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## ***In vitro* Activity of Conventional Antifungal Agents Against *Scedosporium apiospermum* Isolates Recovered from Clinical and Environmental Samples in Nigeria**

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**Abstract:** *In vitro* susceptibilities of *Scedosporium apiospermum* isolates recovered from clinical and environmental samples in Nigeria were tested against ten conventional antifungal agents namely: amphotericin B, nystatin, flucytosine, itraconazole, posaconazole, fluconazole, voriconazole, caspofungin, ketoconazole and miconazole using the CLSI M38- A broth dilution reference method. The isolates showed varied response /sensitivities to the antifungal agents tested. This is probably the first documented testing of *Scedosporium apiospermum* isolates from Africa in general and Nigeria in particular against a broad range of conventional antifungal agents.

**Key words:** Antifungal agents % *Scedosporium* % Clinical % Environmental % Nigeria

### **INTRODUCTION**

*Scedosporium apiospermum* is a filamentous fungi which belong to the class Eucosmomyces, order Microascales and family Microasaceae. The species were originally known to be soil borne organism but have emerged recently as opportunistic pathogens in both immunocompetent and immunocompromised patients [1-2]. They are known also known to be distributed worldwide [3-4]. Despite this, information on the species appears not to be balanced as there are few reports, if any from Africa. Information from other countries indicate that this species show poor response to conventional antifungals [5-6]. Unfortunately strains from African countries have not been tested against conventional antifungal agents to ascertain their specific response to such agents. This is necessary for especially use by Clinicians if the need arises especially in the current era where many patients are immunosuppressed due to HIV/AIDS ravaging the African continent and elsewhere. This study was therefore conducted to determine the *in vitro* susceptibility of *Scedosporium apiospermum* species isolated from clinical and environmental samples in Nigeria against eight antifungal agents.

### **MATERIALS AND METHODS**

**Isolates and inoculums preparation:** The isolates tested were subcultured onto potato dextrose agar (Difco) at 30°C for 7 days to ensure the viability, purity and

sporulation of the inocula. Two quality control strains were included: *Candida albicans* (ATCC 90028) and *Candida parapsilopsis* (ATCC 22019). The drugs were tested in RPMI 1640 medium (Gibco Life Technologies, The Netherlands) buffered to PH 7 with 0.165 M morpholinepropanesulphonic acid (MOPS) (Merck, Germany). Aliquots of 100µl of the different drug dilutions prepared as recommended by the CLSI were inoculated into the wells of microtiter plates containing the inocula using a multichannel pipette. The inocula were prepared by removing the sporulated fungi from the agar slant with a loop and suspending them in 10ml of sterile water. It was filtered with sterile cotton guaze to remove the hyphae. The suspension was adjusted to 68 to 70 % transmittance at 530 nm and diluted ten-fold to yield a working suspension of  $0.4 \times 10^4$  to  $5 \times 10^4$  CFU/ml. One hundred µl of fungal suspensions was added to each well using a multi channel pipette. The concentration of the test ranged from 0.06 to 32 µg/ml for caspofungin and from 0.12 to 64 µg/ml for flucytosine and fluconazole and from 0.03 to 16 µg/ml for the other drugs. The microplates were incubated at 35°C and read at 72hr. The MIC endpoints for the triazoles and amphotericin B were determined as the lowest concentration that produced complete inhibition of growth and those for flucytosine, ketoconazole, fluconazole and micafungin were defined as the well with prominent decrease in turbidity i.e. the lowest concentration that produced 50% growth inhibition relative to the turbidity of the growth control well. The MICs for the quality control strains yielded by

the various methods (broth microdilution and disk diffusion) were all within the reference ranges described in the Clinical Laboratory Scientific Institute (CLSI) (formerly NCCLS) M38A guidelines [7].

## RESULTS

All isolates produced detectable growth after 72 hours of incubation. The MICs were therefore determined at this time interval. The MICs of all the drugs tested are summarized in Table 1. *S. apiospermum* showed variable susceptibility to all the antifungal agents tested. The two control strains, *Candida albicans* (ATCC 90028) and *Candida parapsilopsis* (ATCC 22019) showed MIC values within the recommended range as documented in the CLSI protocol (data not shown)

The Antifungals: itraconazole, nystatin, fluconazole, amphotericin B and flucytosine showed very high MICs against the species. The geometric mean of these drugs against *S. apiospermum* were 14.87, 4.50, >16, 4.00 and >16 respectively. Amphotericin B and ketoconazole showed good antifungal activities on some isolates but this becomes irrelevant with the high geometric mean MICs shown by these drugs..

The newer triazoles, posaconazole and voriconazole were active against *S. apiospermum* with geometric mean of 0.08 and 1.00 µ/ml respectively.

The only echinocandin tested, micafungin generally showed high MICs against the species. The MIC<sub>90</sub> and MIC<sub>50</sub> for all the antifungal agents tested against the isolates were low for some new triazoles. In all cases, posaconazole was more potent than all other antifungal agents tested.

Table 1: *In vitro* susceptibility of *Scedosporium apiospermum* to conventional antifungal agents (µg/ml)

	MIC range	Geometric		
		Mean MIC	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>S. apiospermum</i>				
Amphotericin B (AMB)	2 -32	4.00	4.00	16.00
Nystatin (NYS)	4 - >16	4.50	>16	>16
Itraconazole (ITR)	8 - >32	14.87	>16	>16
Ketoconazole (KET)	0.06 - >16	2.00	2.00	>16
Posaconazole (POS)	0.03- 0.25	0.08	0.03	0.25
Voriconazole (VOR)	0.5- 4	1.00	1.00	2.00
Fluconazole (FLU)	> 16	>16	>16	>16
Micafungin (MIC)	1- >32	>32	>32	>32
Terbinafine (TER)	4 - >64	19.29	32.00	64
Flucytosine (5FC)	>16	>16	>16	>16

Key; MIC- Minimum inhibitory concentration; MIC<sub>50</sub> – MIC of 50% of the isolates;

MIC<sub>90</sub> – MIC of 90% of the isolates

We observed no differences in the antifungal activities shown by environmental and clinical isolates (data not shown). The whole data were therefore presented together as shown in the Table 1.

## DISCUSSION

This is the first data showing the antifungal activities of conventional agents against isolates of *Scedosporium* species from Nigeria and probably from Africa.

The Antifungals: amphotericin B, nystatin, itraconazole and flucytosine showed very high MICs against both species. In most countries, amphotericin B is still the most commonly used conventional antifungal despite its reported side effects. This drug presented geometric mean MICs of 4.00 µg/ml against *S. apiospermum*. A strain dependent *in vitro* response to amphotericin B was observed. However, the MICs for most strains were high. This observation is similar to that reported by other researchers. [8,9]. In contrast, all the strains of *S. apiospermum* tested showed MICs # 4.00µg/ml. The results obtained with the newer triazoles varied according to the strain tested. Voriconazole and posaconazole were active against *S. apiospermum* with geometric mean of 0.08 and 1.0 µg/ml for posaconazole and voriconazole respectively. The information in literature suggests that there is little clinical experience with posaconazole despite its promising activity. Mellinghoff *et al* [10] reported a successful resolution of a brain abscess in a leukemia patient with this drug. The result on voriconazole in this study deviated slightly from the observation made by Carrillo and Guarro [9] who found a lower MIC range of 0.01 to 0.25 µg/ml against *S. apiospermum* isolates. This is understandable as in the said study; they used the 50% reduction endpoint in the determination of the MIC in contrast to the methodology here, which used the criterion of complete inhibition, defining MIC as the lowest concentration that completely inhibited fungal growth. However, the results of Carrillo and Guarro [9] seem to correlate better with available data on *in vivo* outcome with voriconazole in three patients with invasive *S. apiospermum* infections [11-13].

Fluconazole, ketoconazole, terbinafine and micafungin were generally resistant to both species and this observation is in agreement with published studies from other authors who used similar clinical laboratory scientific institute (CLSI) methodology used in this study. This trend seems consistent in clinical practice as itraconazole for instance, was unable to resolve some clinical cases caused by the anamorph of *S. apiospermum* [14-15].The reason for this may be due to its poor

absorption after oral administration. Cuenca-Estrella *et al.* [16] reported some success with miconazole but found them associated to adverse effects [17]. In addition, micafungin and the other echinocandins have been previously reported to have poor activities against *Scedosporium* species [18].

The results of this study have made the search for other antifungal drugs necessary as a way of tackling this poor response of the species to the drugs tested. Our laboratory has initiated a large scale susceptibility study of these species against a variety of medicinal plant materials as a way of finding an alternative and the preliminary data looks promising.

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