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In Vitro Susceptibility Testing of *Aspergillus fumigatus* Against Posaconazole (POS): Comparison of NCCLS M38-P and E-test® Methods

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ABSTRACT

Background: Posaconazole is a second–generation triazole and structural analogue of itraconazole. The drug has fungicidal activity against yeasts and filamentous fungi. The aim of the study was to evaluate E-test® method for *in vitro* susceptibility testing of *Aspergillus fumigatus* isolates against posaconazole.

Methods: A total of 131 isolates of *A. fumigatus* were selected as follows: 106 clinical isolates from colonized patients. 18 from patients with invasive aspergillosis and 7 environmental isolates. Their *in vitro* susceptibility was evaluated by E-test® (AB-Biodisk, Sweden) and compared with NCCLS microdilution reference method (M38-P). Both tests were performed with RPMI 1640 medium at 35°C. MIC values were read after 24h (MIC-24h) and 48h (MIC-48h) incubation time by E-test® method. Two MIC end-points were determined by NCCLS method: 1) No visible growth (MIC-0); 2) 50% reduction (or more) of growth (MIC-2). Three *A. fumigatus* reference strains (IHEM 5734, 6149, and 13935) were included as control.

Results: Geometric mean MICs (µg/mL) were respectively 0.02 for E-test® at 24h and 0.029 at 48h. MIC-0 and MIC-2 values were respectively 0.19 and 0.018 µg/mL. The one correlation between both methods was observed for MICs-24h and MICs-2 (P>0.05). However there was no significant difference according to origin of isolates (P>0.05).

Conclusions: 1) This study assesses the potent role of posaconazole against *A. fumigatus* isolates with very low MICs. 2) MIC values are not predictive of pathogenicity. 3) E-tests® method by reading after 24h-incubation time could easily replace the time-consuming reference NCCLS M38-P method.

INTRODUCTION

New azoles have been recently introduced for the treatment of invasive aspergillosis. Clinical studies are in progress to compare the efficacy and toxicity of these molecules to the gold standard, amphotericin B (AMB). In addition, *in vitro* susceptibility studies are also needed to determine MIC values for these new drugs against emerging pathogens.

In this study we measured the *in vitro* activity of posaconazole (POS) against a collection of *Aspergillus fumigatus* isolates, from diverse origins, using the NCCLS M38-P microdilution reference method. We also compared the NCCLS reference method with the E-test® method. Different paramaters (e.g. incubation time) and end-points were used and the concordance between the 2 methodologies evaluated.

N.B. This work was performed in collaboration with Schering-Plough Research Institute.

MATERIALS AND METHODS

Strains

The 131 isolates of *A. fumigatus* comprised 106 clinical isolates from colonized patients, 18 from patients with invasive aspergillosis and 7 environmental isolates. Three itraconazole-resistant (ITZ-R) *A. fumigatus* reference strains (IHEM-5734, 6149, and 13935) were included as controls (2).

Culture

The strains were grown on Sabouraud dextrose agar (SAB) following the recommendations of NCCLS M38-P microdilution methodology (1).

NCCLS Microdilution

RPMI 1640 medium (Gibco BRL, Life Technologies, USA) buffered with MOPS (Sigma, St Louis, USA) was used. POS, supplied as a powder (Schering-Plough, Kenilworth, USA), was dissolved in 100% DMSO before preparing serial 2-fold dilutions (dilution range was 0.004 to 16 µg/mL) in RPMI. MIC values were determined by visual reading after 48h incubation at 35°C. Two MIC end-points were used: no visible growth (MIC-0); a 50% (or more) reduction in growth (MIC-2) when compared to the no drug control.

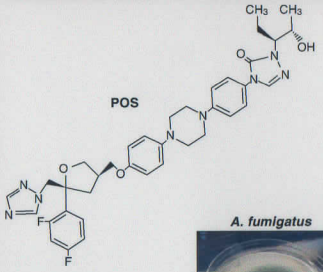
E-test®

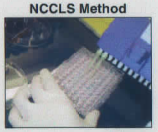
E-test® (AB-Biodisk, Solna, Sweden) was performed on RPMI 1640 buffered agar (1.5%). Readings were made after 24h (MIC-24h) and 48h (MIC-48h) incubation at 35°C.

Statistical Analysis

A generalized linear mixed model was applied.

RESULTS





- The MIC data are summarized in **Tables 1-3**.
- A statistical analysis (**Table 4**) showed that there was a strong correlation between the results from theE-test®, read after 24 hours, and the NCCLS method with an end-point of 50% reduction in growth.
- The origin of the isolates had no impact on the MIC values.

Table 1: MICs and geometric means for all 131 *A. fumigatus* isolates.

Isolates \ MIC (µg/mL)		E-test®		NCCLS M38-P	
		MIC-24h	MIC-48h	MIC-0	MIC-2
A. fumigatus isolates (n=131)	Mean	0.02	0.029	0.19	0.018
	Range	0.002-0.032	0.002-0.125	0.007-1	0.004-0.06

Table 2: MICs and geometric means for *A. fumigatus* isolates from various origins.

Isolates \ MIC (µg/mL)		E-test®		NCCLS M38-P	
		MIC-24h	MIC-48h	MIC-0	MIC-2
Environmental (n=7)	Mean	0.019	0.029	0.18	0.019
	Range	0.012-0.023	0.016-0.047	0.125-0.5	0.015-0.06
Invasive (n=18)	Mean	0.02	0.027	0.21	0.02
	Range	0.002-0.032	0.002-0.047	0.06-0.5	0.007-0.03
Colonization (n=106)	Mean	0.02	0.029	0.19	0.018
	Range	0.006-0.09	0.002-0.125	0.007-1	0.004-0.06

Table 3: MIC_{90} values obtained with the 4 methodologies.

MIC_{90} \ Methods	E-test®		NCCLS M38-P	
	MIC-24h	MIC-48h	MIC-0	MIC-2
MIC_{90} (µg/mL)	0.032	0.047	0.5	0.03

Table 4: Comparison of MIC values by E-test® method, read at 24h (MIC-24h) and 48h (MIC-48h), with NCCLS method, end-points 50% reduction in growth (MIC-2) and no visible growth (MIC-0): statistical analysis.

P-Values \ Methods	Following Origin of the Isolates			Globally for All Strains	
	Colonization	Environmental	Invasive	Method Effect	Origin Effect
MIC-24h / MIC-48h	<0.0001	0.0003	<0.0001	<0.0001	**0.95**
MIC-24h / MIC-0	<0.0001	<0.0001	<0.0001	<0.0001	**0.95**
MIC-24h / MIC-2	**0.12**	**0.96**	**0.92**	**0.15**	**0.96**
MIC-48h / MIC-0	<0.0001	0.0004	<0.0001	<0.0001	**0.98**
MIC-48h / MIC-2	<0.0001	0.048	0.13	<0.0001	**0.98**
MIC-0 / MIC-2	<0.0001	0.0002	<0.0001	<0.0001	**0.75**

DISCUSSION AND CONCLUSIONS

- Consistent with previous reports (3, 4), POS was very active against *A. fumigatus in vitro*.
- POS was active against one of the ITZ-R reference strains. However, further studies are needed to evaluate the clinical efficacy of this drug against azole-resistant isolates.
- MIC values were not predictive of pathogenicity.
- The E-test® method, read after 24h, was as accurate as the more time-consuming NCCLS M38-P reference method. Since the E-test® methodology was easier to perform, and the reading of the MIC did not require prior experience, this method may be preferred to the NCCLS M38-P reference method.

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- **Posaconazole poster compilation booklets are available at the ICAAC Information Kiosk.**

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