Jpn. J. Infect. Dis., 61, 1-4, 2008

Original Article

Speciation of Fecal *Candida* Isolates in Antibiotic-Associated Diarrhea in Non-HIV Patients

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(Received June 5, 2007. Accepted October 3, 2007)

SUMMARY: *Candida* is the most frequently encountered fungal infection of the gastrointestinal tract after antibiotic exposure. The pathogenesis of *Candida* probably varies with each species. The speciation of fecal *Candida* after antibiotic use is not well investigated. One hundred and eleven fecal samples negative for *Clostridium difficile* toxin and for other enteric pathogens formed the basis of our investigation. The diarrheic samples came from patients receiving antibiotics in a hospital setting. In addition, samples from 30 age-matched healthy participants who did not receive antibiotics and did not have diarrhea were also studied. Initially, a Gram stain identification for yeasts was performed for each fecal sample, then each sample was cultured on Sabouraud's dextrose agar. *Candida* was isolated as pure growth (>10^5 cfu/ml) from the stools of 32 (28.8%) patients. The identification of the yeast was done based upon a combination of morphological, physiological and biochemical criteria. The predominant isolates were *C. tropicalis* (n = 16), *C. albicans* (n = 14) and *C. krusei* (n = 2). *Candida* isolated from healthy participants (n = 4) was sparse and therefore not speciated. Different *Candida* spp. may play an important role in precipitating antibiotic-associated diarrhea.

INTRODUCTION

Candida spp. are frequently isolated from the stools of patients with diarrhea, primarily immunocompromised patients including those with AIDS. *Candida* is also the most frequently encountered opportunistic fungal infection of the gastrointestinal tract after antibiotic exposure (1,2). Forbes et al. (3) reported the isolation of predominantly *Candida* spp., the prevalence of which was positively associated with recent antibiotic use in the stools of children with and without diarrhea.

The pathogenesis of *Candida* infections is extremely complex and probably varies with each species. Though over 100 species of *Candida* have been recognized, only a few have been found to cause infections in humans. The role of *Candida* in antibiotic-associated diarrhea (AAD) has been controversial for many years (4). *Candida* spp. commonly colonize the mucosal surfaces of the gastrointestinal tract. The presence of small numbers of *Candida* in stool samples is normal and may not be pathogenic. In patients treated with antibiotics, increased *Candida* counts have been observed and have been linked to the development of diarrhea. However, there is no convincing data that *Candida* may cause AAD in adults.

A variety of *Candida* spp. are responsible for causing opportunistic fungal infections. However *Candida albicans* is the most frequent etiologic agent, followed by *C. tropicalis*, *C. parapsilosis* and *C. glabrata* (5). *C. albicans*, etc., are part of the normal endogenous flora, and these infections are believed to be endogenous in origin. *Candida* infections of the gastrointestinal tract after antibiotic use have not been well investigated. Therefore, the present study was conducted to determine the prevalence of fecal *Candida* in AAD in a premier North Indian hospital and to speciate the fecal *Candida* isolates so that infection control measures can be taken.

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MATERIALS AND METHODS

Study population and samples: Initially, 150 consecutive fecal samples, which were negative for *Clostridium difficile* toxin as well as for other enteric pathogens, were received at the Division of Microbiology, Department of Gastroenterology, Postgraduate Institute of Medical Education and Research, Chandigarh, India, between March 2005 and February 2006. The antibiotic status of 39 of the patients was not known, and they were therefore excluded. The remaining 111 samples were from patients receiving antibiotics, most of whom had diarrhea at the time of the sampling, and those formed the basis of our prospective investigation. These patients were undergoing treatment for various ailments such as chronic diarrhea, dysentery, Crohn's disease, acute myeloid lymphoma, colitis (inclusive of ulcerative colitis and necrotizing enterocolitis), alcoholic liver disease, non-Hodgkin's lymphoma, etc. Patients with AIDS were not included in our study. Apart from the above samples, 30 age-matched healthy participants who had not received antibiotics nor had diarrhea were also included in the study.

***C. difficile* toxin assay**: The *C. difficile* toxin assay was carried out in the supernatant of the fecal contents using a latex agglutination test as described earlier (6). In brief, 50 μl of a 1 in 5 diluted fecal supernatant was placed on a clean glass slide to which ready-to-use *Clostridium sordelli* antitoxin-coated latex beads were added. The slide was gently rocked manually and checked for macroscopic agglutination. A sample was considered to be *C. difficile* toxin-positive when agglutination occurred within 2 min and negative when no agglutination was present. Controls for both positive and negative assays were also included. A known positive fecal sample obtained from a patient with AAD served as the positive con-

trol. The two negative controls consisted of: (i) an unreactive fecal sample from a healthy volunteer who had no antibiotic exposure for 6 weeks prior to testing; and (ii) uncoated latex beads and a diluted test sample. The advantages of using *C. sordelli*-coated latex beads have been described in detail in an earlier report (6).

Gram staining and culture for *Candida* isolates: Initially, a Gram stain identification and scoring for yeasts was made with each fecal sample. Culturing on Sabouraud's dextrose agar (Hi Media, Mumbai, India) for the isolation of the organism was performed by the standard technique. *Candida* overgrowth was defined as growth of 10^5 or more colonies/ml in pure culture from the liquid stool. From the solid stools, routine inoculation was done in order to look for the growth of a sufficient number of colonies. Pure growth of *Candida* ($>10^5$ cfu/ml), when obtained, was further subjected to Gram stain identification of the colonies followed by culture on cornmeal agar (Hi Media) for speciation of the isolates.

Identification of the *Candida* spp.: The identification of *Candida* was based on a combination of morphological, physiological and biochemical criteria (5,7,8). Morphology was primarily used to establish the genera of the yeasts, whereas biochemical and physiological criteria were used to differentiate various species. The morphological criteria that were followed to identify the yeasts included cultural characteristics (shape, size, texture, etc.), asexual structures (shape, size, budding pattern, presence/absence of arthroconidia, ballistoconidia, germ tubes, hyphae or pseudo hyphae formation) and sexual structures (arrangement, cell wall ornamentation, number, shape and size of ascospores). The biochemical and physiological characteristics that were used in identification included assimilation and fermentation of sugars, nitrogen utilization, urea hydrolysis, reduction of tetrazolium medium, cyclohexamide resistance and temperature studies.

RESULTS

Clinical and demographic profile: Of the 111 samples tested, 68 came from males and 43 from females. The age range of the patients was 6 days to 90 years with a median of 34 years. During the time of the assay, diarrhea was present in 106, fever in 50 and abdominal pain in 32 of the patients. The patients were on various antibiotics, the predominant ones in decreasing order being metronidazole, ciprofloxacin, amikacin and vancomycin.

***Candida* isolates**: *Candida* was isolated as pure growth ($>10^5$ cfu/ml) from the stools of 32 (28.8%) patients receiving antibiotics. The predominant *Candida* spp. obtained were *C. tropicalis* ($n = 16$), *C. albicans* ($n = 14$) and *C. krusei* ($n = 2$). *Candida* isolated from the stools of healthy carriers ($n = 4$) was sparse, and therefore no speciation was done. There was a 100% correlation of culture with Gram staining of stool specimens.

Other isolates/toxins: Other organisms isolated from the test samples (data not shown) were non-toxigenic *C. difficile* ($n = 7$) and coagulase-negative *Staphylococcus* spp. ($n = 10$). A lone sample was also positive for *Clostridium perfringens* enterotoxin (data not shown). No other enteropathogen was detected from this series. No *C. difficile* or any other enteropathogen was isolated from the control samples. All of the samples were also negative for *C. difficile* toxin.

Prevalence of *Candida* spp. in relation to clinical symptoms: *Candida* was isolated from 32/106 (30.2%) patients with diarrhea. Fever was present as a symptom in 16/32 (50.0%) of the patients, and abdominal pain was present in 12/32 (37.5%). Table 1 depicts the prevalence of *Candida* spp. isolated in relation to clinical symptoms in 111 patients. The species of *Candida* isolated from the 32 patients who had antibiotic-induced diarrhea along with their underlying disease conditions and antibiotic intake are given in Table 2.

Table 1. Prevalence of *Candida* spp. in relation to clinical symptoms (total no. = 111)

	Diarrhea $n = 106$ (%)	Fever $n = 50$ (%)	Abdominal pain $n = 32$ (%)
C. tropicalis	16 (15.1)	7 (14.0)	6 (18.7)
C. albicans	14 (13.2)	7 (14.0)	5 (15.6)
C. krusei	2 (1.4)	2 (4.0)	1 (3.1)

Relations of antibiotics with *Candida* isolation: *C. tropicalis* isolation was most highly associated with cephalosporin ($n = 9$), followed by vancomycin ($n = 4$) and amikacin ($n = 3$). Similarly, *C. albicans* was most closely associated with ciprofloxacin ($n = 8$), amikacin ($n = 7$) and metronidazole ($n = 4$), and *C. krusei* with cephalosporins ($n = 2$).

DISCUSSION

The prevalence of hospital infections of fungal origin is increasing dramatically. Strategies for the prevention and control of candidiasis depend on whether the mode of transmission is endogenous or exogenous. In AAD precipitated by *Candida* both exogenous and endogenous transmission appear to be important factors. The major endogenous reservoir of the *Candida* group is the gastrointestinal tract (9). Cross transmission from the environment appears to be the exogenous source. Danna et al. (10) studied the role of *Candida* in the pathogenesis of AAD in elderly patients who tested negative for *C. difficile* toxin as well as for other intestinal pathogens and reported the overgrowth of *Candida* in 7/24 of them. Ponnuvel et al. (11) isolated 137 *Candida* spp. from AAD cases in infants and performed a quantitative estimation of the yeast population by a simple Gram stain smear, which revealed that more than 70% of cases had a 3+ score.

Payne et al. (12) studied the persistence of *C. albicans* in the presence of fecal microbiota. They reported that during steady state conditions, overgrowth of *C. albicans* was prevented by commensal bacteria indigenous to the system. However, antibiotics such as tetracycline have the ability to disrupt the bacteria population within the gut. Thus, colonization resistance can be compromised, and overgrowth of undesirable microorganisms like *C. albicans* can then occur. They showed that normal gut flora can exert 'natural' resistance to *C. albicans*; however, this resistance may be diminished during antibiotic intake. Krause et al. (13) reported that the presence of higher *C. albicans* counts in stools from AAD patients than in those from healthy subjects may be due to a reduction in soluble *Candida* inhibitors and an increased availability of growth factors and nutrients. However, in an earlier study (14), they reported no increase in the intestinal *Candida* virulence factor phospholipase in patients with AAD. Kaltenbach and Heitz (2) reported that the elevated fecal counts of *Candida* spp. found in patients treated with antibiotics are the consequence of the therapy rather than the cause of AAD. Samonis et al. (15) studied the effects of vancomycin, teicoplanin and other antibiotics on murine gut colonization by *C. albicans* and found that these animals had higher colony

Table 2. *Candida* spp. isolated from patients having antibiotic-associated diarrhea

Patient no.	Age/sex	Underlying clinical conditions	Antibiotic use	*Candida* spp. isolated
1	35/M	Disseminated Koch	Cephalosporin, amikacin	*C. tropicalis*
2	12/M	AML	Cephalosporin, amikacin, metronidazole	*C. krusei*
3	58/M	DM type 2, HCV positive	Cloxacillin, clindamycin, gentamycin	*C. tropicalis*
4	39/M	Tuberculosis	Anti-tuberculous therapy, cephalosporin	*C. krusei*
5	1.5/M	HUS with sepsis	Metronidazole, vancomycin	*C. albicans*
6	40/M	Ulcerative colitis	Ciprofloxacin, metronidazole	*C. albicans*
7	10/M	Dysentery	Ciprofloxacin, amikacin	*C. albicans*
8	53/F	DM	Ciprofloxacin	*C. albicans*
9	85/F	MND	Piperacillin, ciprofloxacin	*C. albicans*
10	63/M	NHL	Teicoplanin, ciprofloxacin	*C. albicans*
11	21 days/F	Necrotizing enterocolitis	Amikacin, ciprofloxacin	*C. albicans*
12	50/M	PUO with diarrhea	Augmentin	*C. tropicalis*
13	59/M	Drug induced, fever, jaundice	Ciprofloxacin	*C. albicans*
14	11 m/M	Acute dysentery with shock	Cephalosporin	*C. tropicalis*
15	1.5 m/M	Dysentery	Cephalosporin	*C. tropicalis*
16	54/M	NHL with diarrhea	Ciprofloxacin	*C. tropicalis*
17	15.5/F	CAP with diarrhea	Vancomycin, cephalosporin	*C. tropicalis*
18	35/F	SLE with ascitis	Tazobactarcin, piperacillin	*C. tropicalis*
19	59/M	CAP	Amikacin, augmentin, cephalosporin	*C. albicans*
20	28/F	SLE with sepsis with AGE	Metronidazole, amikacin, cloxacillin	*C. albicans*
21	40/M	Cut on antrum	Amikacin, metronidazole	*C. tropicalis*
22	34/M	ALD, UGI bleed	Vancomycin	*C. tropicalis*
23	6/M	GBS	Cephalosporin, sulbactam	*C. tropicalis*
24	30/F	Sepsis, myocarditis	Cephalosporin, vancomycin	*C. tropicalis*
25	30/F	AML	Trimethoprim	*C. albicans*
26	47/M	AML	Tazobactum, tangocid	*C. tropicalis*
27	55/F	DM with sepsis	Amikacin, metronidazole	*C. albicans*
28	2.5/M	Pneumonia	Cephalosporin, amikacin, cloxacillin	*C. tropicalis*
29	4.5 m/F	AAD	Ciprofloxacin, amikacin	*C. albicans*
30	1/F	Chronic meningitis	Vancomycin, sulbactam, cephalosporin	*C. tropicalis*
31	16/F	CRF with broncho pneumonia	Cephalosporin, levofloxacin	*C. tropicalis*
32	46/M	Diabetes with MS	Amikacin, piperacillin, cloxacilin	*C. albicans*

AML, acute myeloid lymphoma; DM, diabetes mellitus; HCV, hepatitis C virus; HUS, hemolytic uremic syndrome; MND, motor neuron disease; NHL, non-Hodgkin's lymphoma; PUO, pyrexia of unknown origin; CAP, community-acquired pneumonia; SLE, systemic lupus erythematosis; AGE, acute gastroenteritis; ALD, alcoholic liver disease; UGI, upper gastrointestinal; GBS, gall bladder stone; AAD, antibiotic-associated diarrhea; CRF, chronic renal failure; MS, mitral stenosis.

counts of yeast in their stools than did control *Candida*-fed mice treated with saline.

Loy (16) investigated 63 feces samples from hospital inpatients with probable AAD who were on multiple antibiotic regimes receiving 'high risk' antibiotics. Of the 71% of cases with a possible pathogen, *Candida* spp. overgrowth was the most common pathogen (44.4%) followed by *C. difficile* toxin (34.9%) and *C. perfringens* enterotoxin (9.5%). Good agreement was reported between significant Gram films and quantitative *Candida* spp. cultures. Loy (16) suggested that quantitative *Candida* spp. culture should be performed on all specimens for which AAD investigations were requested.

In our set-up, *C. tropicalis* was found to be the predominant species (50.0%) isolated from patients with AAD. A few studies have documented the potential for *C. tropicalis* cross-infection in hospital (17). Chowdhary et al. (18) reported an outbreak due to *C. tropicalis* involving 16 neonates receiving hyperalimentation and at least one course of antibiotics. The environmental sampling yielded *C. tropicalis* from each blanket and mattress used for the neonates. *C. tropicalis* is occasionally found in the human gut and is likely to be invasive in neutropenic patients.

The next predominant yeast was *C. albicans*, which comprised 43.7% of the *Candida* isolates in our study. *C. albicans* colonizes and is infectious for the host since both antibody- and cell-mediated immune responses to *Candida* antigens are evoked. An understanding of the host parasite interaction that allows *C. albicans* to switch from a commensal to a pathogen capable of infection is required. *C. albicans* is able to compete with other microbes as well as to adhere to and survive on the mucosal surfaces of hosts with *Candida*-specific antibody and cell-mediated immunity.

Apart from these two predominant species, *C. krusei* was also isolated in two patients. *C. krusei* is intrinsically resistant to fluconazole. In our study it was more closely associated with cephalosporin. Most of our patients received multiple antibiotics, and it is difficult to implicate any one antibiotic as precipitating the candidal diarrhea. However, *C. tropicalis* was also seen to be more closely associated with cephalosporin, followed by vancomycin and amikacin. On the other hand, *C. albicans* was isolated more frequently from patients receiving ciprofloxacin, amikacin and metronidazole.

Two *C. glabrata* and one *C. guilliermondii* were also isolated from the 39 patients whose antibiotic status was not known to us (data not shown). *C. glabrata*, which until recently was considered a nonpathogenic fungal organism, has emerged as an important nosocomial pathogen. *C. glabrata* and *C. parapsilosis* have also been isolated as opportunistic fungal infections (5). We cannot comment on *C. guillermondii* and *C. glabrata* isolates due to the lack of information on

the antibiotic intake of these patients. We did not, however, isolate any *C. parapsilosis* from our study.

Candida strains may survive on environmental surfaces for a very long time. The evidence of exogenous sources for the acquisition of *Candida* spp. continues to increase (20-22). An exogenous source could be the hands of the health-care workers, contaminated infusates, biomaterials, or the inanimate environment (17,19). Strausbaugh et al. (22) reported that 75% of the nurses and 81% of non-nursing personnel harbor yeasts on their hands, with 58 and 38%, respectively, carrying *Candida* spp. Bonassoli and Svidzinski (23) reported that yeasts were colonized in the nasal cavities and on the hands of 68% of nursing students, and that *C. albicans* comprised 59.6% of all the isolates.

Krause et al. (1) studied the role of *Candida* spp. in AAD in 395 subjects and reported that the elevated *Candida* counts were the result of the antibiotic treatment or diarrhea, rather than a cause of AAD. Broad spectrum antibiotics eliminate the endogenous intestinal flora and permit the outgrowth of *Candida* in the gut (15).

The variability in the prevalence of *Candida* spp. may be attributable to differences in the underlying diseases, host-related factors, the types and intensities of the treatment regimens, antimicrobial practices, supportive care measures used, or local factors (24). Our patients with *Candida* overgrowth also showed wide variation in their disease conditions and were on multiple antibiotics.

Speciation of the *Candida* isolates is important mainly because of the organism's different susceptibilities to antifungal agents. The identification of particular species can indicate point source outbreaks for epidemiological purposes. The risk of acquisition of an invasive fungal infection and the interpretation of the results of fecal *Candida* when obtained by other workers differ depending on the species. Thus, the interruption of transmission first requires the recognition of the species.

Candida is the most frequently encountered opportunistic fungal infection of the gastrointestinal tract after antibiotic exposure and may be both endogenous and exogenous in origin depending upon the species. Antifungal prophylaxis may decrease mucosal colonization and prevent endogenous candidiasis, but concern for the development of resistance and the selection of less susceptible species may limit this approach (9). Factors such as fecal incontinence and diarrhea contribute to the subsequent dissemination of pathogens into the health care environment. Selective pressure exerted by antibiotics plays a particularly important role in pathogen colonization and adverse effects associated with these agents often persist beyond the period of treatment. Infection-control measures that are implemented to control individual pathogens may have a positive or negative impact on the effort to control other pathogens that colonize the intestinal tract. Therefore, yeasts recovered in large amounts from any clinical source should be identified for proper control measures to be initiated.

ACKNOWLEDGMENTS

The authors are grateful to Ms. Surinder Kaur for her technical assistance and Monica Sharma for compiling the data.

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