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Fungal diagnosis: from culture to molecular techniques

Clinical characteristics, laboratory identification, and in vitro antifungal susceptibility of *Trichomonascus (Candida) ciferrii* isolates associated with granular myringitis

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Background: *Trichomonascus (Candida) ciferrii* is an emerging opportunistic yeast pathogen that has been increasingly recognised in human infections. *T. ciferrii* has previously been implicated as the cause of tinea pedis, tinea cruris, onychomycosis, and less commonly, fungaemia. Recently, we diagnosed four unusual cases of *T. ciferrii*-associated granular myringitis in Chinese patients in Hong Kong and Shenzhen, China. In this study, we report the clinical characteristics, laboratory identification, and *in vitro* antifungal susceptibility of *T. ciferrii* isolates associated with granular myringitis.

Material/methods: The case records of the patients with *T. ciferrii*-associated granular myringitis were reviewed. Their clinical characteristics and outcome were recorded into a predefined database. We compared the performance of Vitek 2 YST ID Card system, API 20C AUX, and MALDI-TOF MS for laboratory identification of the fungal isolates. We performed internal transcribed spacer (ITS) and 25S nrDNA gene sequencing for genotypic identification. Antifungal susceptibility testing was performed using the Sensititre YeastOne kit.

Results: A total of four patients with *T. ciferrii*-associated granular myringitis were seen at the Queen Mary Hospital and HKU-Shenzhen hospital between 1 January 2015 to 30 June 2015. There were 3 males and 1 female. Their mean age was 38 years (range, 33-42 years). Three patients had no comorbidities and the remaining patient had nasopharyngeal carcinoma, diabetes mellitus, and hyperlipidemia. Their presenting symptoms included obstructive sensation and/or pruritis in their ears, pustular otorrhoea, and otalgia. They all responded to irrigation and topical antifungal (azole) cream. Swabs of the tympanic membrane yielded fungal colonies on Sabouraud agar on day 3 of incubation at 37 °C. Although Vitex 2 YST ID Card system, API 20C AUX, and MALDI-TOF MS all reported the identification of "*T. ciferrii*", none of them contained information on all three species of the *T. ciferrii* complex (namely, *T. ciferrii*, *C. allociferrii*, and *C. mucifera*) in their databases. Therefore, a more appropriate identification result should be "*T. ciferrii* complex". ITS and 25S nrDNA gene sequencing accurately identified all the fungal isolates. All the isolates were susceptible to itraconazole, voriconazole, posaconazole, caspofungin, micafungin, and anidulafungin, but were resistant to fluconazole, amphotericin B, and flucytosine *in vitro*.

Conclusions: *T. ciferrii* may be associated with granular myringitis in patients with or without comorbidities. Vitek 2 YST ID Card system, API 20C AUX, and MALDI-TOF MS can identify *T. ciferrii* complex, but not speciation within the complex. Response to treatment with irrigation and topical antifungal cream are generally good. Antifungal susceptibility tests may aid in optimization of treatment choice.