Effect of fluconazole prophylaxis on *Candida* fluconazole susceptibility in premature infants

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Objectives: Extremely premature infants are at high risk of developing invasive candidiasis; fluconazole prophylaxis is safe and effective for reducing invasive candidiasis in this population but further study is needed. We sought to better understand the effect of prophylactic fluconazole on a selection of fluconazole-resistant *Candida* species.

Methods: We evaluated the susceptibility to fluconazole of *Candida* isolates from premature infants (<750 g birth weight) enrolled in a multicentre, randomized, placebo-controlled trial of fluconazole prophylaxis. *Candida* species were isolated through surveillance cultures at baseline (study day 0–7), period 1 (study day 8–28) and period 2 (study day 29–49). Fluconazole MICs were determined for all *Candida* isolates.

Results: Three hundred and sixty-one infants received fluconazole ($n = 188$) or placebo ($n = 173$). After the baseline period, *Candida* colonization was significantly lower in the fluconazole group compared with placebo during periods 1 (5% versus 27%; $P < 0.001$) and 2 (3% versus 27%; $P < 0.001$). After the baseline period, two infants (1%) were colonized with at least one fluconazole-resistant *Candida* in each group. Median fluconazole MIC was similar in both treatment groups at baseline and period 1. However, in period 2, median MIC was higher in the fluconazole group compared with placebo (1.00 versus 0.50 mg/L, $P = 0.01$). There was no emergence of resistance observed and no patients developed invasive candidiasis with a resistant *Candida* isolate.

Conclusions: Fluconazole prophylaxis decreased *Candida albicans* and 'non-*albicans*' *Candida* colonization and was associated with a slightly higher fluconazole MIC for colonizing *Candida* isolates.

Introduction

Invasive candidiasis affects 7%–10% of infants with an extremely low birth weight (ELBW, <1000 g birth weight).^{1,2} Infants with <750 g birth weight (BW) are at disproportionate risk, with the incidence of invasive candidiasis being 2-fold higher compared with infants with BW of 750–1000 g.³ Consequences are severe, with mortality up to 30% and neurodevelopmental impairment in 30%–70% of affected infants.^{3–5} Fluconazole prophylaxis is suggested in very preterm infants because prophylactic fluconazole reduces the incidence of invasive fungal infection in this

population, particularly in neonatal intensive care units (NICUs) where there is a high incidence of this infection.^{5–7} Although no survival benefit has been observed, fluconazole prophylaxis was shown to be safe and effective in reducing *Candida* colonization and invasive infection in premature infants.^{2,5,7–9} Nonetheless, the effect of prophylactic use of fluconazole on the selection of fluconazole-resistant *Candida* is an ongoing concern since decreased fluconazole susceptibility could limit the efficacy of anti-fungal therapy. To date, controlled trials in premature infants have not demonstrated the emergence of fluconazole-resistant

Candida isolates with prophylactic fluconazole use, although this remains a concern.^{7–10} Furthermore, the effect of fluconazole prophylaxis on fluconazole resistance in the critically important subgroup of infants with <750 g BW alone has yet to be described.

Patients and methods

Study population

We included premature infants previously enrolled in a multicentre, randomized, placebo-controlled trial of fluconazole prophylaxis.⁵ The objective of that initial study was to evaluate the efficacy and safety of fluconazole prophylaxis in preventing death or invasive candidiasis in premature infants with <750 g BW. The study included infants ≤120 h of age at the time of enrolment from 32 NICUs in the USA. Infants were randomly assigned (1:1) to receive either fluconazole (6 mg/kg twice weekly, intravenously or enterally) or placebo for 42 days. The trial did not result in a lower incidence of the composite endpoint of death or invasive candidiasis, but did show a statistically significant reduction of invasive candidiasis alone.

Candida isolates

For all enrolled infants, surveillance cultures for fungal colonization were obtained at baseline (study day 0–7), mid-study (period 1: study day 8–28) and at the end of the dosing phase (period 2: study day 29–49) as part of the protocol. Surveillance cultures were obtained from the umbilical-groin areas, rectum and endotracheal secretions (if endotracheal tube was present) or nasopharynx. In addition, standard-of-care cultures were collected from normally sterile sites (e.g. blood, CSF, urine obtained by sterile catheterization or suprapubic tap, peritoneal fluid) or non-sterile sites [e.g. surface swabs (skin, wound) or urine obtained from a urine bag]. *Candida* isolates were stored at –70°C until speciation and susceptibility testing at the Duke University Mycology Research Unit. Species identification of all *Candida* isolates was performed using an initial screen for germ-tube formation. Isolates that were germ tube negative were identified based on carbon assimilation testing using the API® 20 C Kit (bioMérieux) in conjunction with the Dalmau technique.¹¹ Fluconazole MICs were determined by broth microdilution according to the standards outlined by the CLSI.¹²

Definitions

Candida isolates were defined as resistant to fluconazole based on clinical breakpoints as follows: *Candida albicans*, *Candida tropicalis* and *Candida parapsilosis* were resistant if MIC ≥8 mg/L, susceptible dose-dependent if MIC = 4 mg/L and susceptible if MIC ≤2 mg/L; *Candida glabrata* was resistant if MIC ≥64 mg/L and susceptible dose-dependent if MIC ≤32 mg/L; and *Candida krusei* was assumed to be intrinsically resistant to fluconazole regardless of MIC.¹³ Clinical breakpoints have not been established for uncommon *Candida* species; therefore, we used the same breakpoints as *C. albicans* for *Candida kefyr*, *Candida lusitanae* and *Candida boidinii*, given epidemiological cut-off values of 1–2 mg/L.¹⁴ An infant was considered to have a resistant *Candida* species if colonized with at least one *Candida* isolate resistant to fluconazole. An infant was colonized with a *Candida* isolate when the surveillance culture was positive, regardless of standard-of-care culture results.

The primary endpoint was the proportion of infants colonized with ≥1 *Candida* isolate resistant to fluconazole after the baseline period: period 1 (study day 8–28) and period 2 (study day 29–49). Secondary endpoints included the difference in MIC values between the two treatment groups. We also compared MIC₅₀ and MIC₉₀ of colonizing *Candida*, defined as the MIC required to inhibit 50% and 90% of the isolates, respectively. MIC₉₀ was described for baseline, period 1 and period 2 when >10 isolates of the same species were available for analysis. Secondary endpoints also included the

Table 1. Demographics by treatment group

Characteristic	Fluconazole (n = 188)	Placebo (n = 173)
Gestational age (weeks), median (range)	25 (23–31)	25 (22–30)
Birth weight (g), median (range) ^a	653 (310–794)	640 (350–791)
Female	109 (58)	98 (57)
Maternal ethnicity: Hispanic or Latino	21 (11)	25 (14)
Maternal race		
black	102 (54)	94 (54)
Caucasian	73 (39)	70 (41)
other	13 (7)	9 (5)

Data are n (%) of infants, unless otherwise indicated.

^aSix infants had a birth weight higher than 750 g (four in the fluconazole group and two in the placebo group).

proportion of infants with ≥1 resistant *Candida* obtained from standard-of-care cultures.

Statistical analysis

Demographics, baseline characteristics and endpoints were summarized and compared between the fluconazole and placebo groups. The primary analyses included the comparison of MIC for colonizing *Candida* species obtained from surveillance swabs. Secondary analyses included the standard-of-care cultures. A χ^2 test or Fisher's exact test for discrete variables and Wilcoxon rank-sum test for continuous variables were used to assess any differences between treatment groups. All statistical tests were two-sided with a type I error of 0.05.

Results

A total of 361 infants received either fluconazole (n = 188) or placebo (n = 173). Baseline characteristics were similar in both groups with a median (range) gestational age of 25 weeks (22–31) and BW of 650 g (310–794) (Table 1). Fewer infants developed probable or proven invasive candidiasis in the fluconazole group compared with the placebo group [6 (3%) in fluconazole group compared with 16 (9%) in placebo group, *P* = 0.02].

Candida colonization

A total of 1778 swabs were collected throughout the study period (604, 631 and 543 at baseline, period 1 and period 2, respectively). Overall, 253 (14%) were positive for *Candida* species [112 (19%), 83 (13%) and 58 (11%) at baseline, period 1 and period 2, respectively]. In the fluconazole group, 149 (79%), 148 (79%) and 131 (70%) infants had at least one surveillance swab collected at baseline, period 1 and period 2, respectively. In the placebo group, 153 (88%), 143 (83%) and 135 (78%) infants had at least one surveillance swab collected at baseline, period 1 and period 2, respectively. At baseline, 27/149 (18%) and 35/153 (23%) infants were colonized with a *Candida* species in the fluconazole and the placebo groups, respectively (*P* = 0.31). Among the infants colonized at baseline, *C. albicans* and *C. parapsilosis* were the most frequent species (Table 2). Baseline *C. albicans* colonization occurred in 12% and 15% of fluconazole and placebo infants, respectively. After baseline, overall *Candida* colonization decreased in the

Table 2. Fluconazole MIC (mg/L) for *Candida* isolates obtained from surveillance swabs^a

	Fluconazole (n = 188)			Placebo (n = 173)		
	baseline	period 1	period 2	baseline	period 1	period 2
<i>C. albicans</i> ^b						
no. of isolates	18	5	2	23	27	21
MIC range	0.125–1	0.125–16	0.25–2	0.125–1	0.125–1	0.125–1
MIC ₅₀	0.25	—	—	0.25	0.25	0.25
MIC ₉₀	1	—	—	0.5	0.5	0.5
<i>C. parapsilosis</i> ^b						
no. of isolates	10	1	3	12	13	13
MIC range	0.5–4	2–2	0.5–1	0.5–1	0.5–4	0.25–8
MIC ₅₀	1	—	—	0.5	1	1
MIC ₉₀	1	—	—	1	2	2
<i>C. kefyr</i>						
no. of isolates	0	0	0	1	0	0
MIC range	—	—	—	1	—	—
<i>C. lusitanae</i>						
no. of isolates	3	0	0	1	1	2
MIC range	0.5–1	—	—	0.125	0.5	0.5–0.5
<i>C. boidinii</i>						
no. of isolates	0	0	0	0	1	0
MIC range	—	—	—	—	16	—
<i>C. krusei</i>						
no. of isolates	0	1	1	0	0	0
MIC range	—	32	32	—	—	—
<i>C. glabrata</i>						
no. of isolates	2	0	0	2	1	1
MIC range	16–64	—	—	4–16	16	4

^aWhen an infant had multiple swabs at the same timepoint, the isolate with the highest MIC was included in the analysis.

^bFor the two most common *Candida* species (*C. albicans* and *C. parapsilosis*), the distribution of MICs in period 1 and 2 was not statistically different between the infants in the placebo group compared with infants in the fluconazole group.

fluconazole group compared with the placebo group during period 1 [7/148 (5%) compared with 38/143 (27%); $P < 0.001$] and period 2 [4/131 (3%) compared with 37/135 (27%); $P < 0.001$] (Figure 1). In the fluconazole group, non-*albicans Candida* represented 44%, 29% and 60% at baseline, period 1 and period 2, respectively (Figure 1). In the placebo group, non-*albicans Candida* represented 41%, 34% and 45% at baseline, period 1 and period 2, respectively. More specifically, the most frequent non-*albicans Candida* was *C. parapsilosis* with colonization of 7%, 1% and 2% of infants at baseline, period 1 and period 2, respectively, in the fluconazole group and 8%, 9% and 10% in the placebo group.

Of the 93 infants with positive surveillance swabs, 92 had MIC results available for analysis (Table 2). At baseline, one infant (<1%) was colonized with a fluconazole-resistant *Candida* isolate (*C. glabrata*) in the fluconazole group. After baseline, colonization with ≥ 1 fluconazole-resistant *Candida* isolate occurred in 2/153 (1%) fluconazole patients [*C. albicans* (16 mg/L) and *C. krusei* (32 mg/L)] and 2/148 (1%) of the placebo group [*C. parapsilosis* (8 mg/L) and *C. boidinii* (16 mg/L)]. None of these infants developed an invasive *Candida* infection. Median (range) BW [577 g (540–690)] of infants with a resistant *Candida* was not statistically different from those with no resistant *Candida* [650 g (310–794), $P = 0.44$].

Median (range) fluconazole MIC was similar in both treatment groups at baseline: 0.5 mg/L (0.125–64) in the fluconazole group compared with 0.5 mg/L (0.125–16) in the placebo group ($P = 0.20$) (Figure 2). In period 1, the median (range) MIC was 0.25 mg/L (0.125–32) in the fluconazole group compared with 0.5 mg/L (0.125–16) in the placebo group ($P = 0.58$). Finally, in period 2, median (range) MIC was higher in the fluconazole group [1 mg/L (0.25–32)] compared with the placebo group [0.5 mg/L (0.125–8)] ($P = 0.01$). When excluding *C. glabrata* and *C. krusei* from this analysis, results were similar and median (range) MIC remained one dilution higher in the fluconazole group during period 2 [1 mg/L (0.25–2) in the fluconazole group versus 0.5 mg/L (0.125–8) in the placebo group; $P = 0.04$; Figure S1, available as [Supplementary data](#) at JAC Online]. For the two most common *Candida* species (*C. albicans* and *C. parapsilosis*), the distribution of MICs in period 1 and 2 was not statistically different between the infants in the placebo group and the infants in the fluconazole group (Table 2).

Standard-of-care cultures

Forty infants had ≥ 1 positive standard-of-care culture for *Candida*; 10 and 30 infants in the fluconazole and placebo groups,

Figure 1. *Candida* colonization by treatment group. *Candida* colonization by treatment group at baseline, period 1 and period 2. An infant who was colonized with both a *Candida albicans* and non-*albicans Candida* within a study period was counted twice. F, fluconazole; P, placebo.

Figure 2. Median fluconazole MIC over time for all *Candida* colonization isolates. If an infant had multiple swabs during the same period, the isolate with the highest MIC was used in the analysis. * $P = 0.01$ between groups, using a Wilcoxon rank-sum test.

respectively. Twenty-four (60%) infants had at least one *Candida* isolate with MIC results (56 *Candida* isolates). Of the 56 *Candida* isolates, 48 (86%) were obtained from normally sterile sites. All standard-of-care cultures were positive for either *C. albicans* or *C. parapsilosis*, except for one infant who had positive blood cultures with *C. glabrata* (MIC 16 mg/L) in the placebo group. More infants had a positive standard-of-care culture in the placebo group, compared with the fluconazole group [17 (10%) compared with 7 (4%); $P = 0.02$].

None of the *Candida* isolates obtained in standard-of-care cultures was resistant to fluconazole. MICs were not statistically different between the two treatment groups, with median (range) MICs of 1 mg/L (0.25–4) and 0.25 mg/L (0.125–32) in the

fluconazole and the placebo groups, respectively ($P = 0.13$). Fluconazole MICs appeared to be stable over time in both treatment groups.

Discussion

In this fluconazole prophylaxis study, fluconazole resistance was rare (1.4%) among extremely premature infants (<750 g BW) and did not differ between the treatment groups. The absence of significant resistance emergence with fluconazole prophylaxis is consistent with previous studies in premature infants. Four previous randomized controlled trials of fluconazole prophylaxis in infants born at ≤ 1000 g or ≤ 1500 g reported no development of

resistance in colonizing *Candida*.^{7–10} Among those trials, three were single-centre trials with MICs for colonizing *Candida* that were stable or decreased over the course of prophylaxis (4–6 weeks); one trial was multicentre and showed an increase in MIC₉₀ from 0.125 to 2 mg/L for *C. albicans*.^{7–10} Regarding *Candida* species causing invasive infection, the proportion of fluconazole-resistant *C. parapsilosis* was higher in the fluconazole cohort compared with a historical cohort of 423 ELBW infants, without reaching statistical significance (42% compared with 0%, $P = 0.11$).¹⁵

Although fluconazole resistance was low in our study, we observed a slight increase in MICs in the fluconazole group during period 2, which corresponded to ~1 month of fluconazole prophylaxis. This increase was small and clinically non-significant, given a difference in median MIC of one 2-fold dilution¹⁶ and the fact that it remained well below the fluconazole resistance breakpoint (8 mg/L) for *C. albicans*. Higher MICs in the fluconazole group may be explained by an increasing proportion of non-*albicans* *Candida* among surveillance swabs. However, similar to previous retrospective studies, this increased proportion of non-*albicans* species was due to a reduction in *C. albicans* rather than an absolute increase in non-*albicans* *Candida*.^{17,18} Increased MIC in the fluconazole group may also be explained by low levels of resistance mechanisms, induced by fluconazole exposure.¹⁹

Multiple factors can impact the development of fluconazole resistance, including duration of fluconazole exposure, dosing frequency and level of exposure.²⁰ The longer the exposure (7 days to 2 months), the more likely resistance will develop, as described in immunodeficient adults with prolonged prophylaxis.^{21,22} Duration of prophylaxis in this study was relatively short (42 days), which may contribute to reducing the risk of resistance and the absence of significant resistance emergence in this study. In addition to duration of exposure, drug concentration related to the dosing regimen may also contribute to the development of resistance. Studies of animals infected with both susceptible and resistant strains showed that a daily dosing regimen producing prolonged fluconazole concentrations above the MIC (>40% of the time) prevented the outgrowth of pre-populated resistant strains.^{23,24} Pharmacokinetic studies in premature infants showed that to achieve this target, 3 and 6 mg/kg twice weekly were adequate for MICs of 2 and 4 mg/L, respectively.^{25,26} In this study, the MIC₉₀ values of all colonizing *C. albicans* and *C. parapsilosis* isolates were ≤1 mg/L. Therefore, the twice-weekly dosing used in our study is potentially associated with reduced risk of the development of resistance in premature infants during the prophylaxis period. Moreover, a previous study in infants reported the emergence of one strain of *C. parapsilosis* with decreasing susceptibility to fluconazole, causing bloodstream infections over a 12 year period, during which daily fluconazole prophylaxis of 6–12 mg/kg was administered.²⁷ This again emphasizes the potential benefit of intermittent dosing of 3 and 6 mg/kg twice weekly, targeted to a high-risk population for a specific duration.

Our study must be considered in light of some limitations. First, while we utilized data from a multicentre placebo-controlled, randomized clinical trial, our study was limited by the small sample size of *Candida* isolates in the fluconazole group. This limitation prevented us from adequately comparing the MIC distributions for each *Candida* species separately; however, analysing *Candida* species together for this MIC analysis is reasonable because *C. albicans*, *C. tropicalis* and *C. parapsilosis* all have the same fluconazole

susceptibility breakpoint.¹³ *C. kefyr*, *C. lusitanae* and *C. boidinii* have no established fluconazole susceptibility breakpoint but their epidemiological values are low (1–2 mg/L) and similar to *C. albicans*.¹⁴ *C. glabrata* and *C. krusei* have higher MICs than the rest of the *Candida* species. Nevertheless, when excluding those two *Candida* species from our primary analysis the results were similar. Second, we were limited by the absence of cluster randomization by site. The administration of fluconazole prophylaxis in a subgroup of infants could have affected the susceptibility pattern within a NICU, thereby reducing the observed effect of prophylaxis on MIC. Third, we did not track specific *Candida* isolates, nor did we determine the mechanisms of decreased fluconazole susceptibility; rather, we observed the colonization and susceptibility patterns by treatment group. Finally, results from the standard-of-care cultures should be interpreted with caution since a high proportion (40%) of infants with a positive culture did not have MIC results available. If fluconazole prophylaxis continues to be used in NICUs, then continued surveillance for changes in antifungal susceptibility patterns should be performed.

In conclusion, in premature infants enrolled in a randomized, placebo-controlled trial, fluconazole resistance was rare and we did not observe an emergence of fluconazole resistance in the prophylaxis group. Fluconazole prophylaxis was associated with a slight and clinically non-significant increase in the MICs of colonizing *Candida* species. The true clinical impact of this finding remains to be established. Targeted fluconazole prophylaxis remains safe to use in high-risk preterm infants. As with any antimicrobial prophylaxis, stewardship should continue to include monitoring for significant resistance.

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Supplementary data

Figure S1 and Table S1 are available as [Supplementary data](#) at JAC Online.

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