th September to: Dr Ibrahim Hassan, Consultant Microbiologist, Wythenshawe Hospital Department of Microbiology Southmoor Road Manchester M23 9LT, UK.

An addressed envelope is provided for your convenience.