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Impact of antibiotic use on fungus colonization in patients hospitalized due to fever

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Invasive candidiasis is an opportunistic infection that occurs in medical and surgical patients and carries a high mortality rate. Because its process always follows from colonization to amplification and to invasion, understand the colonization status is important to understanding the likelihood of invasion. Screening for fungal colonization was performed with specimens from urine, throat, anus, and skin in 314 patients admitted for fever both before and after antibiotics treatment was administered. Throat (45%) and anus (43%) had the highest colonization rates. Only 7% of patient had fungal colonization on the skin. *Candida albicans* was the most frequently colonized species in throat (79%) and anus (70%). Colonized fungal species on skin were diverse, including *Candida parapsilosi*s (33%), *C. albicans* (29%), and molds (24%). Sex (M:F ratio, 49.7:50.3) was not a factor in fungal colonization, but aging was associated with increased colonization rate. Forty-five patients received antibiotics treatment for more than the second weeks and second surveillance cultures were taken at the end of 2 week. Antibiotics treatment was associated with increased frequency of colonization (p=0.02), but the fungal species distribution pattern remained unchanged after antibiotic treatment.

Key words: *Candida*, colonization, antibiotic

Candidiasis is an opportunistic fungal infection in hospitalized patients. It has a high mortality rate, which is partly due to difficulty of early diagnosis [1-4]. The reasons for this diagnostic difficulty are diverse. For example, patients with systemic candidiasis sometimes show little objective clinical or laboratory evidence of infection [5-7]. Conversely, positive cultures, even from multiple sites, may only reflect colonization and not tissue invasion [8-11]. Because colonization of mucosal surfaces or skin are the initial steps of infection for most pathogenic *Candida* spp., epidemiologic data on colonization is of great importance for disease prediction and selection of appropriate antifungal agents. Among the many risk factors for invasive *Candida* infection, use of antibiotics is a common and important one [12-14]. This study aimed to determine the epidemiology of fungus colonization at different anatomic sites in patients hospitalized due to febrile diseases. We also examined the impact of antibiotics treatment on the profile of species and sites of *Candida* colonization.

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Materials and Methods

Patient selection

All patients admitted to the infectious disease ward of China Medical College Hospital between October 1, 2000 and January 31, 2001 for evaluation of fever (> 37°C, oral) were enrolled in this study. The exclusion criteria were as follows: patients with neutropenia (white blood cell <1000), receiving parenteral nutrition, with major operation within the previous 2 weeks, and had recently undergone chemotherapy or any form of antibiotics treatment for more than 1 day within 2 weeks prior to enrollment. A total of 314 patients were enrolled in this study.

Microbiology

Surveillance cultures for fungus were done for all patients before antibiotics treatment. Two sets of cultures were done from blood, and 1 each was done from urine, throat, skin, and anus. Blood specimens were inoculated into resin-containing aerobic and anaerobic media and incubated in the BACTEC 9240 System (Becton Dickinson, Sparks, MD, US) for up to 7 days. Well-mixed mid-stream or catheter urine samples were inoculated into a Sabouraud dextrose agar plate with a 0.001 mL loop. Throat swabs were taken

from the posterior pharyngeal wall by a cotton swab. Anal specimens were taken by a cotton swab inserted 2 to 3 cm into the rectum. Skin specimens were taken with a cotton swab scraped on both arms, both thighs, and 20 cm from the abdominal wall. Throat, anus, and skin specimens were all inoculated into Sabouraud dextrose agar plate. All specimens except blood were incubated at 30°C for 2 weeks. Identification of the yeast isolates was carried out by the API Yeast Ident system (Analytab Products, Plainview, NY, US).

Reculture after antibiotics treatment

Surveillance cultures for fungus were taken again if patients had received antibiotics treatment for 14 days. Sites and methods of cultures were as described above.

Statistical analysis

The colonization rate of fungus in patients grouped by age, sex, sites, and before and after antibiotics treatment were examined. Data were analyzed by binomial test for percentage.

Results

The demographic data for the 314 febrile patients enrolled in this study are shown in Table 1. Among the 314 patients, 156 (49.7%) were male. The mean age of the 314 patients was 53.3 years. Sources of fever included musculoskeletal infections in 101 patients, urinary tract infections in 72, intraabdominal infections and infectious diarrhea in 52, respiratory tract infections in 48, central nervous system infections in 5, and infective endocarditis in 3. There were 8 patients with fever of noninfectious causes, and 25 undetermined origins. Underlying diseases included diabetes mellitus in 28 patients, malignancies in 8, end-stage renal disease in 8, and human immunodeficiency virus (HIV) infection in 4. The remaining patients had no aforementioned underlying diseases.

Table 1. Demographic data of patients with fungus colonization admitted due to fever (n = 314)

Characteristic	No. of cases (%)
Male	156 (49.7)
Female	158 (50.3)
Mean age (years, range)	53.3 ± 20.4 (17-89)
Final diagnosis	
CNS infection	5 (1.6)
Infective endocarditis	3 (1.0)
Respiratory tract infection	48 (15.3)
Urinary tract infection	72 (22.9)
Intraabdominal infection	52 (16.6)
Soft tissue, bone, or joint infection	101 (32.2)
Undetermined	25 (8.0)
Other	8 (2.5)
Underlying diseases	
Diabetes mellitus	29 (9.2)
Malignancy	8 (2.5)
Uremia	8 (2.5)
HIV infection	4 (1.3)

Abbreviations: CNS = central nervous system; HIV = human immunodeficiency virus

A total of 306 fungi isolates were found in cultures of specimens collected before antibiotics treatment (Table 2). All patients with positive culture had only 1 fungal isolate per site sampled. Specimens from throat (140/312, 45%) and anus (135/314, 43%) yielded the highest percentage of positive cultures, while skin (21/313, 7%) and urine (9/262, 3%) had relatively low yields. The only patient with positive blood culture to *C. albicans* before antibiotics treatment had hepatitis C virus (HCV)-related cirrhosis of the liver with primary peritonitis. *C. albicans*, a causative pathogen of peritonitis, was isolated from blood, anus, and throat specimens of this patient. Over 70% of the colonized fungi in urine, anus, and throat specimens were *C. albicans*, but the fungi most frequently recovered from skin specimens were *C. parapsilosis* (7/21, 33%), *C. albicans* (6/21, 29%), and mold (5/21, 24%).

Of the 183 patients with fungus colonization before antibiotics treatment, 78 had colonization identified in only 1 site, 89 had colonization identified at 2 sites. Fourteen had colonization at 3 sites and 2 had colonization at 4 sites (Table 3). Pure *C. albicans* colonization or mixed colonization with other yeasts was the major species distribution pattern.

Males had a higher colonization rate (97/156, 62.2%) than females (86/158, 54.4%) (Table 4). The major difference was the positive rate for colonization found from anal swabs (48.7% vs 37.3%). Anal, throat, and total colonization rates all increased with age (Table 5). Skin and urine colonization rates did not differ with age.

Forty-five patients had received antibiotics treatment for more than 2 weeks and second surveillance cultures were taken from these patients at the end of the 2 weeks (Table 6). The total fungal colonization rate and colonization rates of urine, anal swab, skin, and throat after antibiotics treatment were significantly higher 2 weeks after starting antibiotics treatment than before antibiotics treatment. However, the fungal species distribution before and after antibiotics treatment was not different (Table 7). The number of patients with colonization of multiple sites increased after antibiotic treatment, although the percentage of patients colonized with species other than *C. albicans* did not increase after antibiotics treatment (Table 8).

Table 2. Fungus colonization in patients admitted due to fever before antibiotic treatment

Species	Colonization site, no. of isolates (%)			
	Urine n = 262 (%)	Anus n = 313 (%)	Skin n = 314 (%)	Throat n = 312 (%)
Candida albicans	7 (78)	95 (70)	6 (29)	111 (79)
Candida glabrata	0 28 (21)	1 (5)	16 (11)	
Candida parapsilosis	1 (11)	1 (1)	7 (33)	0
Candida tropicalis	1 (11)	2 (1)	0	0
Candida krusei	0	1 (1)	0	0
Other *Candida* spp.	0	5 (4)	2 (10)	5 (4)
Cryptococcus spp.[a]	0	1 (1)	0 4 (3)	
Mold	0	2 (1)	5 (24)	4 (3)
Total	9 (3)	135 (43)	135 (43)	140 (45)

[a]Non-neoformans *Cryptococcus* spp.

Discussion

Candida has become an important isolate pathogen recovered from blood and other sterile site cultures in hospitalized patients [3]. Epidemiological studies have shown that candidal infections occur in both medical and surgical patients, but differences in the species of *Candida* causing infection have been noted between these groups of patients [15]. Data on *Candida* species distribution may be important in determining the choice of empirical antifungal agents because most isolates of *C. albicans*, but not non-*albicans Candida*, are sensitive to fluconazole [16,17]. The pathogenesis of *Candida* infection follows progressively from colonization to local amplification and to invasion [18-20]. Understanding the status of colonization is very important for prediction of infection, especially in high-risk patients.

The gastrointestinal tract is the biggest reservoir of fungus, especially for *Candida* species [18]. Percentages of colonization and distribution of fungal species do not differ between the upper and lower tract [20]. Average colonization rates are around 43% to 45% with 70% of colonizations by *C. albicans*. In spite of the high gastrointestinal tract colonization rate found in this series, invasive fungal infections are rare in patients without any coexisting predisposing factors. In this study, the distribution of invasive fungal species in blood and urine before antibiotics treatment did not differ from gastrointestinal tract colonization, but differed from skin colonization. This finding implies

Table 3. Number of colonization sites and fungus species distribution of 314 patients admitted for fever prior to antibiotics treatment

Fungal species	Multiple sites of fungus colonization, no. of isolates (%)			
	1 site	2 sites	3 sites	4 sites
C. albicans only	53 (67.9)	59 (66.3)	6 (42.9)	0
Other yeasts only	20 (25.6)	10 (11.2)	1 (7.1)	0
Mold only	5 (6.4)	2 (2.2)	0	0
C. albicans + other yeasts	0	16 (18.0)	6 (42.9)	2 (100)
C. albicans + mold	0	1 (1.1)	0	0
Other yeasts + mold	0	1 (1.1)	1 (7.1)	0
Total	78	89	14	2

Table 4. Sites of fungus colonization in male and female patients admitted for fever prior to antibiotics treatment

Site of survey	Female n = 158 (%)	Male n = 156 (%)	*p*
Blood	0/156 (0)	1/156 (0.6)	0.158
Urine	4/136 (2.9)	5/127 (3.9)	0.330
Anal swab	59/158 (37.3)	76/156 (48.7)	0.020
Skin	9/157 (5.7)	12/156 (7.7)	0.244
Throat	69/156 (44.2)	71/156 (45.5)	0.410
Total	86/158 (54.4)	97/156 (62.2)	0.102

Table 5. Sites of fungus colonization according to age group in patients admitted for fever before antibiotics treatment

Age, years	No. of colonization (%)				
	Urine	Anus	Skin	Throat	Total
10-20 (n = 16)	0	4 (25)	0	2 (13)	4 (25)
21-30 (n = 47)	1 (2)	13 (28)	3 (6)	8 (17)	16 (34)
31-40 (n = 39)	1 (3)	6 (15)	1 (3)	8 (21)	11 (28)
41-50 (n = 28)	0	9 (32)	3 (11)	14 (50)	14 (50)
51-60 (n = 37)	2 (5)	19 (51)	4 (11)	15 (41)	24 (65)
61-70 (n = 62)	1 (2)	34 (55)	2 (3)	33 (53)	40 (63)
>70 (n = 85)	5 (6)	50 (59)	8 (9)	59 (69)	65 (76)

that the gastrointestinal tract is not only a reservoir of colonization, but is likely to be the most important source of infection.

Skin has a relatively low colonization rate of fungus (7%). The species distribution on skin in this study did not include invasive isolates and gastrointestinal tract colonization. *C. parapsilosis* was the most prevalent species on skin, followed by *C. albicans*, mold, and other *Candida* species. This species distribution pattern is more similar to the fungal infection pattern in patients with chemotherapy than in those with surgery [15]. This finding suggests that skin flora may play a more important role in invasive fungal infection in chemotherapy patients than in surgical patients. The high colonization rate of *C. parapsilosis* in skin can also explain the high reported rate of *C. parapsilosis* infection of intravascular devices [8,21-23].

In this series, the fungal colonization rate did not differ between the 2 sexes, but increased with age. The increase of the colonization rate with age was in parallel with the increase of the gastrointestinal tract colonization rate. Because colonization is always the first step in many fungal infections, aging may be a potential risk factor for invasive fungal infection. Most of the patients colonized with fungi in this series only had colonization identified at 1 or 2 sites. Multiple site colonization was rare. In patients with more than 3 sites of colonization, the percentage of pure *C. albicans* colonization decreased from 66% to 42%. This finding suggests that the cause of multiple site colonization may not be spread from a single colonized source. Acquisition of fungi from different sources seems to be a rational explanation for multiple sites colonization.

Molds were rarely found in this surveillance study. This result is compatible with the hypothesis of mold infection. Because most mold infections are exogenous without preceding colonization and amplification, low colonization rates do not represent low infection rates. On the contrary, if molds are isolated from clinical specimens, such as sputum, throat swab, or anal swab, or if molds are isolated from an immunocompromised host, clinical judgment should be cautious in ruling out infection. Environment disinfection procedures, not

Table 6. Fungus colonization rate at various sites before and after antibiotics treatment for 14 days

Specimens	No. isolate/total isolated (%)		*p*
	Before antibiotics	After antibiotics	
Urine	2/40 (5)	5/37 (13.5)	0.098
Anus	31/45 (68.9)	40/45 (88.9)	0.008
Skin	4/44 (9.1)	10/45 (22.2)	0.041
Throat	31/45 (68.9)	35/45 (77.8)	0.169
Total	37/45 (82.2)	43/45 (95.6)	0.020

Table 7. Percentage of *Candida albicans* colonization at various sites before and after antibiotics treatment for 14 days

Specimens	*Candida albicans* isolate/total isolated (%)		*p*
	Before antibiotics	After antibiotics	
Urine	2/2 (100%)	3/5 (60%)	0.382
Anus	23/31 (74.2%)	28/40 (70%)	0.347
Skin	2/4 (50%)	7/10 (70%)	0.549
Throat	25/31 (80.6%)	30/35 (85.7%)	0.292
Total	52/68 (76.5%)	68/90 (75.6%)	0.447

Table 8. Number of colonization sites and fungus species distribution of 45 patients hospitalized due to fever before and after antibiotics treatment

Fungal species	No. of cases with fungus colonized before/after antibiotics treatment				
	0 site	1 site	2 sites	3 sites	4 sites
C. albicans only	-	8/8	15/15	2/4	0/0
Other yeasts only	-	3/1	3/3	1/1	0/0
Mold only	-	0/0	0/0	0/0	0/0
C. albicans + other yeasts	-	-	3/5	2/3	0/2
C. albicans + mold	-	-	0/0	0/1	0/0
Other yeasts + mold	-	-	0/0	0/0	0/0
Total	8/2	11/9	21/23	5/9	0/2

intestinal tract antifungal prophylaxis, are mandatory for the prevention of mold infections in an immunocompromised host.

Antibiotics treatment is a well-known risk factor for invasive fungal infection. The mechanism of increased fungal infection is through suppression of intestinal bacteria flora and the proliferation of yeasts [13,14,24]. When the population of *Candida* increases to a significant level, persorption [25,26] can let *Candida* become invasive [25,26]. Antibiotics exposure can increase the colonized fungal density by 10- to 100-fold in the gastrointestinal tract [13,14,24], but does not increase the number of colonization sites [13]. In this study, antibiotics treatment can slightly increased the number of sites of colonization, although the pattern of species distribution remained unchanged. The post-treatment species distribution pattern in patients with multiple site colonization before antibiotics treatment showed a shift from *C. albicans* to non-*C. albicans* species after antibiotics treatment. A reasonable explanation for the post-antibiotics treatment increase of colonization sites with preservation of species distribution found in this study is colonial spread after antibiotics amplification.

Candida colonization is the first step in invasive infection. The species distribution pattern of colonization can always predict the pattern of invasive infection and may assist in the choice of empirical antifungal agents [15,27]. A previous study found that in 2 risk groups of invasive fungal infection, fungal infections in patients undergoing abdominal operation were always gastrointestinal in origin [15]. The choice of fluconazole as an empirical antifungal agent in deep-seated *Candida* infection is rational because most infecting species are *C. albicans* and other fluconazole-sensitive species [28, 29]. Sources of invasive fungal infection in cancer patients are diverse, including gastrointestinal tract, skin, and environmental molds. The choice of empirical antifungal agents should therefore be prudent and considerate. Guidelines for choosing antifungal agents in cancer patients should be settled in the near future after more studies.

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