Research Article

Fluconazole Susceptibility of Candida Species

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Abstract

Aim: The aim of this study was to evaluate the susceptibility profile of Candida species isolated from clinically relevant specimens by micro broth dilution method to fluconazole.

Setting of the study: The study was conducted in Department of Microbiology, Govt. Medical College, Kozhikode, Kerala over a period of one year.

Materials and Methods: Antifungal susceptibility of 117 relevant Candida isolates were done by microbrothdilution methods according to CLSI guide lines

Results: 96.6% of all Candida isolates were sensitive to fluconazole and 3.4% were found to be resistant.

Keywords: Candida albicans, Non albicans Candida, Fluconazole.

Introduction

Candida causes a diverse spectrum of opportunistic infections ranging from mild superficial mucocutaneous infections to life threatening invasive candidiasis.¹ The major pathogens include C.albicans, C.tropicalis, C.parapsilosis, C.kefyr, C.krusei, C.glabrata, C.guillermondi, C.famata, C.lusitaniae and C.lipolytica. With widespread use of antibiotic since 1940s, infections due to Candida came to be noticed. Most important predisposing factor for Candida infection and especially to disseminated candidiasis is iatrogenic. Candida species have been recognised as the fourth common cause of nosocomial invasive infections.² Among Candida species C.albicans is the most common pathogen, but Non albicans Candida species infections are increasing.³ Polyenes, azoles, flucytosine, echinocandins are the major groups of antifungals used for the treatment of candidiasis. But indiscriminate use may lead to emergence ofazole resistant species by selective pressure. In contrast to primary resistance, strain to strain variations in antifungal susceptibility profiles and cross - resistance are always possible. So, it is of great importance to know the species of Candida responsible for the infection as well as its susceptibility patterns. Conventional methods like
Macrobroth dilution method, microbroth dilution or disk diffusion methods and commercially available systems like Sensitive yeast one test panel (Trek Diagnostics systems Inc, Westlake, OH), Fungitest (sanofi Dignostics), E-test, VITEK R 2 antifungal susceptibility methods are used for antifungal susceptibility testing.

This study was undertaken to know the antifungal susceptibility of clinically relevant Candida isolates to fluconazole by broth microdilution methods.

Materials and Methods

Susceptibility testing was carried out on 117 Candida isolates from a variety of sources (blood, urine, body fluids, swabs, nail clippings, skin scrapings, gastric aspirates etc) were included in this study. These consisted of Candida albicans (46 isolates), Candida tropicalis (57 isolates), Candida parapsilosis (12 isolates), Candida kefyr (1 isolate) and one unidentified species.

The culture was done on Sabouraud’s dextrose agar (SDA) in accordance with the standard methods. Yeast isolates were identified on the basis of colony characteristics and further by germ tube production, morphology on corn meal agar, Hi Chrome Candida agar (Hi Media), urease test, carbohydrate fermentation and assimilation tests as per standard recommended procedures (Forbes et al., 2002; Koneman et. al., 1997). Azoles, especially fluconazole is the most widely used antifungal agent in our institution, so the susceptibility of all Candida isolates to fluconazole was determined to formulate an empirical therapy. Antifungal susceptibility testing was performed by broth micro dilution modification method as per CLSI M 27-A3 Document, Third edition Vol.28, No: 14.

Serial dilutions of fluconazole (from 128 μg/ml to 0.25μg/ml) were prepared in RPMI 1640 broth and then dispensed into micro-dilution test panels. Inoculum was prepared by picking 5 distinct colonies from SDA culture, and the turbidity adjusted to 0.5 McFarland standards. Each well is inoculated with100μL of inoculum suspension. The trays were incubated at 35°C in ambient air for 24 to 48hrs and the plates read both at 24 and 48hrs for the presence and absence of visible growth. MICs for fluconazole were recorded as the lowest concentrations in which prominent decrease in turbidity was observed.

Results

96.6% of all Candida isolates were sensitive to fluconazole and 3.4% were found to be resistant. Among the different species, 100% of C.albicans., 96.5%.C.tropicalis and 91.7% of C.paraspilosis were sensitive to fluconazole. One unidentified Non albicans Candida species was resistant to fluconazole.

Table: 1 Percentage of clinically relevant Candida species isolated

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>46</td>
<td>39.3</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>57</td>
<td>48.7</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>12</td>
<td>10.2</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>Non albicans Candida-un identified</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>Total</td>
<td>117</td>
<td>100</td>
</tr>
</tbody>
</table>

Table: 2 Fluconazole susceptibility patterns of Candida species:

<table>
<thead>
<tr>
<th>Species</th>
<th>Fluconazole susceptibility</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive (S)</td>
<td>Resistant (R)</td>
</tr>
<tr>
<td>C. albicans</td>
<td>46(100%)</td>
<td>0</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>55(96.5%)</td>
<td>2(3.5%)</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>11(91.7%)</td>
<td>1(8.3%)</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>1(100%)</td>
<td>0</td>
</tr>
<tr>
<td>Unidentified</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Discussion

Fluconazole is the most widely used triazole since its approval in 1990. Its availability in both oral and parenteral formulations, led to widespread use of fluconazole for both treatment and prophylaxis in candidiasis. The increased use of this drug has caused increasing rate of resistance among Candida spp, mainly C. glabrata and C. krusei isolates. Use of prophylaxis with azoles remains controversial in most high risk population. Empirical use of fluconazole in febrile patients at
high risk for invasive candidiasis is a common therapeutic strategy, there should be concern about the extensive use of fluconazole due to a possible shift towards non albicans species as the cause of infection. A study by Garnacho Montero et al, 2010 reported that prior fluconazole treatment is an independent risk factor for candidemia caused by microbiologically confirmed fluconazole resistant species.

Although, fluconazole is most frequently used as an antifungal agent in the treatment of systemic yeast infections, resistance rates have been reported for *C. albicans* (5.7–5.8 %) and *C. tropicalis* (6.2–9.8 %) by Fothergill AW, Zhang L and Lockhart SR. Globally, *C. glabrata* showed the higher resistant rates (7.7–11.9 %) than other *Candida* species.

In the present study 96.6% of the total Candida isolates were sensitive to fluconazole and 3.4% were found to be resistant. 100% of *C.albicans* and *C. kefyr* was susceptible to fluconazole. Fluconazole resistance in *C.tropicalis* was 3.5% and in *C.parapsilosis* 8.3%. In a study by Sahar Ali Mohamed and Ziab Zakey Al Ahamedey, 2013 reported the resistance rate of 22.5% in Non albicans Candida species and 5% in *C.albicans* isolates. Study by Adhikari et al also showed increase susceptibility to fluconazole by candida. The variation in fluconazole susceptibility of Candida species observed in different studies may be due to the difference in institutional based protocol for the usage of antifungal agents and diversity in the study population. In the present study most of the Candida isolates (96.6%) in general, and 100% isolates of *C.albicans* were susceptible to fluconazole. Species which are intrinsically resistant to fluconazole were not prevalent in our institution, so fluconazole can be used for empirical therapy in clinically susceptible cases of candidiasis.

**Conclusion**

Fluconazole can be used as an empirical therapy in clinically susceptible cases of candidiasis. But the indiscriminate use of fluconazole should be controlled by good clinico microbiological correlation in order to prevent the emergence of azole resistant species by selective pressure.

**Competing interests**

There are no competing interests.

**Ethical approval and consent to participate:**

The ethical approval for study was taken from Institutional Research committee, Govt. Medical College, Kozhikode, Kerala

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