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CASE REPORTS

Molecular Characterization of Fluconazole Resistance in a Case of *Candida albicans* Ocular Infection

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Ocular yeast infections in diabetics are a therapeutic challenge. Drug resistance and reduced azole susceptibility are major concerns. The case we describe characterizes a *Candida albicans* strain from a vitrectomy specimen that was susceptible to fluconazole by in vitro testing but recalcitrant to therapy. Molecular studies revealed transient overexpression of *CDR1* and *ERG11* mRNA in the presence of fluconazole that may have contributed to poor clinical response in this patient.

CASE REPORT

A 48-year-old human immunodeficiency virus-negative woman with a past medical history of insulin-dependent diabetes mellitus, congenital absence of one kidney, recurrent pyelonephritis, and gastric bypass surgery presented to the emergency department with pain and visual loss in her right eye. She responded poorly to treatment with prednisone (60 mg daily) for presumed sarcoid uveitis. A *Candida* spp. (designated NYP-1) was isolated from a vitrectomy culture. She received an intravitreal injection of amphotericin B and then fluconazole (FLU; 400 mg daily) for 4 months. Her FLU dose was subsequently lowered to 200 mg daily secondary to clinical stability. On follow-up 1 week later, she developed a new choroidal lesion adjacent to the optic disk in her left eye. Treatment was changed to caspofungin and liposomal amphotericin B (5 mg/kg of body weight daily). Although the initial lesions regressed, new satellite lesions appeared during this therapy. Caspofungin was subsequently discontinued secondary to concerns regarding pharmacologic antagonism between the antifungal agents, and the dose of amphotericin B was increased (7.5 mg/kg daily) to maximize penetration into the eye. Her left eye lesions resolved with this treatment, and no further infections were noted in the left eye.

Candida dubliniensis has been reported to respond poorly to azole drugs (7). NYP-1 was characterized by both phenotypic and genotypic methods to definitively differentiate *Candida albicans* from *Candida dubliniensis* and to determine drug resistance mechanisms that could account for therapeutic failure. Phenotypically, the isolate was identified as *C. albicans* by formation of germ tubes at 37°C in horse serum (Life Technologies, Grand Island, N.Y.), production of chlamydospores on cornmeal agar with polysorbate 80 (Becton Dickinson Microbiology Systems, Cockeysville, Md.), substrate assimilation with the API 20C AUX (bioMérieux Inc., Hazelwood, Mo.; bionumber 2174174) and Rapid Yeast Identification Panel (Dade Microscan, Sacramento, Calif; bionumber 745032001), colorimetric growth on CHROMagar *Candida* plates (DRG International, Mountainside, N.J.), and growth at 42°C. Despite advances in phenotypic detection methods, results can be problematic (1, 8), so molecular evaluation of the ribosomal ITS2 region of the NYP-1 isolate was used to independently verify the species identification. PCR-restriction enzyme analysis (REA) and DNA sequence-based studies were performed utilizing the species-specific ITS2 spacer region of rRNA genes (ribosomal DNA) (5). Fragment analysis showed the NYP-1 ITS2 region to be ~300 bp, similar in size to control ITS2 DNA of *C. albicans*, *C. dubliniensis*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis*. Results showed that only the *C. albicans* ITS2-specific restriction enzyme AlwI digested the NYP-1 ITS2 region (Fig. 1). All control DNA was appropriately digested by species-specific ITS2 region restriction enzymes. Based on ITS2 region fragment and PCR-REA, the isolate NYP-1 was confirmed as *C. albicans* (Fig. 1).

Susceptibility testing guidelines for broth microdilution were followed according to NCCLS Standard M27-A (4), using the yeast 1 colorimetric panel (Trek Diagnostics, Westlake, Ohio) containing the following antifungal drugs at the indicated MIC ranges: amphotericin B, 0.008 to 16 μg/ml; FLU, 0.125 to 256 μg/ml; itraconazole, 0.008 to 16 μg/ml; ketoconazole, 0.008 to 16 μg/ml; 5-flucytosine (5-FC), 0.03 to 64 μg/ml. *C. krusei* ATCC 62258 and *C. parapsilosis* ATCC 22019 were used as quality control strains. Results showed NYP-1 to be susceptible to FLU (MIC, 0.25 μg/ml), itraconazole (MIC, ≤0.008 μg/ml), 5-FC (MIC, 0.24 μg/ml), and amphotericin B (MIC, 0.128 μg/ml). The MIC of voriconazole was reported as ≤0.125 μg/ml (data not shown).

NYP-1 was characterized for known azole resistance mech-

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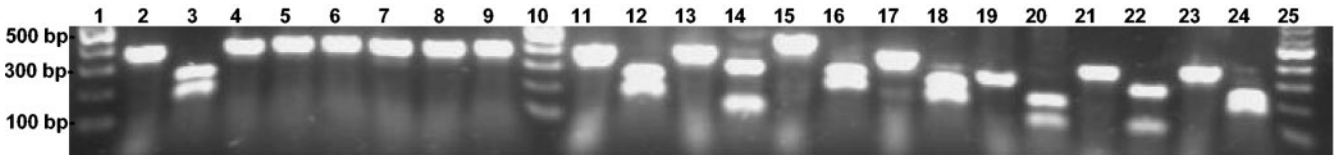

FIG. 1. NYP-1 and reference *Candida* strain ITS2 region fragment and REA. The ITS2 region was amplified by PCR with universal fungal primers ITS3 and ITS4. Lane 2, NYP-1 ITS2 PCR amplification product. The products were digested with restriction enzymes AlwI (*C. albicans* specific; lane 3), BsmAI (*C. dubliniensis* specific; lane 4), HpaI (*Candida glabrata* specific; lane 5), FspI (*C. krusei* specific; lane 6), BsmI (*Candida lusitaniae* specific; lane 7), RsaI (*C. parapsilosis* specific; lane 8), and SmaI (*C. tropicalis* specific; lane 9). The REA patterns were compared to those for known control strains. These included ATCC 90028 (*C. albicans*; ITS2 region only; lane 11), ATCC 90028 (*C. albicans*; ITS2 region plus AlwI fragment; lane 12), NCPF 3949 (*C. dubliniensis*; ITS2 region only [lane 13] and ITS2 region plus BsmAI fragment [lane 14]), ATCC 90030 (*C. glabrata*; ITS2 region only; lane 15), ATCC 90030 (*C. glabrata*; ITS2 region plus Hpa1 fragment; lane 16), ATCC 6258 (*C. krusei*; ITS2 region only; lane 17), ATCC 6258 (*C. krusei*; ITS2 region plus Fsp1 fragment; lane 18), ATCC 200950 (*C. lusitaniae*; ITS2 region only; lane 19), ATCC 200950 (*C. lusitaniae*; ITS2 region plus BsmI fragment; lane 20); ATCC 90018 (*C. parapsilosis*; ITS2 region only; lane 21), ATCC 90018; *C. parapsilosis*; ITS2 region plus RsaI fragment; lane 22); ATCC 750 (*C. tropicalis*; ITS2 region only; lane 23), and ATCC 750 (*C. tropicalis*; ITS2 region plus SmaI fragment; lane 24). Lanes 1, 10, and 25, DNA markers. The ITS2 PCR products and restriction fragments were run on a 1.2% agarose gel and stained with GelStar (FMC Bioproducts).

anisms (2). The *ERG11* gene, encoding lanosterol 14-α-demethylase, was amplified by PCR, and the DNA sequence of the ~1.5-kb coding region was determined to see if point mutations known to cause resistance were present. Azole-susceptible strain *C. albicans* ATCC 90028 was used as a control (NCCLS Standard M27-A). NYP-1 and the control strain showed a sequence identity of >99% (data not shown). Neither strain contained any point mutations that correlated with azole resistance. Molecular Beacon quantitative reverse transcription-PCR (MB QRT-PCR) was performed to probe for overexpression of *ERG11* and drug efflux pump genes *CDR1* (ABC transporter class), *CDR2*, *FLU1*, and *MDR1* as described previously (3). RNA was extracted from NYP-1 and ATCC 90028 cultures grown at 8, 24, and 48 h in the absence and presence of FLU (32 μg/ml) for MB QRT-PCR transcript analysis. Copy numbers of the known drug resistance genes relative to that of *PMA1*, a highly and constitutively expressed gene encoding the essential plasma membrane proton pump, were measured. Without the addition of FLU, no noticeable differences (defined as greater than fivefold) in *CDR1*, *CDR2*, *ERG11*, *FLU1*, and *MDR1* copies were observed for NYP-1 and ATCC 90028. However, at 8 h of FLU exposure, twofold increases in *ERG11* copy numbers were observed for NYP-1. After 24 h of FLU exposure, a 10-fold increase in *CDR1*, a multidrug efflux pump gene, and a 7-fold increase in *ERG11* were observed. At 48 h of FLU exposure, *CDR1* mRNA levels were reduced to the pre-drug-induced state, yet higher (approximately an eightfold increase) *ERG11* copy numbers were maintained. *FLU1* and *MDR1*, FLU-specific efflux pump genes (multifacilitator class), levels were not changed in both strains.

In conclusion, the patient isolate was genotypically confirmed as *C. albicans* and not *C. dubliniensis*. Despite in vitro susceptibility to fluconazole, the initial therapeutic failure with this drug may have resulted from either inadequate drug penetration to the infected eye site or drug-induced overexpression of *CDR1* and *ERG11* in NYP-1. *CDR1* and *ERG11* overexpression has been linked to resistance in other clinical strains of *C. albicans* (2, 6, 7, 9). Further research correlating genetic and phenotypic susceptibility test results with clinical outcomes is warranted.

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