

Candida Isolates from Neonates: Frequency of Misidentification and Reduced Fluconazole Susceptibility

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Systemic candidiasis affects 1.6 to 4.5% of very low birth weight ($\leq 1,500$ g) infants. A specimen archive of *Candida* strains from intensive care nurseries was created; it currently houses 98 isolates from 17 institutions. Eight isolates (8.2%) were misidentified at the referring institution. The MICs of fluconazole for seven isolates (7.1%, all non-*C. albicans* species, one misidentified initially) were >8 $\mu\text{g/ml}$.

Very low birth weight (VLBW) ($\leq 1,500$ g) infants develop late-onset sepsis (variably defined as sepsis occurring >3 , >4 , or >7 days after birth) with high frequency. Two multicenter studies described late-onset sepsis in 16 and 25% of VLBW infants (6, 19). Nine percent of the bloodstream isolates in one study were fungi, predominantly *Candida* species (19). Systemic candidiasis in neonates is not limited to bloodstream infection: meningitis, urinary tract infection, and deep skin infection are other manifestations (4, 12, 17). The incidence of systemic candidiasis in this population is rising. In the 1980s, 1.6 to 4.5% of VLBW infants developed this infection (1, 2, 5, 22), whereas a recent study found that the incidence in one nursery had increased 11-fold between 1981 and 1995 (9).

Appropriate diagnosis and management of systemic candidiasis in neonates are controversial (16). The Neonatal Candidiasis Study Group (NCSG) is a consortium of pediatric infectious-disease specialists and neonatologists interested in exploring the epidemiology, treatment, and prophylaxis of this infection. The NCSG established a specimen archive to house *Candida* isolates involved in neonatal disease. Isolates were submitted to the archive before therapeutic or prophylactic trials were launched to provide a baseline of susceptibility

patterns as a means to allow detection of shifts in these patterns following the introduction of new therapies. Evaluation of submitted isolates revealed that species identification was frequently incorrect and that resistance to fluconazole (MIC >8 $\mu\text{g/ml}$) was already present in nursery isolates.

Participants in the NCSG submitted at least five *Candida* isolates to the archive. Isolates were required to be from patients in the intensive care nursery. However, the isolates were not required to be from VLBW infants or from infants suffering from invasive disease. The specimens were streaked onto Sabouraud glucose agar slants and mailed to the Medical Mycology Research Center at the University of Texas Medical Branch in Galveston. Upon receipt, the isolates were checked for purity. Germ tube formation in plasma was assessed to identify *Candida albicans*. Non-*C. albicans* species were further identified by inoculation of either a Vitek yeast biochemical card or an API 20C strip and a Dalmau plate. Fluconazole MICs were determined by the M27 standard of the National Committee for Clinical Laboratory Standards (7).

A total of 98 isolates from 90 patients at 17 institutions were submitted. The median gestational age of the patients was 26 weeks (range, 23 to 41 weeks), the median birth weight was 820 g (range, 426 to 4,885 g), and the median age at the time of culture was 24 days (range, 1 to 198 days). Most of the isolates represented invasive disease. The source of the culture was blood in 56 samples, urine in 14, skin or soft tissue in 9, sputum or tracheal aspirates in 6, cerebrospinal fluid in 4, catheter tips in 3, and other sources in 6. Species included *C. albicans* (56%); *C. parapsilosis* (34%); *C. tropicalis* (5.3%); and *C. guilliermondii*, *C. glabrata*, and *C. lusitaniae* (collectively comprising the remaining 4.7%).

Seven institutions referred a total of eight isolates (8.2%) that were misidentified in their clinical laboratories (Table 1). Table 2 provides information about the seven isolates for which the fluconazole MICs were >8 $\mu\text{g/ml}$. Two of these isolates were from the blood and cerebrospinal fluid of the same patient. Two specimens with *C. albicans* contained two morphotypes, for which the fluconazole MICs were different (1 and 0.5 $\mu\text{g/ml}$ and 0.25 and 2 $\mu\text{g/ml}$). The fluconazole MICs for the *C. albicans* and *C. parapsilosis* strains (the most common species isolated) are summarized in Fig. 1.

The NCSG specimen archive contains a large number of invasive isolates from infants, with a wide geographic distribution (see footnote \dagger to byline of this article). The National Epidemiology of Mycoses Survey, the only other multicenter study of *Candida* isolates from infants, included 147 isolates from intensive care nurseries, but this survey studied only 34

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TABLE 1. *Candida* isolates misidentified by the referring institution

Assigned species	Correct species identification	Source of isolate	Fluconazole MIC, $\mu\text{g/ml}$
<i>C. albicans</i>	<i>C. parapsilosis</i>	Eye	2.0
<i>C. albicans</i>	<i>C. parapsilosis</i>	Blood	2.0
<i>C. albicans</i>	<i>C. parapsilosis</i>	Blood	1.0
<i>C. albicans</i>	<i>C. parapsilosis</i>	Blood	4.0
<i>C. parapsilosis</i>	<i>C. albicans</i>	Right atrial mass	0.5
<i>C. tropicalis</i>	<i>C. albicans</i>	Groin	0.5
<i>C. parapsilosis</i>	<i>C. tropicalis</i>	Blood	2.0
<i>C. albicans</i>	<i>C. glabrata</i>	Blood	16

patients from six institutions (11). Much of the data from that study is presented in aggregate with isolate data from surgical intensive care units. Thus, the NCSG specimen archive is an excellent companion resource focusing on neonatal infection.

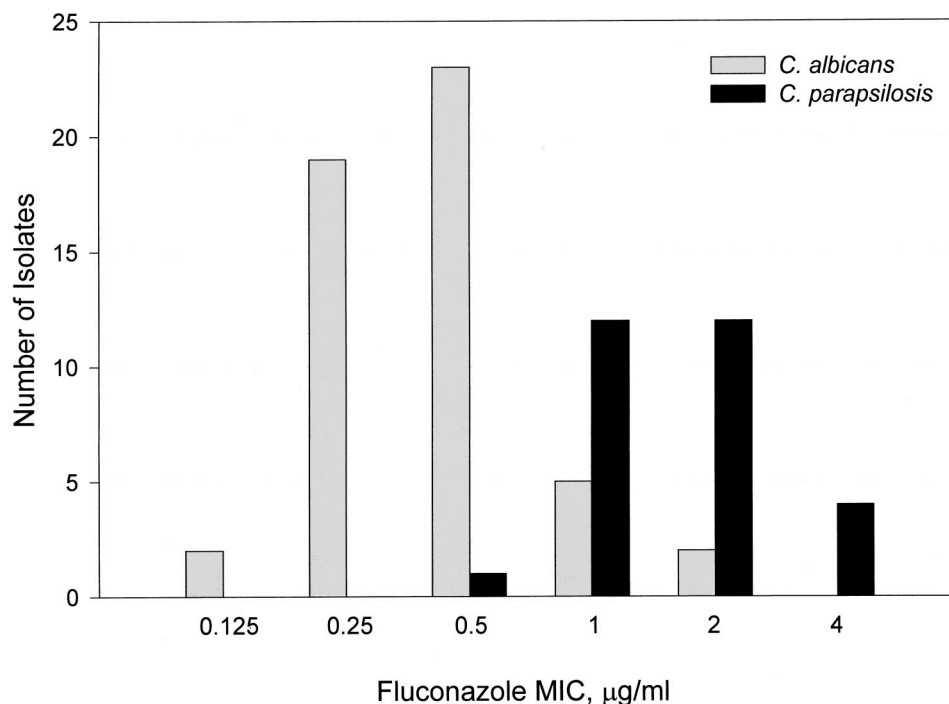
Although amphotericin B is considered the treatment of choice in neonatal systemic candidiasis, fluconazole is used by a substantial number of practitioners (16). Breakpoints for fluconazole susceptibility testing have been established through correlation with clinical outcomes (15). Isolates for which the MIC is $\leq 8 \mu\text{g/ml}$ are considered susceptible, whereas an MIC of $\geq 64 \mu\text{g/ml}$ indicates resistance. When the MIC is 16 or 32 $\mu\text{g/ml}$, the isolate has dose-dependent susceptibility. Data on fluconazole pharmacokinetics in VLBW infants is sparse, and no data exist concerning the safety of increasing the dose when the MIC is elevated (18, 20). In the specimens described in this report, elevated fluconazole MICs were found only for non-*C. albicans* species (Table 2). Although fluconazole resistance in *C. albicans* has been described, higher MICs are more common in non-*C. albicans* strains (10, 14). There is no published data correlating outcome to MIC in fluconazole-treated neonates; treatment failures, mostly due to *C. albicans*, have been de-

TABLE 2. Isolates for which the fluconazole MICs were $>8 \mu\text{g/ml}$

Species	Source of isolate	MIC, $\mu\text{g/ml}$
<i>C. glabrata</i>	Blood	16
	Tracheal aspirate	16
	Blood	16
<i>C. tropicalis</i>	Blood	>64
	Urine	>64
<i>C. guilliermondii</i>	Blood	32
	Cerebrospinal fluid	16

scribed, but no MICs are included in the reports (3, 8, 21). The distribution of MICs shown in Fig. 1 is comparable to the distribution published in another large study of adult patients, suggesting that intensive care nursery isolates reflect those present in the community at large (15).

Several isolates in the archive were incorrectly identified at the referring institution's clinical laboratory (Table 1). In a recent survey of clinical laboratories in New York, blinded proficiency testing revealed misidentification of *C. parapsilosis*, *C. tropicalis*, and *C. glabrata* in 15% of specimens and *C. albicans* in 11% (13). Thus, the 8.2% error rate in this archive is not surprising. However, it does raise the specter of possible misidentification of isolates as *C. albicans* and subsequent complacency about the likelihood of resistance to azoles. Only 28% of clinicians obtain antifungal susceptibility testing data when treating neonates with invasive disease (16). We recommend that clinicians verify the competency of their clinical laboratories for species identification and for susceptibility testing if azole therapy is to be used. Additionally, whenever species identification is potentially critical (e.g., in treatment trials), we recommend that all isolates be submitted to a mycology specialty laboratory.

FIG. 1. Distribution of fluconazole MICs for the *C. albicans* and *C. parapsilosis* strains submitted to the archive.

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