Faecal candida and diarrhoea

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Abstract

Background—*Candida* species are frequently isolated from stools of children with diarrhoea but are not proven enteropathogens. It is hypothesised that faecal candida causes diarrhoea.

Aims—To determine the prevalence of faecal candida in childhood diarrhoea and the relation between faecal yeasts and diarrhoea.

Methods—Comparison of clinical and laboratory data, including quantitative stool culture for yeasts from 107 children hospitalised with diarrhoea and 67 age matched controls without diarrhoea.

Results—Yeast species, predominantly candida, were identified in the stools of 43 children (39%) with diarrhoea and 26 (36%) without diarrhoea. The concentration of candida was positively associated with recent antibiotic use ($p = 0.03$) and with the presence of another enteric pathogen ($p < 0.005$), but not with patient age, nutritional status, or duration of diarrhoea.

Conclusion—*Candida* species do not cause childhood diarrhoea in well nourished children.

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Keywords: diarrhoea; candida; yeasts

Despite the availability of effective therapy, diarrhoea still kills millions of children each year.^{1–3} Given the difficulties of bringing about social and economic changes necessary to reduce the incidence of gastroenteritis, there is a need to identify the microbial causes of the disease. In up to 40% of children with presumed infectious diarrhoea, no recognised pathogen can be identified.^{4–5} This may be due to our failure to appreciate the significance of certain intestinal microorganisms, such as yeasts.

Candida species form a ubiquitous genus of yeast present throughout the environment. They are part of the normal flora in the alimentary tract and on mucocutaneous membranes.⁶ *C. albicans* is the most common yeast species isolated from human faeces, being identified in 65% of stool samples from healthy adults.⁷ Nevertheless, several reports have suggested that it may cause diarrhoea. These studies have identified candida, but not other enteric pathogens, in the stools of patients with diarrhoea and have reported symptom resolution following treatment.^{8–10} Candida has been identified in high concentrations in the stools of malnourished children, frequently with associated diarrhoea,^{11–12} and it has been reported as the sole “pathogen” in the stools of

children with diarrhoea.^{13–14} It has also been suggested as a cause of antibiotic associated diarrhoea in infants.¹⁵ The role of candida is therefore potentially important as the disease is common and potentially treatable.

A prospective, hospital based study was carried out to assess the prevalence and concentrations of candida and other yeast species in the stools of hospitalised children with and without diarrhoea. An attempt was made to identify factors predisposing to intestinal carriage of yeasts.

Materials and methods

The study was undertaken at Princess Margaret Hospital for Children, Perth, Western Australia, the tertiary paediatric centre for Western Australia. It was approved by the institution's Research and Ethics Committee.

PATIENT SELECTION

Stool specimens were obtained from children who were admitted to Princess Margaret Hospital for Children with diarrhoea or who developed diarrhoea while in hospital (diarrhoea group, DG). Control patients without diarrhoea were matched for age and selected from patients admitted to the same ward, and at the same time as the DG subjects (control group, CG). The majority of the CG children had acute respiratory infections. Clinical data collected prospectively included patient age, race, nutritional state (percentage weight for age), history of recent antibiotic usage, hydration status, duration of diarrhoea, stool characteristics, and evidence of oral or cutaneous candidiasis.

SPECIMEN ANALYSIS

All faecal specimens were examined for ova, cysts, parasites, fat globules, white and red blood cells, and cultured for salmonella, shigella, aeromonas, vibrio, campylobacter, and *Clostridium difficile*. *C. difficile* cytotoxin assay was carried out on cell culture using *C. sordelli* antitoxin to neutralise the cytopathic effect. Specimens from children with diarrhoea were also examined for rotavirus and adenovirus using latex particle agglutination (Diarlex, Australian Diagnostics). Culture of *C. difficile* or identification of *C. difficile* toxin was accepted as evidence of *C. difficile* infection.

All specimens were cultured for candida by diluting 0.2 g of faeces in 1.8 ml of sterile saline solution. A 10 µl aliquot was then plated on Sabouraud's dextrose agar (Oxoid CM41) containing 300 µg/ml chloramphenicol (Parke Davis Australia) and 10 µg/ml gentamicin (Rousell Laboratories Limited, England). Plate cultures for yeasts were incubated in air at 35°C for 48 hours before counting and identi-

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Table 1 Characteristics of diarrhoea and control patients

	Diarrhoea group (DG) n (%)	Control group (CG) n (%)
Number of subjects	107	67
Race		
White	81 (76)	52 (78)
Aboriginal	17 (16)	6 (9)
Other	8 (8)	7 (10)
Unknown	1	2 (3)
Age		
0–6 mth	24 (22)	25 (37)
>6–12 mth	20 (19)	16 (24)
>12–60 mth	57 (53)	21 (31)
>60 mth	1 (1)	1 (2)
Unknown	5 (5)	4 (6)
% weight for length	97 (SD 15%)	99.8 (SD 14)
Antibiotic use	48 (45)	19 (28) (p = 0.013)

Table 2 Enteric pathogens isolated from diarrhoea and control patients

	Diarrhoea group n (%)	Control group n (%)
	107	67
Bacteria		
<i>C. difficile</i> culture and/or toxin	28 (26)	6 (9)
<i>Campylobacter jejuni</i>	4 (4)	—
Viruses		
Adenovirus	4 (4)	—*
Rotavirus	20 (19)	—*
Parasite		
<i>Giardia intestinalis</i>	6 (6)	2 (3)
Total	62 (58)	8 (12)

*Rotavirus/adenovirus testing not performed on non-diarrhoeal stools.

fying the colonies. *C. albicans* was identified by the production of germ tubes and confirmed by the production of chlamydoconidia.¹⁶ Species identification of germ tube negative yeasts was carried out using the commercial API C20 AUX yeast kit (Blackaby Diagnostics, Australia). Yeast counts were determined by colony counting 48 hours after incubation.

STATISTICAL ANALYSIS

The significance of differences between the DG and CG patients was determined using the χ^2 test for categorical variables and Student's *t* test for continuous variables. Analyses are reported with all yeasts included together in one group.

Results

Stool samples were collected from 107 children with diarrhoea and 67 children without diarrhoea. Both groups were similar in age, race, and percentage weight for height (table 1). The duration of diarrhoea in the DG patients ranged from less than one day to 480 days, with a median of one day. The duration of diarrhoea in DG patients with and without stool yeasts was not significantly different (median: one day in both groups). More of the DG children had recent exposure to antibiotics than CG children (n = 51, 45% compared with n = 19, 28%; p = 0.013).

Enteric pathogens were isolated from the stools of 47 of 107 (44%) DG and seven of 67 (10%) CG children (table 2). Twenty three of 66 patients with enteric yeasts also had other pathogens isolated from their stools (18 from DG and five from CG). There were five subjects in the DG in whom rotavirus and another pathogen was isolated. Identification

Table 3 Age distribution of patients with faecal yeasts

	Diarrhoea group		Control group	
	Total patients n	Patients with stool yeasts n (%)	Total patients n	Patients with stool yeasts n (%)
Patient age				
<1 year	46	19 (37)	41	17 (24)
1–2 years	31	17 (39)	11	4 (18)
>2 years	30	7 (20)	15	5 (27)
Total	107	43	67	26

Table 4 Concentration of faecal yeasts

	Diarrhoea group 107 subjects (%)	Control group 67 subjects (%)
Yeast count		
<10 000 cfu/g stool	9 (8.4)	14 (20.9)
>50 000 cfu/g stool	34 (31.8)	10 (14.9)*

*p = 0.009.

of an enteric pathogen was significantly associated with the identification of faecal yeasts ($\chi^2 = 25.1$, p < 0.005).

There was no difference in the isolation of yeasts between DG (n = 43, 39%) and CG (n = 26, 35%) children. *C. albicans* was the most commonly identified yeast and was isolated from 35 of 107 (35%) and 18 of 67 (27%) DG and CG children, respectively. Other yeasts isolated included other candida species (*C. parapsilosis*, *C. guilliermondii*, *C. lipolytica*, *C. humicola*) as well as *Torulopsis glabrata* (n = 2), *Trichosporon beigeli* (n = 1), and *Rhodotorula glutinis* (n = 1). All of the isolated yeast species were grouped together in subsequent analyses, and referred to collectively as yeasts.

Table 3 shows the age distribution of patients with yeasts in the stool. There was no significant association between age and identification of faecal yeasts.

Table 4 shows the concentration of stool yeasts. Patients with diarrhoea were more likely to have higher concentrations of faecal yeasts than the CG patients. The CG children tended to have more counts of less than 10 000 yeasts per g of faeces ($\chi^2 = 9.687$, p = 0.017). The concentration of faecal yeasts was significantly associated with use of antibiotics ($\chi^2 = 12.515$, p < 0.005).

Patients with yeasts in their stools were more likely to have had recent antibiotic treatment than those without yeasts, although this was not significant (48% v 35%, $\chi^2 = 2.74$, p = 0.095). The density of candida was however related to recent antibiotic use, with higher concentrations of faecal candida more likely to occur with recent antibiotic use ($\chi^2 = 11.687$, p = 0.009).

Stool characteristics recorded included the presence of white blood cells (18 patients), red blood cells (two patients), fat globules (28 patients), lactose intolerance (17 patients), and monosaccharide intolerance (six patients). None of these characteristics were associated with the presence of stool yeasts.

Discussion

Yeasts and fungi, especially candida species, are present in the faeces of healthy individuals. Candida colonises the oral cavity of up to three

quarters of preterm infants within the first week of life,^{17,18} and can be found in the oropharynx of nearly 20% of healthy infants.¹⁹ The gut is colonised more slowly, with 11% of preterm infants having positive faecal cultures for candida at 4 weeks of age.²⁰ Up to a quarter of healthy, non-hospitalised infants are reported to have candida in their faeces by 5 to 12 months of age.²¹ During childhood 12 to 16% of children carry candida in the stool, and the proportion increases to 80% in adulthood.^{7,22-23} Hospitalised children appear to have higher carriage rates of candida than children in the community, and the proportion increases with duration of hospitalisation.^{22,24}

This study confirms that yeasts, predominantly candida species, are common in the faeces of hospitalised patients in a paediatric hospital. Candida species were identified in the stools of approximately 40% of all patients studied. Although higher concentrations of candida were found in patients with diarrhoea it was not possible to prove a causative relation between the yeasts and diarrhoea.

A number of reports have suggested that candida might cause diarrhoea in adults and children. Discontinuation of antibiotics and the use of nystatin in adults with persistent diarrhoea and "heavy" faecal growth of yeasts resulted in a return to normal stool patterns within days.^{8,10} Gupta and Ehrinpreis identified heavy faecal growth of candida in a group of elderly, malnourished, and critically ill patients with diarrhoea, but were unable to find any other microbiological, inflammatory, or chemical cause for their symptoms.⁹ With anti-candida therapy, the diarrhoea resolved. Danna *et al* reported resolution of antibiotic associated diarrhoea in elderly patients with intestinal overgrowth of candida, after treatment with nystatin.¹⁰

Bishop and Barnes identified *C. albicans* in the stools of one third of children with acute gastroenteritis who were aged less than 1 year, compared with just 15% of controls.²⁵ They suggested that *C. albicans* was an opportunistic pathogen, causing prolonged diarrhoea after mucosal injury by another pathogen.²⁵ Fungal mycelia in the stools were reported as a marker of invasive enteric candida in a group of patients, some of who had persistent diarrhoea following other infections.²⁶ In these patients there was resolution of the diarrhoea after treatment with nystatin. In a group of Nigerian children, 77% had fungi isolated from the stools, and candida, the predominant isolate, was strongly associated with diarrhoea.¹³ In a large Indian study fungal proliferation was noted in the stools of 55% of children and adults with acute and chronic diarrhoea.²⁷ The prevalence of faecal fungi in children peaked at the same time as the peak prevalence of bacterial diarrhoea. A subsequent study in India reported candida as the sole pathogen in 15% of diarrhoeal stool specimens.¹⁴ Prevalence was highest among infants aged 0–12 months, with a summer peak in prevalence. Several authors have reported small intestinal overgrowth with candida in malnourished children.^{11,12}

In contrast to these previous studies, our study did not document an association between faecal candida species and diarrhoea. There are several explanations for this discrepancy. Firstly, earlier studies did not include control groups. Secondly, it is possible that candida may have a different effect in patients who are malnourished. Malnutrition may encourage proliferation of yeast species, and the association with diarrhoea may be coincidental.

It is not surprising that recent antibiotic use was associated with higher candida counts, given that antibiotic therapy encourages proliferation of candida in the intestine.^{28–30} Our finding that the identification of another pathogen was strongly associated with higher counts of faecal candida is also consistent with other reports.^{12–14,26,31}

Despite previous reports to the contrary, we have not confirmed an association between faecal candida or other yeasts and diarrhoea. Faecal concentrations of candida were however higher in patients with diarrhoea. While candida did not contribute to diarrhoea in our population it is possible that in malnourished children with chronic diarrhoea, candida species may assume a significant role.

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Commentary

Does candida cause diarrhoea?

Despite significant progress over the years, no causative agent can be identified in a substantial proportion of infants and children with acute diarrhoea. In one study of over 4600 such children in Australia, no known enteric pathogen was found in 44% of cases.¹ The search for possible pathogens goes on. In this study the authors asked whether *Candida* spp. or other yeasts might be an important and unrecognised cause of diarrhoea.

They analysed stool specimens from well nourished children in a tertiary referral hospital in Western Australia. These included children admitted with diarrhoea, children who developed diarrhoea while in hospital, and children without diarrhoea who were in hospital with various other disorders (disease controls). Although some of the subjects had chronic diarrhoea (the duration ranged from less than 24 hours to 480 days), the median duration was just one day. Yeasts (most often *C. albicans*) were identified in stool samples from 39% of those with diarrhoea and 35% of the controls (no significant difference). Higher yeast concentrations were reported in those with diarrhoea and in those with a history of recent antibiotic treatment. However, there was a higher incidence of antibiotic exposure in those with diarrhoea, and this may have explained that difference in concentration.

Here again the principle applies that "coincidence is no proof of causality".² The association between antibiotic usage and diarrhoea is no surprise, and the link between antibiotic usage and faecal candida has been well documented. This, of course, is no proof that antibiotic associated diarrhoea is caused by candida. Nevertheless, there are anecdotal reports of successful treatment of this condition with nystatin.

Patients infected with human immunodeficiency virus often suffer from diarrhoea. *C. albicans* is commonly isolated from the stool in these patients, but is just one of many possible agents that might be responsible for the diarrhoea.

As the authors indicate, studies from Pakistan and Nigeria have revealed increased carriage of *Candida* spp. in the stools of malnourished children with diarrhoea. This does not confirm an aetiological role for candida, even in those populations. *Candida* spp. are frequently identified in the absence of diarrhoea as the present study confirms.

If "coincidence is not causality", neither does the failure of this study to show a significant association with diarrhoea disprove the role of yeasts as potential pathogens. There may be specific clinical circumstances in which they can be responsible for diarrhoea. In addition, there are more than 80 candida species, as well as other yeast species, and these may vary in their pathogenicity. Even within species certain strains may have different properties. Using restriction enzyme analysis and a *C. albicans* specific DNA probe, Mathaba et al recently identified specific genetically distinct strains that may be associated with diarrhoea.³ Increased levels of secretory proteinase production were noted in isolates from patients with diarrhoea. Variation in adherence properties was shown in different isolates, and they reported that reduced and increased adherence were associated with acute and chronic diarrhoea respectively.

The role of candida and other yeast species as enteric pathogens may yet prove to be a complex one.

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