


7. Senda K, Arakawa Y, Ichiyama S et al. PCR detection of metallo-

-lactamase gene (blaIMP) in gram-negative rods resistant to broad-spectrum 


RESEARCH NOTE

In vitro susceptibility of Cryptococcus gattii clinical isolates

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ABSTRACT

The data available in the literature concerning Cryptococcus gattii in vitro antifungal susceptibility are contradictory. We have analyzed the activity of eight antifungal agents against 23 C. gattii clinical isolates and compared the susceptibility profiles with those of C. neoformans. MIC analysis (mg/L) revealed that C. gattii isolates were more susceptible to amphotericin B and flucytosine than were C. neoformans isolates. Fluconazole and other azole compounds showed high MIC values for C. gattii. Posaconazole displayed good activity. Further studies are required to ascertain the predictive value of the in vitro data presented here.

Keywords Antifungal susceptibility testing, Cryptococcus gattii, emerging yeast, EUCAST method, posaconazole

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The Cryptococcus neoformans species complex comprises basidiomycetous yeasts that are able to

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cause life-threatening disease of the central nervous system, lung and skin. These species have been traditionally classified into three varieties, *C. neoformans* var. *neoformans*, *C. neoformans* var. *grubii* and *C. neoformans* var. *gattii*, which are morphologically undistinguishable and differ in epidemiological and clinical presentations [1]. *C. neoformans* var. *gattii*, recently raised to species level as *C. gattii* [2], has until now been considered to be restricted to tropical and subtropical climates, and may cause infection in hosts with normal immunity. However, the outbreak of *C. gattii* infection on Vancouver Island (Canada, 2001) [3] demonstrated the importance of this species as an infectious agent in temperate climates.

In Spain, this species is uncommon, although several autochthonous *C. gattii* strains were isolated from goats with pulmonary disease between 1990 and 1994 [4] and from a brain abscess in a Spanish immunocompetent patient in 2003 [5].

Regarding the *C. gattii* antifungal susceptibility profile, the reported data are contradictory. To allow adequate management of emerging infections caused by this organism, we have analyzed the antifungal susceptibility profile of *C. gattii* clinical isolates. A review of the literature concerning the susceptibility of this species is also presented.

Twenty-three *C. gattii* strains were included in the study. Most of them were clinical isolates provided by colleagues: 19 isolates were from cerebrospinal fluid and blood of Brazilian patients, deposited at the Instituto Adolfo Lutz, Sao Paulo, Brazil; three isolates were provided by A. Casadevall (New York, NY, USA), including NIH isolates 191 (serotype C, ATCC 32608), 198 and 34 [3,6]; and one isolate was from cerebrospinal fluid of a Spanish farmer with underlying lupus erythematosus. The *in vitro* susceptibilities of 340 *C. neoformans* strains identified at the Instituto de Salud Carlos III between 1995 and 2006 were also determined and compared.

Susceptibility testing was performed according to the recommendations proposed by the European Committee for Antibiotic Susceptibility Testing of fermentative yeasts (APST-EUCAST, definitive document 7.1) [7]. To improve the growth of the strains, minor modifications were made [8].

The antifungal agents used were amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, ravuconazole, posaconazole and caspofungin. Statistical differences between MIC values were assessed using Student’s *t*-test. P-values <0.01 were considered to be significant.

Geometric mean (GM), MIC ranges and MICs inhibiting 90% (MIC90) and 50% (MIC50) of the isolates are shown in Table 1.

High susceptibility to amphotericin B was observed among the *C. gattii* strains studied (GM 0.09, range 0.03–0.25 mg/L). Only one strain showed *in vitro* resistance to flucytosine (MIC ≥32 mg/L). On the other hand, fluconazole showed poor activity, with MIC values higher than 4 mg/L for 21 of 23 isolates (91%). Among the newazole compounds, posaconazole displayed what was perhaps the highest activity, with MICs ranging between 0.03 and 0.5 mg/L (Table 1).

*C. neoformans* isolates showed higher MIC values for amphotericin B and flucytosine than *C. gattii* isolates (GM of 0.24 and 4.47 mg/L respectively, *P* <0.01). Twenty strains (5.8%) showed MIC values for amphotericin B higher than 1 mg/L, and 6% (21/340) were resistant to flucytosine (MIC ≥32 mg/L). However, the *C. neoformans* isolates exhibited MIC values for itraconazole, voriconazole, ravuconazole, posaconazole, and even fluconazole, that were significantly lower than those of *C. gattii* (Table 1). The

### Table 1. Geometric means (GMs) of MIC values (mg/L), MIC ranges and MICs inhibiting 90% (MIC90) and 50% (MIC50), respectively, of the isolates included in this study

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>Cryptococcus gattii (n = 23)</th>
<th>Cryptococcus neoformans (n = 340)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GM</td>
<td>Range</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.09</td>
<td>0.03–0.25</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>1.52</td>
<td>4 to &gt;64</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.44</td>
<td>0.12–2</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.47</td>
<td>0.03–1</td>
</tr>
<tr>
<td>Ravuconazole</td>
<td>0.41</td>
<td>0.03–2</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>0.26</td>
<td>0.03–0.3</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>17.51</td>
<td>8 to &gt;16</td>
</tr>
</tbody>
</table>
former showed MIC values of >4 mg/L for 68% of the isolates (232/340). Caspofungin was inactive against both species.

In this study, C. gattii was more susceptible than C. neoformans to amphotericin B and flucytosine. Comparative studies performed to determine differences in in vitro antifungal susceptibilities are scarce, and the results are contradictory. Major discrepancies were identified concerning amphotericin B and flucytosine.

Eight reports were found in the literature, including findings with clinical and environmental isolates of C. gattii [9–16]. Some studies found very similar MICs for both species [9,13]. Others reported C. gattii as less susceptible to some antifungals than C. neoformans [10–12,14,16].

The method used for MIC determination influences the in vitro susceptibility pattern observed. Although the standardized CLSI methodology for Candida spp. has proved to be reliable, there are still technical problems with Cryptococcus. For this reason, MIC data obtained from different laboratories cannot be directly compared, because of the variability in methods used and the absence of standardized approaches, especially with respect to the growth medium. Odds et al. showed that oxygen is absolutely required for adequate growth of Cryptococcus in RPMI medium [17] and that agitation led to a substantial improvement in the growth rate of the yeast. This indicates that methods providing better growth rates facilitate endpoint determination and MIC reproducibility.

The Etest has proven to be an excellent method for Cryptococcus spp. susceptibility testing. In agreement with our findings, Tay et al. showed a collection of C. gattii strains to be highly susceptible to amphotericin B (MIC <0.5 mg/L) using the Etest, and also found a high percentage of C. gattii isolates with higher resistance to fluconazole, when compared with C. neoformans [15]. The susceptibility testing method used here included a larger inoculum as well as agitation, and may represent a good alternative for Cryptococcus susceptibility testing, as it allows faster determination of the MIC endpoint.

However, with use of the Etest, contradictory results have also been reported [11]. The reason for these discrepancies could be related to differences in the origins of the yeasts. Some environmental C. gattii strains produced more rapid and intense pigmentation than did C. neoformans [18], which correlated with higher resistance to amphotericin B and fluconazole. Previous reports have demonstrated that melanization reduces susceptibility to amphotericin B [19]. In addition, clinical antifungal resistance during therapy or prophylaxis has been reported [20].

In summary, it is shown in this study that amphotericin B and flucytosine have potent activity against C. gattii clinical isolates. It is also interesting to point out the occurrence of low MICs of posaconazole. Some modifications of the susceptibility testing method are provided that could allow more accurate determination of the susceptibility profile of C. neoformans, although further studies are needed to validate the clinical predictive value of the in vitro data presented.

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REFERENCES