JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2008, p. 2659–2664 Vol. 46, No. 8
0095-1137/08/$08.00+0 doi:10.1128/JCM.00803-08

Geographic Distribution and Antifungal Susceptibility of the Newly Described Species *Candida orthopsilosis* and *Candida metapsilosis* in Comparison to the Closely Related Species *Candida parapsilosis*▽

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Received 28 April 2008/Returned for modification 5 June 2008/Accepted 10 June 2008

***Candida orthopsilosis* and *Candida metapsilosis* are recently described species, having previously been grouped with the more prevalent species *Candida parapsilosis*. Current literature contains very little data pertaining to the distributions and antifungal susceptibilities of these *Candida* species. We determined the species and antifungal susceptibilities of 1,929 invasive clinical isolates from the ARTEMIS antifungal surveillance program collected between 2001 and 2006 and identified as *C. parapsilosis* using Vitek and conventional methods. Of the 1,929 isolates of presumed *C. parapsilosis* tested, 117 (6.1%) were identified as *C. orthopsilosis* and 34 (1.8%) as *C. metapsilosis*. The percentage of presumed *C. parapsilosis* isolates found to be *C. orthopsilosis* varied greatly by region, with the highest percentage (10.9%) from South America and the lowest (0.7%) from Africa. The MIC distributions of the *C. orthopsilosis* and *C. metapsilosis* isolates were statistically significantly lower than those of *C. parapsilosis* for all drugs except fluconazole, for which they were significantly higher ($P < 0.001$ for all). No *C. orthopsilosis* or *C. metapsilosis* isolates were fluconazole resistant, and all were susceptible to caspofungin, anidulafungin, and micafungin.**

Candida parapsilosis is a cause of serious nosocomial infections and is the second most common *Candida* species isolated from bloodstream infections in many regions of the world (1, 21, 25, 29), particularly in Latin America (2, 4). Of further concern are recent reports of reduced azole and echinocandin susceptibilities in this species (1, 2, 3, 14, 19, 20, 21, 26).

C. parapsilosis has long been considered a three-species complex. Since the early 1990s, it has been shown by randomly amplified polymorphic DNA analysis (10), karyotyping (13), multilocus enzyme electrophoresis and ribosomal internal transcribed spacer sequencing (11), DNA reassociation analysis (24), complex DNA probe pattern by Southern blot analysis (6), and multigenic sequence analysis (5) that the phenotypic and genotypic patterns fall into three distinct groupings. Tavanti and coworkers (27) used multigenic sequence analysis and internal transcribed spacer sequencing to define the *C. parapsilosis* complex as the three separate species, *C. parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis*.

Despite the fact that the *C. parapsilosis* complex has been recognized for a number of years, very little is known about the epidemiology of the two rare species within the complex, *C. orthopsilosis* (formerly *C. parapsilosis* group II) and *C. metapsilosis* (formerly *C. parapsilosis* group III). There have been at least two outbreaks reported in which *C. orthopsilosis* (*C. parapsilosis* type II) was reported to be part of the outbreak. The first was an outbreak in a hospital in San Antonio reported by Lin and coworkers (11). In 2000, Zancope-Oliviera and coworkers (31) described a small nosocomial outbreak of *C. parapsilosis* group II in a hospital in Brazil. There are other outbreaks that were reported as being caused by *C. parapsilosis* in which a distinction was not made between the types, and thus they could have been caused by either *C. orthopsilosis* or *C. metapsilosis*. No other epidemiological data appeared until Tavanti and coworkers (28) described a number of *C. orthopsilosis* strains from 13 patients in Pisa, Italy.

In this study, we used a large worldwide collection of presumed *C. parapsilosis* isolates, collected as part of the ARTEMIS Global Surveillance study, to screen for *C. orthopsilosis* and *C. metapsilosis*. This is the first global description of the prevalence, distribution, and antifungal susceptibility of *C. orthopsilosis* and *C. metapsilosis*.

MATERIALS AND METHODS

Study collection and test sites. The isolates were those previously analyzed as part of the ARTEMIS Global Surveillance study (19). One hundred thirty-four medical centers submitted isolates over the course of the study, and at least 101 of these hospitals submitted both adult and pediatric isolates. Yeasts from all body sites and tissues that were considered pathogens responsible for invasive disease were submitted. Yeasts considered by the collecting physicians to be colonizers were excluded, as were duplicate isolates from a single patient as determined locally. Identification was performed locally and confirmed at the University of Iowa using the Vitek Yeast Biochemical Card (bioMérieux Vitek, Durham, NC).

DNA isolation and amplification. Isolates were stored as water-based stocks, passaged on potato dextrose agar, and then plated fresh on potato dextrose agar and incubated at 30°C for 24 h. A 3-μl equivalent of yeast was scraped from the plate and resuspended in 20 μl of sterile water. The yeast was "shocked" by heating it to 95°C for 8 min and was then placed in a −70°C freezer for >1 h.

Taq DNA polymerase reaction mixtures were prepared as suggested by the manufacturer (New England Biolabs, Ipswich, MA). Each 50-μl reaction mixture contained 4 μl of the prepared yeast supernatant. Primers S1F and S1R, which

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▽ Published ahead of print on 18 June 2008.

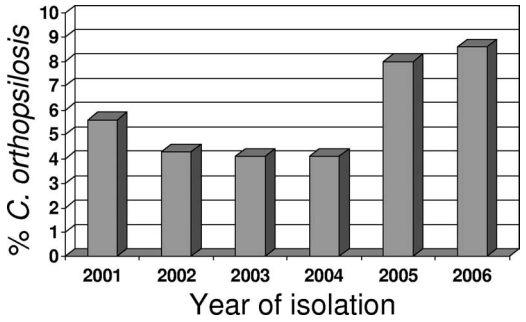

FIG. 1. Percentages of *C. parapsilosis* isolates that were *C. orthopsilosis* by year ($P = 0.01$ for an increasing trend over time in the proportion of all isolates that were *C. orthopsilosis*).

amplify the secondary alcohol dehydrogenase gene (27), were used for the PCR. The reaction consisted of one 5-min cycle at 95°C, followed by 40 cycles as follows: 1 min at 92°C, 1 min at 45°C, and 1 min at 68°C. The reaction was terminated by one 7-min cycle at 68°C. Following completion of the cycle, 1 μl of BanI (New England Biolabs, Ipswich, MA) was added directly to the reaction mixture, and the mixture was incubated for an additional 2 hours at 37°C. Isolates of *C. parapsilosis*, *C. orthopsilosis*, *C. metapsilosis*, and *Lodderomyces elongisporus* were identified by the size of the major restriction fragment on an agarose gel following digestion. All isolates identified as *C. orthopsilosis*, *C. metapsilosis*, and *L. elongisporus* were amplified and digested a second time for confirmation. ATCC isolates 96139, 96144, and 22688 were used as positive controls for *C. orthopsilosis*, *C. metapsilosis*, and *L. elongisporus*, respectively.

Antifungal agent susceptibility testing. Standard antifungal susceptibility testing was performed as described previously (22) and in the Clinical and Laboratory Standards Institute (CLSI) document M27-A2 (15). The final concentration ranges were 0.007 to 8.0 μg/ml for anidulafungin, caspofungin, and micafungin and 0.12 to 128.0 μg/ml for fluconazole. Amphotericin B Etest concentrations ranged from 0.002 to 32 μg/ml. The MIC was defined as the lowest concentration at which there was a visually prominent reduction in growth (approximately 50%) relative to the drug-free growth control after 24 h of incubation (anidulafungin, caspofungin, and micafungin) (17) or after 48 h of incubation (fluconazole). The interpretive criteria for fluconazole were those published by the CLSI (15) and by Rex et al. (23). Isolates for which the fluconazole MICs were ≤8.0 μg/ml were susceptible, those for which the MICs were 16 to 32 μg/ml were susceptible dose dependent, and those for which the MICs were ≥64 μg/ml were resistant. Interpretive criteria for the echinocandins (anidulafungin, caspofungin, and micafungin) were those recently established by the CLSI (isolates with an MIC of ≤2.0 μg/ml to the echinocandins were considered susceptible [minutes of the June 2007 meeting of the CLSI Antifungal Subcommittee {unpublished}]). Interpretive criteria for amphotericin B have not yet been established by the CLSI. However, for purposes of comparison, isolates with amphotericin B MICs of ≤1.0 μg/ml were considered susceptible. Quality control was performed using *Candida krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 for each batch of isolates tested.

Statistical analyses. Differences in antifungal MIC distributions were examined using the Wilcoxon rank sum test. Differences in species distribution by year were examined using a chi-square test. Alpha was set at 0.05, and all *P* values were two tailed. SPSS version 15.0 (Chicago, IL) was used for all statistical analyses.

RESULTS

Demographic distribution of *C. orthopsilosis* isolates. Between 2001 and 2006, 1,976 isolates of *C. parapsilosis* were banked at the University of Iowa as part of the ARTEMIS Global Surveillance study. Of those isolates, 1,929 could be recovered and species identification could be performed. These isolates came from 89 study centers in 29 countries on six continents. *C. parapsilosis* accounted for 1,762 (91.3%) of the isolates, while 117 (6.1%) were *C. orthopsilosis*, 34 (1.8%) were *C. metapsilosis*, and 16 (0.8%) were *L. elongisporus* (described previously [12]). The percentage of *C. parapsilosis* complex isolates that were *C. orthopsilosis* increased longitudinally during this surveillance study (Fig. 1). While in the first 4 years of the study the average percentage of the isolates that were *C. orthopsilosis* was 4.5%, the average percentage over the last 2 years was 8.3% ($P = 0.01$).

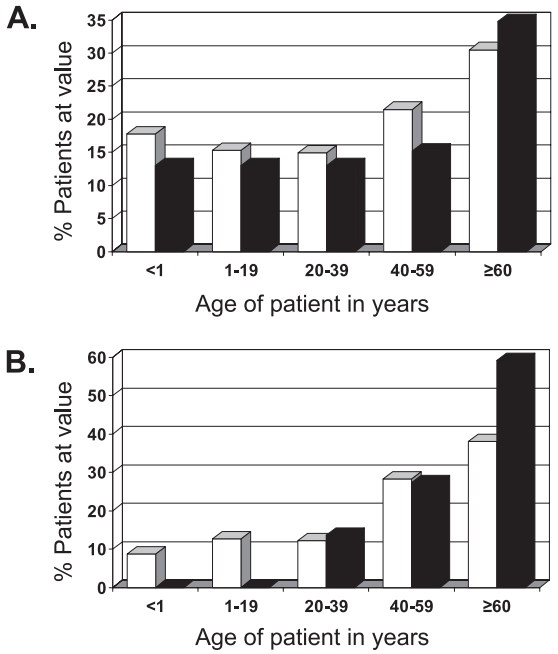

FIG. 2. Distribution by age of patients with *C. parapsilosis* (white) and *C. orthopsilosis* (black) globally (A) and in the United States (B).

Of the isolates for which the site of isolation was provided, 77% of *C. orthopsilosis* isolates and 60% of *C. metapsilosis* isolates were from bloodstream infections compared to 79% of *C. parapsilosis* isolates. *C. orthopsilosis* was also isolated from ascites fluid, abscesses, catheters, cerebrospinal fluid, and pulmonary sources (e.g., pleural and bronchial alveolar lavage fluids). *C. metapsilosis* was also isolated from abscesses, ascites fluid, bronchial alveolar lavage fluid, and joint fluid.

C. parapsilosis is often associated with infections of neonates and infants (1, 8). In this study, the median age range for patients with *C. parapsilosis* isolates was 40 to 49 years old, with the highest percentage of isolates (30.5%) falling into the ≥60-year-old age group (Fig. 2A). Likewise, the median age range for patients with *C. orthopsilosis* isolates was 40 to 49 years old, and the highest percentage of isolates (34.8%) fell into the ≥60-year-old age group. Interestingly, when only the *C. parapsilosis* and *C. orthopsilosis* isolates from the United States were analyzed, there was a difference between the two groups (Fig. 2B). While 33.6% of the patients with *C. parapsilosis* isolates were in the ≤39-year-old age group, only 13.6% of the patients with *C. orthopsilosis* isolates fell into this age group, and while only 38.1% of the patients with *C. parapsilosis* isolates were in the ≥60-year-old age group, 59.1% of the patients with *C. orthopsilosis* isolates were in this age group.

Regional distribution of *C. orthopsilosis* and *C. metapsilosis*. *C. orthopsilosis* isolates were identified from all six of the continents from which isolates were received, but the isolates were not evenly distributed by continent or by country (Table 1).

TABLE 1. Regional distribution of *C. parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis*

Country or region	No. of isolates by species (%)[c]			
	C. parapsilosis	*C. orthopsilosis*	*C. metapsilosis*	*L. elongisporus*
United States	506	26	2	2
Canada	52		2	
Mexico	42	6		8
North America	600	32 (5.0)	4 (0.6)	10
Venezuela	94	19		2
Chile	7			
Ecuador	45	2		
Brazil	80	16	4	
Argentina	62	2	1	
Columbia	49	3		
South America	337	42 (10.9)	5 (1.3)	
Finland	12			
Netherlands	2	1		
Hungary	16			
Czech Republic	27	2		
Slovak Republic	44	3		
United Kingdom	25			
Spain	46	2	1	
Turkey	72	1	2	
Poland	25		4	
Portugal	66		1	
Italy	86	8	5	1
Russia	18			1
Israel	15		1	
Europe and Middle East	454	17 (3.5)	14 (2.9)	2
Thailand	2	1		
Malaysia	38	15[b]		2
South Korea	72	2		
Taiwan	29		2	
China	22	3	3	
Asia	163	21 (11.0)	5 (2.6)	2
Australia	64	4 (5.5)	5 (6.8)	
Africa[a]	144	1 (0.7)	1 (0.7)	

[a] All African isolates in this study came from the country of South Africa.
[b] Five isolates were not included in the perceived outbreak.
[c] The percentages of *C. orthopsilosis* and *C. metapsilosis* in each geographic region are indicated in parentheses.

Asia had the highest percentage of isolates that were *C. orthopsilosis* (11%). However, if a series of 10 isolates from a single pediatric ward in one hospital, a possible outbreak, was removed, the percentage in Asia dropped to 6.1%. Of the isolates from South America, 10.9% were *C. orthopsilosis*. The distribution on this continent varied from 16.5% and 16.0% *C. orthopsilosis* isolates from Venezuela and Brazil, respectively, to 4.3% and 3.1% *C. orthopsilosis* isolates from Ecuador and Argentina, respectively. In North America, 5.0% of the *C. parapsilosis* complex isolates were *C. orthopsilosis*. In the United States, 4.9% of the isolates were *C. orthopsilosis*, while in Mexico, 10.7% of the isolates were *C. orthopsilosis*. None of the 52 isolates from Canada were *C. orthopsilosis*. Overall, Europe and the Middle East had 17 (3.5%) *C. orthopsilosis* isolates, with almost half of them coming from Italy, where 8.0% of the isolates were *C. orthopsilosis*. No *C. orthopsilosis* isolates were recovered from Finland, the United Kingdom, Poland, Portugal, Russia, Hungary, and Israel, despite their combined total of 177 isolates of *C. parapsilosis*. Interestingly, although there were 146 *C. parapsilosis* complex isolates from multiple centers in South Africa, only a single isolate of *C. orthopsilosis* was identified from that country.

Like *C. orthopsilosis*, *C. metapsilosis* isolates could be found on all six continents. Only 4 of 646 (0.6%) North American isolates and only 1 of 146 (0.7%) South African isolates were *C. metapsilosis*. Although no *C. orthopsilosis* isolates came from Canada, two *C. metapsilosis* isolates did. Europe had almost as many *C. metapsilosis* isolates, 14 (2.9%), as *C. orthopsilosis* isolates. Poland had the highest percentage with 13.8% of all *C. parapsilosis* complex isolates being *C. metapsilosis*. *C. metapsilosis* isolates also came from Spain, Portugal, Turkey, and Italy. Australia had more *C. metapsilosis* isolates than *C. orthopsilosis* isolates, and 6.8% of all *C. parapsilosis* complex isolates from Australia were *C. metapsilosis*. Both China and Taiwan had *C. metapsilosis* isolates, and 2.6% of all Asian isolates were *C. metapsilosis*. Despite the very high number of *C. orthopsilosis* isolates from South America, only 1.7% of South American *C. parapsilosis* complex isolates were *C. metapsilosis*: four from Brazil and one from Argentina.

Antifungal susceptibility testing. Susceptibility test results are summarized in Fig. 3 and Table 2. All of the *C. orthopsilosis* isolates were fluconazole susceptible. There were no resistant or susceptible dose-dependent *C. metapsilosis* isolates, and the highest fluconazole MIC was 4 μg/ml (Table 2 and Fig. 3A). By comparison, 0.6% of *C. parapsilosis* isolates were resistant and 4.0% were susceptible dose dependent. The mean MIC of fluconazole against *C. parapsilosis* was higher than the mean MICs of fluconazole against *C. orthopsilosis* and *C. metapsilo-*

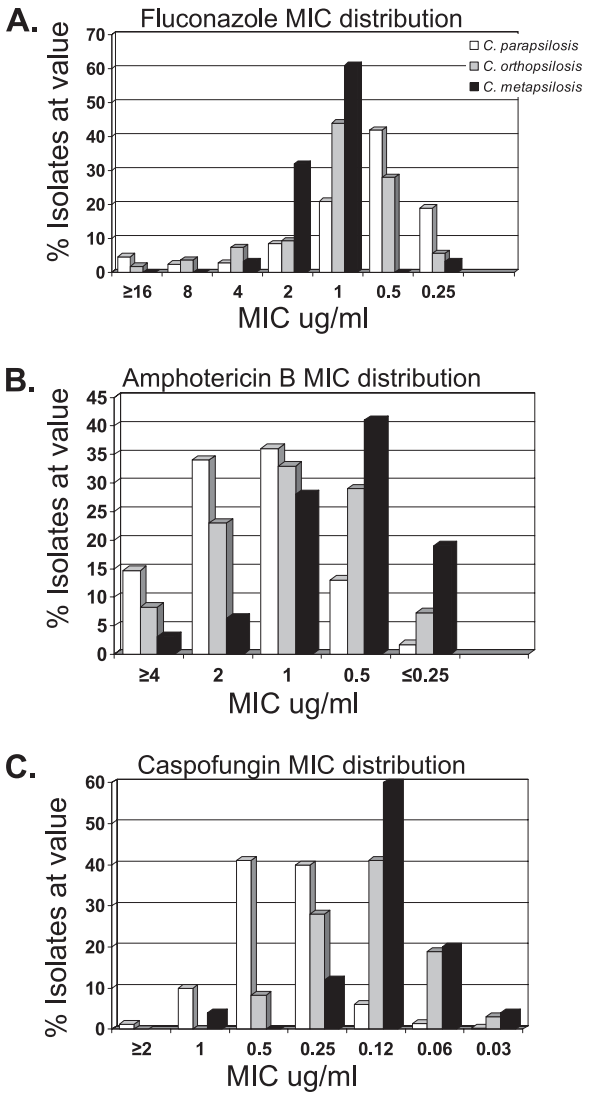

FIG. 3. MIC distributions of *C. parapsilosis* (white), *C. orthopsilosis* (gray), and *C. metapsilosis* (black) isolates against fluconazole (A), amphotericin B (B), and caspofungin (C).

sis. However, both the MIC_{90}s and the overall MIC distributions were higher for *C. orthopsilosis* and *C. metapsilosis* than for *C. parapsilosis* (Table 2 and Fig. 3A).

The MICs of *C. orthopsilosis* and *C. metapsilosis* to amphotericin B were lower than those of *C. parapsilosis* (Table 2 and Fig. 2B). While 14.7% of *C. parapsilosis* isolates had an amphotericin B MIC of ≥4 μg/ml, only 8.3% of *C. orthopsilosis* isolates and 3.1% of *C. metapsilosis* isolates had MICs of ≥4 μg/ml. While only 15% of *C. parapsilosis* isolates had MICs of ≤0.5 μg/ml, 36.3% of *C. orthopsilosis* isolates and 60% of *C. metapsilosis* isolates had MICs of ≤0.5 μg/ml.

Recent reports have indicated that the MICs of the echinocandins for *C. parapsilosis* isolates are elevated compared to those for *C. albicans* (18, 20, 21). As is demonstrated in Fig. 3 and Table 2, the echinocandin MICs of *C. parapsilosis* were higher than those of *C. orthopsilosis* or *C. metapsilosis*. In this study, the MIC_{90}s for *C. parapsilosis* against caspofungin, anidulafungin, and micafungin were 1 μg/ml, 2 μg/ml, and 2 μg/ml, respectively. By contrast, the MIC_{90}s for *C. orthopsilosis* against caspofungin, anidulafungin and micafungin were 0.25 μg/ml, 2 μg/ml, and 0.5 μg/ml, respectively. Similar results were seen for *C. metapsilosis*, with MIC_{90} values of 0.25 μg/ml, 1 μg/ml, and 0.5 μg/ml against caspofungin, anidulafungin, and micafungin, respectively.

DISCUSSION

This is the most comprehensive study of the antifungal susceptibilities of *C. orthopsilosis* and *C. metapsilosis* to date. There have been reports of smaller numbers of isolates, some dating to before they were formally separated into species. In 1995, Lin and coworkers (11) reported that two isolates of *C. metapsilosis* had MICs to amphotericin B that were lower on average than those of *C. parapsilosis* in the same study. Similar results were reported for two isolates of *C. metapsilosis* from Hungary (9). In a study of 27 isolates of *C. orthopsilosis* from Pisa, Italy, by Tavanti and coworkers (28), the mean MICs for fluconazole (5.89 μg/ml) were higher than we report here (2.1 μg/ml). The same study reported mean MICs of 0.045 μg/ml for amphotericin B and 0.18 μg/ml for caspofungin. The caspofungin MIC is similar to what we report here (0.17 μg/ml), but the mean MIC for amphotericin B is more than fourfold lower that the global average of 1.1 μg/ml reported here.

To date, there have been very few studies looking at the prevalence of *C. orthopsilosis* and *C. metapsilosis* among *C. parapsilosis* complex isolates. The most recent data, a population-based study from Spain (7), found that *C. metapsilosis* comprised 6.9% (6/87) and *C. orthopsilosis* comprised 5.7% (5/87) of all *C. parapsilosis* complex isolates collected between 2002 and 2003. A survey of *Candida* bloodstream infections in Scotland during 2005 and 2006 reported 35 isolates of *C. parapsilosis* but none of *C. orthopsilosis* or *C. metapsilosis* (16).

TABLE 2. MIC_{50}/MIC_{90} and mean MIC of *C. parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis* isolates

Drug	*C. parapsilosis*		*C. orthopsilosis*[a]		*C. metapsilosis*[a]	
	MIC_{50}/MIC_{90}	Mean MIC	MIC_{50}/MIC_{90}	Mean MIC	MIC_{50}/MIC_{90}	Mean MIC
Fluconazole	0.5/2	2.3	1/4	2.1	1/2	1.4
Amphotericin B	1/4	1.5	1/2	1.1	0.5/1	0.7
Caspofungin	0.5/1	0.4	0.12/0.25	0.2	0.12/0.25	0.2
Anidulafungin	2/2	1.9	1/2	1.0	0.5/1	0.6
Micafungin	1/2	1.1	0.25/0.5	0.4	0.25/0.5	0.4

[a] $P < 0.001$ for the difference in MIC distribution compared with *C. parapsilosis* for all drugs tested. For fluconazole, *C. orthopsilsosis* and *C. metapsilosis* MIC distributions were higher than those of *C. parapsilosis*, while for amphotericin B and all the echinocandins, *C. orthopsilosis* and *C. metapsilosis* MIC distributions were lower than those of *C. parapsilosis*.

We likewise report no *C. orthopsilosis* or *C. metapsilosis* isolates among 25 *C. parapsilosis* complex isolates from the United Kingdom. A similar survey in Hungary of isolates collected in 2004 and 2005 found that out of 22 *C. parapsilosis* complex isolates, 2 were *C. metapsilosis* and none were *C. orthopsilosis* (9). We found no *C. orthopsilosis* or *C. metapsilosis* isolates among 16 *C. parapsilosis* complex isolates from Hungary. Tavanti and coworkers (28) reported that 4.5% of all *C. parapsilosis* complex isolates from Pisa, Italy, were *C. orthopsilosis*, and none were *C. metapsilosis*. The actual incidence in that study may have been somewhat lower, as all 33 *C. orthopsilosis* isolates came from only 13 patients. Although our results were pooled from eight different sites in Italy, we found 8% *C. orthopsilosis* and 5% *C. metapsilosis*, similar to the numbers reported for Spain (7). There is also a very recent report of *C. orthopsilosis* causing a bloodstream infection in Malaysia (30), another country from which we have isolated a number of *C. orthopsilosis* isolates.

Although we do not have the clinical data from the hospital or the fingerprint patterns of the isolates, there were 10 isolates of *C. orthopsilosis* from a pediatrics unit isolated over the course of a year in a single hospital in Malaysia. If this was an outbreak, it would bring the number of known nosocomial outbreaks of *C. orthopsilosis* to three.

In conclusion, we report the species distribution and antifungal susceptibilities of a large global collection of *C. parapsilosis* complex isolates. We found that *C. orthopsilosis* and *C. metapsilosis* together account for fewer than 10% of *C. parapsilosis* complex infections overall, with substantial regional variation noted. However, the proportion of isolates represented by *C. orthopsilosis* may have been increasing in recent years. Both *C. orthopsilosis* and *C. metapsilosis* have amphotericin B and echinocandin MICs lower than those of *C. parapsilosis*. Although they have slightly higher fluconazole MICs overall than *C. parapsilosis*, all of the *C. orthopsilosis* and all of the *C. metapsilosis* isolates were susceptible to fluconazole. The differences in antifungal susceptibility that we describe are therefore not categorical differences, suggesting that routine discrimination among these *C. parapsilosis* complex species is not necessary for the clinical laboratory. However, ongoing surveillance is needed to monitor changes in the species distribution and antifungal susceptibility within this species complex. In addition, discrimination of species within the complex may be important for future clinical trials of candidemia treatment, in order to determine if the MIC differences noted by our group and others are associated with differences in clinical outcomes.

ACKNOWLEDGMENTS

We thank Shailesh Tendolkar and Richard Hollis for their contributions to the manuscript.

This study was funded by grants from Merck & Co., Inc., and Pfizer, Inc.

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