Survey of Vaginal-Flora *Candida* Species Isolates from Women of Different Age Groups by Use of Species-Specific PCR Detection[▽]

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A retrospective survey of 93,775 samples testing positive in *Candida* species-specific PCR tests performed on cervicovaginal swabs over a 4-year period demonstrated consistent yearly distributions of *Candida albicans* (89%), *C. glabrata* (7.9%), *C. parapsilosis* (1.7%), and *C. tropicalis* (1.4%). However, the species distributions among different age groups revealed increases in the percentages of non-*albicans* species with increases in age.

Vulvovaginal candidiasis (VVC) is a common fungal infection that affects healthy women of all ages. At least 75% of women will develop one or more infections once during their lifetime, with 5 to 8% of those individuals developing recurrent infections (5, 7). Current literature examining the species distribution of *Candida* isolates involved in VVC is limited; however, several important observations have been made. For example, one study shows that *Candida albicans* accounts for 70 to 90% of all VVC cases, with a recent emergence of non-*albicans* species (10). The rise in VVC infections, more specifically in those caused by non-*albicans* species, could be due to several factors, ranging from an increase in over-the-counter antifungal use to an increase in high-risk patient populations (i.e., diabetics and menopausal women). *Candida glabrata* is the primary non-*albicans* species emerging in VVC, accounting for up to 14% of infections in immune-competent women (9, 10).

In addition to an increase in non-*albicans* species overall, it is becoming clear that certain patient populations may experience higher risks of infection from these non-*albicans* species, often leading to limited treatment options. Interestingly, in a few small studies, *C. glabrata* was found to be the primary species isolated from diabetic (61.3%) and elderly (51.2%) patients with VVC (2, 4, 6, 11). Often, these non-*albicans* species are associated with elevated MIC levels for the azoles, the most commonly prescribed class of antifungal drugs. It has been well documented that *C. glabrata* demonstrates both intrinsically low susceptibility to the azoles and the ability to develop frank resistance (8, 12, 13, 14, 15, 16). Moreover, a recent increase in the trailing phenotype, with low-level resistance to the azoles, has been observed for the *Candida tropicalis* isolates (1, 3). This highlights the importance of identifying *Candida* species within clinical samples in order to provide physicians with information concerning the proper treatment for their patients.

The purpose of this study was to examine the species distribution from a large collection of *Candida* isolates ($n = 93,775$) from cervicovaginal swabs of women suspected of having VVC in the United States. The species distributions among different age groups were also examined. The retrospective survey was performed on clinical cervicovaginal swab samples submitted to our clinical laboratory for PCR-based *Candida* identification between March 2003 and March 2007. All four *Candida* species-specific tests were performed on each sample, with informed consent as ordered by the evaluating physician, following the guidelines of the laboratory's federal, state, and Clinical Laboratory Improvement Amendments (CLIA) certifications. Internal validation procedures for the sensitivity, specificity, concordance, interference, accuracy, and precision of the individual and multiplexed PCR tests were performed. These validation procedures included comparison to culturing and determination of concordance with other published *Candida* species-specific and pan-*Candida* PCR tests. Patient anonymity was strictly protected in accordance with the federal Health Insurance Portability and Accountability Act (HIPPA) of 1996. Subjects were informed of the results of the testing procedures by their physicians. None of the samples were accompanied by information regarding the medical history or clinical presentation of the participant at the time of specimen collection. Only the patient's state of residence and age were obtained for the purposes of this retrospective study. The specimens were collected from a cervicovaginal sampling performed by the evaluating physician with a Cellmatics swab (BD, Sparks, MD) or OneSwab (Medical Diagnostic Laboratories L.L.C., Hamilton, NJ). Upon receipt, swabs were immediately processed for PCR analysis. The reference strains were obtained from the American Type Culture Collection (Manassas, VA) and include *C. glabrata* 66032, *C. tropicalis* 750, *C. albicans* 90028, and *Candida parapsilosis* 22019. Statistical analyses of the annual percent distribution of species-specific-PCR-positive patient samples were performed using Student's *t* test.

The evaluation of the prevalence of the four most common candidiasis-causing *Candida* species, *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*, within the cervicovaginal environments of patients suspected of VVC was performed. In addition, the survey evaluated potential changes of *Candida*

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TABLE 1. Survey of samples testing positive in *Candida* species-specific PCR tests by species, annual distribution, and geographic location

Species	No. of positive samples	Annual distribution (%)	Distribution (%) by region (United States)			
			Northeast	South	Midwest	West
C. albicans	83,407	89.0 ± 2.0	17,065 (90.5)	52,932 (88.1)	8,626 (90.0)	4,784 (91.1)
C. glabrata	7,401	7.9 ± 0.26	1,224 (6.5)	5,128 (8.5)	671 (7.0)	378 (7.2)
C. parapsilosis	1,604	1.7 ± 1.0	362 (1.9)	1,041 (1.8)	155 (1.6)	46 (0.9)
C. tropicalis	1,363	1.4 ± 1.3	217 (1.1)	971 (1.6)	132 (1.4)	43 (0.8)
Total isolates	93,775		18,868	60,072	9,584	5,251

species prevalence with age. The 93,775 samples testing positive in *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* species-specific PCR tests demonstrated a consistent yearly distribution of these *Candida* species over 4 years (Table 1). This distribution of *Candida* species is consistent with previously published surveys (8). Additionally, the distribution of *Candida* species encompassing the 48 continental states was consistent among the Northeast, South, Midwest, and West regions as defined by the U.S. Census Bureau (Table 1). Moreover, the distributions of *Candida* species-positive samples were consistent from year to year within the different age groups (Fig. 1). However, one interesting trend was noticed upon comparing the distributions of *Candida* species for the different age groups. The data showed significant ($P \leq 0.05$) increases in the prevalences of non-*albicans* species, along with steady decreases in *C. albicans*, with increases in age. *C. glabrata* accounted for >18% of isolates in women of menopausal age and older (≥51 years), compared to <4.8% for young adults (<30 years) and <11.8% for middle-aged women (31 to

50 years). Significant increases in the prevalences of *C. parapsilosis*-positive samples were also observed with increases in age (Fig. 1). This increase in non-*albicans* species, specifically *C. glabrata*, is an important observation that was reported in smaller studies of the elderly (4). Similar increases in non-*albicans* species have been reported for diabetic patients, in which *C. glabrata* was the primary species isolated (2, 6, 11). Understanding the changes that occur in patient populations which exhibit higher prevalences of non-*albicans* species is warranted (i.e., menopausal and postmenopausal women and diabetic patients). Possible explanations include changes in patient physiology, hormone balance, and decrease in immune function.

This large set of *Candida* species-specific PCR tests performed on cervicovaginal swabs over a 4-year period allowed us to investigate the species distribution of *Candida* isolates throughout the United States from women suspected of having VVC. Although *C. albicans* still remains the primary causative agent of VVC, this study shows a rise in the non-*albicans*
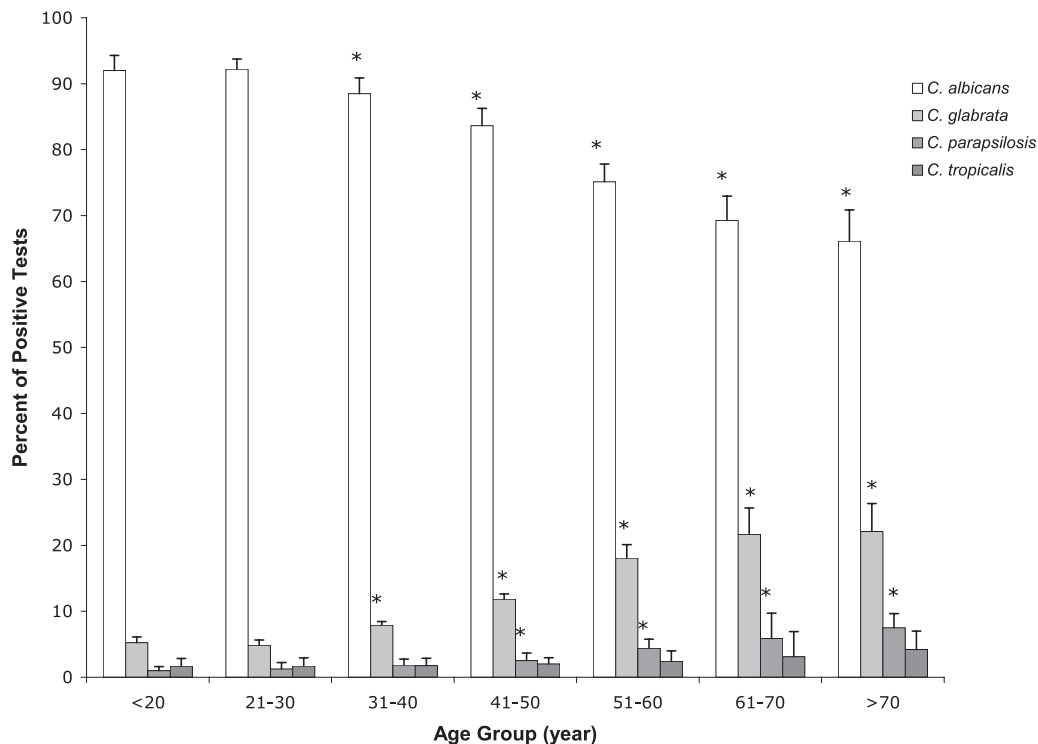
FIG. 1. Distribution of 93,775 *Candida* isolates by prevalence of species versus age. ∗, *P* was <0.05 for comparison to the <20 and 21-to-30 age groups. Error bars represent the standard deviations of the percentages of *Candida* species detected per year within each age group.

species, which correlates with an increase in patient age. Given that non-*albicans* species, such as *C. glabrata*, have reduced susceptibilities to the azoles, a common VVC treatment, the importance of early species identification is necessary for proper treatment. The use of nonazole antifungals, such as boric acid and flucytosine, has been shown to be effective in treating non-*albicans* species, especially *C. glabrata*, which can often resist azole antifungal therapy.

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