In Vitro Antifungal Susceptibilities of Five Species of Sporothrix

Rita Marimon, Carolina Serena, Josepa Gené, Josep Cano, and Josep Guarro*

Unitat de Microbiologia, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, Reus, Spain

Received 1 August 2007/Returned for modification 10 October 2007/Accepted 10 November 2007

Ninety-two isolates belonging to five species of the Sporothrix schenckii complex were tested in vitro against 12 antifungal agents, using a reference microdilution method. There were significant differences among the species; Sporothrix brasiliensis was the species that showed the best response to antifungals, and S. mexicana had the worst response. In general, terbinafine was the most active drug, followed by ketoconazole and posaconazole.

Sporotrichosis is a worldwide subacute or chronic infection caused by the dimorphic fungus Sporothrix schenckii, affecting both animals and humans. This disease is characterized by nodular cutaneous and subcutaneous lesions, which may involve the adjacent lymphatic system (2, 18). A saturated solution of potassium iodide has been used as an effective therapy for localized sporotrichosis. Other drugs commonly used are itraconazole (ITC) for the treatment of lymphocutaneous infections (1, 11, 20) and amphotericin B (AMB) for severe infections or when ITC therapy fails (9). Although these drugs are generally effective, the long duration of therapy and the toxicity of AMB make it necessary to explore new alternatives for the treatment of severe infections.

Some in vitro studies have demonstrated variable results among the strains tested, and some authors have concluded that antifungal susceptibility is strain dependent (7, 14, 21). This could be explained by the fact that S. schenckii does not represent a single species; instead it is a complex of cryptic species. Recently, using a multilocus sequence analysis, we have demonstrated that at least six phylogenetic species are included in the complex (13), several of these species being phenotypically characterized (12). Since the antifungal susceptibility of these species is unknown, we have evaluated the in vitro activity of 12 drugs against the mycelial phase of 92 strains representing five species of the complex (Table 1), using a reference microdilution method (15). The isolates were selected to represent the widest variety of geographical regions possible.

The isolates tested in this study were stored on potato dextrose agar (PDA) plates (Difco Laboratories, Detroit, MI) covered with paraffin oil, subcultured on PDA, and incubated at 30°C for 5 to 6 days. Candida krusei ATCC 6258 and Candida parapsilosis ATCC 22019 were used as control strains.

The antifungal agents were obtained as pure powders. AMB (USP; Rockville, MD), ITC and ketoconazole (KTC) (Janssen Pharmaceutica, Beerse, Belgium), albaconazole (ABC) (J. Uriach & Cía, Barcelona, Spain), voriconazole (VRC) (Pfizer, Inc., NY), posaconazole (PSC) (Schering-Plough, Kenilworth, NJ), ravuconazole (RVC) (Bristol-Myers Squibb Company, New Brunswick, NJ), ebeconazole (EBC) (Laboratorios Salvat, S.A., Barcelona, Spain), and terbinafine (TRB) (Novartis, Basel, Switzerland) were diluted in dimethyl sulfoxide (Panreac Química S.A., Barcelona, Spain), and micafungin (MFG) (Astellas Pharma, Inc., Tokyo, Japan), flucytosine (5FC) (Sigma-Aldrich Corp., St. Louis, MO), and fluconazole (FLC) (Pfizer, Inc., Madrid, Spain) in sterile distilled water. Microplates were prepared as described in NCCLS standard M38-A (15). Final drug concentrations ranged from 12.5 to 0.25 μg/ml for MFG, from 64 to 0.12 μg/ml for FLC and 5FC, and from 16 to 0.03 μg/ml for the other drugs. The inoculum was prepared as recommended by the CLSI (formerly NCCLS) (15), by flooding the surface of the agar plate with sterile saline, scraping the sporulating mycelium with a culture loop, and drawing up the resultant suspension with a sterile Pasteur pipette. The suspensions were then filtered once through sterile gauze to remove hyphae. The numbers of conidia in the suspensions were adjusted to optical densities that ranged from 0.09 to 0.11, which corresponded to final concentrations of 1 × 10⁵ to 5 × 10⁶ CFU/ml. The viabilities of these inocula were verified by plating dilutions of the suspensions on PDA plates. The microplates were incubated at 30°C and read at 72 h. The MIC endpoint for the triazoles, AMB, MFG, and TRB was defined as the lowest concentration that produced complete inhibition of growth and for FLC, KTC, and 5FC as the lowest concentration that produced 50% growth inhibition. Approximately 80% of the tests were repeated and showed the same tendency (data not shown). However, in the few cases that did not coincide, the test was repeated and a modal MIC of the three values was considered.

The results are shown in Table 1. The MICs for the control strains agreed with the CLSI guidelines (15). TRB was the most active drug, showing a geometric mean (GM) MIC of 0.23 μg/ml for all the strains tested, followed by KTC with a GM MIC of 0.84 μg/ml. However, this latter drug was less active against Sporothrix mexicana (GM MIC of 4 μg/ml) and Sporothrix albicans (GM MIC of 3.2 μg/ml) than it was against the other species of the complex. The activity of KTC was more variable than that of TRB and depended on the species tested. PSC was the third-most active antifungal drug tested, with a total GM MIC of 1.59 μg/ml, and the most active of the drugs for systemic use.

Although we could test only two isolates of S. mexicana, this...
was the species that responded least well to antifungals and
only TRB showed a relatively low MIC (0.5 μg/ml) against this
species.

FLC and MFG were not active against any of the isolates
tested, as had already been demonstrated by other authors (11,
21). VRC showed poor activity, in agreement with the results
of McGinnis et al. (14), who also obtained a high GM MIC
(6.50 μg/ml) against strains of *S. brasiliensis*.

RVC and ITC only showed good activity against *Sporothrix
brasiliensis*, whereas, for the other species tested, both drugs
showed high MICs. Other authors (14) had also demonstrated
that *S. brasiliensis* is a species that responded least well to
antifungals and only TRB showed a relatively low MIC (0.5 μg/ml)
in vitro against this species.

Although in vitro results do not always correlate with in vivo
outcome, the drugs tested showed in general very poor activity
against *S. albicans*, *Sporothrix globosa*, and *S. mexicana*. It
would be interesting to know if any drug combinations exert
any activity against such species. However, no data are so far
available on the activity of combined drugs against *S. schenckii*
sensu lato.

In recent years, application of the phylogenetic species con-
cept in different biological species of pathogenic molds has
revealed different lineages that reflected species divergence (3,
10, 16). The delineation of these phylogenetic groups and the
development of easy methods for their identification are cru-
cial, since they can show different pathological behaviors and
different antifungal responses (4). This study has demonstrated
that *S. schenckii* constitutes a clear example of the latter.

Since clinical information on these new species does not yet
exist, the significance of our findings is unknown. However, it
seems that proper identification of the species of the *S.
schenckii* complex involved in a given infection could be im-
portant for the appropriate treatment. For instance, in the case
of a systemic infection, if the species causing the infection was
*S. mexicana*, it is likely that the response to treatment with ITC
or PSC would be poorer than if the species was *S. brasiliensis*.

We are indebted to the curators of the Centraalbureau voor
Schimmelcultures (Utrecht, The Netherlands), BCCM/IHEM Biomedical Fungi and Yeasts Collection (Brussels, Belgium), A. Espinosa (Centro de Investigaciones en Ciencias Microbiológicas, Universidad Autónoma de Puebla, Mexico), J. M. Torres (IMIM, Hospital del Mar, Barcelona, Spain), C. Rubio (Hospital Universitario Lozano Blesa, Zaragoza, Spain), R. Negroni (Hospital de Infecciosas Francisco Javier Muñiz, Buenos Aires, Argentina), L. Trilles (Servicio de Micrología Médica, Instituto Evandro Chagas, Fiocruz, Rio de Janeiro, Brazil), P. Godoy (Escola Paulista de Medicina, Universidade Federal
de São Paulo, Brazil), M. Kawasaki (Department of Dermatology, Kanazawa Medical University, Ishikawa, Japan) and D. A. Sutton (Department of Pathology, University of Texas Health Science Center, San Antonio, TX) for supplying many of the strains used in the study.

This study was supported by the Spanish Ministerio de Ciencia y Tecnología, grants CGL 2004-00425/BOS and CGL 2005-07394.

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