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Neurotropic black yeast *Exophiala dermatitidis* in children's gut microbiota

Abstract

Exophiala is a heterogeneous genus implicated in a variety of illnesses. The aim was to investigate the presence of *Exophiala* species in the gastrointestinal tract from a pediatric population. Strains from stool samples were isolated and identified by sequencing internal transcribed spacer region. Results showed a prevalence of 13% of *Exophiala dermatitidis*. This is the first report of *E. dermatitidis* in the gastrointestinal microbiota from a pediatric population in a Latin-American country.

Keywords: molecular diagnosis, gastrointestinal microbiota, fungal infection, neurotropism, phaeohyphomycosis

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Abbreviations: SDA, Sabouraud's dextrose agar; CFU, colony forming units; DNA deoxyribonucleic acid; ITS, internal transcribed spacer region; Exd, *Exophiala dermatitidis;* PCR, polymerase chain reaction; ADS, autism spectrum disorder; DM1, diabetes type 1; GI, gastrointestinal

Introduction

Exophiala is a heterogeneous genus of dematiaceous (darkly pigmented fungus) taxonomically positioned in the family *Herpotrichiellaceae*, order *Chaetothyriales*, class *Ascomycete.*[1] It is responsible for subcutaneous and invasive human infections, can cause phaeohyphomycosis and chromoblastomycosis by traumatic inoculation and systemic invasions mostly in immunocompromised hosts.[2–4] *Exophiala dermatitidis* is implicated in severe illnesses in humans, such as pneumonia and keratitis and might lead to fatal brain infections. It has been reported as colonizer of the lungs of cystic fibrosis patients.[5] This specie seems to have neurotropism, with significant morbidity and mortality.[6,7] It has also been isolated from a wide range of surfaces such as soil, glacier water, bathrooms, dishwashers, steam baths, contaminated areas with aromatic hydrocarbons, indoor habitats connected to water sources and high temperatures.[8–11] In the human intestinal tract there are only a few reports from Germany, Nigeria, Netherlands, Slovenia and United States.[4,12,13] The aim was to investigate the presence of *Exophiala* species in the gastrointestinal tract from a pediatric population in Venezuela.

Material and methods

A total of forty (n=40) stool samples were collected from children in Zulia state, with ages from 4 to 16 years old. A 100µL of each dilution starting with a 1:10 (0,5 gr of feces in 4,5 mL of distilled water) was cultured on plates with Sabouraud's dextrose agar (SDA) with chloramphenicol and incubated for 7 days at 37°C. The quantification scale of the culture-based method by colony forming units (CFU) was used according to Adams, 2011.[14] Brown; black and olivaceous colonies were counted, isolated and purified. Identification was performed with the physiological assimilation and growth temperature protocol described by de Hoog in 1993.[15] Ethical clearances was obtained by the Research Division of Zulia University and every participant's parent signed informed consent.

DNA extraction was performed using Wizard® Genomic DNA Purification Kit (Promega®). Species identification was done by sequencing internal transcribed spacer region (ITS) ITS1 [5′-TCCGTAGGTGAACCTGCGG-3′] and ITS4 [5′-TCCTCCGCTTATTGATATGC- 3′] to identify *Exophiala* genus obtained from GenBank (http://ww.ncbi.nlm.nih.gov/entrez). The novel primer pair, forward–Exdf [5′-CCGCCTATTCAGGTCC-3′] and reverse–Exedra [5′-TCTCTCCCACTCCCGC-3′] was designed which targeted conserved regions of the partial ITS1 region–complete 5.8S rDNA–partial ITS2 region of *E. dermatitidis*. Species identification by ITS1/ITS4 for *Exophiala* genus with 643 bp products and Exdf/Exdr for ITS1-ITS2 region of *E. dermatitidis* with 455bp.[16]

Results

Morphologic observation showed that 13% (n=5) of the samples were positive for black yeast. Colonies waxy, smooth and olivaceous black, were cautiously identified as *Exophiala* species is Table 1 show strains' physiological profile. All analyzed sequences were probed to be *E. dermatitidis* by multiplex PCR (Figure 1).

Samples 1 and 3 belong to one child with Autism Spectrum Disorder (ASD) and the other with diabetes type 1 (DM1), respectively. Both follow a refined sugar & casein diet free and have unremitting gastrointestinal (GI) symptoms such as abdominal pain, occasional vomiting, nausea, flatulence, incomplete bowel movements and lumpy stools with mucus. However, neither had diarrhea the day of collection the sample. Individuals 2, 4 and 5 did not have GI symptoms.

E. dermatitidis strain from individual 2 was unable to assimilate D-galactose, maltose, cellobiose and sucrose. Patient 1 strain did not assimilate myo-Inositol but assimilated cycloheximide. Black yeast wuantification in patients 1, 4 and 5 was classified as rare (0 to 10^3 CFU) and patients 2 and 3 had few (10^3 to 10^4 CFU).[17]

Table 1 Physiological test quantification of clinical isolations of black yeast in stool

Physiological profile and assimilation	1	2	3	4	5	*Exophiala dermatitidis*[15]
37°C	+	+	+	+	+	+
40°C	+	+	+	+	+	+
D-Glucose	w	+	+	+	w	+
Lactose	w	-	+	-	-	w/-
Nitrate	-	-	+	-	-	- (+)
Nitrite	-	-	-	-	-	-
Melobiose	-	-	-	-	+	+ (-)
myo-Inositol	-	w	+	w	+	+ (w)
D-Galactose	+	-	+	+	+	+
Maltose	+	-	+	+	+	+
Cellobiose	+	-	+	+	+	+
Sucrose	+	-	-	+	+	+
Cycloheximide 0,01%	+	-	-	w	w	-
Ethanol 0,1%	-	-	-	-	-	?
Ethanol 0,5%	-	-	-	-	-	?
Ethanol 1%	-	-	-	-	-	?
CFU/mL	$2x10^2$	$2x10^4$	$1x10^4$	$5x10^2$	$2x10^2$	

w=weak growth reaction.
+/w or -/w several reactions scored as weak; between brackets single isolate deviating.
?=not reported.

CFU/mL=Colony Forming Units per milliliter.

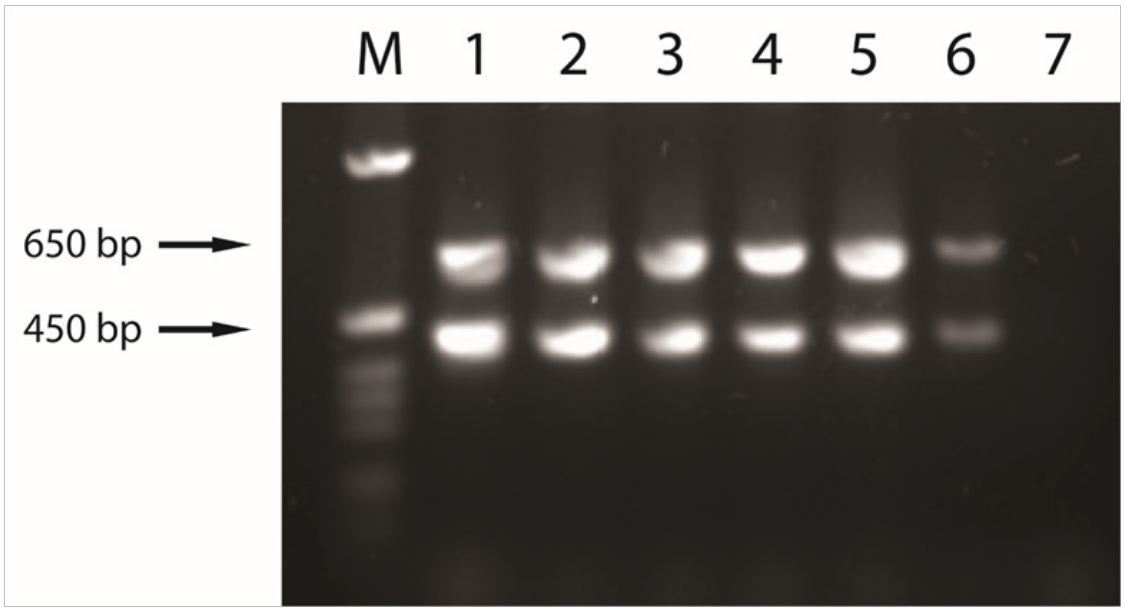

Figure 1 Multiplex PCR banding pattern of *Exophiala dermatitidis* of the 5 clinical's black yeast strains. M: 1 kb DNA Ladder. 1-5: clinical's black yeast strains 6: *Exophiala dermatitidis* reference strain. 7: H_2O as negative control.

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Discussion

Exophiala spp. are among the black yeast responsible for human infections. Authors recommended molecular methods for species identification.[3] We found a high prevalence (13%) of *E. dermatitidis*, comparatively with publishing data in Europe (0,52%),[12] Nigeria (3,5%)[13] and (1%) in EEUU.[3] A case of *E. dermatitidis* peritonitis was recently reported in a pediatric patient on peritoneal dialysis.[18] Patients N°2 and N°3 live together, as well as N°4 and N°5, this may support the proposed that water and maybe tropical fruits[8] (as pineapple and mango) can have a place in the transmission and it is possible that the transit through the gastrointestinal tract as *E. dermatitidis* may have thermo & acid tolerance and external mucopolysaccharides that allows avoid the phagocytosis.[4,8,11,19]

Patient with DM1 diagnosis had a high CFU/mL (2x104) suggesting that may be related to the inherited innate immune disorders.[3,6,7,19] Strains were isolated consecutively during a period of 3 weeks, supporting what de Hoog already proposed that *E. dermatitidis* is able to persist in gut for prolonged periods instead of being a transitory microbiota,[12] however, the clinical significance of the gastrointestinal carriage is still unknown.

Although this is the first report of *Exophiala dermatitidis* in the gastrointestinal microbiota from a Latin American pediatric population, we thought, as Zeng was suggested, *Exophiala* spp. infections are severely under diagnosed.[3] Due to the lack of information about infection pathways and neurotropism implications, we suggested the study of black yeast on the lab's test of stools samples. Further research needs to be performed with special emphasis in tropical countries with epidemiological conditions such as Zulia state, which is an oil region with aromatic hydrocarbon pollution of water and soil, high temperatures from 28 to 42°C and elevated humidity that fulfills the conditions were *E. dermatitidis* has been isolated previously.[8,9,19]

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Conflicts of interest

Authors declare that there is no conflict of interest.

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