CASE REPORT: *Trichoderma longibrachiatum*
INFECTIONS IN A PEDIATRIC PATIENT WITH PERITONEAL DIALYSIS

(Reporte de caso: Infección por *Trichoderma longibrachiatum* en un paciente pediátrico en peritoneodílisis)

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Key words: *Trichoderma longibrachiatum*, pediatric infections, peritoneal dialysis.

ABSTRACT

A case report of an intrahospitalary *Trichoderma longibrachiatum* peritonitis in a female 13-year-old pediatric patient who had been previously diagnosed a terminal chronic renal insufficiency while being under peritoneal dialysis is presented. Nowadays literature recognizes certain members of the genus *Trichoderma* to be opportunistic pathogens of increasing occurrence in immunocompromised patients. The above girl received a satisfactory Amphotericin B treatment yet she died due to an hypertensive emergency appearing secondarily to her basic pathology.

Increase of fungal infections in immunocompromised patients continues being higher in frequencies as well as in variety at the early stage of our 21 century (Santos et al., 2004; Richter et al., 1999; Rex et al., 1998; Guarro et al., 2003; PengNg et al., 2003). The internist during his routine clinic work has not only faced the presence of unknown exogenous agents but has also to improvise many times in an empirical way their treatment with available antifungal drugs, failing to know generally the sensibilities of these new agents in their field of work (Steinbach & Perfect, 2003). Several members of the genus *Trichoderma*, a filamentous ascomycete microfungus bearing a wide cosmopolitan distribution and which rarely causes localized or disseminated infections in man, have become more frequently involved in serious clinic cases (Muñoz et al., 1997; Guarro et al., 1999; Richter et al., 1999; Chouaki et al., 2002) include most of cases in literature. *Trichoderma longibrachiatum* is the most commonly involved in these infections, especially responsible for continuous ambulatory or hospitalary peritoneal dialysis or invasive infections in immunocompromised patients.

RESUMEN

Se reporta un caso clínico de peritonitis intrahospitalaria por *Trichoderma longibrachiatum* en una paciente pediátrica de 13 años de edad, con antecedentes de insuficiencia renal crónica terminal, en tratamiento con peritoneodílisis. La literatura actual reconoce a ciertos integrantes del género *Trichoderma* como patógenos oportunistas de creciente frecuencia en pacientes con compromiso inmunológico. La paciente fue tratada satisfactoriamente con Amfotericina B, sin embargo, falleció a causa de una emergencia hipertensiva secundaria a su patología de base.

Case report

A female 13-year-old patient with focal and segmented glomerulosclerosis, chronic renal insufficiency, secondary arterial hypertension antecedents, in peritoneal dialysis since may 2004 and two events of multiresistant *Enterobacter cloacae* and *E. aerogenes* intrahospitalary peritonitis, respectively. She was treated with Ciprofloxacin, Imipenem and Vancomycin in the pediatric intensive care ward at the Hospital Carlos van Buren, fifth Region, Chile.
Since 23/7/04 she exhibits *Acinetobacter baumannii* sepsis and is received Ciprofloxacin and Imipenem. After a 10-day treatment, she shows a satisfactory evolution and recovers her conscience.

On 10/8/04 she shows turbid peritoneal fluid with purulent sediment, 76mg/l reactive C protein (PCR), 14300 blood leukocyte and peritoneal fluid cytochemical with 300 leukocytes and 2 blood red corpuscle. Later on she undergoes an intense abdominal pain and fever, and then Ciprofloxacin, Imipenem and Fluconazol are empirically suggested. Based on a nephrology evaluation, an hemodialysis is recommended together with her inclusion in the renal transplant program. With a 162 mg/l PCR, an abdominal scan is carried out resulting a chronic bilateral renal nephropaty and the absence of other pathological findings. The patient exhibits a regular evolution with an increased abdominal pain (PCR:191mg/l) and a peritoneal fluid cytochemical of 1450 leucocytes, 83% polymorphonuclear and 50 erythrocytes.

According to the laboratory report (17/08), a growth of a green filamentous fungus, possibly similar to a strain of *Aspergillus* was detected in samples of peritoneal fluid whereby an initial Amphotericin B treatment in addition to indovenous Fluconazol is prescribed together with the suspension of the antibacterial treatment. Culture samples were sent to the Cátedra de Micología de la Escuela de Medicina de la Universidad de Valparaíso in order to undergo a mycological diagnosis which revealed the presence of *Trichoderma longibrachiatus*.

On 19/8/04 the patient shows an intrahospitalary pneumonia, Vancomycin and Imipenem are indicated while the antymycotic treatment with Amphotericin B 1mg/kg plus daily indovenous Fluconazol 200mg is maintained. Successive samples of peritoneal fluid revealed a decrease in the number of *T. longibrachiatus* colonies with time. On 13/9/04 a double catheter is inserted in the jugular for hemodialysis, the peritoneal catheter is withdrawn, the antibiotic and antymycotic treatment is maintained with a good clinic response, an enteral feed probe is installed due to a severe malnutrition, gustatory disorders and dysphagia. However, the patient continued with high arterial tension readings despite the treatment with sodium nitroprusside, propanolol and nifedipine.

On 1/10/04 the patient suffers an hypertensive emergency, convulsions and undergoes a cardiorespiratory arrest from which she could not recover in spite of
the efforts of the personnel belonging to the ICU of the hospital.

**Mycology**

The first fungal isolates were cultured in Sabourad dextrose agar and Blood agar at 37°C and visually confirmed in the hospital laboratory to be similar to *Aspergillus* species and sent for their identification to the Catedra de Micologia de la Escuela de Medicina de la Universidad de Valparaiso where they were subcultured in PDA at 30°C and CMA at 40°C to measure colony radius in 65 h. In PDA colonies grew rapidly, over 70mm dia. and over 40mm in CMA. In PDA conidia covered the entire colony surface tending to form concentric rings or forming crusts dark in colour (Fig.2) yellow to greenish yellow pigments diffusing through the agar (not forming at 40°C) (Samuels et al., 1998).

Conidiophores in CMA are smooth, confluent pulvinate aggregates, with short secondary branches, with primary branches long and secondary ones usually short and rarely rebranched. Phialides typically cylindrical to lageniform, solitary, paired or 3 verticillate, intercalary phialides frequently produced (5.8-11 x 2-3.3 μm). Conidia in CMA, pale to medium green, oblong to ellipsoid, smooth walled, with a truncate base (3.3-5.1 x 2-4-3.2 μm) (Fig.1). Chlamydospore frequent in old culture (CMA), terminal or intercalary, globose to subglobe (4-12 μm).

This clinical strain was identified as *T.longibrachiatum* Rifai, and a living culture (CMEM 201) has been deposited in the mycology laboratory at the Escuela de Medicina de la Universidad de Valparaiso.

**DISCUSSION**

Localized and disseminated infections caused by members of the genus *Trichoderma* have been sporadically registered in literature as the first case described in a patient with chronic lung disease (Escudero et al., 1979). Most of existing cases have been reported in immunocompromised patients such as peritonitis under a recurrent peritoneal dialysis (Tanis et al., 1995), liver (Jacobs et al., 1992), bone marrow transplant (Richter et al., 1999), brain abscess (Seguin et al., 1995), skin infections, invasive sinusitis (Furukawa et al., 1998), necrotizing stomatitis (Myoken et al., 2002), renal transplant (Guarro et al., 1999), aplastic anemia (Muñoz et al., 1997) and invasive infections in different organs (Chouaki et al., 2000).

Allergic features of mitospores and meiospore of many fungi can cause at the same time allergy symptoms, mainly fungal sinusitis, in which *T.longibrachiatum* may become involved in those patients bearing atopy and asthma histories. Based on studies performed to characterize allergen release from spore before and after germination of different fungi such as *Trichoderma viride*, it is concluded that not all ungerminated spores release allergens yet all of them release them from their hyphae. Critical events that determine exposure to allergen might be an opportunity for spore to germinate after inhalation and increase of severity of lower respiratory tract disease (Tang et al., 2003).

Identification of *Trichoderma* species based on macro and micromorphological character remains problematic and difficult without experience in the genus, mainly the colony morphology which can be difficult to interpret as a taxonomic character (Gams & Bisset, 1998). At present, the genus has been subdivided into 5 sections (Hypocreanum, Longibrachiatum, Pachybasium, Saturnisporum and Trichoderma) based primarily on micromorphological features, growth rates and growth at different temperatures (Bisset, 1984, 1991 a,b,c). However in spite of continuous progress in the genus since the contemporary findings of Rifai monography (1969) with his 9 "species aggregates", its taxonomy is still problematic at species level and morphological characters are not always useful in resolving evolutionary relationships between a teleomorph and an anamorph (*Hypocreanum* (*Trichoderma*) (Bisset, 1984, 1991 a,b,c, Gams & Bisset, 1998; Kinderman et al., 1998). Modern keys and taxonomic descriptions are disperse in literature, (Rifai 1969; Bisset, 1984, 1991; Gams & Bisset 1998 among others) yet considering its usefulness in our case report, we can mention those of Samuels et al. (1998), that by means of morphological and physiological characteristics make it possible to identify the *Hypocreanum schizophrenia* complex and its anamorphs closely related to the species of the Section *Longibrachiatum* and *Saturnisporum* (the latter included in the Section *Longibrachiatum* by Gams & Bisset, 1998).

*T.longibrachiatum* and *Hypocreanum schizophrenia* or *T.citrinoiride* are the two most common species in the *T.schewitschi* complex and they are difficult to be separated by morphological distinctions and *T.longibrachiatum* might be considered to be a clonal derivate of *T.orientalis* (Samuels et al., 1998). At present, in addition to current morphological methods of identification, molecular schemes to differentiate the members of the Section *Longibrachiatum* can be used (Kuhl et al., 1997).

The isolation of *Trichoderma* species from diverse habitats is easy to be carried out through many cultural methods because of their rapid growth and capacity to produce a great number of conidia in a few days. The production of conidia (mitospores) from
effused conidiophores or aggregates in fascicles or
pustules is observed in many general culture media that
are commonly used in the clinic laboratory (Sabouraud,
PDA, MEA) yet they can also be obtained from other
selective media (Gams & Samuel, 1998 page 5).

**Trichoderma longibrachiatum** has very diverse
habitats, soil in their variable substrata, wood, tree roots,
herbs, senescent tissues, air and sometimes it is
associated with the commercial growth of *Agaricus
bisporus* (Seab,1996); Samuels *et al.*, 1988, 2002).

Despite the ability of *Trichoderma* species to
produce diverse metabolites and to exhibit an aggressive
behaviour in nature, strains of this fungus are rarely
associated with any type of disease in living plants.
Mainly *T. harzianum* aggregates cause a significant
loss of the commercial mushroom (*Agaricus bisporus*)
(Samuels, 1966).

It can not be assured that the patient had not been
at some moment, prior to the beginning of the infection,
exposed to some short contacts with soil particles or
vegetal material. The entry of this agent to the patients
peritoneal fluid can be explained in different ways: the
presence of its mitospores on the skin by aerial
contamination, as a result of the personnel in charge of dialysis
handling material contaminated with soil-dust microparticles
and as an accidental contamination during the
preparation of dialysis liquid. This latter situation has been
reported in literature by introducing different fungal
agents (Tanis *et al.*, 1995; Fevre *et al.*, 1999). In this case
the base clinical condition must be taken into account since
it gave rise to an immunodepression, the use of long
antimicrobials range, the intrahospital peritoneal
dialysis and the secondary malnutrition, all of which
contributed to a peritoneal infection caused by a fungal
opportunistic. In spite of her deteriorated overall
condition, the patient showed a satisfactory evolution after
the indovenous antifungal treatment yet her sudden
deceased was due to a hypertensive emergency.

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