

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

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

**This is the author's manuscript**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/98007> since 2020-08-31T12:44:47Z

*Published version:*

DOI:10.1179/joc.2011.23.6.379

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

This is the author's final version of the contribution published as:

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

The publisher's version is available at:

<http://www.tandfonline.com/doi/full/10.1179/joc.2011.23.6.379>

When citing, please refer to the published version.

Link to this full text:

<http://hdl.handle.net/2318/98007>

1 **LETTER**

2  
3 **A case of fluconazole, voriconazole-resistant *Cryptococcus neoformans* isolated**  
4 **from an immunocompetent patient.**

5  
6 **N. MANDRAS - J. ROANA -V. TULLIO - V. ALLIZOND - G. BANCHE - D. SCALAS - G.**  
7 **FUCALE<sup>1</sup> - A. M. CUFFINI.**

8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21 Department of Public Health and Microbiology, Microbiology Section, University of Turin, Italy.

22 <sup>1</sup>Analysis Laboratory and Microbiology, C.T.O./C.R.F. Hospital, Turin, Italy

23  
24  
25 *Correspondence:* Vivian Tullio, PhD, Professor of Microbiology, Department of Public Health and  
26 Microbiology, Microbiology Section, University of Turin, Via Santena 9, 10126 Turin, Italy. Tel  
27 +390116705637; Fax +390112365637. E-mail: vivian.tullio@unito.it

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/mm<sup>3</sup> 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 <sup>4,21</sup>.  
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 <sup>42</sup>. Fluconazole and voriconazole resistance was confirmed by disk  
58 diffusion method in accordance with CLSI guidelines <sup>21</sup>; 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 <sup>12,3</sup>. The source  
61 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 <sup>3</sup>.

This agent is not adequate 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 <sup>4</sup>.

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 <sup>5</sup>. 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 <sup>4</sup>.

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 <sup>6</sup>. *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* <sup>7</sup>.

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

## References

<sup>21</sup>Clinical and Laboratory Standards Institute (CLSI). Method for antifungal disk diffusion susceptibility testing of yeasts; approved guideline 2004; M44-A 2004. Wayne, PA, USA.

91 <sup>42</sup>McTaggart L, Richardson SE, Seah C, Hoang L, Fothergill A, Zhang SX. Rapid Identification of  
92 *Cryptococcus neoformans* var. *grubii*, *C. neoformans* var. *neoformans*, and *C. gattii* by use of rapid  
93 biochemical tests, differential media, and DNA sequencing. J Clin Microbiol 2011;49(7):2522-2527.

94 <sup>3</sup>Espinel-Ingroff A, Canton E, Gibbs D, Wang A. Correlation of Neo-Sensitabs tablet diffusion assay  
95 results on three different agar media with CLSI broth microdilution M27-A2 and disk diffusion M44-A  
96 results for testing susceptibilities of *Candida spp.* and *Cryptococcus neoformans* to amphotericin B,  
97 caspofungin, fluconazole, itraconazole, and voriconazole. J Clin Microbiol 2007;45(3):858-864.

98 <sup>4</sup>Brzankalski GE, Najvar LK, Wiederhold NP, Bocanegra R, Fothergill AW, Rinaldi MG et al.  
99 Evaluation of aminocandin and caspofungin against *Candida glabrata* including isolates with reduced  
100 caspofungin susceptibility. J Antimicrob Chemother 2008;62(5):1094-1100.

101 <sup>5</sup>Voelz K, May RC. Cryptococcal interactions with the host immune system. Eukaryot Cell 2010;9(6):  
102 835-846.

103 <sup>6</sup>Johnson E, Espinel-Ingroff A, Szekely A, Hockey H, Troke P. Activity of voriconazole, itraconazole,  
104 fluconazole and amphotericin B *in vitro* against 1763 yeasts from 472 patients in the voriconazole  
105 phase III clinical studies. Int J Antimicrob Agents 2008;32(6):511-514.

106 <sup>7</sup>Pfaller MA, Messer SA, Boyken L, Rice C, Tendolkar S, Hollis RJ et al. Global trends in the  
107 antifungal susceptibility of *Cryptococcus neoformans* (1990 to 2004). J Clin Microbiol  
108 2005;43(5):2163-2167.