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Malassezia pachydermatis fungemia in a preterm neonate resistant to fluconazole and flucytosine

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ABSTRACT

A case of *Malassezia pachydermatis* fungemia in a preterm neonate is described. The isolate was identified by rDNA sequencing and was resistant to fluconazole and flucytosine. Since *M. pachydermatis* does not require lipid supplementation for growth, it can be misidentified as a *Candida* species. The report highlights *M. pachydermatis* as a cause of late onset sepsis in preterm neonates and emphasizes the need for prior antifungal susceptibility testing.

1. Introduction

The genus *Malassezia*, currently comprises 14 lipophilic yeast species [1–3]. Among them, *M. pachydermatis* is uniquely placed, primarily being a zoopathogen with ability to grow without oil enrichment. The species forms normal microbiota of the skin and ear canal of dogs, cats, and other canines, where it causes dermatitis and otitis externa [4,5]. *M. pachyderamtis* has also been isolated from healthy and diseased human skin [6], whereas in neonates, it is associated with fungemia [7–9]. Here, we describe a case of *M. pachydermatis* fungemia in a pre-term neonate.

2. Case

At 26 weeks of gestation, a baby boy, weighing 710 g, was delivered by emergency caesarian section to a 30-year-old Egyptian female. On day 0 (day of birth), the baby had symptoms of respiratory distress syndrome, although the mother was on oral steroids. His Apgar score was 5 at one minute after birth and 9 after 5 min. The mother had previous history of multiple abortions and neonatal deaths. The baby was admitted to Neonatal Intensive Care Unit (NICU) and put on ventilator and umbilical venous catheter was inserted for giving total parenteral nutrition (TPN). Since he had abdominal distension, a vacuum-assisted closure device was also used. His cardiovascular and central nervous systems were functioning normally. Although there were no positive blood cultures, the baby was prescribed ampicillin and amikacin for 7 days to prevent sepsis due to necrotizing enterocolitis. On day 18, as the baby became febrile, the treatment was switched to Tazocin® (piperacillin and tazobactam) for 10 days. Blood culture taken on day 22, however, grew a yeast (isolate no. Kw1247/13) in BACTEC blood culture bottle after nearly 5 days of incubation. Consequently, on day 26, the baby was started on liposomal amphotericin B (Abelcet). The subsequent blood cultures taken on days 30 and 33 after birth became negative for the yeast and the patient recovered completely and was discharged.

The subculture from BACTEC blood culture bottles yielded slow-growing, cream-colored colonies on Sabouraud dextrose agar (SDA) and blood agar without lipid supplementation. On SDA at 30 °C, colonies were convex, cream-colored, 2–3 mm in diameter with entire margins. Microscopically, yeast cells were ovoid to elongated with monopolar budding, measuring 4–6 μm × 2.8–4 μm in size. No hyphal forms were observed. The identity of the isolate was determined as *Malassezia furfur* by Vitek 2 Yeast identification system (bioMeriux, France) with 98% probability (excellent identification, bionumber 4000100002000100). Since the isolate grew without lipid enrichment, it required molecular identification. Sequencing of the ITS and D1/D2 regions of rDNA was carried out according to previously described methods [10,11] to confirm the identity of the isolate (Kw1247/13). The DNA sequencing data for the D1/D2 region showed only three nucleotide differences with reference strain *M. pachydermatis* CBS1879. Based on the observations that conspecific strains exhibit < 1% sequence difference in the D1/D2 region of 28S rRNA, the identity of the isolate was established as *M. pachydermatis* [11,12]. The

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Table 1
Antifungal susceptibility of *M. pachydermatis* strain by Etest.

Medium	Minimum inhibitory concentrations (μg/ml) read at 48 h					
	AP	VO	POS	FL	FC	CS
RPMI medium 1640[a]	0.19	0.012	0.016	≥256	≥32	≥32
Sabouraud dextrose Agar	0.25	0.19	0.016	≥256	≥32	≥32

[a] Supplemented with 2% glucose and overlaid with olive oil. Abbreviations: AP—amphotericin B, VO—voriconazole, POS—posaconazole, FL—fluconazole, FC—flucytosine, CS—caspofungin.

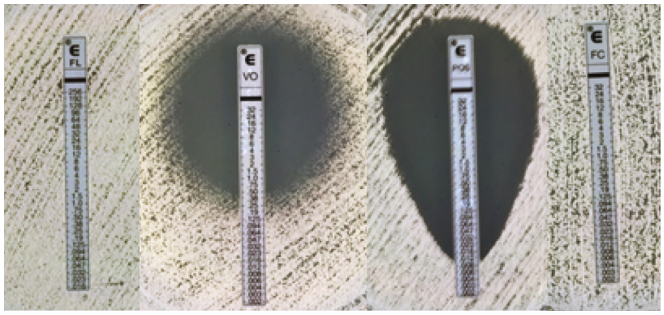

Fig. 1. EtestS on Sabouraud dextrose agar showing minimum inhibitory concentrations for fluconazole (FL, ≥256 μg/ml), voriconazole (VO, 0.012 μg/ml), posaconazole (POS, 0.016 μg/ml) and flucytosine (FC, ≥32 μg/ml) read after 48 h of incubation at 35 °C.

identity of the isolates was also confirmed by matrix-assisted laser desorption and ionization–time-of-flight mass spectrometry (MALDI–TOF MS; bioMeriuex) as *M. pachydermatis* with 99.9% confidence value.

Minimum inhibitory concentrations (MICs) for antifungal drugs were determined by Etest on RPMI 1640 medium supplemented with 2% glucose as described previously [10] after swabbing the surface with olive oil. The MICs read at 48 h of incubation at 35 °C and were scored as susceptible to amphotericin B, 0.19 μg/ml; voriconazole, 0.012 μg/ml and posaconazole, 0.016 μg/ml; but showed reduced susceptibility (resistance) to fluconazole, ≥256 μg/ml; flucytosine, ≥32 μg/ml; and caspofungin, ≥32 μg/ml (Table 1, Fig. 1).

3. Discussion

Malassezia spp. are known etiologic agents of pityriasis versicolor and are normally present on skin without clinical manifestations [1–3]. However, in view of their close association with human skin, *M. furfur* and some other species of the genus may cause systemic infection, particularly in severely ill patients receiving TPN with lipid enrichment [3]. Although, *M. pachydermatis* does not require lipid supplementation for growth, it is lipophilic and like other members of the genus, has the potential to cause systemic infection in preterm neonates and adults [1,8,13,14]. There are several reports of *M. pachydermatis* fungemia in preterm, low birth-weight neonates, who received TPN through central venous catheter [7–9]. The other risk factors associated with *M. pachydermatis* fungemia include increased median neonatal acute physiology score, >9 days of arterial catheterization and contact with healthcare staff harboring the organism [15].

Although *M. pachydermatis* is primarily a zoopathogen, there is evidence to suggest that *M. pachydermatis* strains may be introduced in NICU through the hands of health care staff who own dogs [15]. In a recent study, using PCR-based method, 93% of dog owners had *M. pachydermatis* carriage on their hands [16]. We have not investigated the source of origin of our isolate. In Kuwait, population density of dogs is low and keeping them as pets is rare, whereas domestic and stray cats are not uncommon. A recent study from north-west India (Punjab) reported that about 5% of the isolates of *M. pachydermatis* were obtained from the back of healthy individuals without evidence of pityriasis versicolor [17]. This observation is noteworthy as it suggests that *M. pachdermatis* fungemia may also have similar mode of acquisition as for candidemia.

Because of the ability of *M. pachydermatis* to grow without oil enrichment, it can be misidentified as a *Candida* species, which may lead to inappropriate antifungal therapy, particularly when *Malessezia* spp. are known to be intrinsically resistant to echinocandins. As observed in our isolate, most strains of *M. pachydermatis* exhibit reduced susceptibility to fluconazole and flucytosine [7,18–23]. However, in contrast to some other studies, where cross-resistance between azoles has been reported among veterinary strains [19,22,23], our isolate appeared susceptible to voriconazole and posaconazole (Table 1).

A limitation of the study is that we determined MICs by Etest and not by CLSI microdilution method, although both the tests have shown high categorical agreement [18]. Our isolate showed suboptimal growth on RPMI 1640 medium even with lipid supplementation, hence MIC readings were taken after 48 h [24]. Since our isolate did not grow on Mueller–Hinton medium (with or without oil supplementation), MICs were also determined on SDA for confirming results (Table 1). Optimal conditions for determining susceptibility and antifungal breakpoints for *Malassezia* spp. are not yet established.

In conclusion, a case of *M. pachydermatis* fungemia is described. The identity of the isolate was confirmed by sequencing of rDNA. The isolate was resistant to fluconazole and flucytosine. The report highlights the need of species-specific identification and prior antifungal susceptibility testing for appropriate management. This appears to be the first report of *M. pachydermatis* fungemia from Kuwait and the Middle East.

Nucleotide sequence accession numbers

Sequences obtained for the ITS and D1/D2 regions of rDNA of our isolate were deposited in GenBank under accession numbers HG529981 and HG532009, respectively.

Conflict of interest

There are none.

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