Evaluation of Efficacy of Probiotics in Prevention of *Candida* Colonization in a PICU—A Randomized Controlled Trial*

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Objective: To evaluate the efficacy of probiotics in prevention of *Candida* colonization in a PICU.

Design: Prospective double blinded, randomized controlled trial.

Setting: PICU of a tertiary care teaching hospital in north India.

Subjects: One hundred fifty children (106 boys, 44 girls), 3 months to 12 yrs old, on broad spectrum antibiotics for at least 48 hrs were randomized using computer-generated random numbers to receive probiotic mix (EUGI) ($n = 75$) or placebo ($n = 75$).

Intervention: Patients received one sachet twice a day of either probiotics or placebo for 7 days. Probiotics contained *Lactobacillus acidophillus*, *Lactobacillus rhamnosum*, *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Saccharomyces boulardi*, *Saccharomyces thermophilus*, fructo-oligosaccharides; and placebo-contained lactose packed in similar-looking sachets. Rectal swabs for fungal culture were taken at day 0, 7, and 14 of enrollment. Primary outcome measure was prevalence of rectal colonization with *Candida* on day 14 postenrollment; secondary outcomes were growth of *Candida* in urine (candiduria) and blood (candidemia). Patients were followed until completion of 14 days study period or death of patient.

Results: Demographic and clinical variables were comparable in two groups. Prevalence of *Candida* colonization on day 0 was similar (15 of 75) in both the groups. On day 7, 27.9% (19 of 68) patients in the probiotic group and 42.6% (29 of 68) patients in the placebo group were colonized (relative risk 0.65; 95% confidence interval 0.41–1.05; $p = 0.07$), whereas, on day 14, colonization was observed in 31.3% (21 of 67) patients in the probiotic group and 50% (34 of 68) in the placebo group (relative risk 0.63; 95% confidence interval 0.41–0.96; $p = 0.02$). Thus, the relative reduction in prevalence of *Candida* colonization on day 7 and 14 in the probiotic group was 34.5% and 37.2%, respectively. The increase in number of colonized patients from day 0 to 7 and day 0 to 14 was significant in the placebo group ($p = 0.004$ and 0.001, respectively) but not in the probiotic group ($p = 0.30$ and 0.19, respectively; McNemar test). Candiduria was significantly less common in the probiotic group than in the placebo group (17.3% vs. 37.3%; relative risk 0.46; 95% confidence interval 0.26–0.82; $p = 0.006$). However, prevalence of candidemia did not differ significantly in two groups (1.6% in the probiotic group vs. 6.35% in placebo group; relative risk 0.46; 95% confidence interval 0.08–2.74; $p = 0.39$).

Conclusions: Supplementation with probiotics could be a potential strategy to reduce gastrointestinal *Candida* colonization and candiduria in critically ill children receiving broad spectrum antibiotics. (*Crit Care Med* 2013; 41:565–572)

Key Words: candida; candidemia; candiduria; colonization; pediatric intensive care unit; probiotics

Candida is the third most common agent responsible for healthcare-associated bloodstream infections and is the most common agent responsible for invasive fungal infections in children (1, 2). Candidemia is associated with a high mortality and morbidity among hospitalized patients as well as high healthcare costs (3). Prevalence of candidemia has dramatically increased in the past decade (4, 5). *Candida* species is implicated in 8%–10% of bloodstream infections in PICUs in various studies (6–8). In recent years, we have also witnessed and alarming rise in the prevalence of *Candida* colonization and candidemia. Colonization by *Candida* species was seen in 69.2% of patients receiving broad spectrum antibiotics for sepsis or septic shock in our PICU (9), and candidemia was seen in 4.3% of patients (10).

* **See also p. 689.**

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Several factors are known to predispose critically ill children to *Candida* colonization and candidemia. These include broad spectrum antibiotic therapy, use of invasive cannula, invasive hemodynamic monitoring, indwelling urinary catheter, endotracheal intubation, mechanical ventilation, presence of multiple organ failure, use of antacids and H_2 blockers, and malnutrition (10, 11). *Candida* colonization is most frequently reported to be associated with an increased risk of development of candidemia in critically ill patients in medical, surgical, and neonatal ICUs (9, 12–14). The gastrointestinal tract (GIT) has been implicated as the major site of colonization (15, 16).

Normal intestinal microbiota is composed of a complex collection and balance of microorganisms that normally inhabit the healthy gastrointestinal tract (17). Endogenous flora, gastric acidity, peristalsis, and secretion of antibacterial substances are important for maintenance of microbial balance in the GIT. These local defense mechanisms (colonization resistance) may be altered in critically ill patients, thus facilitating *Candida* colonization and overgrowth (18).

Achieving a reduction in rates of fungal colonization by using systemic antifungal drugs has been proven to be effective in preventing invasive fungal infection in the pediatric setting (19–21). However, prophylaxis with antifungal agents raises concern about their tolerability, cost, and potential selection of resistant strains, and it is not yet implemented as a standard of care (22–24). Reinforcement of the intestinal mucosal barrier with administration of commensal bacteria (probiotics) as drug or food supplements can act as an effective tool for prevention of nosocomial fungal infections. Probiotics modify the enteric microflora by colonizing GIT and reduce overgrowth of pathogens, hence colonization and invasive infection (25). Few trials have reported beneficial effect of probiotics in the prevention of the enteric colonization by *Candida* in preterm newborns (26, 27), but no such trial exists in critically ill pediatric patients. The objectives of this study were 1) to evaluate the hypothesis that supplementation with probiotics may reduce the *Candida* colonization in critically ill children on broad spectrum antibiotics and 2) to determine the effect of probiotics on prevalence of candiduria and candidemia in these patients.

MATERIALS AND METHODS

This was a double blind randomized controlled trial conducted in the PICU of a tertiary care teaching hospital in north India during a 1-yr period from November 2007 to October 2008. The ethics committee of the institute approved the study. Informed written consent was obtained from the parents.

Children (3 months to 12 yrs) admitted to the PICU who had received broad spectrum antibiotics for a minimum of 48 hrs were eligible for inclusion. Children with known chronic illness, immunodeficiency states, and on prior antifungal therapy and probiotics were excluded from the study.

The sample size was calculated on the basis of outcome on day 14 with the assumption that pretrial prevalence of gastrointestinal *Candida* colonization in children in our PICU after receiving 2 wks of broad spectrum antibiotic therapy was 43% (9), and use of probiotics will reduce it to 25%. Considering a 5% level of significance, 80% power, and 10% dropout, the minimum sample size was calculated as 72 children in each of the two groups.

All patients satisfying inclusion criteria were enrolled consecutively and randomized by using a computer-generated randomization table to either receive probiotics EUGI (Wallace pharma, Goa, India) (*Lactobacillus acidophilus* [0.24 billion CFU], *Lactobacillus rhamnosum* [0.24 billion CFU], *Bifidobacterium longum* [0.24 billion CFU], *Bifidobacterium bifidum* [0.24 billion CFU], *Saccharomyces boulardii* [0.05 billion CFU], *Saccharomyces thermophilus* [0.24 billion CFU], fructo-oligosaccharides [300 mg], and lactose as base) or placebo, which contained only lactose. Both probiotics and placebo were packed in similar-looking sachets, and these sachets were placed in similar-looking envelopes, each containing 14 sachets. All patients received one sachet twice a day for 7 days. Neither the treating physician nor the patients/parents were aware of the nature of the drug used. A person not involved with patient assignment, data collection, and analysis prepared the packets. Randomization codes were broken after the initial analysis was done.

Rectal swabs for fungal cultures were taken on day 0, 7, and 14 of enrollment. Urine was submitted for fungal culture twice a week, and blood culture was sent for fungal cultures if clinically indicated as per unit's protocol (10). Rectal swabs were immediately transported to a Mycology laboratory at room temperature. Each swab was transferred into two culture tubes containing Sabouraud agar media with antibiotics (Chloramphenicol 50 mg/L and Gentamicin 20 mg/L of media). The culture tubes were then incubated at 22°C and 37°C separately for 7 days. *Candida* growth was identified by morphological and biochemical tests such as a germ tube test, urease test, sporulation on corn meal agar, sugar fermentation test, and sugar assimilation test. Colonization was defined as positive culture for *Candida* species from the surveillance site (i.e., rectum). Candidemia was defined as isolation of *Candida* species in blood culture. Candiduria was defined as isolation of *Candida* species from urine.

Antifungal drugs were used as per our PICU protocol. Children who had grown *Candida* in blood culture (candidemia) and who had features of severe sepsis despite being on broad spectrum antibiotics for 5–7 days were given empirical antifungal therapy. These children were started on intravenous Amphotericin-B. Clinically stable patients with candiduria were treated with Fluconazole. Ten patients in the probiotic group and 15 patients in the placebo group received antifungal drugs ($p = 0.27$; **Table 1**). These patients remained enrolled in the study until the end point, and were not excluded.

Outcome

Primary outcome measure was prevalence of *Candida* colonization (isolation of *Candida* from rectal swab) on day 14 postenrollment. Secondary outcomes were growth of *Candida* in urine (candiduria) or blood (candidemia). Patients were followed until completion of the 14–day study period or death.

Data Analysis

Appropriate data entry and statistical analysis were performed on Microsoft Excel 2007 (Microsoft, Redmond, WA) and SPSS

TABLE 1. Demographic and Clinical Characteristics, Interventions, and Antibiotic and Antifungals Usage in Probiotic and Placebo Group

Characteristics	Probiotic Group (*n* = 75)	Placebo Group (*n* = 75)	*p*[a]
Age in yrs, mean (±SD)	3.07 (2.15)	2.78 (2.04)	0.45[b]
Boys:girls	52:23	54:21	0.72
Primary diagnosis at admission			
Community-acquired pneumonia, (*n*) %	34 (45.3)	34 (45.3)	0.71
Central nervous system infections, (*n*) %	22 (29)	17 (22.6)	
Septic shock, (n) %	6 (8)	12 (16)	
Cardiac disease, (*n*) %	3 (4)	5 (6.6)	
Others, (*n*) %	10 (13)	7 (9.3)	
Length of PICU stay before enrollment in days, mean (±SD)	3.4 (1.64)	3.3 (1.35)	0.60
Pediatric Risk of Mortality III, median (10th–90th percentile)	9 (2–16)	9 (1.2–18)	0.30[b]
Glasgow Coma Scale, median (10th–90th percentile)	7 (3–12.2)	8 (4–15)	0.13[b]
Central line, (*n*) %	42 (56)	41 (54.7)	0.87
Mechanical ventilation, (*n*) %	61 (81.3)	65 (86.6)	0.51
Urinary catheter, (*n*) %	54 (72)	62 (82.6)	0.12
Antibiotics usage:			
Duration in days prior to enrollment, mean (±SD)	3.51 (1.62)	3.25 (1.25)	0.22
Total duration (in days), mean (±SD)	11.19 (6.73)	12.01 (9.23)	0.53
Numbers that received following antibiotics:			
Ampicillin and gentamicin, *n*	25	22	0.94
Ceftriaxone, *n*	25	23	
Ceftriaxone and amikacin, *n*	15	20	
Cloxacillin, *n*	22	24	
Metronidazole or clindamycin, *n*	13	10	
Meropenem or pipercillin–tazobactum, *n*	10	12	
Vancomycin, *n*	10	9	
Antifungal agents usage:			
Number of patients who received antifungals, *n* (%)	10 (13.3)	15 (20)	0.27
Day of PICU stay when antifungals were started, mean (±SD)	6.9 (3.6)	6.60 (2.87)	0.77
Total duration of therapy in days, mean (±SD)	11.3 (2.71)	11.87 (3.16)	0.65
Antifungal agents used:			
Amphotericin-B IV (1 mg/kg/day)	7	10	0.86
Fluconazole IV (5 mg/kg/day)	3	5	
Indications:			
Empirical	6	6	0.35
Candidemia	1	4	
Candiduria	3	5	

[a]Chi-square test.
[b]Mann–Whitney test.

software version 15 (SPSS, Inc, Chicago, IL). Descriptive statistics (mean, SD, median, range, and percentages) were used for baseline variables. The analysis has been done on intention-to-treat basis. Dichotomous outcomes were compared by chi-square test or Fisher's exact test as applicable. Continuous variables were compared by Student *t* test. Change in pattern of colonization from day 0 to 7 and from day 0 to 14 in both the groups was determined by using McNemar test. Seven patients who died in each group between day 0 and 7 were not included in further analysis as no colonization data were available for these patients. All tests were two-tailed and p value <0.05 was taken as significant.

RESULTS

The study flow chart, recruitment, and randomization are depicted in **Figure 1**. Of 150 patients' enrolled in the study, 75 were randomized into group A and group B. Two groups were similar with respect to demographic and clinical characteristics, known risk factors for fungal colonization and

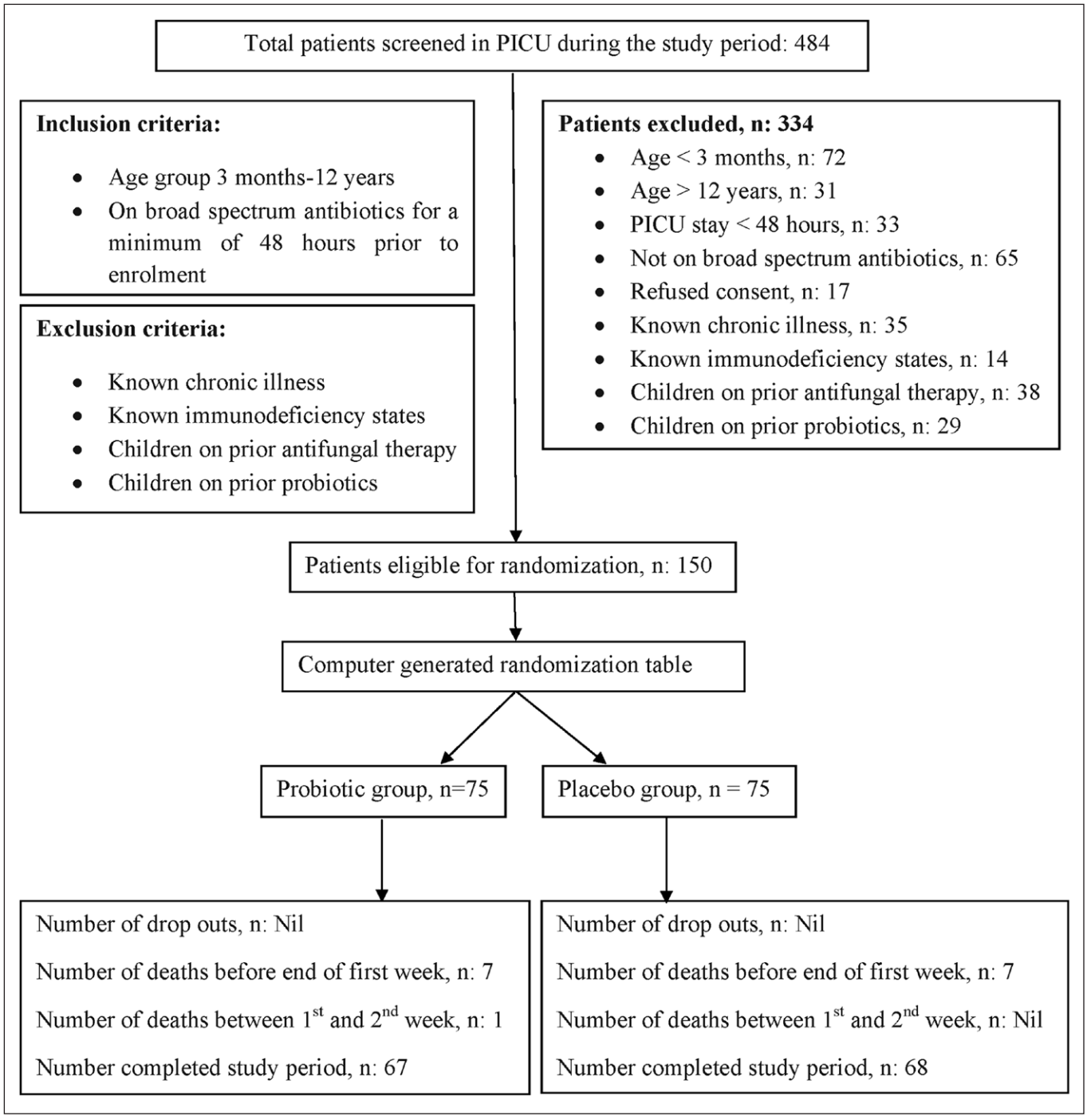

Figure 1. Study flow chart.

invasive fungal infection, and antibiotics and antifungals usage (Table 1).

Candida Colonization

Prevalence of *Candida* species colonization on day of enrollment (day 0) was similar in both the groups; 15 patients (20%) were colonized in each group. Seven patients died in each group between day 0 and 7, and one patient died in probiotic group between days 7–14. On day 7 of enrollment, 27.9% of patients (19 of 68) in probiotic group and 42.6% of patients (29 of 68) in the placebo group were colonized (relative risk 0.65; 95% confidence interval [CI] 0.41–1.05; $p = 0.07$). On day 14, *Candida* colonization was observed in 31.3% of patients (21 of 67) in the probiotic group and 50% of patients (34 of 68) in the placebo group (relative risk 0.63; 95% CI 0.41–0.96; $p = 0.02$; **Table 2**). Thus, the relative reduction in prevalence of *Candida* colonization in probiotics users was 34.5% and 37.2% on day 7 and day 14, respectively.

The change in number of colonized patients in the two groups on day 7 and day 14 with respect to initial colonization status (on day 0) is shown in **Table 3** and **Figure 2**. In the placebo group, there was a significant increase in *Candida* colonization from day 0 to 7 and day 0 to 14 ($p = 0.004$ and 0.001, respectively), but the change was not significant in the probiotic group ($p = 0.30$ and 0.19, respectively). The total number of patients colonized at any time during the study period were significantly lower in the probiotic group than in the placebo group (32 vs. 45; $p = 0.03$; Fig. 2). The total number of rectal swabs taken in the probiotic group were 210, out of which 55 (26.2%) were positive for *Candida*. In contrast, in the placebo group, 78 (37%) of 211 rectal swabs

TABLE 2. Outcomes: Frequency of Colonization With *Candida* on Days 0, 7, and 14; Overall Colonization During Study Period as Well as Number of Positive Rectal Swabs for *Candida*; Number of Patients With Candiduria and Candidemia; and Final Outcome in Probiotic and Placebo Group

Results	Probiotic Group ($n = 75$)	Placebo Group ($n = 75$)	p[a]
Patients colonized on day 0, *n* (%)	15 (20)	15 (20)	1
Patients colonized on day 7, *n* (%)[b]	19 (27.9)	29 (42.6)	0.07
Patients colonized on day 14, *n* (%)[c]	21 (31.4)	34 (50)	0.02
Patients colonized during study period, *n* (%)	32 (42.6)	45 (60)	0.03
Number of rectal swabs positive for *Candida*[d]	55/210	78/211	0.01
Patients with candiduria, *n* (%)	13 (17.3)	28 (37.3)	0.006
Patients with candidemia, *n* (%)[e]	1 (1.61)	4 (6.35)	0.36
Outcome			
Number that died, *n* (%)	8 (10.7)	7 (9.3)	0.78

[a]Z test for proportions.
[b]number of patients sampled in both group were 68.
[c]number of patients sampled in probiotic group and placebo group were 67 and 68, respectively.
[d]number of positive results/total number of rectal swabs taken for culture.
[e]blood for fungal culture was taken from 62 and 63 patients in probiotics and placebo group, respectively.

TABLE 3. Increase in Frequency of *Candida* Colonization in Two Groups From Day 0 to 7 and From Day 0 to 14 With Respect to Colonization Status at Enrollment

			Day 7[a]			Day 14[c]		
Group	Day 0		Sterile, *n*	Colonized, *n*	p[b]	Sterile, *n*	Colonized, *n*	p[b]
Probiotic group	Sterile, *n*	53	43	10	0.30	38	14	0.19
	Colonized, *n*	15	6	9		8	7	
Placebo group	Sterile, *n*	53	33	20	0.004	27	26	0.001
	Colonized, *n*	15	6	9		7	8	

[a]Seven patients died in each group between day 0 and 7. Therefore, data from 68 patients were analyzed by using McNemar test.
[b]McNemar test.
[c]one patient died in probiotic group between day 7 and 14.

showed growth of *Candida* (relative risk 0.71; 95% CI 0.53–0.94; $p = 0.01$; Fig. 2 and Table 2).

Candiduria

Candiduria was found in a significantly lower number of patients in the probiotic group (17.3%, 13 of 75) as compared to the placebo group (37.3%, 28 of 75) (relative risk 0.46; 95% CI 0.26–0.82; $p = 0.006$; Table 2).

Candidemia

Blood for fungal culture was obtained from 62 patients in the probiotic group and 63 patients in the placebo group. Candidemia (due to *Candida albicans*) was found in one patient (1.6%) in the probiotic group as compared to four patients (6.3%) (*C. albicans* in two and *Candida tropicalis* and *Pichia anomala* from one patient each) in the placebo group (relative risk 0.25; 95% CI 0.03–2.21; $p = 0.36$).

The predominant colonizing species in GIT was *C. albicans* followed by *C. tropicalis*. Similarly, *C. albicans* was also the most common species responsible for candiduria followed by *C. tropicalis*. The distribution of individual species causing colonization and candiduria was similar in the two groups (**Table 4**). There was no difference in final outcome in the two groups ($p = 0.78$).

DISCUSSION

The present study demonstrates that the concurrent administration of probiotics for 1 wk in critically ill children receiving broad spectrum antibiotic therapy decreased the prevalence of *Candida* colonization by 34.5% on day 7 and 37.2% on day 14. These results are similar to the ones obtained on preterm neonates (26, 27) and suggest that supplementation of probiotics could be a potentially effective strategy in reducing *Candida*

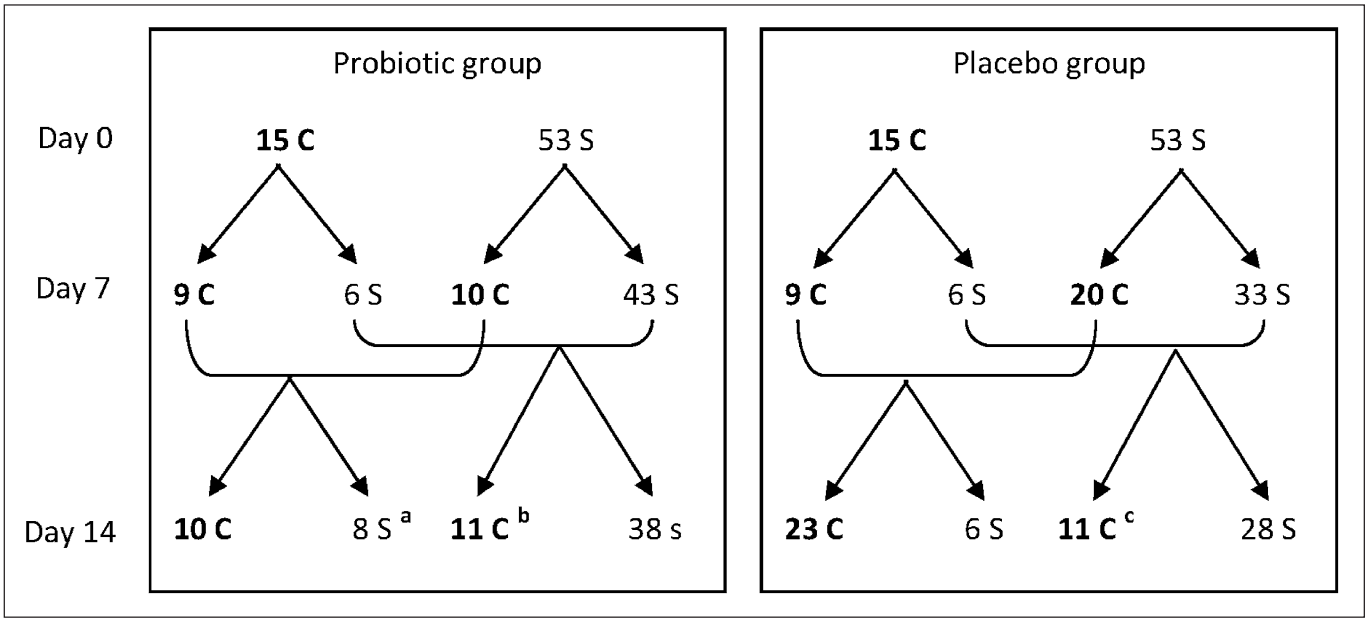

Figure 2. Flow chart showing colonization of patients with *Candida* species during study period from day 0 to 14. C = colonization; S = sterile. [a]One patient died in probiotic group between day 7 and 14; [b]out of 11 newly colonized patients from day 7 to 14 in probiotic group, four were already colonized on day 0. Total patients colonized in probiotic group during the study period were 32. Total swabs positive for *Candida* were 55; [c]out of 11 newly colonized patients from day 7 to 14 in placebo group, one patient was already colonized on day 0. Total patients colonized in placebo group during study period were 45. Total swabs positive for *Candida* were 78.

TABLE 4. *Candida* Isolates From Rectal Swabs and Urine

Group	Probiotic Group	Placebo Group	p^a
Isolates from rectal swab			0.31
Candida albicans, *n* (%)	50 (90)	65 (83.3)	
Candida tropicalis, *n* (%)	5 (10)	13 (16.7)	
Isolates from urine			0.58
Candida tropicalis, *n* (%)	9 (69.2)	20 (71.4)	
Candida tropicalis, *n* (%)	4 (30.8)	8 (28.6)	

[a]Chi-square test.

colonization in critically ill children in the PICU receiving broad spectrum antibiotics.

The use of broad-spectrum antibiotics alters the intestinal milieu, favoring colonization and infections with pathogenic bacteria and fungi (28), which is a known risk factor for invasive candidiasis (12). In a previous study, we had reported that the prevalence of *Candida* colonization and candidemia in children admitted in the PICU for treatment of severe sepsis and septic shock was 69.2% (45 of 65) and 30.7% (20 of 65), respectively; 90% of patients (18 of 20) who developed candidemia were colonized with *Candida* (9). Tortorano et al (29) also found that mucous membrane colonization preceded fungemia in 83% of patients.

A reduction in *Candida* colonization has been suggested as a strategy to reduce prevalence of invasive candidiasis. Preventive use of systemic antifungal agents has shown promising results (22, 23). Wainer et al (21) described a two-fold reduction in gut colonization by fungi with the use of a miconazole oral gel in high-risk preterm neonates. Using prophylactic intravenous fluconazole, a three-fold reduction in rectal colonization was achieved in preterm neonates (19). However, the prophylactic use of antifungal drugs is associated with selection of resistant strains, increased cost, and adverse effects (22). Therefore, there is a need to test other safer strategies to reduce *Candida* colonization.

During the past decade, several in vitro and in vivo studies have explored the possible role of probiotics in prevention of *Candida* colonization and invasive candidiasis. Wagner et al (30) demonstrated that oral and anal inoculation of probiotics (*L. acidophilus*, *Lactobacillus reuteri*, *Lactobacillus casei GG*, *and Bifidobacterium animalis*) in immunodeficient mice reduced the density of *C. albicans* in GIT, prevalence of systemic candidiasis, and prolonged the survival of adult and neonatal mice. Zwolinska-Wcislo et al (31) found that *L. acidophilus* therapy shortened the duration of fungal colonization of gastrointestinal mucosa and attenuated the effect of fungal colonization on process of ulcer healing in patients with gastric ulcer and ulcerative colitis. Manzoni et al (27) showed that orally administered *L. casei* subspecies *rhamnosus* significantly reduced the prevalence (23.1% in probiotic group and 48.8% in placebo group) and the intensity of enteric colonization by *Candida* species among very low birth weight neonates. Romeo et al (26) also found that the use of probiotics seems to be effective in the prevention of gastrointestinal colonization by *Candida* apart from reducing the prevalence of late-onset sepsis.

Normally intestinal bacterial flora inhibits *Candida* growth by competing for both adhesion sites and nutrients (32). Probiotics modify gut ecology and prevent fungal colonization by directly competing with pathogenic microorganisms and indirectly by enhancing the function of the intestinal barrier and the modification of the immune response (33). Probiotics activate mucosal immunity and stimulate cytokine production, immunoglobulin A secretion, and phagocytosis (33, 34). In vitro studies have clearly shown inhibitory effect of probiotics individually and as mix on germination of *Candida* (conversion of yeast to germ). Tang et al (35) assessed 16 strains of probiotics (*L. rhamnosus* ATCC 53103, *Lactobacillus johnsonii* JCM1022, *L. reuteri* JCM1081, *Lactobacillus gasseri* JCM1130, *L. acidophilus* 1.1878, *L. rhamnosus* 1.0120, *Lactobacillus plantarum* LA, *Lactobacillus bulgaricus* LB, *B. Longum* bif.l-1, *B. Longum* bif. l-2, *Bifidobacterium adolescentis*, *Enterococcus faecalis, Enterococcus faecium, Bacillus subtilis* and *Bacillus licheniformis*, and *S. thermophilus*) and found that each one of these significantly decreased germination of *Candida*. Five of the probiotic strains (except *S. boulardii*) used by us were among the 16 strains studied by Tang et al.

A significant finding of our study was almost 50% reduction in prevalence of candiduria in the probiotic group as compared with the placebo group. Candiduria is considered as a marker of heavy colonization (36) and a useful indicator of systemic candidiasis (37) particularly in critically ill children (38). A reduction in candiduria with the use of probiotics therefore is a good indicator that probiotics may offer a protection against invasive candidiasis.

The major strengths of our study are that this was a double blind, randomized controlled trial with an adequate sample size for the primary outcome. Also all patients completed the study. The results of the study can be generalized to pediatric patients receiving broad spectrum antibiotics in a PICU similar to ours. A confirmation of our findings and evaluation of the effectiveness of probiotics to reduce invasive candidiasis is a multicentric study covering different geographical location is needed. In the present study, there was a trend toward lower rate of candidemia in the probiotic group compared to the placebo group, though the difference was not statistically significant as the sample size was not adequate to study this outcome.

We conclude that probiotics were effective in reducing *Candida* colonization in GIT and candiduria in critically ill children receiving broad spectrum antibiotic therapy. Concurrent use of probiotics with antibiotics should be evaluated as a strategy to reduce the *Candida* colonization and prevalence of candidemia in critically ill children, in a multicentric study.

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REFERENCES

1. Steinbach WJ: Epidemiology of invasive fungal infections in neonates and children. *Clin Microbiol Infect* 2010; 16:1321–1327
2. Zaoutis T: Candidemia in children. *Curr Med Res Opin* 2010; 26:1761–1768
3. Singhi S, Deep A: Invasive candidiasis in pediatric intensive care units. *Indian J Pediatr* 2009; 76:1033–1044
4. Neu N, Malik M, Lunding A, et al: Epidemiology of candidemia at a Children's hospital, 2002 to 2006. *Pediatr Infect Dis J* 2009; 28:806–809
5. Filioti J, Spiroglou K, Panteliadis CP, et al: Invasive candidiasis in pediatric intensive care patients: Epidemiology, risk factors, management, and outcome. *Intensive Care Med* 2007; 33:1272–1283
6. Wisplinghoff H, Bischoff T, Tallent SM, et al: Nosocomial bloodstream infections in US hospitals: Analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis* 2004; 39:309–317

7. Pappas PG, Rex JH, Lee J, et al; NIAID Mycoses Study Group: A prospective observational study of candidemia: Epidemiology, therapy, and influences on mortality in hospitalized adult and pediatric patients. *Clin Infect Dis* 2003; 37:634–643
8. Zaoutis TE, Argon J, Chu J, et al: The epidemiology and attributable outcomes of candidemia in adults and children hospitalized in the United States: A propensity analysis. *Clin Infect Dis* 2005; 41:1232–1239
9. Singhi S, Rao DS, Chakrabarti A: Candida colonization and candidemia in a pediatric intensive care unit. *Pediatr Crit Care Med* 2008; 9:91–95
10. Singhi SC, Reddy TC, Chakrabarti A: Candidemia in a pediatric intensive care unit. *Pediatr Crit Care Med* 2004; 5:369–374
11. Zaoutis TE, Prasad PA, Localio AR, et al: Risk factors and predictors for candidemia in pediatric intensive care unit patients: Implications for prevention. *Clin Infect Dis* 2010; 51:e38–e45
12. Saiman L, Ludington E, Dawson JD, et al; National Epidemiology of Mycoses Study Group: Risk factors for Candida species colonization of neonatal intensive care unit patients. *Pediatr Infect Dis J* 2001; 20:1119–1124
13. Agvald-Ohman C, Klingspor L, Hjelmqvist H, et al: Invasive candidiasis in long-term patients at a multidisciplinary intensive care unit: Candida colonization index, risk factors, treatment and outcome. *Scand J Infect Dis* 2008; 40:145–153
14. Charles PE, Dalle F, Aube H, et al: Candida spp. colonization significance in critically ill medical patients: A prospective study. *Intensive Care Med* 2005; 31:393–400
15. Issa SY, Badran EF, Aqel KF, et al: Epidemiological characteristics of Candida species colonizing oral and rectal sites of Jordanian infants. *BMC Pediatr* 2011; 11:79
16. Kicklighter SD, Springer SC, Cox T, et al: Fluconazole for prophylaxis against candidal rectal colonization in the very low birth weight infant. *Pediatrics* 2001; 107:293–298
17. Salminen S, Benno Y, de Vos W: Intestinal colonisation, microbiota and future probiotics? *Asia Pac J Clin Nutr* 2006; 15:558–562
18. Pfaller MA: Nosocomial candidiasis: Emerging species, reservoirs, and modes of transmission. *Clin Infect Dis* 1996; 22 (Suppl 2):S89–S94
19. Kaufman D, Boyle R, Hazen KC, et al: Fluconazole prophylaxis against fungal colonization and infection in preterm infants. *N Engl J Med* 2001; 345:1660–1666
20. Ben-Ari J, Samra Z, Nahum E, et al: Oral amphotericin B for the prevention of Candida bloodstream infection in critically ill children. *Pediatr Crit Care Med* 2006; 7:115–118
21. Wainer S, Cooper PA, Funk E, et al: Prophylactic miconazole oral gel for the prevention of neonatal fungal rectal colonization and systemic infection. *Pediatr Infect Dis J* 1992; 11:713–716
22. Austin NC, Darlow B: Prophylactic oral antifungal agents to prevent systemic candida infection in preterm infants. *Cochrane Database Syst Rev* 2004;(1):CD2003478
23. McGuire W, Clerihew L, Austin N: Prophylactic intravenous antifungal agents to prevent mortality and morbidity in very low birth weight infants. *Cochrane Database Syst Rev* 2004;(1):CD003850. Update in: *Cochrane Database Syst Rev* 2007;(4):CD003850
24. Neely MN, Schreiber JR: Fluconazole prophylaxis in the very low birth weight infant: Not ready for prime time. *Pediatrics* 2001; 107:404–405
25. Agarwal R, Sharma N, Chaudhry R, et al: Effects of oral Lactobacillus GG on enteric microflora in low-birth-weight neonates. *J Pediatr Gastroenterol Nutr* 2003; 36:397–402
26. Romeo MG, Romeo DM, Trovato L, et al: Role of probiotics in the prevention of the enteric colonization by Candida in preterm newborns: Incidence of late-onset sepsis and neurological outcome. *J Perinatol* 2011; 31:63–69
27. Manzoni P, Mostert M, Leonessa ML, et al: Oral supplementation with Lactobacillus casei subspecies rhamnosus prevents enteric colonization by Candida species in preterm neonates: A randomized study. *Clin Infect Dis* 2006; 42:1735–1742
28. Kaufman D, Fairchild KD: Clinical microbiology of bacterial and fungal sepsis in very-low-birth-weight infants. *Clin Microbiol Rev* 2004; 17:638–680
29. Tortorano AM, Biraghi E, Astolfi A, et al; FIMUA Candidemia Study Group: European Confederation of Medical Mycology (ECMM) prospective survey of candidaemia: Report from one Italian region. *J Hosp Infect* 2002; 51:297–304
30. Wagner RD, Pierson C, Warner T, et al: Biotherapeutic effects of probiotic bacteria on candidiasis in immunodeficient mice. *Infect Immun* 1997; 65:4165–4172
31. Zwolinska-Wcislo M, Brzozowski T, Mach T, et al: Are probiotics effective in the treatment of fungal colonization of the gastrointestinal tract? Experimental and clinical studies. *J Physiol Pharmacol* 2006; 57 (Suppl 9):35–49
32. Kennedy MJ, Volz PA: Ecology of Candida albicans gut colonization: Inhibition of Candida adhesion, colonization, and dissemination from the gastrointestinal tract by bacterial antagonism. *Infect Immun* 1985; 49:654–663
33. Chermesh I, Eliakim R: Probiotics and the gastrointestinal tract: Where are we in 2005? *World J Gastroenterol* 2006; 12:853–857
34. Buts JP, Bernasconi P, Vaerman JP, et al: Stimulation of secretory IgA and secretory component of immunoglobulins in small intestine of rats treated with Saccharomyces boulardii. *Dig Dis Sci* 1990; 35:251–256
35. Tang H, Jiong R, Jing Y, et al: An in vitro assessment of inhibitory effect of 16 strains of probiotics on the germination of Candida albicans. *Afr J Microbiol Res* 2012; 4:1252–1256
36. Toya SP, Schraufnagel DE, Tzelepis GE: Candiduria in intensive care units: Association with heavy colonization and candidaemia. *J Hosp Infect* 2007; 66:201–206
37. León C, Ruiz-Santana S, Saavedra P, et al; EPCAN Study Group: A bedside scoring system ("Candida score") for early antifungal treatment in nonneutropenic critically ill patients with Candida colonization. *Crit Care Med* 2006; 34:730–737
38. Chakrabarti A, Reddy TC, Singhi S: Does candiduria predict candidaemia? *Indian J Med Res* 1997; 106:513–516