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Candida orthopsilosis and *Aureobasidium pullulans*: Rare Fungal Pathogens Causing Persistent Skin Infection

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Abstract: Rare fungal human skin pathogens, identified as *Candida orthopsilosis* and *Aureobasidium pullulans* were co-isolated from patient with persistant cutaneous skin infection in Singapore. This significant discovery offers knowledge to the scientific and medical community in order to stimulate research interest and to address treatment regimes of fungal infection.

Key words: Skin infection, *Candida orthopsilosis*, *Aureobasidium pullulans*, anphotericin B, primary aldosteronism

In recent years, cutaneous fungal infections are believed to affect 20-25% of the world's population and their incidences continue to increase (Ameen, 2010). Clinical fungal infections are divided into four types which are superficial, cutaneous, subcutaneous and systemic (Schwartz, 2004). We present here the case of a 38-year-old male hypertensive patient from National University Hospital of Singapore with persistent cutaneous infection on his skin. The patient had been treated for more than three years with various types of antifungal medications, either prescribed or non-prescribed from various medical practitioners and clinics. Despite the treatment, the patient did not show sign of recovery. The persistent localized cutaneous fungal infection resulted in skin at the infected area of the limbs to harden, dry up and crack (Fig. 1a, b). The patient also experiences deep itching sensation. Interestingly, this patient also suffers from primary aldosteronism, hypertension and hypertriglyceridemia for more than three years. He is currently being treated with spironolactone, metformin hydrochloride, atenolol, simvastatin and amlodipine besilate.

The infected area was swabbed with alcohol and the skin of foot and finger were scrapped and inoculated onto Potato-Dextrose-Agar (PDA). The plates were incubated overnight at 30°C for 5 days. Fungal strains, designated AY2 and AY4, shown in Fig. 1c and d were isolated and there colony morphology was examined. These isolates were characterized by 18S rRNA gene identification. The 18S rRNA gene of 1.8 kb were amplified and sequenced using NS1 and NS8 primers (White *et al*., 1990). The 18S rRNA gene sequences are available in GenBank database under accession numbers HQ215535 and HQ215536, respectively. The sequences were aligned with sequences from related fungi available from GenBank database by using ClustalW. MEGA version 4.1 (Beta 3) was used for construction of Neighbor-Joining phylogenetic tree with bootstrap values calculated based on 1000 replicates. The fungal strains were identified as *Candida orthopsilosis* and *Aureobasidium pullulans*.

Candida orthopsilosis is a new species of pathogenic yeast from genus *Candida*. *C. orthopsilosis* was recently identified in 2005 and is a new designation for *C. parapsilosis* Group II (Tavanti *et al*., 2005; Yong *et al*., 2008). In Malaysia, this species has been isolated from bloodstream of two leukaemic patients and from a pediatrics unit (Lockhart *et al*., 2008; Yong *et al*., 2008). *Candida orthopsilosis* has been clinically prevalent and was recovered from nails, skin, lung, urine, catheter, blood, sputum, bronchial aspirate and wound (Gomez-Lopez *et al*., 2008; Tavanti *et al*., 2007). Gacser *et al*. (2007) reported on vilrulence of *C. orthopsilosis* on reconstituted human tissue models that revealed severe attenuation, morphological changes and cellular damage (Gacser *et al*., 2007). Some *C. orthopsilosis* strains were reported on their biofilm-forming ability on silicone elastomer discs (Lattif *et al*., 2010). Former studies on the pathogenic significance of *C. orthopsilosis* remain inconclusive as the classification was probably under *C. parapsilosis*. *C. orthopsilosis* is

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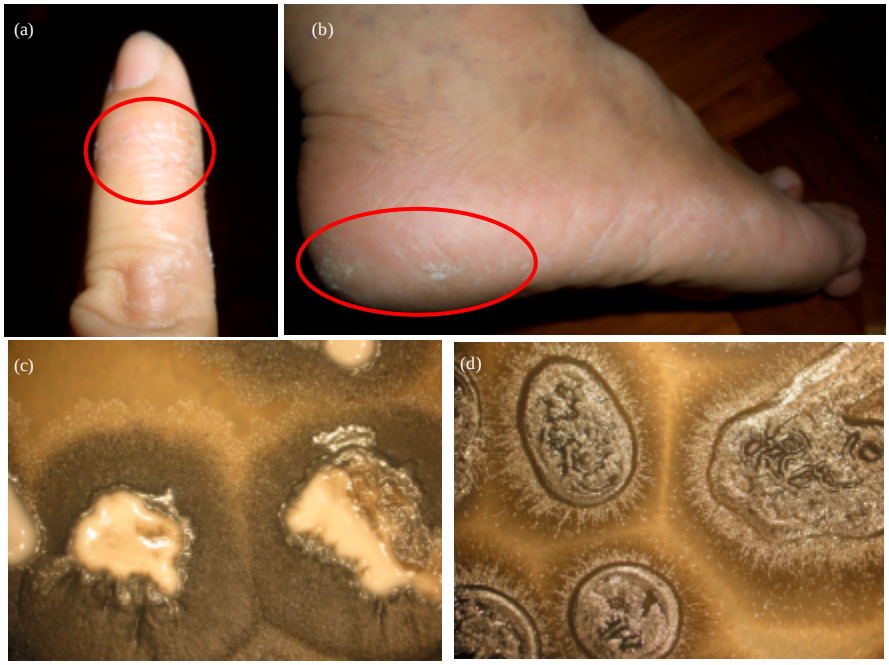

Fig. 1: Skin lesion of fungal infection on (a) finger and (b) foot. Colony morphology of fungal strains grown on potato-dextrose-agar: (c) *Candida orthopsilosis* and (d) *Aureobasidium pullulans*

susceptible to amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, ravuconazole, posaconazole, caspofungin, micafungin and anidulafungin (Canton *et al*., 2010; Gomez-Lopez *et al*., 2008; Lockhart *et al*., 2008; Tavanti *et al*., 2007).

A. pullulans is a dematiaceous yeast-like fungus, which is popularly known as black yeast due to its melanin production (Chi *et al*., 2009). It could be categorized into three distinctive forms, namely elongated branched septate filaments, large chlamydospores and smaller elliptical yeast-like cells. The colour of its colony progresses from yellow, cream, light pink, or light brown to blackish at a later stage due to chlamydospore production (Chi *et al*., 2009). *A. pullulans* is well reported for various biotechnological applications such as production of pullulans, extracellular polysaccharide and hydrolytic enzymes including amylases, proteases, esterases, pectinases, xylanases and mannanases (Chi *et al*., 2009; Ravella *et al*., 2010; Rumbold *et al*., 2003). Various strains of *A. pullulans* were mainly isolated from soil, plants, wood, damp indoor surface and indoor air environment (Hawkes *et al*., 2005; Joshi *et al*., 2010; Prasongsuk *et al*., 2005).

A. pullulans has been reported to cause nosocomial infection, abscess in the spleen, invasive pulmonary infection, fungemia, peritonitis (among patients on peritoneal dialysis), pneumonia, meningitis, corneal ulcer, catheter-related septicemia and scleral infection (Bolignano and Criseo, 2003; Clark *et al*., 1995; Hawkes *et al*., 2005; Huang *et al*., 2008; Jones and Christensen, 1974; Salkin *et al*., 1986). Though regarded as rare cause of cutaneous infection in human, pathological significance of *A. pullulans* has been reported lately (Joshi *et al*., 2010; Pikazis *et al*., 2009). However, certain *A. pullulans* are considered to be of low virulence, when isolated from skin scrapping of healthy individuals. According to the 5-year review of 556 dematiaceous hyphomycetes, 75 isolates were *Aureobasidium* spp. and most of these (91%) are unlikely to be pathogenic (Pritchard and Muir, 1987). Until today, there is no standard treatment of infection caused by *A. pullulans* (Hawkes *et al*., 2005; Joshi *et al*., 2010). Amphotericin B alone and a combination with other drugs had been used with variable success (Clark *et al*., 1995; Hawkes *et al*., 2005; Huang *et al*., 2008; Joshi *et al*., 2010; Pikazis *et al*., 2009).

To the best of our knowledge, this is the first report of *C. orthopsilosis* isolated from fungal infection in Singapore and the co-infection of *C. orthopsilosis* and *A. pullulans* on skin of patient suffering from primary aldosteronism. Skin infection by individual strain of either *C. orthopsilosis* or *A. pullulans* has been reported. Extensive literature search reveals no human case of infection caused by both of these fungal strains on any

patient group. To date, there is only a report by Ravella *et al*. (2010) in which *C. orthopsilosis* and *A. pullulans* were among the isolates from laboratory scale biogas reactors. Is such partnership of *C. orthopsilosis* and *A. pullulans* common in nature and in the pathogenesis of infection? Does this partnership enhance its virulence in skin infection? Does the infection by these fungal strains affect the prognosis of patient with primary aldosteronism? What are the appropriate drugs to be used for antifungal treatment on patient with primary aldosteronism? These questions remain to be answered.

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