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National mycology laboratory diagnostic capacity for invasive fungal diseases in 2017: Evidence of sub-optimal practice

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SUMMARY

A survey of laboratory testing capabilities for systemic fungal pathogens was undertaken in the UK, to identify where improved compliance with published standards and guidelines is required and to inform antifungal stewardship (AFS).

The survey captured information from laboratories in the UK on diagnostic capacity for invasive fungal diseases (IFD), including identification, serology, molecular diagnostics and susceptibility testing. The survey was circulated in March 2017 through key networks.

Of 154 laboratories providing diagnostic mycology services in the UK, 80 (52%) responded to the survey. Results indicated that 85% of respondents identified fungal isolates from high risk patients to species level, and that many laboratories (78%) could access local susceptibility testing for yeasts, whereas 17% could for *Aspergillus* species. However, direct microscopy was only used in 49% as a first line investigation on samples where it would be appropriate. A low number of respondents identified yeasts cultured from intravascular line tips to species level (63%) and even fewer fully identified urine isolates from critically ill patients (42%) or the immunocompromised (39%). Less than half of respondents advised therapeutic drug monitoring (TDM) for flucytosine. Few laboratories had access to local β-glucan (4%) or galactomannan (20%) testing.

The survey highlights that the current level of fungal diagnostics in the UK is below accepted best practice with an urgent need to improve across many diagnostic areas including the timely accessibility of fungal biomarkers, susceptibility testing and provision of TDM testing. Improvements are important to facilitate the delivery of diagnostic driven AFS strategies as well as appropriate management of IFD.

Introduction

Invasive fungal diseases (IFD) are a major cause of morbidity and mortality despite medical and diagnostic advances. Severely immunocompromised patients including those with

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haematological malignancies and those undergoing transplantation continue to be at high risk of IFD.[1–3] Recent reports of invasive aspergillosis following influenza infection highlight there is also need for greater vigilance and diagnostic support in other patient groups such as intensive care patients.[4,5] To improve outcomes for patients with IFD, a comprehensive diagnostic mycology work-up is vital. Revised best practice recommendations for the diagnosis of IFDs have been published in the past.[6–9]

Increasing antifungal resistance as well as emergence of new fungal species causing healthcare-associated outbreaks and infections, such as *Candida auris*, pose a diagnostic challenge. The clinical need for antifungal drug susceptibility testing not just for yeasts but also for moulds has increased demand on laboratories.[10–12] High rates of treatment failure and associated mortality are particularly noted in infections due to azole resistant *Aspergillus fumigatus* isolates which are emerging in several countries including the UK.[13,14] These reports highlight the importance of monitoring antifungal resistance in clinically relevant fungal isolates at a national level to provide the evidence base for antifungal guidance and policies.[15]

Antifungal stewardship (AFS) aims to minimize adverse events related to antifungal use, to reduce the emergence of antifungal resistance and to improve outcomes for patients with IFD. Timely provision of mycological diagnosis is vital for AFS as it can be used to guide the rational use of antifungal agents.

The increasing utility of fungal biomarkers for the investigation of IFD and implementation of AFS require a timely availability of non-culture based diagnostics test results, such as for (1–3) β-D-glucan (BDG), *Aspergillus* galactomannan (GM),[6] and cryptococcal antigen (CRAG).

In order to assess laboratory capacity in the UK, to identify areas where improved compliance with published standards is required and to support AFS activities, Public Health England's (PHE) English Surveillance Programme for Antimicrobial Utilisation and Resistance (ESPAUR) undertook a national survey of laboratory testing capabilities for clinically significant fungal pathogens in collaboration with the British Society for Medical Mycology (BSMM) and endorsed by the UK Clinical Mycology Network (UKCMN).

Methods

The survey was developed by the ESPAUR antifungal consumption and resistance surveillance subgroup based on the BSMM's 2009 survey of mycology laboratory practice, the updated BSMM best practice guidance for patients with invasive fungal infections, the BSMM's guidance on therapeutic drug monitoring (TDM) and PHE's guidance on diagnosis of *C. auris*.[6,7,16,17] The survey was launched via digital SelectSurvey v4 platform on 27 March 2017 for 6 weeks and was distributed by email through clinical networks and accessible through websites and newsletters from PHE, UKCMN, the British Infection Association (BIA), BSMM and the Royal College of Pathologists (RCPath). Senior microbiologists/laboratory managers of all 154 UK laboratories providing mycology services that were either UK Accreditation Service (UKAS) accredited or reporting to PHE's laboratory surveillance system were asked to complete the survey questionnaire. Some participants were member laboratories of the UKCMN. All electronic responses received were independently validated by two of the authors (SS and KO). This included checking data for completeness and duplication. Duplicate responses were removed from analysis. Respondents were able to select more than one answer for some questions and could opt not to respond to all questions. Results reflect the percentage of total responses received for each question on the survey. Results for the PHE mycology reference laboratory were excluded from analysis that assessed compliance with BSMM best practice recommendations as they in their role already comply. Results for the NHS Mycology Reference Laboratory in Manchester were included in the analyses in line with the audit in 2009.

Table 1
Identification of yeasts from urine samples, and fungi isolated from sterile sites.

Identification of Yeast to species level from urine samples	Laboratories that identify and culture urine isolates/respondents (%)
All critical care patients	29/69 (42%)
Immunocompromised	27/69 (39%)
Variable	29/69 (42%)
Sterile sites	Laboratories that identify fungi isolated from sterile sites/respondents (%)
Blood cultures	59/60 (98%)
CAPD fluid	54/60 (90%)
IV line tips	38/60 (63%)
Bronchoscopy specimens[a]	42/60 (70%)
Sinus materials	49/60 (82%)

[a] Bronchoscopy fluid and paranasal sinus material are regarded as sterile for all fungi except *Candida spp.*

Results

Response rate and awareness of the BSMM diagnostic best practice

A total of 80 responses were received from clinical laboratories across the UK (England $n=72$, Wales $n=2$, Scotland $n=4$, Northern Ireland $n=1$ and Isle of Man $n=1$), representing a response rate of 52%. Of survey returns that provided information on hospital type, 54% (43/79) were from district general hospitals, 37% (29/79) from teaching hospitals and 6% (5/79) from specialist hospitals. Eleven responses were received from national and regional UKCMN mycology centres (14%). Seventy-five percent of the 75 responding laboratories were aware of the BSMM's best practice guidance.

Identification of yeast and mould species

Nearly all responding laboratories (99%; 68/69) indicated that, as per the BSMM best practice guidance, they identify to species level yeast isolates from clinical samples obtained from patients at high risk of IFD, and of these respondents, 94% (65/69) could perform the testing locally; 16 (23%) of these laboratories also indicated that they referred yeast isolates for confirmation. Three (3%) responding laboratories indicated that they referred yeast isolates to a reference laboratory or regional mycology centre for either species identification or confirmation, with no onsite testing.

Most laboratories indicated that they identified all fungi as recommended (both yeast and/or mould isolates) when isolated from blood cultures (98% 59/60), continuous ambulatory peritoneal dialysis (CAPD) fluid (90%), vascular line tips (63%), sinus material (82%) or non-*Candida* species from bronchoscopy specimens (70%). When assessing clinical samples, 42% (29/69) of laboratories identified yeast species detected from urine samples in critical care patients and 39% from immunocompromised patients (Table 1).

As a first line investigation, 49% (33/68) laboratories performed microscopy for the identification of moulds and yeasts directly from specimens or cultures. The use of other methods for fungal species identification, either onsite or by referral, included Matrix Assisted Laser Desorption/Ionization (MALDI-ToF; 83%; 57/69), VITEK® 2 system (43%; 30/69), analytical profile index (API) identification (32%; 22/69), chromogenic agar (65%; 45/69) and molecular sequencing (Table 2).

With respect to the globally emerging *C. auris*, 98% (62/63) of respondents confirmed their awareness of this new pathogen.

Table 2
Fungal species identification tests performed and site of testing for responding laboratories.

Tests used for fungal species identification (other than microscopy)	Laboratories that use test for species identification/respondents[a] (%)	
	Onsite or Centralised	Other External or Private
MALDI	46/69 (67%)	4/69 (6%)
VITEK®	29/69 (42%)	0/69 (0%)
API	21/69 (30%)	1/69 (1%)
Chromogenic agar	44/69 (64%)	1/69 (1%)
Reference laboratory referral	N/A	49/69 (71%)
Other[b]	6/69 (19%)	1/69 (1%)
At least one of the above tests	67/69 (97%)	21/69 (30%)
No response	10/79 (13%)	

[a] Respondents were able to select more than one response.
[b] Free text included 18S PCR, Morphological examination, refer where appropriate, fungal species identification for yeast only.

Table 3
Candida auris species identification testing and reference laboratory confirmation.

Candida auris species identification	Laboratories that identify to species level/respondents (%)
Onsite	
MALDI	24/51 (47%)
VITEK	2/51 (4%)
API	0/51 (0%)
Chromogenic Agar	0/51 (0%)
PCR/Sequencing	1/51 (2%)
Other	1/51 (2%)
Reference Laboratory Referral	23/51 (45%)
Species Confirmation	Laboratories that refer to a Reference Laboratory or mycology centre for confirmation/respondents (%)
No	7/25 (28%)
Yes	18/25 (72%)

The survey also revealed that 45% (23/51) responding laboratories referred suspected *C. auris* isolates to a reference laboratory for species identification, and 55% (28/51) of laboratories could discriminate *C. auris* from other *Candida* species locally, with the majority using MALDI-ToF for this (Table 3). Of those testing onsite, 72% (18/25) indicated that they referred suspected *C. auris* isolates to a reference or regional mycology centre for species confirmation. Compared to yeasts, fewer laboratories identified clinically relevant moulds to species level onsite (77%; 53/69). A large proportion (41%; 28/69) referred their mould isolates to a reference laboratory for identification or confirmation.

Antifungal susceptibility testing

Fifty-five percent (36/65) of laboratories responded that they perform susceptibility testing of yeasts onsite or at their centralised hub. The majority (77%; 43/56) of the respondents performed susceptibility testing for *Candida* spp. isolated from sterile sites (and patients failing therapy) locally. In contrast, 81% (34/42) referred isolates to a reference laboratory or a regional mycology centre for *Aspergillus* susceptibility testing, where clinically relevant. Only 18% (10/55) of all responding laboratories performed antifungal susceptibility testing locally for antifungal agents used for treatment, on *A. fumigatus* isolates.

Most respondents indicated that they did not perform antifungal susceptibility testing for *Aspergillus* species isolated from patients with various forms of aspergillosis where treatment has been initiated, with only 13% (7/55), 16% (9/58) and 22% (12/55) testing antifungal agents used for treatment in allergic bronchopulmonary aspergillosis (ABPA), aspergilloma and chronic aspergillosis respectively (Table 4). Only 17% (18/47) of those not undertaking susceptibility testing stored clinically relevant *Aspergillus* isolates for up to 6 months.

Table 4
Patients for which Aspergillus susceptibility testing for antifungals used in treatment if therapy is initiated.

Aspergillosis patients	Laboratories that perform susceptibility testing for drugs used for treatment/respondents (%)
Allergic bronchopulmonary aspergillosis (ABPA)	7/55 (13%)
Aspergilloma	9/55 (16%)
Chronic Aspergillosis	12/55 (22%)

Table 5
Antifungal susceptibility testing performed or requested by laboratories.

Antifungal susceptibility tests	Laboratories that test for antifungal susceptibility/respondents to the question[a] (%) Onsite, Centralised or submission to a reference laboratory/mycology centre
Yeasts[b]	
Disk	19/65 (29%)
Microdilution	37/65 (57%)
E-Test	21/65 (32%)
VITEK®	28/65 (43%)
Aspergillus[c]	
Microdilution	33/42(51%)
E-Test	20/42 (31%)
Sequencing	18/42 (43%)
Other	21/42 (50%)
Other Moulds[d]	
Any testing	28/30 (93%)

[a] Respondents were able to select more than one response; denominator is total laboratories responding to that section.
[b] 18% (14/79) laboratories did not enter anything for this question.
[c] 47% (37/79) provided no response to this question.
[d] 62% (49/79) laboratories provided no response to this question.

Thirty laboratories answered the survey questions related to susceptibility testing of moulds other than *Aspergillus* species, 17% (5/30) indicated that susceptibility testing was undertaken locally but the majority (77%; 23/30) referred isolates to a reference laboratory or a regional mycology centre (Table 5).

Non-culture based fungal diagnostics

Laboratories reported that BDG testing locally was only available in 5% (3/63) of responding laboratories (Table 6). Similarly, *Aspergillus* GM testing is offered locally by 21% (13/63) of laboratories for serum and 16% (10/63) of laboratories for bronchoalveolar lavage (BAL) specimens. These tests were predominantly performed in teaching hospitals (40%; 8/20) and specialist hospitals (75%; 3/4). *Aspergillus* antibody testing is mainly indicated for the investigation of chronic or allergic aspergillosis; most laboratories (63%; 40/63) responded that they refer serum samples to a reference laboratory for antibody testing (Table 6).

Similarly, the majority of responding laboratories (63%; 40/63) refer samples to a reference laboratory or a regional mycology centre for CRAG testing. Fourteen percent (9/63) of laboratories and 24% (15/63) of laboratories perform CRAG testing onsite using lateral flow or agglutination technique respectively (Table 6), two of the laboratories reported that they performed both testing methods.

The application of molecular methods such as specific PCR tests or pan-fungal PCR followed by sequencing on patient specimens remains mainly a domain of mycology reference laboratories or academic research facilities (Table 6). However, molecular methods

Table 6
Non-culture based fungal diagnostics.

Fungal testing	Laboratories the use test serum or patient specimens/respondents[a] (%)		
	Provision Onsite	Sent to a Reference Laboratory/mycology centre	Not Applicable/not provided
Serology[b]			
Beta-D Glucan	3/63 (5%)	53/63 (84%)	0/63 (0%)
Serum Galactomannan	13/63 (21%)	47/63 (75%)	0/63 (0%)
BAL Galactomannan	10/63 (16%)	41/63 (65%)	1/63 (2%)
Mannan Antibodies	0/63 (0%)	23/63 (37%)	6/63 (10%)
Cryptococcal Antigen Lateral Flow	9/63 (14%)	27/63 (43%)	3/63 (5%)
Cryptococcal Antigen by Agglutination	15/63 (24%)	31/63 (49%)	4/63 (6%)
Aspergillus Antibody Test	14/63 (22%)	40/63 (63%)	1/63 (2%)
Other[c]	0/63 (0%)	28/63 (44%)	3/63 (5%)
Molecular tests[d]			
Aspergillus PCR	4/62 (6%)	48/62 (77%)	4/62 (6%)
Candida PCR	0/62 (0%)	38/62 (61%)	7/62 (11%)
18 s PCR	1/62 (2%)	43/62 (69%)	5/62 (8%)
PCP PCR	13/62 (21%)	36/62 (58%)	5/62 (8%)
Other	0/62 (0%)	8/62 (13%)	6/62 (10%)

[a] Respondents were able to select more than one response.
[b] 80% (63/79) of responding laboratories answered serology diagnostic provision and availability.
[c] Free text included dimorphic fungi serology, Histoplasma, blastomyces, Candida antibody and coccidiodes antibody.
[d] 78% (62/79) of responding laboratories answered molecular diagnostic provision and availability for patient specimens (tissues, fluid or blood).

Table 7
Therapeutic Drug Monitoring (TDM) advised for antifungal agents, both BSMM recommended and antifungals with no recommendation.

BSMM Guidance	Drugs	Drugs TDM advised for/respondents (%)
Recommended	Flucytosine	20/48 (42%)
	Itraconazole	28/48 (58%)
	Voriconazole	34/48 (71%)
	Posaconazole	21/48 (44%)
No Recommendation	Amphotericin B	2/48 (4%)
	Echinocandins	0/48 (0%)
	Isavuconazole	3/48 (6%)
	Other[a]	2/48 (4%)

[a] Free text responses, “depends on the clinical picture” and “fluconazole when appropriate”.

Table 8
Clinical circumstances in which TDM is advised.

Recommended TDM for the following	TDM advised for recommended antifungal agents/respondents (%)
Repeat TDM after dose changes	22/47 (47%)
Shift from IV to oral treatment	17/47 (36%)
Optimisation during long term therapy of fungal disease	30/47 (64%)
Other[a]	7/47 (15%)

[a] Treatment failure, compliance, toxicity, drug-drug interactions, haematology patients on prophylaxis, clinical picture.

were often used for the diagnosis of suspected *Pneumocystis jirovecii* pneumonia (PCP), with 55% (31/56) using PCP PCR (albeit only 21% (13/62) laboratories could do so locally), and 21% (12/56) using direct fluorescent antibody testing (DFA) as a diagnostic tool.

Three laboratories (3/52; 6%) indicated that there were no processes in place to actively communicate positive fungal biomarker results to clinicians within 2 h of availability.

Therapeutic drug monitoring

TDM is recommended for a number of triazole antifungal agents and for 5-fluocytosine in order to optimise dosing and to reduce toxicity.[17] Our survey showed that 74% (40/54) of laboratories were aware of the recommendations on TDM testing. The majority of laboratories provide adequate advice on TDM testing for itraconazole (58%; 28/48) and voriconazole (71%; 34/48) and appropriately did not recommend TDM for amphotericin B or echinocandins (Table 7). There was poor compliance with TDM recommendation for flucytosine and posaconazole with only 42% (20/48) and 44% (21/48) of laboratories respectively providing adequate recommendations for testing (Table 7).

Forty-seven percent of responding laboratories advised TDM after dosage change (22/47), 36% (17/47) following a shift from intravenous to oral treatment, and 64% (30/47) for optimisation during long term therapy (Table 8). Thirteen percent (7/47) also provided responses for other situations in which they would advise TDM, including treatment failure, toxicity/compliance issues and drug-drug interactions.

Discussion

This survey describes the status of fungal diagnostic capacity in the UK in 2017 and compliance with UK best practice guidelines for the diagnosis of serious fungal diseases.[9,18–20]. Compared to a previous audit of BSMM diagnostic standards in 2009 there was a much higher response rate of laboratories participating in the survey which may reflect an increased interest in fungal diagnostics in general.[16]

Identification of yeast and mould species

Our survey demonstrates that most UK laboratories identify isolates of both yeast and mould from deep sites to species level with the highest level of 98% for blood culture isolates, which is similar to a survey in 2007.[16] Fewer laboratories identify moulds to species level onsite compared to yeast (82% versus 94% respectively) and a large proportion (41% versus 28%) were more likely to refer their mould isolates to a reference laboratory or a regional mycology centre.

Local use of MALDI-ToF methodology, which is now considered by many to be the gold standard yeast identification method, was

the most prevalent method applied. Prompt identification is especially important as it gives an early indication of the likely susceptibility profile, guiding the most suitable empirical antifungal treatment. Moreover, species identification is essential in order to assess the correct species-specific breakpoints to apply to the resulting MICs.[21]

Antifungal susceptibility testing

Compared to a mycology diagnostic survey in 2007, in 2017 there has been an improvement in the number of laboratories providing local susceptibility testing of yeasts (38% vs 55%). Susceptibility testing of *Aspergillus* species and other mould genera unfortunately remains a diagnostic challenge, with most laboratories (81%) referring these to reference laboratories where turnaround times in 2014 were reported as a minimum of 5 days (mean 9.6 days) in 2014 by one reference laboratory.[16,22]

Very few laboratories arrange susceptibility testing for isolates from cases of chronic aspergillosis (<20%), even though development of resistance to azole antifungal agents during therapy is a well-established cause of treatment failure in patients treated with long-term azole therapy.[23] From a resource perspective, it may not be feasible for laboratories to perform antifungal susceptibility testing on all clinical *Aspergillus* isolates. However, our survey has demonstrated that only 24% of participating laboratories retained *Aspergillus* isolates for the six months as recommended to allow retrospective testing in cases of poor clinical response.[6]

Many fungal species have now been demonstrated to have either innate resistance or the propensity to develop resistance to various antifungal classes during treatment. There is particular concern due to the recent spread of azole resistant *Aspergillus fumigatus* following the extensive use of environmental azole compounds for the control of fungal infections in crops.[24] Whilst there are commercial PCR tests available to detect the key azole resistance mechanisms new molecular diagnostic tools are urgently required.

For now, most treatment guidelines recommend conventional antifungal susceptibility testing of isolates from IFD or recalcitrant localised infection for both yeasts and moulds. There are reference methods for yeasts and moulds published by the CLSI and EUCAST but these can be labour intensive and unwieldy and may not be suitable for most laboratories.[25] However, the additional time for transport of isolates to a reference laboratory and return of the results adds to the turnaround time which may lead to a longer period of inappropriate or more costly therapy. To reduce this additional time in transit there are several commercial methods now available which have led to many laboratories performing at least some onsite testing, although this is largely for yeasts. Care should be taken to ensure any commercial method is validated in the user's hands against one or more of the reference standards. This could be achieved by sending isolates to a reference laboratory for a period of duplicate testing. There has been an attempt to harmonise breakpoints as far as possible and recent years have seen closer agreement between the clinical and epidemiological breakpoints published by the laboratory standards setting organisations.[26–28] There remain discrepancies between the breakpoints and it is important to remember that the breakpoint that is applied to any given MIC result will depend on the method used to generate that result.

Non-culture based fungal diagnostics

Up to date standards of care increasingly incorporate new non-culture based fungal diagnostics. The evolution of nucleic acid amplification technologies (NAAT) has been slow but the development of new standardised techniques (Fungal PCR Initiative (FPCRI), International Society for Human and Animal Mycology) and platforms is encouraging.[29] The routine application of fungal PCR based methods provided locally has remained low over the last 10 years with 6% of laboratories providing such tests, except for the PCP diagnostics in 2017 rising to 21% of laboratories now applying this method.

Serological fungal biomarkers such as BDG quantification, although non-specific, are capable of detecting organisms other than *Candida*, and are an alternative technique for detecting candidaemia and invasive candidosis (IC). A positive BDG test is one of the EORTC/MSG (European Organization for Research and Treatment of Cancer/Mycosis Study Group) accepted diagnostic criteria for IFD. The European Society for Clinical Microbiology and Infectious Diseases (ESCMID) and the U.S. Food & Drug Administration (FDA) recommend BDG quantification for ruling out candidaemia or IC in adult patients at risk of infection due to its high negative predictive value. Recent studies have demonstrated that implementation of an AFS programme including adherence to ESCMID candidaemia guidelines and use of BDG testing was successful in reducing the number of inappropriate initiations of antifungal therapy in an ICU by 90%.[30] Concurrently, mortality due to IC was reduced by 58% suggesting that BDG testing can guide safe cessation of antifungal therapy in ICU patients. Unfortunately, local GM testing ability of laboratories in England has not improved much in the last 10 years which was up to 17% in 2007 and reached 20% by 2017. The lack of implementation of theses fungal biomarkers and molecular tests may currently be hindered by costs, lack of expertise and poor infrastructure.

Candida auris

It has become increasingly important to identify yeast isolates for infection control purposes following the global spread of the emerging pathogenic yeast *C. auris*.[11,31] This *Candida* is often misidentified as other closely-related species by some commercially available yeast identification methods. While awareness of this pathogen was good (98% of respondents), the survey showed that only 54% of laboratories could discriminate *C. auris* from other *Candida* species locally, with the majority using MALDI-ToF, which together with molecular identification methods, is currently the most reliable commercial system. Forty-six percent of laboratories referred suspected isolates to a reference laboratory for species identification.

Non-identification remains an issue as isolates are not referred to reference laboratories or reported in surveillance datasets. The misidentification of the East Asian *C. auris* clade as *C. duobushaemulonii* using the updated VITEK® 2 system in a recent study is concerning and highlights the on-going need for caution in result interpretation.[32]

Therapeutic drug monitoring

In the UK, the routine use of antifungal TDM is advised by the BSMM for certain azoles, including itraconazole, posaconazole and voriconazole, both for prophylaxis and treatment purposes, to reduce toxicity and to assure appropriate drug levels are achieved. TDM is also advised for 5-flucytosine.[17] In 2007 only 6% of laboratories provided TDM testing locally and 23% of services did not even refer serum samples for TDM testing. Our survey shows that local antifungal TDM testing guidance has much improved with more than half of respondents recommending itraconazole TDM, nearly half for posaconazole and nearly three-quarters of respondents recommending voriconazole TDM. However, due to the known concentration-toxicity relationship for this antifungal

drug, the low adherence to the BSMM's recommendation for using TDM for 5-flucytosine treatment management (47% of non-reference laboratory respondents) is of concern.

Isavuconazole, a newer azole, licensed for the treatment of invasive mould infections, was not available when the TDM guidelines were published by the BSMM in 2014 but TDM may well be indicated in specific clinical scenarios.[33] Also, the recent availability of the oral posaconazole tablet preparation, with a better oral bioavailability profile when compared to the oral suspension, may reduce the need for posaconazole TDM. Pea & Lewis (2018) have suggested that we are facing a 'silent epidemic' of under-dosing of antifungal drugs in the management of IC and this might suggest that a new approach to antifungal TDM is required for this indication.[34] Interestingly, none of the current Summary of Product Characteristics (SPC) for posaconazole, voriconazole and isavuconazole advise the use of TDM.[35]

Surveillance

This survey highlights the difficulties in assessing the incidence of IFD at a national level and identifying the emergence of antifungal resistance, due to diagnostic and methodological limitations which compromise laboratory reporting and therefore the quality of data captured by surveillance.

Surveillance programmes such as PHE's voluntary laboratory surveillance system and the Surveillance Collaboration on Aspergillus Resistance in Europe (SCARE) network,[36] will have critical roles in strengthening the quality and transparency of antifungal surveillance data to monitor levels of antifungal resistance and to detect emergence of new resistance patterns.

Other considerations

A major determinant in the successful management of IFD is the speed with which appropriate antifungal therapy can be initiated. This is reliant on rapid, accurate and easily accessible diagnostic testing, timely access to results and their interpretation and access to clinical advice when necessary.[37] The emergence of new fungal species with resistance to some classes of antifungal drugs and the emergence of resistance in previously susceptible organisms also highlights the need for accurate and predictive susceptibility testing.[25] Moreover, the availability of new classes of antifungal agents and new agents in existing classes has increased the choice for clinicians necessitating guidance in the selection of the most suitable agent.

This survey sought to elucidate the current state of fungal diagnostic services in the NHS and it has detected some deficiencies. However, it did not address the important question of the utilisation of existing services. Clinical behaviour is profoundly influenced by practical issues such as ready availability of tests, turnaround times and access to expert advice; if these elements are lacking, then widespread empirical treatment of at-risk patients, and therefore overuse of antifungal agents, will remain typical. Remedies include development of a system for rapid sample transportation from geographically disparate sites, concentrating technical staff and mycological expertise in a network of hub laboratories, and having the Information Technology (IT) systems for electronic reporting and remote access to results. If these issues can be addressed, it will result in a more focussed, diagnostic-driven approach to fungal disease leading to decreased drug usage, increased detection of fungal infections and an overall decrease in healthcare costs.

Pathology consolidation has occurred to varying degrees of success throughout the UK, and there are potentially significant savings to be made from a centralised regional 'hub' model for mycology services, allowing higher numbers of samples to be processed with a rapid throughput. Kits for fungal antigen testing and fungal nucleic acid detection are commercially available for diseases such as candidosis, aspergillosis, pneumocystis and cryptococcosis. However, test interpretation still requires a high level of competency and understanding of mycoses. Many newer tests have not been standardised and validated clinically, and a lack of consensus on their performance characteristics remains an issue.

Table 9
External Quality Assurance schemes.

Laboratory test	EQA Scheme
Yeast identification	UKNEQAS, Instand e.V.
Mould identification	UKNEQAS, Instand e.V.
Susceptibility testing	UKNEQAS
Antifungal assay	UKNEQAS, Instand e.V., KKGT
Antibody testing: *Aspergillus*	UKNEQAS
Antibody testing: *Candida*	UKNEQAS, Instand e.V.
Cryptococcal antigen	UKNEQAS, Instand e.V.
Aspergillus antigen (galactomannan)	UKNEQAS
Candida antigen (mannan)	UKNEQAS, Instand e.V.
PCR: *Aspergillus, Candida, Pneumocystis*	QCMD
Interpretative comments	UKNEQAS (occasional fungal cases)

UKAS = http://www.ukneqas.org.uk; Instand e.V. = http://www.instandev.de; KKGT = http://www.kkgt.nl; QCMD = http://www.qcmd.org.

One area our survey has not addressed is the importance of external quality assurance (EQA) schemes which are essential tools to enhance a laboratory's confidence in its results. They form an important component in the accreditation of the clinical service and the ultimate goal of improvement in quality of patient care. However, at present there are no EQA schemes available for BDG detection, direct microscopy of samples and histology, dimorphic serology, panfungal and tissue PCR and no schemes send out simulated specimens to test ability to isolate pathogens from clinical samples and detect mixed cultures. Many countries provide their own regional and national EQA schemes; table 9 lists some medical mycology EQA schemes that are commercially available internationally which may help laboratories to monitor their performance.

Other areas of importance are the on-going problems at hubs without a functioning single Laboratory Information Management System (LIMS) will impact on the commissioning for quality and innovation (CQUIN; NHS quality improvement programme) indicators for diagnostic driven AFS. Failure to provide adequate laboratory IT will also have a significant negative impact on efficiency, including turnaround times.

Conclusion

This survey has provided an overview of the current fungal diagnostic landscape in the UK. Whilst some diagnostic aspects such as species identification of yeasts have improved over the last 10 years, mainly because of the introduction of updated technologies (e.g. MALDI-ToF) our findings highlight some diagnostic gaps that need improving to support optimal management of IFD. Our survey highlights specifically the need to improve access to timely provision of fungal biomarkers, susceptibility testing and TDM which have a crucial impact on the delivery of diagnostic driven AFS strategies as well as the appropriate management of IFD. A focus on closing the diagnostic gaps has the potential to promote greater rational use of antifungal agents, reduce the development of antifungal resistance and ultimately improve patient outcomes.

Whether tests are carried out locally or at central specialist hub laboratories will depend on several factors including the local availability of fungal expertise, whether there are economies of scale and on improvements in intra-laboratory sample transportation and the development of improved IT systems allowing immediate electronic access to results. On-going surveillance programmes are also required in order to monitor incidence and the

emergence or detection of antifungal resistance for a range of fungal diseases, both nationally and globally.

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Competing interests

SS has consulted for Pfizer, Gilead and Astellas. DAE has received funding to attend conferences from MSD, Gilead and Astellas, and has consulted for Astellas and Pfizer. RJM has been a principle investigator for an Astellas funded clinical trial, and has received honoraria for lectures from MSD. CM received travel grants to attend conferences from Astellas, Gilead, Pfizer and Novartis, educational grants from Pfizer and Novartis, attended a Pfizer Advisory Board Meeting and consulted for Astellas. MDR has consulted for Gilead Sciences, Pfizer and MSD. RR has consulted for Astellas, Gilead Sciences, Pfizer and MSD and is involved with a Scynexis Inc. clinical trial.

All remaining authors have no competing interests to declare

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