B39: Investigation of dermatological specimens for superficial mycoses

Ijeoma Ezeajughi, Ayuen Lual, Ruhi Siddiqui, Clare Harris
Public Health England, London, UK

Introduction

The term 'superficial mycoses' refers to fungal infections usually confined to the outer layers of the skin, hair and nails. Superficial fungal infections are chronic and recurring conditions and are caused by dermatophytes as well as non-dermatophytes.

Infection by dermatophytes is cutaneous and generally restricted to the non-living cornified layers in patients who are immunocompetent. This group of fungi are generally unable to penetrate tissues which are not fully keratinised (deeper tissues and organs). Reactions to such infections can range from mild to severe depending upon the host's immune response, the virulence of the infecting species, the site of infection and environmental factors. The dermatophytes are classified in three genera: *Epidermophyton* species, *Microsporum* species and *Trichophyton* species.

There are non-dermatophyte moulds that can infect healthy skin, nails damaged by physical trauma, or pre-existing infection with a dermatophyte. These include *Neoscytalidium dimidiatum*, *Neoscytalidium hyalinum*, *Phaeoannellomyces werneckii*, *Piedraia hortae*, etc.

Discussion

Aimed at practising professionals in the field of microbiology, B39: Investigation of dermatological specimens for superficial mycoses is a diagnostic tool which describes and recommends the procedures that can be used to visualise and isolate dermatophytes, non-dermatophyte moulds and other fungi from skin, nail and hair specimens.

B39 is developed by professionals in the field with specialist input and is intended as a general resource for laboratory staff, clinicians and healthcare commissioners. This document focuses on how detection and accurate identification can aid the management and control of superficial mycoses.

Conclusions

B39 provides guidance on the best minimum practice when investigating infection caused by dermatophytes, non-dermatophytes and other fungi. It also provides information on rapid molecular methods and culture testing for these fungi. In conclusion, this has been proven to be a useful resource tool to access information on detection and identification techniques in clinical laboratories across the NHS and it aims to help drive pathology modernisation by recommending new technologies.
Sharing and developing a consensus approach to antifungal stewardship in London - a proof of concept

Charles Parkinson¹, Mark Gilchrist¹, Darius Armstrong-James¹, Laura Whitney², Tihana Bicanic²

¹Imperial College Healthcare NHS Trust, London, UK
²St George’s Healthcare NHS Trust, London, UK

Introduction

There are many patient groups who are vulnerable to high-mortality fungal infections. Despite the low incidence of invasive fungal disease (IFD) compared to invasive bacterial infections, current antimicrobial expenditure is dominated by antifungal use in the majority of tertiary centres.

Whilst strategies have focused on the prudent use of antibiotics, antifungal stewardship has been less well defined. This has been primarily due to a lack of:

- Effective diagnostics to guide antifungal treatment
- Therapeutic drug monitoring (TDM) to guide antifungal dosing
- Expertise to guide therapeutic decision making
- Knowledge of the NHS anti-fungal financial arrangements

In October 2010, St Georges Healthcare NHS Trust (SGHNT) introduced an antifungal stewardship programme with the principal aim of identifying, optimising and managing patients with an IFD. Since inception, the service has reviewed 195 patients with interventions including tailoring therapy, recommending TDM and further diagnosis/imaging together with monitoring cost.

Scientific findings

In November 2013, Imperial College Healthcare NHS Trust (ICHNT), started to examine the feasibility of initiating a weekly ward round based on the experiences, aims and objectives of the SGHNT programme. In January 2014, ICHNT utilising NHS England monies employed an embedded pharmacist who has become involved in the programme. This allowed further work on guideline development, utilisation and formulary choices. In March 2014, ICHNT started its antifungal stewardship programme.

A review of 6 months of ICHNT data was undertaken and compared to historic SGHNT data over 3 years. In addition it provided a proof of concept methodology where the round could be reproducible outside of SGHNT.

Within ICHNT, between March and August 2014 there were 22 rounds in which 100 patients (157 patient encounters) were reviewed. This compared to 114 rounds with 195 patients (370 encounters) at SGHNT over 36 months.

Comparison of patient speciality and interventions made on the rounds are described below:

<table>
<thead>
<tr>
<th>Speciality</th>
<th>ICHNT</th>
<th>SGHNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematology</td>
<td>51%</td>
<td>29%</td>
</tr>
<tr>
<td>Renal</td>
<td>19%</td>
<td>3%</td>
</tr>
<tr>
<td>General surgery</td>
<td>11%</td>
<td>8%</td>
</tr>
<tr>
<td>Cardiothoracic surgery</td>
<td>7%</td>
<td>12%</td>
</tr>
<tr>
<td>Others</td>
<td>12%</td>
<td>48%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interventions (per 100 encounters)</th>
<th>ICHNT</th>
<th>SGHNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostics</td>
<td>34.9</td>
<td>10.6</td>
</tr>
<tr>
<td>Step down</td>
<td>25.5</td>
<td>14.3</td>
</tr>
<tr>
<td>Step up</td>
<td>3.2</td>
<td>3.8</td>
</tr>
<tr>
<td>IV – PO switch</td>
<td>21.0</td>
<td>18.9</td>
</tr>
</tbody>
</table>
Discussion

The highest proportion of patients seen at both sites was haematology patients. This was expected as these patients are often heavily immunosuppressed and at high risk of developing an IFD.

There were more diagnostic recommendations at ICHNT; this could be due to a high level of observed empiric therapy. At both trusts interventions stepping down and stopping unnecessary empiric therapy were common.

Whilst the number of patients seen per round was greater within ICHNT (7.1 vs 3.2) similar themes were echoed. These included: optimising antifungal pharmacokinetics and management in particular TDM, education and engagement of medical teams around antifungal therapy.

Conclusions

Overall the service has been very well received and shows that the SGHNT model of antifungal stewardship is practically useful and reproducible in other centres. A business case at ICHNT is currently being written to address the need for molecular diagnostic testing with a view to more targeted antifungal therapies and management.
Isolation of *Aspergillus fumigatus* from sputum samples of cystic fibrosis patients: comparison between potato-dextrose agar and Sabouraud-dextrose agar

Sook Fong Sharon Koo, Deborah Modha, Christopher Holmes

*University Hospitals of Leicester NHS Trust, UK*

**Introduction**

*Aspergillus fumigatus* (*A. fumigatus*) is the commonest filamentous fungus isolated from lower respiratory samples of cystic fibrosis (CF) patients. The reported prevalence varies significantly between centres and is thought to be related to differences in fungal culture methodology. There are two national guidelines on mycological processing of respiratory samples from CF patients and these allow for considerable variation especially in the duration of incubation and concentration of inoculum used. This was noticeable from results of a national survey that we undertook previously with laboratories processing paediatric CF respiratory samples. We aim to evaluate if the combination of neat sputum plug sample with potato dextrose agar (PGCF) improved the yield of *A. fumigatus* isolation in CF patients when compared to the modified Public Health England UK Standards for Microbiology Investigations (PHE SMI) using Sabouraud dextrose agar (SDA-C).

**Scientific findings**

A total of 25 sputum samples were analysed prospectively. Individual plates of PGCF and SDA-C media were inoculated with 10 µL of sputum plug. 10 µL and 100 µL of homogenised and dilute-homogenised sputum samples were also inoculated onto the respective media. These were incubated at 37°C and inspected for any growth at 48 hours and at 5 days. Growth on the media was identified using macroscopic and microscopic methods (Sellotape method with Lactofuschin stain for filamentous fungi and gram stain for non-filamentous fungi and bacteria).

Filamentous fungal growth was observed in 15 sputum specimens (60%). All filamentous fungi isolated were identified as *A. fumigatus*. Growth of *A. fumigatus* was highly dependent on the concentration of inoculum (SDA-C, Q=14.923, p<0.001; PGCF, Q=10.182, p<0.0001). Higher volumes of inoculum yielded more *A. fumigatus* positive cultures but this did not reach statistical significance. *A. fumigatus* detection was not improved using the novel methodology when compared to the modified national recommendations (10 µL loopful instead of 1 µL) ($\chi^2_{\text{McNemar}}=0.8$, p=0.371). The combination of neat sputum plug and homogenized samples inoculated onto SAB-C media incubated for five days at 37°C in air detected the highest number of *A. fumigatus* positive samples. At 48 hours, this combination detected an additional five *A. fumigatus* positive samples which would have been missed if processed according to the modified PHE SMI ($\chi^2_{\text{McNemar}}=4.167$, p=0.041). Gram negative bacteria (GNB) were present exclusively in PGCF plate in three samples. Six out of seven samples which grew GNB had no fungal growth detected.

**Discussion**

A pre-study telephone survey undertaken with 22 microbiology laboratories processing paediatric CF respiratory specimens revealed that most laboratories were using a 10 µL loop to inoculate the fungal culture media. This was in contrast to the 1 µL loopful recommended by PHE SMI. The 10 µL inoculum used in this study simply reflected the practice in the majority of laboratories across the UK. Despite using a higher volume of inoculum, it failed to detect five *A. fumigatus* positive samples. This number is expected to be higher if 1 µL inoculum is used.

Earlier studies have shown that the use of PGCF media with neat sputum plug sample increased the isolation of *Aspergillus fumigatus* in COPD patients compared to the national PHE SMI used throughout the UK. Unfortunately, we were unable to reproduce this result in the CF cohort. The reason for this is unclear. We postulate that this could be due to presence of multi/pan-resistant GNB such as *Pseudomonas aeruginosa* and *Bukholderia* species which occurs more frequently in CF sputum compared to COPD patients.
to other lung conditions. Growth of these bacteria has been shown to be inhibited by high concentrations of antibiotics under experimental conditions. Low concentration of antibiotics in PGCF media may have led to the exclusive isolation of GNB in three samples which may in turn have an inhibitory effect on fungal growth. This could also be the reason why the majority of samples with GNB growth did not yield any fungi.

Conclusions

We believe that a standardized methodology that is sensitive enough to detect filamentous fungi, particularly *A. fumigatus*, in respiratory samples of CF patients should be adopted by all laboratories. This study demonstrated that PGCF did not improve *A. fumigatus* yield in sputum samples of CF patients. We propose that > 10 µL of neat sputum plug and homogenized sputum samples of CF patients should be inoculated onto SDA-C and incubated at 37°C in air for 5 days in order to increase sensitivity of *A. fumigatus* detection.
A pseudo-outbreak of *Fusarium oxysporum* associated with bronchoscopy

Edward Barton¹², Andy Borman¹, Elizabeth Johnson¹, James Sherlock², Annette Giles²

¹Public Health England, Bristol, UK
²University Hospitals Bristol NHS Foundation Trust, UK

**Introduction**

Bronchoscopy and bronchoalveolar lavage (BAL) is a procedure used to investigate patients with respiratory conditions and obtain samples for microbiological tests. Endoscopes, such as those used in bronchoscopy, have been implicated in outbreaks of nosocomial infection. *Fusarium* is a genus of environmental moulds, mostly plant pathogens, which can be found in soil and water - these fungi are an emerging cause of opportunistic infection in immunocompromised patients. Nosocomial outbreaks of mould infection, such as infection with *Aspergillus* associated with ventilation systems, have been reported, but outbreaks of *Fusarium* infection are relatively infrequently reported in the medical literature, except as a cause of keratitis. Here we report a pseudo-outbreak of two isolates of *F. oxysporum* in BAL samples from two patients. Neither isolate was associated with a compatible clinical syndrome in the patient. On sampling, *Fusarium* could not be found in the automated endoscope reprocessors or the endoscope storage/drying cabinets. Contamination was traced to a single 3.8mm bronchoscope that was found to have damage to its internal channel - this device remained persistently colonised with *Fusarium* despite decontamination and refurbishment work. The affected bronchoscope was discarded and no further isolates of *Fusarium* were detected.

**Scientific findings**

Two BAL samples taken from two patients on different days and in different procedure rooms grew *Fusarium*. Both isolates were identified as *Fusarium oxysporum* species complex (FOSC) sequence type (ST) 33. This is one of the most commonly isolated sequence types found within this complex so does not necessarily prove a point source. However, the endoscope used to obtain both samples was found to be the same: a Fujinon EB-470P bronchoscope and it yielded FOSC ST 33 on brush sampling of the internal lumen. *Fusarium* was not found on sampling of the automated endoscope reprocessors or the endoscope storage/drying cabinets.

**Discussion**

At refurbishment damage was found to the internal lumen which presumably allowed *Fusarium* biofilm formation and colonisation to persist despite decontamination processes in line with current best practice. The device remained persistently colonised so was removed from service. The initial source of contamination of the bronchoscope with *Fusarium* was not identified, but these organisms are widespread in the environment, especially water sources, and may be transiently associated with human hosts, so numerous potential sources exist. Bronchoscopes colonised with *Fusarium* may be a potential reservoir for nosocomial infection of immunocompromised hosts, although clinical infection did not occur in our patients.

**Conclusions**

Pseudo-outbreaks of *Fusarium* associated with bronchoscopy are only very rarely reported in the medical literature although they may occur more frequently in clinical practice. Established biofilms of *Fusarium* on medical devices may be resistant to conventional decontamination processes.