Aspergillus Section Fumigati: Antifungal Susceptibility Patterns and Sequence-Based Identification

Laura Alcazar-Fuoli, Emilia Mellado,* Ana Alastruey-Izquierdo, Manuel Cuenca-Estrella, and Juan L. Rodriguez-Tudela

Servicio de Micología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain

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This study analyzed 28 Aspergillus strains belonging to the section Fumigati that were isolated from clinical samples in Spain. All isolates sporulated slowly and were unable to grow at 48°C. Phylogenetic analysis based on sequencing of partial sequences of the β-tubulin and rodlet A genes was used to classify the 28 strains into six different clades (Neosartorya hiratsukae, Neosartorya pseudofischeri, Aspergillus viridinutans, Aspergillus lentulus, Aspergillus fumigatiatifinis, and Aspergillus fumisynnematus). Antifungal susceptibility testing showed heterogeneous patterns and grouped the strains together by species. Most A. lentulus and A. fumigatiatifinis isolates showed high MICs of amphotericin B (geometric mean [GM] MICs, $>4.5$ μg/ml), itraconazole (GM MICs, $>6$ μg/ml), voriconazole (GM MICs, $>3$ μg/ml), and ravuconazole (GM MICs, $>3$ μg/ml); N. pseudofischeri and A. viridinutans showed high MICs of itraconazole (GM MICs, $>8$ μg/ml), voriconazole (GM MICs, $>3.33$ μg/ml), and ravuconazole (GM MICs, $>2$ μg/ml); and N. hiratsukae and A. fumisynnematus were susceptible to all the antifungals tested. In conclusion, a number of different species whose morphological features resemble those of Aspergillus fumigatus could succeed in producing invasive infections in the susceptible host. In addition, some of them showed high MICs for most of the antifungals available for the treatment of patients infected with these organisms. The epidemiology and clinical relevance of these species should therefore be addressed.

The incidence of invasive aspergillosis continues to increase, due to the rising number of patients undergoing bone marrow or solid organ transplantation or corticosteroid treatment and those with hematological malignancies or pulmonary disease (18).

Invasive aspergillosis is mainly caused by Aspergillus fumigatus, although other species, such as Aspergillus terreus, Aspergillus niger, and Aspergillus flavus can also cause invasive infections (10, 15, 22). Furthermore, recent studies have reported on cases of aspergillosis caused by other Aspergillus species that belong to Aspergillus section Fumigati (4, 12, 14, 17).


In the section Fumigati, besides A. fumigatus, other species, such as Neosartorya fischi, Neosartorya pseudofischi, Neosartorya hiratsukae, and A. lentulus, have been reported to be human pathogens (3, 4, 12, 14, 17). This implies that in the appropriate human host, all of them could cause disease.

The conventional means of identification of A. fumigatus is based on its morphological characteristics and microscopic features. Several morphological characteristics for differentiation between species of the Aspergillus section Fumigati have been described. However, many species sporulate very slowly, and an extremely high level of expertise and long-term observation are also required to identify the species. Other species, such as those of the genus Neosartorya, are able to produce ascospores; however, a considerable length of time is usually required for the production of ascospores, and ascospore production is not a practical method of identification for clinical microbiology laboratories (14, 16, 17, 36). Although for the very experienced taxonomist the species included in the section Fumigati are not morphologically uniform, morphological observation is not sufficient to distinguish between them. This fact has led to species misidentification and also to the discarding of organisms as contaminants (6, 44).

In order to resolve this issue, a number of different techniques have been developed and used to identify the species belonging to this section. These include analysis of the profiles of secondary metabolites, isozyme electrophoretic pattern analysis (23, 27, 33, 38, 39, 40), and molecular data analysis (41, 44).

Although secondary resistance to azole drugs has been described in A. fumigatus strains (7, 8, 11, 25, 26, 28, 42), most A. fumigatus strains are susceptible to the antifungals available for the treatment of patients with invasive infections (13). Because few clinical laboratories routinely perform antifungal susceptibility testing of molds and resistance in the section Fumigati has already been reported (5, 24), the misidentification of these species is a matter of concern.
TABLE 1. Origin, molecular identification, MICs, and MECs of different antifungal against clinical isolates of Aspergillus section Fumigati

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Molecular identification</th>
<th>MIC (µg/ml)</th>
<th>MEC (µg/ml)</th>
<th>CAS MICA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 2004305</td>
<td>Reference strain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNM-CM-1290 Sputum</td>
<td>A. lentulus</td>
<td>4.5</td>
<td>0.25–1.0</td>
<td>0.12–0.5</td>
</tr>
<tr>
<td>CNM-CM-3134 Sputum</td>
<td>A. lentulus</td>
<td>10</td>
<td>0.125–1.0</td>
<td>0.03–0.25</td>
</tr>
<tr>
<td>CNM-CM-3364 BAL</td>
<td>A. lentulus</td>
<td>6.6</td>
<td>0.25–1.0</td>
<td>0.03–0.25</td>
</tr>
<tr>
<td>CNM-CM-3537 Sputum</td>
<td>A. lentulus</td>
<td>7.25</td>
<td>0.25–1.0</td>
<td>0.03–0.25</td>
</tr>
<tr>
<td>CNM-CM-3583 Sputum</td>
<td>A. lentulus</td>
<td>8</td>
<td>0.25–1.0</td>
<td>0.03–0.25</td>
</tr>
<tr>
<td>CNM-CM-3599 Sputum</td>
<td>A. lentulus</td>
<td>8</td>
<td>0.25–1.0</td>
<td>0.03–0.25</td>
</tr>
<tr>
<td>CNM-CM-4330 Sputum</td>
<td>A. lentulus</td>
<td>10.66</td>
<td>0.25–1.0</td>
<td>0.03–0.25</td>
</tr>
<tr>
<td>CNM-CM-4370 Sputum</td>
<td>A. lentulus</td>
<td>12</td>
<td>0.25–1.0</td>
<td>0.03–0.25</td>
</tr>
<tr>
<td>CNM-CM-4420 Nail</td>
<td>A. lentulus</td>
<td>12</td>
<td>0.25–1.0</td>
<td>0.03–0.25</td>
</tr>
<tr>
<td>CNM-CM-4426 Sputum</td>
<td>A. lentulus</td>
<td>12</td>
<td>0.25–1.0</td>
<td>0.03–0.25</td>
</tr>
<tr>
<td>CNM-CM-4428 Skin</td>
<td>A. lentulus</td>
<td>12</td>
<td>0.25–1.0</td>
<td>0.03–0.25</td>
</tr>
<tr>
<td>CNM-CM-4063 BAS</td>
<td>A. fumigatus</td>
<td>10</td>
<td>0.125–1.0</td>
<td>0.03–0.25</td>
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<tr>
<td>CNM-CM-2280 Sputum</td>
<td>A. fumigatus</td>
<td>10</td>
<td>0.125–1.0</td>
<td>0.03–0.25</td>
</tr>
<tr>
<td>CNM-CM-3277 BAL</td>
<td>A. fumigatus</td>
<td>10</td>
<td>0.125–1.0</td>
<td>0.03–0.25</td>
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<tr>
<td>CNM-CM-3303 Skin</td>
<td>N. hiratsukae</td>
<td>0.5</td>
<td>0.03–0.25</td>
<td>0.03–0.25</td>
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<tr>
<td>CNM-CM-3305 Skin</td>
<td>N. hiratsukae</td>
<td>0.31</td>
<td>0.03–0.25</td>
<td>0.03–0.25</td>
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<td>CNM-CM-3764 OPE</td>
<td>N. hiratsukae</td>
<td>1</td>
<td>0.03–0.25</td>
<td>0.03–0.25</td>
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<td>CNM-CM-3740 OPE</td>
<td>N. hiratsukae</td>
<td>0.66</td>
<td>0.03–0.25</td>
<td>0.03–0.25</td>
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<td>CNM-CM-4328 Corneal</td>
<td>N. hiratsukae</td>
<td>0.75</td>
<td>0.03–0.25</td>
<td>0.03–0.25</td>
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<tr>
<td>CNM-CM-4270 Sputum</td>
<td>N. pseudofischeri</td>
<td>1</td>
<td>0.03–0.25</td>
<td>0.03–0.25</td>
</tr>
<tr>
<td>CNM-CM-4370 Sputum</td>
<td>N. pseudofischeri</td>
<td>0.17</td>
<td>0.03–0.25</td>
<td>0.03–0.25</td>
</tr>
<tr>
<td>CNM-CM-4060 Sputum</td>
<td>N. pseudofischeri</td>
<td>0.25</td>
<td>0.03–0.25</td>
<td>0.03–0.25</td>
</tr>
<tr>
<td>CNM-CM-3914 Nail</td>
<td>N. pseudofischeri</td>
<td>0.25</td>
<td>0.03–0.25</td>
<td>0.03–0.25</td>
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<tr>
<td>CNM-CM-3147 OPE</td>
<td>N. viridinutans</td>
<td>0.39</td>
<td>0.03–0.25</td>
<td>0.03–0.25</td>
</tr>
<tr>
<td>CNM-CM-4518 Nail</td>
<td>N. viridinutans</td>
<td>0.37</td>
<td>0.03–0.25</td>
<td>0.03–0.25</td>
</tr>
</tbody>
</table>

The aim of this study was to analyze clinical strains of Aspergillus section Fumigati. To date, we have analyzed a collection of 28 Aspergillus clinical strains that had previously been identified as atypical A. fumigatus isolates. We report on the results of the molecular identification by sequencing of both the β-tubulin and the rodlet A genes and the antifungal susceptibility testing profiles of the strains.

MATERIALS AND METHODS

Fungal strains and media. A total of 37 strains were included in this study; 28 Aspergillus section Fumigati strains (Table 1) were independent clinical isolates from different patients and belong to the Mold Collection of the Centro Nacional de Microbiología (CNM). Control strains included a set of five A. fumigatus strains belonging to the CNM (strains CNM-CM-3248, CNM-CM-3254, CNM-CM-3258, CNM-CM-3652, and CNM-CM-3722) and four strains obtained from the Centralbureau voor Schimmelcultures (CBS) (N. hiratsukae CNM-CM-4551, N. pseudofischeri CNM-CM-4487, and N. viridinutans CNM-CM-4488). The fungi were grown at 37°C in potato dextrose agar (Oxoid, Madrid, Spain); 2 mM MgCl2 (Applied Biosystems); 250 ng of each primer (قدمية 2004305 was used as the quality control strain to validate the MICs and MECs (reference values are shown in Table 1). Antifungal susceptibility testing was repeated at least three times on different days.

PCR amplification and sequencing. Partial sequences of the β-tubulin and the rodlet A genes were amplified with primer set βtub3 (5′-TTCACCTTCAGAC CGGT-3′) and βtub2 (4′) and primer set RodA1 and RodA2, respectively (4). All primers were synthesized by Sigma Genosys (Madrid, Spain). PCRs were carried out with a 50-μl volume containing 1× PCR buffer (Applied Biosystems, Madrid, Spain); 2 mM MgCl2 (Applied Biosystems); 250 μM each of dATP, dGTP, dCTP, and dTTP (Applied Biosystems); 1 μM of each primer; 2.5 U of Taq DNA polymerase (Applied Biosystems); and 25 to 50 ng of A. fumigatus genomic DNA. Amplification was performed in a thermal cycler (GeneAmp PCR system 9700; Applied Biosystems) for 1 cycle of 5 min at 94°C and then 30 cycles of 30 s at 94°C, 45 s at 55°C, and 1 to 2 min at 72°C, followed by 1 final cycle of 10 min at 72°C. The PCR products were analyzed by electrophoresis on 0.8% agarose gels and were visualized by transillumination (Gel Doc 2000; Bio-Rad Laboratories, Madrid, Spain). PCR products were sequenced using primers described in Table 1.
Sequence analysis. The sequences were assembled and edited with the SeqMan II and EditSeq software packages (Lasergene; DNASTar, Inc., Madison, WI). Sequence analysis was performed by comparing the DNA sequences with those of the control strains included in this study and with the sequences obtained from the GenBank database. Fourteen β-tubulin gene partial sequences and nine rodlet A gene partial sequences were used and are listed in Table 2.

Phylogenetic analysis. All phylogenetic analyses were conducted with InfoQuest FP software (version 4.50; Bio-Rad Laboratories). The methodology used was maximum-parsimony clustering. Phylogram stability was assessed by parsimony bootstrapping with 2,000 simulations. The Aspergillus clavatus β-tubulin and rodlet A gene sequences were used as the outgroups (Table 2).

Nucleotide sequence accession numbers. The following are the GenBank accession numbers for the β-tubulin and rodlet A gene sequences from all the strains used in this work: for the β-tubulin gene fragments, CM-1290, EU310839; CM-2270, EU310840; CM-2280, EU310841; CM-3134, EU310842; CM-3147, EU310843; CM-3227, EU310844; CM-3248, EU310845; CM-3254, EU310846; CM-3258, EU310847; CM-3303, EU310848; CM-3305, EU310849; CM-3364, EU310850; CM-3377, EU310851; CM-3385, EU310852; CM-3583, EU310853; CM-3599, EU310854; CM-3652, EU310855; CM-3722, EU310856; CM-3740, EU310857; CM-3764, EU310858; CM-3769, EU310859; CM-3914, EU310860; CM-4065, EU310861; CM-4063, EU310862; CM-4328, EU310863; CM-4430, EU310864; CM-4430, EU310865; CM-4437, EU310866; CM-4445, EU310867; CM-4440, EU310868; CM-4447, EU310869; CM-4448, EU310870; and CM-4518, EU310871; for the rodlet A gene fragments, CM-1290, EU310860; CM-2270, EU310861; CM-2280, EU310862; CM-3134, EU310863; CM-3147, EU310864; CM-3227, EU310865; CM-3248, EU310866; CM-3254, EU310867; CM-3258, EU310868; CM-3303, EU310869; CM-3305, EU310870; CM-3364, EU310871; CM-3377, EU310872; CM-3385, EU310873; CM-3583, EU310874; CM-3599, EU310875; CM-3652, EU310876; CM-3722, EU310877; CM-3740, EU310878; CM-3764, EU310879; CM-3769, EU310880; CM-3914, EU310881; CM-4065, EU310882; CM-4063, EU310883; CM-4328, EU310884; CM-4430, EU310885; CM-4437, EU310886; CM-4445, EU310887; CM-4440, EU310888; CM-4447, EU310889; CM-4448, EU310890; and CM-4518, EU310891.

RESULTS
Clinical isolate morphology and growth temperature. The clinical origins of the 28 Aspergillus section Fumigati strains are shown in Table 1. These were isolated from 21 respiratory specimens, 1 ocular swab specimen, and 6 skin or nail samples.

All strains were first identified as atypical A. fumigatus strains by conventional macroscopic and microscopic morphological analysis methods. Identification to the Aspergillus genus level was straightforward; however, we were not able to discriminate between the different species of Aspergillus section Fumigati. While A. fumigatus grew at 37°C and 48°C, all the other strains analyzed in this study grew at 37°C but were not able to grow at 48°C.

Molecular identification of Aspergillus section Fumigati. Partial DNA sequences of the β-tubulin and rodlet A genes were obtained and analyzed. Four CBS strains were amplified with the primers described above in order to compare the 28 atypical Aspergillus with members of the section Fumigati. We also included a set of β-tubulin and rodlet A sequences from members of Aspergillus section Fumigati available from GenBank (Table 2).

The phylogenetic tree produced by maximum parsimony of the β-tubulin sequences (Fig. 1) grouped the 28 clinical isolates into six different clades (N. hiratsukae, N. pseudofischeri, A. viridinutans, A. lentulus, A. fumigatiavus, and A. fumisy mennatus).

On the basis of these results, 14 atypical A. fumigatus strains were identified as A. lentulus, supported by bootstrap values from 70% to 99%. Five isolates were identified as N. hiratsukae with a bootstrap value of 100%, four were identified as N. pseudofischeri (bootstrap value, 100%), two were identified as A. viridinutans (bootstrap value, 97%), two were identified as A. fumigatiavus (bootstrap value, 92%), and one was identified as A. fumisynnematus (bootstrap value, 98%). None of the Aspergillus section Fumigati sequences analyzed matched the A. fumigatus sequences.

Figure 2 shows the results of a phylogenetic analysis obtained by maximum parsimony of the rodlet A gene sequences. According to those results, the 28 clinical strains fell into the same six clades. Fourteen samples were grouped with A. lentulus with bootstrap values of 63% and 81%. Five isolates were identified as N. hiratsukae with a bootstrap of 100%. Four isolates were identified as N. pseudofischeri, two were identified as A. viridinutans, two were identified as A. fumigatiavus, and one was identified as A. fumisy mennatus, supported by bootstrap values of 99%, 100%, 46%, and 83%, respectively. All A. fumigatus sequences were divided into a single group according to their rodlet A sequences.

The identities and classification results for the Aspergillus section Fumigati strains on the basis of their β-tubulin and rodlet A sequences are summarized in Table 1.

Antifungal susceptibility testing. The MICs and MECs of the antifungal agents for the collection of clinical isolates are shown in Table 1. All are expressed as geometric means (GMs). The species were identified by β-tubulin and rodlet A gene sequencing. Analyses of the susceptibility phenotypes (Table 1) resulted in clear differences between Aspergillus species of the section Fumigati. We were able to differentiate three different antifungal phenotypes. Twelve of the 14 A. lentulus isolates and the two A. fumigatiavus isolates showed high MICs of amphotericin B (GM MICs, 4.5 μg/ml to 16 μg/ml), itraconazole (GM MICs, 6 μg/ml to 10.25 μg/ml), voriconazole (GM MICs, 3 μg/ml to 7.5 μg/ml), and ravuconazole (GM MICs, 3 μg/ml to 7.5 μg/ml), and ravuconazole (GM MICs, 3 μg/ml to 7.5 μg/ml), and ravuconazole (GM MICs, 3 μg/ml to 7.5 μg/ml), and ravuconazole (GM MICs, 3 μg/ml to 7.5 μg/ml).
FIG. 1. Phylogenetic tree obtained by maximum-parsimony phylogenetic analysis with 2,000 bootstrap simulations on the basis of the β-tubulin sequences from all the strains included in the study.
MICs, 1.5 μg/ml to 7 μg/ml). Although strains CNM-CM-3583 and CNM-CM-3599 did not follow this pattern for itraconazole (GM MICs, 0.43 μg/ml and 0.5 μg/ml, respectively), they retained the high MICs of amphotericin B (GM MICs, ≥2.66 μg/ml), voriconazole (GM MICs, ≥3.5 μg/ml), and ravuconazole (GM MICs, 2 μg/ml). A second profile was related to the species N. pseudofischeri and A. viridinutans. They showed high MICs of itraconazole (GM MICs, ≥8 μg/ml), voriconazole (GM MICs, ≥3.33 μg/ml), and ravuconazole (GM MICs, ≥2 μg/ml) but were susceptible to amphotericin B (GM MICs, ≤1 μg/ml).
μg/ml). In contrast, strains identified as N. hiratsukae and A. fumisynnematus were more susceptible in vitro to all the antifungal compounds tested.

Among the azoles, posaconazole showed better activity in vitro (GM MICs, 0.26–0.75 μg/ml) against all clinical isolates analyzed in this study. Moreover, all strains were susceptible to terbinafine (GM MICs, ≤1.75 μg/ml) and the echinocandins, showing GM MECs of caspofungin and micafungin of ≤1.3 μg/ml and ≤0.26 μg/ml, respectively.

**DISCUSSION**

The present study highlights the limitations of phenotypic methods for the identification of some genera of molds. The use of molecular methods to partially sequence the β-tubulin and rodlet A genes enabled us to identify to the species level all clinical strains included in the study and previously classified as “atypical” A. fumigatus isolates. The majority of molecular methods use either specific probes or universal primers that are normally directed to conserved regions of the rRNA gene, particularly to the internal transcribed spacer regions (32, 35). However, internal transcribed spacer regions do not have enough phylogenetic strength to resolve the evolutionary relationship with strong bootstrap support for Aspergillus species from the section Fumigati (4, 5, 24, 41). Sequence analysis of both the β-tubulin and the rodlet A genes revealed that this method accurately differentiated the non-A. fumigatus isolates from the A. fumigatus isolates (4, 5). Therefore, all clinical isolates used in this study were identified to the species level by maximum-parsimony analysis of the β-tubulin and the rodlet A gene sequences.

On the basis of the identities of β-tubulin and rodlet A DNA sequences, the 28 strains were divided into six monophyletic clades supported by bootstrap values that differentiated the species (Fig. 1 and 2). The clades identified the isolates as belonging to the following species: 14 strains were A. lentulus, 5 strains were N. hiratsukae, 4 strains were N. pseudofischeri, 2 strains were A. viridinutans, 2 strains were A. fumigatiaffinis, and 1 strain was A. fumisynnematus.

A. lentulus has been isolated from soil and air, and it has also been isolated from patients with invasive infections (4). In this regard, most A. lentulus isolates recovered in this study were cultured from respiratory samples. This fact demonstrates the potential invasiveness of this pathogen in a susceptible host because A. fumigatus is able to colonize the human respiratory tract. A. viridinutans, N. hiratsukae, and N. pseudofischeri have also been isolated from humans (44), although only N. hiratsukae and N. pseudofischeri have been associated with human invasive fungal infections (3, 14, 17).

A. fumisynnematus was described as a separate taxon on the basis of the partial cytochrome b gene sequences of the species (43). However, this species was not included in the last classification of Aspergillus section Fumigati because the type strain of the species was not available for phenotypic characterization (36). To our knowledge, A. fumisynnematus and A. fumigatiaffinis had always been isolated from environmental sources. We describe for the first time the isolation of A. fumisynnematus and A. fumigatiaffinis strains from human hosts, which raises the intriguing issue of whether these species should be considered pathogenic fungi.

The common antifungal susceptibility phenotype of A. fumigatus is characterized by low MICs of theazole drugs, amphotericin B, and echinocandins. A. fumigatus strains showing resistance to azole drugs have been reported, although they have always maintained low amphotericin B MICs (11, 25, 26). In contrast, most A. lentulus and A. fumigatiaffinis isolates analyzed here showed extremely high MICs of amphotericin B, itraconazole, voriconazole, and ravuconazole.

Although 2 of the 14 A. lentulus isolates seemed to be susceptible to itraconazole, it is important to emphasize that we have not observed a uniform itraconazole susceptibility pattern, even after repeating the susceptibility testing more than eight times (data not shown). Due to the slow growth of most of these species, endpoint reading was easier at 72 h than at 48 h.

Since it has been demonstrated that elevated MICs of amphotericin B are associated with poor clinical outcomes (19, 30), the high MICs of amphotericin B for A. lentulus and A. fumigatiaffinis could have a remarkable clinical impact that merits research. In addition, it is well known that A. terreus strains have higher amphotericin B MICs than A. fumigatus strains, and this fact has been associated with a poorer response to amphotericin B in patients infected with this species (21, 37).

Even though A. fumisynnematus seems to be very closely related to A. lentulus, it had a different profile of susceptibility to all drugs tested. However, more isolates need to be analyzed in order to establish the antifungal susceptibility profile for this species.

Consistent with previous data (3, 44), the antifungal phenotypes of N. pseudofischeri and A. viridinutans showed that they had high itraconazole, voriconazole, and ravuconazole MICs. All N. hiratsukae isolates were susceptible to all the antifungal drugs tested. There has been only one report of cerebral aspergillosis caused by N. hiratsukae (14), in which the strain cultured from that patient showed a pattern of MICs similar to the patterns described in this study.

Among the azoles, posaconazole showed the highest level of activity against all species analyzed. Terbinafine also had good activity in vitro against all clinical isolates compared with the MICs for A. fumigatus.

Finally, our antifungal susceptibility testing results for the echinocandins showed that all strains were susceptible to both caspofungin and micafungin (MECs, ≤1.3 μg/ml). This result contradicts the findings of other authors, but since a reference method for testing the susceptibilities of filamentous fungi to echinocandins has not yet been defined, conclusions about the echinocandin susceptibility or resistance of Aspergillus spp. should be made carefully.

This study emphasizes that molecular methods are needed for the correct identification of members of Aspergillus section Fumigati to the species level. Moreover, the members of this section have different antifungal susceptibility profiles. The identification of species or isolates showing high MICs of the antifungals used clinically is therefore mandatory. It should be noted that high in vitro MICs may not necessarily reflect decreased susceptibility in vivo, especially because these species have growth differences that could affect the results. Therefore, to ascertain the true clinical importance of these species, epidemiological studies must be performed together with in
vitro-in vivo correlation studies. In the meantime, it is difficult to give practical advice for clinical laboratories, but we suggest that those isolates which appear to be *A. fumigatus* under the microscope but which have poor sporulation or slow growth be sent to reference laboratories. An alternative approach could be to send any *A. fumigatus*-like strain which does not grow at 48°C to a reference laboratory. Due to the shortage of data and the unpredictable susceptibility profiles of some isolates, we highly recommend that antifungal susceptibility testing be performed by a standardized methodology for all isolates associated with human infections.

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REFERENCES


