A case of fluconazole, voriconazole-resistant Cryptococcus neoformans isolated from an immunocompetent patient [*V.Tullio is the corresponding author]

Mandras N; Roana J; Tullio V; Allizond V; Banche G; Scalas D; Fucale G; Cuffini AM. A case of fluconazole, voriconazole-resistant Cryptococcus neoformans isolated from an immunocompetent patient. JOURNAL OF CHEMOTHERAPY. 23 (6) pp: 379-380.
DOI: 10.1179/joc.2011.23.6.379

LETTER

A case of fluconazole, voriconazole-resistant *Cryptococcus neoformans* isolated from an immunocompetent patient.

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A healthy 22-year-old male, following an accident by a car, was admitted to CTO/CRF Hospital (Turin, Italy) and his right leg was subamputated. The patient's temperature was 39.6°C. Therapy with ticarcillin and clavulanic acid (3.2g/day/4 days) was started. Laboratory data revealed WBC count of 21.100/mm^3 with 86.4% neutrophils and 7.5% lymphocytes. Haemoglobin was 10.4g/dL and creatinine 0.83 mg/dL. HIV serotypes 1 and 2 were negative, while Hepatitis B core Antibody (HBcAb)-IgG was positive. Following two ischemic crisis at the right foot, vancomicin (500mg/day/2days) was added. Flogosis and increasing temperature were detected. A second amputation was undergoing at the proximal third leg. After two days, an infection occurred on the postsurgical wound and a *Staphylococcus capitis spp.ureolyticus* strain was detected. A therapy with meropenem (2g/day/3days) and vancomicin (1g/day/2days) was initiated. Patient became afebrile and clinical conditions improved. Therapy with meropenem was kept. After 3 weeks, the patient developed new fever (38.8°C). Three blood cultures, with automated systems (BACTEC, Becton Dickinson Diagnostic Instrument Systems, Madrid, Spain), were performed. These three blood cultures on Sabouraud dextrose agar yielded a yeast strain; the strain was isolated in pure culture and identified on CHROMagar Candida as non-*Candida albicans.*

Antifungal susceptibility was determined by Etest (Biolife,Milan,Italy) on RPMI-1640 agar supplemented with 2% glucose. The isolate was amphotericin B susceptible but fluconazole and voriconazole resistant, with following MICs: fluconazole >256 mg/L; voriconazole >32 mg/L and amphotericin B=0.75 mg/L. CLSI interpretive criteria recommended for *Candida spp*. were used [1,2]. Before biochemical strain identification, an empirical antifungal therapy with intravenous caspofungin was established by hospital clinicians. Meanwhile the yeast strain was sent to the Mycology Laboratory, Public Health and Microbiology Department, University of Turin for final identification. At Department of Public Health and Microbiology the isolate was identified by its typical microscopic morphology showing encapsulated yeast cells and by biochemical characteristics, employing the ID32C identification system (bioMérieux,Rome,Italy), as *Cryptococcus neoformans*. The variety (*C.neoformans* var.*neoformans*) was determined by the color reaction test on L-canavanine-glycine-bromothymol blue medium [1]. Fluconazole and voriconazole resistance was confirmed by disk diffusion method in accordance with CLSI guidelines [2]; caspofungin susceptibility was performed by Etest (MIC value obtained was >32 mg/L). In the absence of a susceptibility breakpoints for *Cryptococcus spp*., CLSI interpretive criteria recommended for *Candida spp*. were used [1,3]. The source of the infection was unknown; the patient was neither exposed to potential environmental sources nor

to bird feces; he had never been outside Europe and had not received fluconazole therapy. Moreover no skin lesions were noted and reported. There was no known percutaneous inoculation. In the meantime, the patient showed clinical improvement; repeated blood cultures showed no fungal growth and laboratory tests values were within a normal range.

As expected caspofungin showed no activity against *C.neoformans in vitro*, confirming literature data [3]. This agent is not adeguate in cryptococcosis, but it was administered based on CHROMagar identification before biochemical assay, because *in vitro* and *in vivo* studies have demonstrated excellent potency and efficacy of caspofungin against the Candida species [4].

Patient conditions improved probably because in an immunocompetent host the immune system is able to eliminate most of the initial number of *C.neoformans* or to maintain the yeast in a latent state [5]. In fact, *in vivo* several factors play an important role at the fungal site together with the fungicidal activity of human serum, the normal host-defence mechanisms and the immune response [4].

This case underlines that resistance may appear for new drugs like voriconazole without previous azoles exposure, although voriconazole is more potent than fluconazole *in vitro* against *C.neoformans* and strains resistant to fluconazole are generally susceptible to voriconazole [6]. *Cryptococcus spp*. rarely causes infection in immunocompetent host and *in vitro* resistance to antifungal agents like fluconazole and voriconazole remains uncommon among *C.neoformans*. The resistance to azoles initially described in patients with AIDS is becoming important in immunocompetent patients in critical conditions. It has been suggested that the widespread use of fluconazole could bring about selective pressure leading to the emergence of less-susceptible strains of *C.neoformans* [7].

This case suggests that a continuous surveillance of antifungal treatment as well as introduction of drug prescribing control is important for an accurate infection treatment, mainly when new drugs are used.

Acknowledgments

This work was supported by ARTEMIS Global Antifungal Surveillance Program

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