Antifungal susceptibility testing practices in mycology laboratories in France, 2018

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Abstract

A survey of mycology laboratories for antifungal susceptibility testing (AFST) was undertaken in France in 2018, to better understand the difference in practices between the participating centers and to identify the difficulties they may encounter as well as eventual gaps with published standards and guidelines. The survey captured information from 45 mycology laboratories in France on how they perform AFST (number of strains tested, preferred method, technical and quality aspects, interpretation of the MIC values, reading and interpretation difficulties). Results indicated that 86% of respondents used Etest as AFST method, with a combination of one to seven antifungal agents tested. Most of the participating laboratories used similar technical parameters to perform their AFST method and a large majority used, as recommended, internal and external quality assessments. Almost all the participating mycology laboratories (98%) reported difficulties to interpret the MIC values, especially when no clinical breakpoints are available. The survey highlighted that the current AFST practices in France need homogenization, particularly for MIC reading and interpretation.

Key words: antifungal susceptibility testing; Etest; MIC value interpretation; laboratory practice

Introduction

In vitro antifungal susceptibility testing (AFST) is required to determine the best treatment for a specific fungal species [1-3] and to detect resistance. Two reference techniques (Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST)), that are both microdilution methods, are available [4-7] but rarely incorporated in the routine hospital workflow because they are time-consuming and laborious [8]. Commercial techniques (microdilution systems or strip test on solid media), which are simple, rapid and cost effective, are generally the AFST methods applied in routine daily practice by mycology laboratories.

The main difficulties encountered by the microbiologists while performing AFST are i) the choice of the clinical breakpoints (CBs) which are validated for the reference techniques (CLSI and EUCAST) but not for the commercial techniques; ii) the CBs availability only for a few species, recognized as the most frequently isolated (such as *Candida albicans*, *C. glabrata, C. krusei, C. parapsilosis, C. tropicalis,* and *Aspergillus fumigatus*) and only for a few antifungal agents (fluconazole, posaconazole, itraconazole, voriconazole, and echinocandins, mostly); iii) the possible confusion between CBs and epidemiological cut-off values (ECOFFs/ ECVs), and thus interpretation difficulties.

In this context, a survey was undertaken in 2018 to clarify the current practices of the mycology laboratories for AFST: type of method used, criteria to perform and interpret AFST, detailed technical parameters and quality aspects. The preliminary results of this survey were presented and discussed during a national meeting organized by the French Society of Medical Mycology in Paris in November 2018.

Methods

In November 2018, a meeting dedicated to AFST was organized by the French Society of Medical Mycology. Before the meeting, in September 2018, an online survey was performed to assess the practices of AFST in mycology laboratories in France. All the mycology laboratories within University Hospitals in France were contacted. Additionally, all microbiologists registered to the meeting were also enrolled in the survey. The original questionnaire was designed by the organizers of the meeting and first beta-tested by two mycologists not involved in its set-up. After modifications, the final questionnaire was uploaded in a web-based electronic platform. The electronic questionnaire consisted of 43 questions divided into 16 sections (Suppl Material 1). Briefly, the survey included questions on i) the type of center (type of hospital, number of hospital beds), ii) the mycology laboratory activity (number of fungal isolates tested, type of method used for AFST, antifungal agents systematically tested for yeast and filamentous fungi, way of interpreting the MIC value, screening for emerging resistant species), iii) technical aspects such as method for inoculum preparation, temperature of incubation, incubation time and rules used for reading MICs and iv) quality aspects (internal and external quality assessments). Laboratories failing to respond initially were contacted by personalized emails. All data were downloaded from the platform and analyzed in Microsoft Excel (Office 365).

Results

Study participants and AFST activity

Among the 48 French mycology laboratories contacted, a total of 45 answered the e-questionnaire (overall response rate of 94%). A majority (82%) of the participants were University Hospital mycology laboratories. The size of the participating hospitals varied from less than 500 beds (7%), between 500 and 1500 beds (41%), to more than 1500 beds (52%).

A majority (77%) of the participating mycology laboratories (PML) performed antifungal susceptibility testing (AFST) on more than 150 fungal isolates per year. A ratio was calculated to assess the number of fungal isolates tested according to the size of the hospital (number of beds). Most of the PML (72%) tested annually 100 to 500 fungal isolates per 1000 beds, 14% tested less than 100 fungal isolates per 1000 beds and 14% more than 500 fungal isolates per 1000 beds. All the PML performed AFST on both yeasts and filamentous fungi except five centers which tested only yeasts, all of which but one, were non-university hospitals. AFST was more often performed on yeasts than on filamentous fungi with 60% of the PML testing 100 to 500 yeasts per year and 58% testing at least 50 filamentous fungi. Among the PML that tested both yeasts and filamentous fungi, the percentage of AFST performed against filamentous fungi ranged from 1 to 39%.

The three situations mainly triggering AFST were "yeast isolates from deep sites" (86%), "isolates from blood cultures" (91%) and "case-by-case decision of the microbiologist" (79%) (Table 1).

Methods of AFST

The Etest was the most frequently used method (86%) to perform AFST. The laboratories performing AFST with Etest tested from one to seven antifungal drugs against yeasts, whereas the five Sensititre YeastOne users tested nine drugs. For yeasts (Figure 1A), all centers using Etest tested fluconazole, 97% at least one echinocandin and 41% tested 5-fluorocytosine (5FC). Among the PML using Etest for AFST, 85% of them used routinely a combination of at least four antifungal molecules to test yeasts (one echinocandin, fluconazole, voriconazole and amphotericin B). Among the PML testing only one echinocandin (n=20), 50% chose caspofungin, 15% micafungin, and 35% anidulafungin. Among the PML testing two echinocandins (n=13), 85% associated caspofungin and micafungin. The four molecules more

often tested against filamentous fungi by Etest (Figure 1B) were voriconazole (100%), amphotericin B (97%), posaconazole (86%) and itraconazole (71%).

Technical parameters of AFST

Concerning the technical aspects of AFST, the PML generally used a spectrophotometrically adjusted inoculum for yeasts (78%) and filamentous fungi (76%) (Table 2). A large majority of the PML used an incubation at 35°C ± 2°C, both for yeasts (95%) and filamentous fungi (84%) (Table 2). A majority of PML declared reading the MIC results first after 24h of incubation, and reading them again after 48h, both for *Candida* spp. (67%) and *Aspergillus* spp. (58%) (Table 2). Among the 39 PML using Etest on yeasts, 100% read amphotericin B (AMB) MIC at complete inhibition, 97% read azole MIC at partial inhibition, 53% and 47% read echinocandin MIC at complete or partial inhibition, respectively (Figure 2A). Concerning the reading of the MIC against filamentous fungi, 97% of the PML using Etest read AMB MIC at complete inhibition, 73% read azole MIC at complete inhibition, and 79% read echinocandin MIC at partial inhibition (Figure 2B).

Internal quality assessment (IQA) and external quality assessment (EQA) (Table 3)

A majority of the PML used an IQA (78%) made of an ATCC strain (76%), that was performed either for each new batch delivery (42%) or at least monthly (52%) (Table 3). The two most frequently used ATCC strains used were *Candida krusei* ATCC 6258 (76%) and *C. parapsilosis* ATCC 22019 (67%). Seventy percent of the PML subscribed to at least one of the several EQA available in France (UKNEQAS, ABP, Prospective biology, AGLAE, LABQUALITY and RCPA QAP). Overall, 89% of the PML used either an IQA or an EQA and 62% used both IQA and EQA.

MIC interpretation and reporting of results

Forty-six percent of the PML used the EUCAST clinical breakpoints (CBs), when available, to interpret the MIC values (as Susceptible/Intermediate/Resistant), and a large majority (89%)

declared reporting the interpretation of the MIC values on the medical report (Table 4). In the absence of CBs for the species tested, 58% of the PML declared reporting the MIC values without interpretation, while others interpret MIC values based either on CBs from other species or based on ECOFF/ECVs (Figure 3). Most of those trying to interpret the MIC values in absence of CBs discussed the case directly with the physician in charge (53%) (Figure 3). Almost all the PML (98%) declared that the interpretation of MIC values was their main problem encountered during AFST and 40% reported difficulties with MIC values reading (Table 4).

Screening of resistant strains

Considering emerging antifungal resistant species, only a minority (11%) of the PML had already incorporated into their routine work flow the screening for azole-resistant *Aspergillus fumigatus* strains and even less of them (7%) used molecular techniques to detect antifungal resistance in yeasts.

Discussion

This survey showed that AFST is performed by a majority of the PML both on yeasts and filamentous fungi. The present survey highlighted some common practices: similar commercial method used (Etest), similar AFST indication criteria (0% systematically, 86% yeast isolates from deep sites, 91% isolates from blood culture, 79% case-by-case discussion), similar panels of antifungal agents tested (a combination of four antifungals for more than 80% of the PML), similar applied technical parameters (inoculum preparation and temperature of incubation).

This survey also showed that the mycologists face some difficulties while performing AFST. The first issue is the reading of MIC values: the Etest manufacturer is unclear about the fact that the reading should be made at 24h or at 48h. In the present survey, 67% of the PML declared reading twice the MIC values for yeasts, 1st at 24h and a 2nd time at 48h. Considering that the

MIC value read at 48h may change the categorization (Susceptible / Intermediate / Resistant) of the microorganism compared to the MIC value read at 24h, the optimal time for MIC reading remains to be defined. This variation of the incubation time (e.g. 24h vs 48h) may explain some low essential agreements between Etest and reference methods reported in the literature [9]. Therefore, new studies for determination of the optimal reading time are warranted. Moreover, the time of reading probably differs according to the type of fungal microorganisms, depending on their ability to grow more or less rapidly (yeasts *vs* molds for example). The manufacturer and/or the French Society of Medical Mycology (or other international societies) should provide clear recommendations on the optimal time of reading, detailed per group of fungal species. In the same way, only 40% of the PML reported that the reading of the MIC value was a problem. Nevertheless, they reported that MICs were read according to different criteria, especially for the echinocandins against yeasts (about half/half of the PML read these MICs either at complete inhibition or at partial inhibition). Reading at partial inhibition is more complex and more subjective, as some parameters, such as the size of the colonies, may influence the MIC value. It should be noted that there is clear guidance, supplied by the manufacturers of Etest, for reading of micro- versus macro-colonies within the zone of inhibition to aid with result interpretation [10]. Again, more detailed recommendations provided by both the manufacturer and the French Society of Medical Mycology (or other international societies) would be helpful to standardize MIC values determination.

The second issue is the choice of the drug to be tested for echinocandins. Caspofungin is the most often tested molecule, while some PML are testing two molecules (mostly caspofungin and micafungin). It has been clearly shown that caspofungin should not be tested with CLSI or EUCAST methodology due to inter-laboratory variability [11]. Therefore, micafungin or anidulafungin should be used as a marker of class for echinocandin susceptibility when using these reference microbroth dilution techniques. Although, there are few evidences that this

problem also applied with Etest, a recent literature analysis showed that anidulafungin is probably the best choice for testing echinocandins against *Candida* spp. by Etest [12].

The third main issue is that the interpretation of MICs is not always performed according to recommendations: while about 86% of the PML use Etest as routine AFST method, only 38% use the recommended CLSI CBs to interpret MIC values [10], while 46% use the EUCAST CBs. This discrepancy may be explained by the fact that more data are available with EUCAST in terms of species diversity and antifungal agents. It should be highlighted that CBs are method-specific, and laboratories should not choose to use alternative CBs either due to better availability or comfort. Applying non-CLSI breakpoints to MICs obtained by Etest may result in some mis-interpretations of MICs (as the two sets of CBs are different), therefore, this practice should be avoided. In contrast, when no CBs are available, about 31% of the PML already use the ECOFFS/ECVs, showing that they are familiar with these criteria. Recently published works proposing specific ECOFFS/ECVs, for Etest, for a large panel of species, should be helpful to extend this attitude and help to categorize an isolate as wild-type or non-wild-type [13-15].

Another point is that this survey, performed in 2018, showed that a large majority (89%) of the PML used either an IQA or an EQA, many of them (62%) using both. This demonstrates that quality assessment is a major preoccupation of mycologists performing AFST. In contrast, this survey showed that systematic routine screening of resistance was performed by very few PML (≈10%) in 2018. As early detection of azole resistance in *Aspergillus fumigatus* is now recommended [3] and may be important for management of patients with aspergillosis, measures should be taken to improve a more widespread use of resistance screening.

Conclusion

A majority of French mycology laboratories are routinely using Etest as AFST method, both for yeasts and filamentous fungi. If several parameters are similar between the 45 PML, the two main issues that highlighted this survey were: the reading and the interpretation of MIC value. Detailed guidelines and instructions are needed to standardize AFST practices, which could be implemented by both the manufacturer and the French Society of Medical Mycology (or other international societies).

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Conflict of interest

During the past 5 years:

Eric Dannaoui has received research grants from MSD and Gilead: travel grants from Gilead, MSD, Pfizer, & Astellas; speaker's fee from Gilead, MSD and Astellas. Anne-Pauline Bellanger has received travel grants from Gilead and MSD. Florence Persat has received travel grants from Gilead. Yohann Le Govic has received travel grants from Gilead. Stéphane Ranque has received travel grants from LDBio, Pfizer, and MSD. Kevin Brunet has received travel grants from Gilead and MSD. Jacques Guillot has received research grants from MSD and speaker's fee from Gilead and MSD. Céline Nourisson has received travel grants from Gilead, MSD, and Pfizer. Arnaud Fekkar has received research grants from MSD and Astellas, travel grants from Gilead, MSD, Pfizer, and Astellas, speaker's fee from Gilead and MSD. Adela Angoulvant has received a travel grant from Pfizer. MEB has received research grants from Astellas: travel grants from Gilead, MSD, Pfizer, & Astellas; speaker's fee from Gilead, MSD

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Table 1. Responses to the questionnaire considering AFST strategy (several answers by PML were possible).

In which situation do you perform AFST?	**%**
For all isolates systematically	0
For all isolates from blood cultures	**91**
For all patients treated with ATF	20
For all yeast isolates from deep sites	**86**
Decided by the microbiologist, on a case-by-case basis	**79**

AFST: Antifungal susceptibility testing; ATF: antifungal; PML: participating mycology laboratories.

Table 2. Responses to the questionnaire considering technical parameters of AFST by Etest

How do you prepare your inoculum for AFST for yeasts?	**%**
Mc Farland spectrometer	**78**
Mc Farland by eye	15
Inoclic® (I2A)	2
Densitometer	5
How do you prepare your inoculum for AFST for filamentous fungi?	
Mc Farland spectrometer	**76**
Mc Farland by eye	19
Inoclic® (I2A)	3
Densitometer	2
At which temperature do you incubate your AFST method for yeasts?	
35 ± 2°C	**95**
30 ± 2°C	5
At which temperature do you incubate your AFST method for filamentous fungi?	
35 ± 2°C	**84**
27 ± 2°C	8
30 ± 2°C	5
27°C if non thermophilic species and 35°C if thermophilic species	3
When do you read the AFST results for yeasts?	
First reading at 24h, then second reading at 48h	**67**
A 24h reading only	20
A 48h reading only	13
When do you read the AFST results for filamentous fungi?	
First reading at 24h, then second reading at 48h	**58**
A 24h reading only	27
A 48h reading only	15

Table 3. Responses to the questionnaire considering quality aspect of AFST

Do you use an intern quality assessment (IQA)?	**%**
Yes	**78**
No	22
Do you use an ATCC strain as IQA?	
Yes	**76**
No	24
More specifically, which ATCC strain do you use as IQA?*	
Candida krusei ATCC 6258	**76**
C. parapsilosis ATCC 22019	**67**
C. albicans ATCC 90028	35
C. krusei ATCC 12243	3
C. tropicalis ATCC 1369	3
Aspergillus fumigatus ATCC 204305	44
Aspergillus flavus ATCC 204304	6
At which frequency do you test the IQA?	
At each change of reagent batch	**42**
Monthly (at least)	52
Less frequently than monthly	6
Do you use extern quality assessment (EQA)?	
Yes	**70**
No	30

*Several strains used per PML

Table 4. Responses to the questionnaire considering MIC interpretation

How do you interpret the MIC value to classify the strain S/I/R	
EUCAST clinical breakpoints (CB)	**46**
CLSI CB	27
CB recommended by the manufacturer (CLSI for Etest)	11
ECOFFs /ECVs	4
Other	16
Do you write the interpretation of the MIC values on the medical report?	
Yes	**89**
No	11
What is the main problem that you encounter while performing ATFS testing?	
Reading	40
MIC interpretation	**98**

Figure legends

Figure 1: Antifungal tested against yeasts (1A) and filamentous fungi (1B) by laboratories using Etest for AFST

Figure 2: Type of reading of the MIC value for yeasts (2A) and fungi (2B) for azole, echinocandins and amphotericin B

Figure 3: Details of MIC interpretation in absence of clinical breakpoints available.

Figure 1

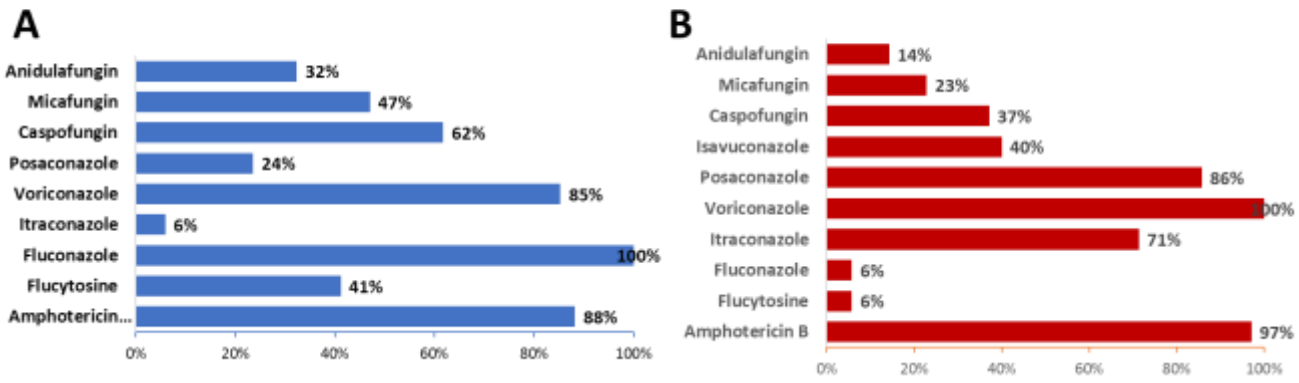

A
Anidulafungin
Micafungin
Caspofungin
Posaconazole
Voriconazole
Itraconazole
Fluconazole
Flucytosine
Amphotericin...
32%
47%
62%
24%
85%
6%
100%
41%
88%
0%
20%
40%
60%
80%
100%
B
Anidulafungin
Micafungin
Caspofungin
Isavuconazole
Posaconazole
Voriconazole
Itraconazole
Fluconazole
Flucytosine
Amphotericin B
14%
23%
37%
40%
86%
100%
71%
6%
6%
97%
0%
20%
40%
60%
80%
100%

Figure 2

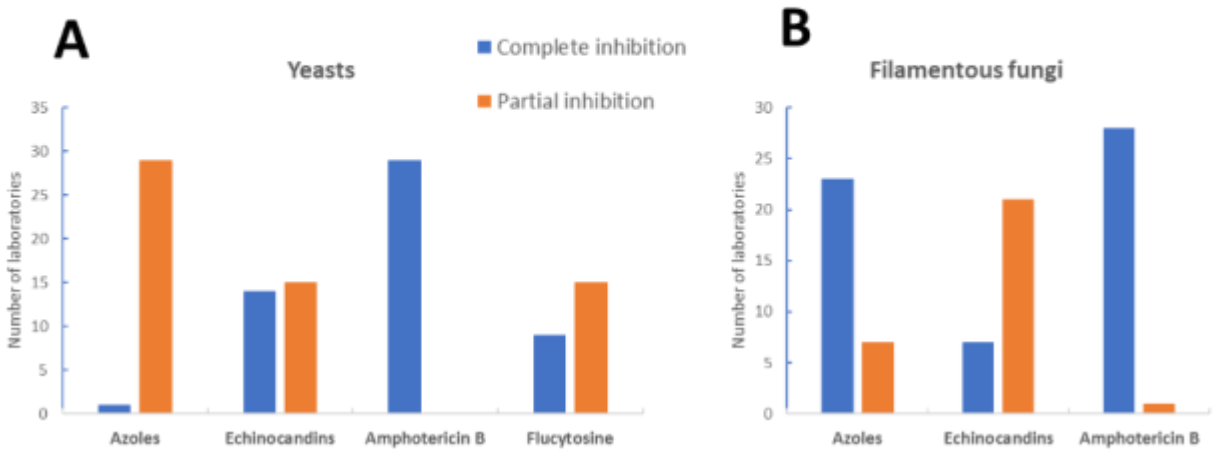
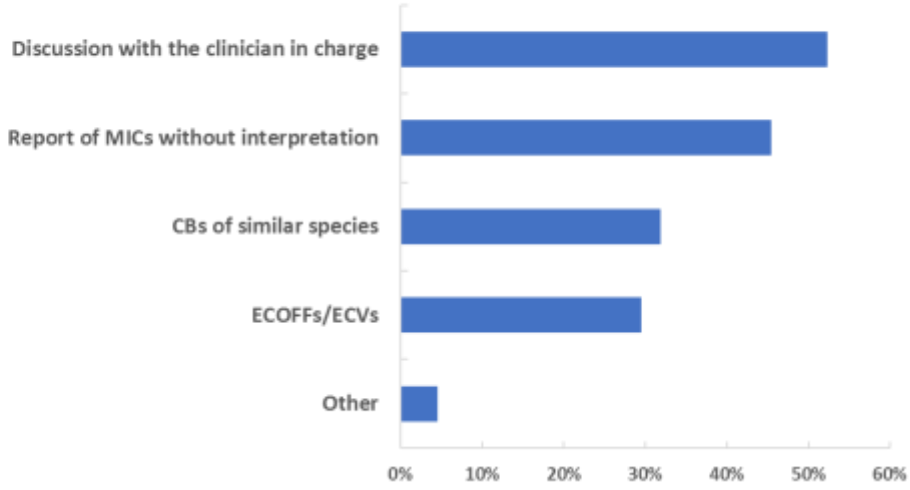

A
Yeasts
Complete inhibition
Partial inhibition
Number of laboratories
35
30
25
20
15
10
5
0
Azoles
Echinocandins
Amphotericin B
Flucytosine
B
Filamentous fungi
Number of laboratories
30
25
20
15
10
5
0
Azoles
Echinocandins
Amphotericin B

Figure 3


Discussion with the clinician in charge
Report of MICs without interpretation
CBs of similar species
ECOFFs/ECVs
Other
0%
10%
20%
30%
40%
50%
60%

Supplementary Material 1

Questionnaire concernant vos pratiques des tests de sensibilité aux antifongiques

Ceci est une enquête des pratiques et pas une évaluation de la qualité des laboratoires. Nous souhaiterions donc des réponses reflétant les pratiques quotidiennes même si celles-ci ne sont pas en accord avec les recommandations des fournisseurs ou les recommandations de la littérature. Les réponses seront anonymisées et les résultats traités de façon confidentielle.

Informations - Hôpital - Laboratoire

Pour l'hôpital, ne pas comptabiliser les lits de long séjour et de soins de suite

1. Hôpital Nom

2. Hôpital Type

Une seule réponse possible.

- CHU
- CHG
- Autre :

3. Hôpital, Nombre de lits (hospitalisation conventionnelle et HDJ) (approximatif)

4. Laboratoire, Nom

5. Activité en B+BHN / an (approximatif)

6. Nom du biologiste ayant complété le questionnaire

7. Email du biologiste ayant complété le questionnaire

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