NEONATAL SEPTICAEMIA IN A PREMATURE INFANT DUE TO CANDIDA DUBLINIENSIS

Candida dubliniensis is a recently described species that shares many features with Candida albicans. There are very few reports of isolation of this species from bloodstream in adults and paediatric population. Here we report a case of neonatal septicaemia produced by C. dubliniensis in a premature infant admitted to neonatal intensive care unit. The preterm male neonate with a gestational age of 30 weeks and a birth weight of 1.2 kg presented with respiratory distress syndrome for which mechanical ventilation was provided. In spite of receiving antibiotics, the patient developed fever. C.dubliniensis was repeatedly isolated from the blood culture of the patient collected aseptically from different sites. The patient was successfully treated with amphotericin B.

**Key words:** Candida dubliniensis, neonatal septicaemia, blood culture

Candida spp. are known to cause neonatal sepsis particularly in premature infants. Most of these cases are produced by Candida albicans followed by other non albicans species such as Candida tropicalis, Candida glabrata and Candida parapsilosis. Candida dubliniensis has emerged as a new pathogen prevalent in HIV population, causing oral lesions and bloodstream infections. Many times identification of this species is missed, as it shares many features with C. albicans. In clinical microbiology, this organism has not been known to be significant pathogen in paediatric cases till recently. However, now this species has been reported from paediatric population. In the case presented here we have isolated...
C. dubliniensis from blood stream of a premature infant suffering from neonatal sepsis. To the best of our knowledge, this is first of such reports from India.

Case Report

A male preterm baby of 30 weeks of gestational age was born on 17th June 2007 to gravida 2, para 1 mother on by spontaneous vaginal delivery. His birth weight was 1.2 kg, length 36 cm, head circumference 26 cm. Soon after birth, the baby developed respiratory distress needing mechanical ventilation. Venous catheterisation was performed and antibiotics (gentamicin and ampicillin) were started. The patient was febrile. There was no cyanosis and the abdomen was soft on palpation with no palpable enlargement of the liver and spleen. On the sixth day the patient became tachypnoeic and pale. His haematological profile revealed low haematocrit with normal blood cells and platelet counts. CSF findings revealed increased WBC counts of 120 cells/mm³, high protein of 200 mg/dL and normal sugar. Bacterial culture of CSF, urine and gastric lavage did not grow any organisms. Blood (2 mL) was collected in brain heart infusion broth under aseptic precautions and was transported to the microbiology laboratory as such. It was incubated for 48 hours at 37°C. After 48 hours, subcultures were done on blood agar, chocolate agar, MacConkey’s agar and Sabouraud dextrose agar with antibiotics. On blood agar, chocolate agar and Sabouraud dextrose agar white opaque colonies were obtained. Secondary smears were prepared from colonies on blood agar, chocolate agar and Sabouraud dextrose agar, which showed budding yeast cells, on the basis of which a provisional diagnosis of sepsicaemia due to Candida spp. was made. Repeated blood cultures were performed under aseptic precautions from different arms, which showed similar findings. The patient was started on intravenous amphotericin B initially at 0.5 mg/kg and gradually increased to 1 mg/kg within one week to which the baby responded well with the subsidence of fever.

In the laboratory further tests were performed for identification. The organism showed a positive germ tube test and growth on cornmeal agar after 48 hours revealed blastoconidia, pseudohyphae and plenty of chlamydospores. Hence we suspected the species as either C. albicans or C. dubliniensis as both may show similar findings on germ tube test and cornmeal agar. We further identified the strain as C. dubliniensis by testing growth at 45°C (Fig.) xylose assimilation (Fig. 1) test[5] growth pattern on cornmeal agar[6] and tobacco agar.[7] The isolate did not grow at 45°C and xylose was not assimilated. The growth on tobacco agar[7] showed peripheral fringes, microspores, pseudohyphae, blastoconidia and plenty of chlamydospores, which were arranged in clusters. We also tested chlamydospores formation on cornmeal agar plates[6] by streaking inoculum and putting coverslips to create microaerophilic conditions. One was incubated at room temperature and the other at 30°C. It was observed that the strain produced colonies with abundant chlamydospores (more than one terminal chlamydospore). The filaments carrying chlamydospore appeared shorter. It produced chlamydospores at room temperature as well as at 30°C, even on the plate without coverslip (microaerophilic conditions). Based on these phenotypic methods the isolate was identified as C. dubliniensis.

Discussion

Candida spp. are capable of causing both local and systemic infections. The incidence of candidaemia has increased over several decades. Candidaemia usually occurs at the rate of 0.5-1% cases per 1000 patient admissions of neonatal intensive care units. Candida albicans is the species most commonly isolated from clinical materials and accounts for 50-70% of cases of invasive candidiasis, followed by C. parapsilosis, C. tropicalis and C. glabrata.[1]

Neonatal sepsis occurs in the presence of several predisposing factors such as long-term administration of antibiotics, low birth weight, prematurity, intravenous hyperalimentation, patients on ventilation and endotracheal intubation.

In the present report, prematurity, low birth weight and ventilation were probably the risk factors involved. Prematurity is a very important risk factor for neonatal candidal sepsis. The incidence of candidaemia in very low birth weight infants (<1500g) is reported to be 3-5%. Prematurity acts as risk factor because of immature immune system, which mainly involves T cells and neutrophils.

Before 1995, all germ tube test positive Candida spp. were identified as C. albicans. In 1995, a second germ tube positive Candida spp., C. dubliniensis was reported to have colonized oral cavity and caused oral candidiasis in HIV positive patients in Ireland.[2] It appears to be a minor component of normal flora of human beings. Fewer than 10% of germ tube positive cases in culture collections or
from oral cavity of healthy individuals have been identified as *C. dubliniensis.* The organism was isolated from from blood, urine, bile, synovial fluid, sputum, bronchial washings and abscess fluids apart from oral cavity.\[4\]

There have been small case series of reports of paediatric *C. dubliniensis* in immunocompromised hosts. These patients were undergoing chemotherapy for malignancy or were infected with HIV.\[3,4\] The data in immunocompromised patients were undergoing chemotherapy for malignancy or *C. dubliniensis* in immunocompromised hosts. These from oral cavity of healthy individuals have been identified as *C. albicans* in absence of other tests required for identification.

In this case we repeatedly isolated and identified it from blood cultures, the sample being collected aseptically from different arms at different intervals, on several occasions, ruling out contamination or colonization of the blood sampling site. The clinical correlation also showed that following the isolation of *C. dubliniensis,* amphotericin B was given to the patient, who responded well with the subsidence of fever. This signifies the association of *C. dubliniensis* isolate with neonatal septicaemia, which may be in part due to misreporting of the strains as *C. albicans* in absence of other tests required for identification.

In our patient we could not find the source of infection. *C. dubliniensis* is reported as part of normal flora of gut and break in mucosal barriers is reported as one of the sources of candidaemia. Probably all the predisposing factors had aided in the colonization of the gut with the strain, from where the spill over occurred into the blood stream leading to septicaemia.

*C. dubliniensis* is differentiated in the laboratory by phenotypic tests i.e., testing growth at high temperature (45°C), xylose assimilation and patterns of chlamydospores formation.

In recent years the chlamydospore formation has also been used as a method to distinguish the two closely related species *C. albicans* and *C. dubliniensis.* *C. dubliniensis* produces two or three chlamydospores on the tip of longer hyphae or pseudohyphal forms. *C. dubliniensis* forms rough colonies with mycelia and abundant chlamydospores on Staib agar (colonies have peripheral fringes) where as *C. albicans* forms smooth colonies. Staibs agar and tobacco agar are widely used for identification of *Cryptococcus neoformans,* any further applicability of this medium appears to be of clinical diagnostic value and epidemiological value. Staib agar\[6\] has been replaced by sunflower seed agar, tobacco agar and tomato juice agar. In addition, incubation at room temperature might be a specific requirement for many of the conditions, which have been documented to favour species specific chlamydospores induction in *C. dubliniensis.*\[6\]

We found that *C. dubliniensis* produced multiple chlamydospores on corn meal agar even without coverslip and also on tobacco agar it produced rough colonies with peripheral fringes. The lactophenol cotton blue (LPCB) preparation from tobacco agar showed clusters of chlamydospores [Fig. 2].

It has been demonstrated that *C. dubliniensis* is less virulent than *C. albicans* in healthy mice suggesting that *C. dubliniensis* is more susceptible to phagocytic action of neutrophils. This may be one of the reasons for lower incidence of *C. dubliniensis* infection than *C. albicans.* The majority of *C. dubliniensis* isolates have been susceptible in vitro to commonly used antifungal agents. However, isolates with reduced susceptibility to flucanazole have been reported. The isolates were sensitive to amphotericin B to which our patient also responded.

Thus, *C. dubliniensis* is an emerging opportunistic fungal pathogen that can cause invasive disease in patients with a variety of clinical conditions, including cancer, HIV infection, neonatal septicaemia etc. It appears that clinical spectrum of *C. dubliniensis* resembles that of *C. albicans.* However the in vitro studies have shown that *C. dubliniensis* may be able to develop resistance to azoles. Further studies need to be conducted to find out the pathogenicity, clinical spectrum, outcome and resistance in patients with *C. dubliniensis.* Every laboratory should keep in mind that, all germ tube positive *Candida* spp. are not *C. albicans.*
References


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