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

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1 **LETTER**

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

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31 A healthy 22-year-old male, following an accident by a car, was admitted to CTO/CRF Hospital (Turin,
32 Italy) and his right leg was subamputated. The patient's temperature was 39.6°C. Therapy with
33 ticarcillin and clavulanic acid (3.2g/day/4 days) was started. Laboratory data revealed WBC count of
34 21.100/mm³ with 86.4% neutrophils and 7.5% lymphocytes. Haemoglobin was 10.4g/dL and creatinine
35 0.83 mg/dL. HIV serotypes 1 and 2 were negative, while Hepatitis B core Antibody (HBcAb)-IgG was
36 positive. Following two ischemic crisis at the right foot, vancomycin (500mg/day/2days) was added.
37 Flogosis and increasing temperature were detected. A second amputation was undergoing at the
38 proximal third leg. After two days, an infection occurred on the postsurgical wound and a
39 *Staphylococcus capitis spp.ureolyticus* strain was detected. A therapy with meropenem (2g/day/3days)
40 and vancomycin (1g/day/2days) was initiated. Patient became afebrile and clinical conditions improved.
41 Therapy with meropenem was kept. After 3 weeks, the patient developed new fever (38.8°C). Three
42 blood cultures, with automated systems (BACTEC, Becton Dickinson Diagnostic Instrument Systems,
43 Madrid, Spain), were performed. These three blood cultures on Sabouraud dextrose agar yielded a
44 yeast strain; the strain was isolated in pure culture and identified on CHROMagar Candida as non-
45 *Candida albicans*.

46 Antifungal susceptibility was determined by Etest (Biolife,Milan,Italy) on RPMI-1640 agar
47 supplemented with 2% glucose. The isolate was amphotericin B susceptible but fluconazole and
48 voriconazole resistant, with following MICs: fluconazole >256 mg/L; voriconazole >32 mg/L and
49 amphotericin B=0.75 mg/L. CLSI interpretive criteria recommended for *Candida spp.* were used ^{1,21}.
50 Before biochemical strain identification, an empirical antifungal therapy with intravenous caspofungin
51 was established by hospital clinicians. Meanwhile the yeast strain was sent to the Mycology
52 Laboratory, Public Health and Microbiology Department, University of Turin for final identification.
53 At Department of Public Health and Microbiology the isolate was identified by its typical microscopic
54 morphology showing encapsulated yeast cells and by biochemical characteristics, employing the
55 ID32C identification system (bioMérieux,Rome,Italy), as *Cryptococcus neoformans*. The variety
56 (*C.neoformans* var.*neoformans*) was determined by the color reaction test on L-canavanine-glycine-
57 bromothymol blue medium ⁴². Fluconazole and voriconazole resistance was confirmed by disk
58 diffusion method in accordance with CLSI guidelines ²¹; caspofungin susceptibility was performed by
59 Etest (MIC value obtained was >32 mg/L). In the absence of a susceptibility breakpoints for
60 *Cryptococcus spp.*, CLSI interpretive criteria recommended for *Candida spp.* were used ^{12,3}. The source
61 of the infection was unknown; the patient was neither exposed to potential environmental sources nor

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

66 As expected caspofungin showed no activity against *C.neoformans in vitro*, confirming literature data ³.

67 This agent is not adequate in cryptococcosis, but it was administered based on CHROMagar
68 identification before biochemical assay, because *in vitro* and *in vivo* studies have demonstrated
69 excellent potency and efficacy of caspofungin against the Candida species ⁴.

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

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

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

84

85 **Acknowledgments**

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