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Species Identification and Antifungal Susceptibility of *Candida* Bloodstream Isolates from Population-based Surveillance in Two US Cities: 2008-2011

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ABSTRACT

Between 2008 and 2011, population-based candidemia surveillance was conducted in Atlanta, GA and Baltimore, MD. Surveillance had been previously performed in Atlanta in 1992-1993 and in Baltimore in 1998-2000, making this the first population-based candidemia surveillance conducted over multiple time points in the US. From 2,675 identified cases of candidemia in the current surveillance, 2,329 *Candida* isolates were collected. *Candida albicans* no longer comprised the majority of isolates but remained the most frequently isolated species (38%), followed by *C. glabrata* (29%), *C. parapsilosis* (17%) and *C. tropicalis* (10%). The species distribution has changed over time; in both Atlanta and Baltimore the proportion of *C. albicans* decreased and the proportion of *C. glabrata* increased, while the proportion of *C. parapsilosis* increased in Baltimore only. There were 98 multi-species episodes, with *C. albicans* and *C. glabrata* the most frequently encountered combination. The new species-specific CLSI *Candida* MIC breakpoints were applied to these data. With the exception of *C. glabrata* (11.9% resistant), resistance to fluconazole was very low (2.3% for *C. albicans*, 6.2% for *C.tropicalis* and 4.1% for *C. parapsilosis*). There was no change in the proportion of fluconazole resistance between surveillance periods. Overall echinocandin resistance was low (1%) but was higher for *C. glabrata* isolates, ranging from 2.1% resistant to caspofungin in Baltimore to 3.1% resistant to anidulafungin in Atlanta. Given the increase at both sites and the higher echinocandin resistance, *C. glabrata* should be closely monitored in future surveillance.

INTRODUCTION

Candida species are a leading cause of healthcare-associated bloodstream infections (BSIs) in the U.S., accounting for approximately 10% of healthcare-associated BSIs and one of the highest crude mortality rates (37). Candidemia infections increase the risk of patient mortality and increase both the length of stay and cost associated with hospitalization (11,17). To implement appropriate control measures, it is important to understand the epidemiology of BSIs caused by *Candida* species. Although the species distribution has changed over the last three decades, more than 95% of candidemia infections have been caused by the five most common species: *Candida albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* (4,26,36). The most dramatic temporal change has been the shift from *C. albicans* to non-*C. albicans* species as causative agents of the majority of BSIs (1,4,10,12,16,26,36). The significance of this trend is that non-*C. albicans* species, especially *C. glabrata*, can be less susceptible to fluconazole, the most inexpensive and readily available antifungal agent used to treat candidemia (10,25,28,31). The shift to non-*C. albicans* species is also significant because the newest class of antifungal agents, the echinocandins, are not currently recommended as primary therapy against *C. parapsilosis*, the third most common species in the US (10,16,20,25).

Sentinel and population-based surveillance play complementary roles in our understanding of candidemia. Sentinel surveillance can encompass many facilities over broad geographic areas but the makeup of individual institutions may not be representative of whole populations. The advantage of population-based surveillance is that it allows the calculation of population-based incidence rates (9,10,12,16,25,28,29). Antifungal susceptibility testing of *Candida* isolates collected as part of surveillance has played an increasingly important role in surveillance strategies for epidemiological evaluation of antifungal resistance

(2,6,9,10,12,28,33,34). With the advent of standardized susceptibility testing methodology (7), past and present surveillance can be directly compared to monitor temporal trends in antifungal resistance. Global sentinel surveillance revealed an increasing trend in resistance to fluconazole among *C. parapsilosis*, *C. guilliermondii* and *C. lusitaniae,* while the resistance rate among *C. albicans*, *C. glabrata* and *C. tropicalis* fluctuated slightly or remained stable (28). Because of the recent appearance of echinocandins in clinical practice, temporal surveillance data for susceptibility to these drugs are limited, but an early report indicated that susceptibility rates were stable (23). Temporal changes in antifungal susceptibility for *Candida* species to any antifungal agent have not yet been described using population-based surveillance in a single US city.

The Centers for Disease Control and Prevention (CDC) and state partners in the Emerging Infections Program (EIP) have undertaken two active, population-based surveillance studies to determine the incidence of candidemia, the distribution of *Candida* species causing BSI, and the prevalence of antifungal drug resistance (10,12). In both studies, surveillance was conducted in two geographic areas: Atlanta, GA (1992-1993); and Baltimore city and county, MD (1998-2000). CDC and EIP partners again conducted population-based surveillance for candidemia in 2008-2011, returning to the previous areas of Atlanta, GA and Baltimore, MD. By returning to previous areas of surveillance, changes in species distribution and antifungal susceptibility can be described. Here we report the laboratory results of that surveillance including molecular determination of *Candida* species and antifungal susceptibility testing against nine antifungal agents. This is the first time that susceptibility to echinocandins has been determined within a US population-based collection of isolates.

MATERIALS AND METHODS

Case and isolate definitions. Isolates were obtained from persons with an incident episode of candidemia (defined below) identified between March 1, 2008 and February 28, 2011 for residents of Georgia Health District 3 (the eight counties comprising metropolitan Atlanta, GA)(population=3.8 million) and between June 1, 2008 and May 31, 2011 for residents of Baltimore City or Baltimore County, Maryland (population=1.4 million). The isolate retrieval rate from identified incident cases in Atlanta was 71% and in Baltimore it was 92%. An incident episode of candidemia was defined as the 30 days following the first positive blood culture for *Candida* species in a resident of the surveillance area. Positive cultures of the same species within the 30-day period were considered part of the incident case episode and were not captured but positive cultures of a different species during a single episode were captured. Cultures drawn more than 30 days after the incident case were considered a new incident episode and assigned a new case number. Isolates were received from 17 hospitals in Baltimore with an average of 87 isolates per hospital (range of 1-562) and from 24 hospitals in Atlanta with an average of 63 isolates per hospital (range of 2-310).

Isolate storage, DNA extraction, PCR amplification and sequencing. Prior to use, all isolates were stored in glycerol at -70° C. Isolates were identified by both conventional biochemical means at the referring institutions and molecular means using a Luminex assay or DNA sequencing of the D1/D2 subunit of the 28S rDNA at the CDC as previously described (8).

Antifungal susceptibility testing. Antifungal susceptibility testing was performed by broth microdilution with fluconazole, itraconazole, voriconazole, posaconazole, flucytosine, anidulafungin, caspofungin, and micafungin as described by the Clinical and Laboratory Standards Institute (CLSI) M27-A3 document guidelines (7) using frozen RPMI microbroth

trays custom manufactured by TREK Diagnostics (Cleveland, OH). Susceptibility testing for amphotericin B was performed by Etest as per the manufacturer's instructions (bioMérieux, Durham, NC). Results were read visually after 24 h for fluconazole, voriconazole, posaconazole (24), caspofungin, micafungin, anidulafungin, amphotericin B and flucytosine (14) and 48 h for itraconazole. For all but amphotericin B, the MIC was read as the lowest concentration of drug that caused a significant decrease in growth as compared to the control well. Recently approved CLSI 24 h resistance breakpoints for fluconazole, voriconazole and the echinocandins were used (21,22,27): isolates of *C. albicans, C, tropicalis* and *C. parapsilosis* with an MIC ≥8 µg/ml and isolates of *C. glabrata* with an MIC ≥64 µg/ml were considered resistant to fluconazole; *C. krusei* was considered intrinsically resistant to fluconazole; isolates of *C. albicans, C, tropicalis* and *C. parapsilosis* with an MIC ≥1 µg/ml and *C. krusei* isolates with an MIC ≥2 µg/ml were considered resistant to voriconazole; isolates of *C. albicans*, *C. tropicalis* and *C. krusei* with an MIC ≥1 µg/ml were considered resistant to caspofungin, micafungin and anidulafungin; isolates of *C. parapsilosis* with an MIC ≥8 µg/ml were considered resistant to caspofungin, micafungin and anidulafungin; isolates of *C. glabrata* with an MIC ≥0.5 µg/ml were considered resistant to caspofungin and anidulafungin, while those with an MIC ≥0.25 µg/ml were considered resistant to micafungin. For *C. dubliniensis,* the *C. albicans* breakpoints were used. All other species were considered to have no current breakpoints. Amphotericin B susceptibility was performed using Etest as per the manufacturer's instructions and MIC values were read at 24 h. Quality control isolates *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were included on each day of testing. As prior population-based surveillance was conducted in both Atlanta (1992-1993)(12) and Baltimore (1998-2000)(10) and susceptibility to fluconazole was previously

determined for all isolates, we applied the new breakpoints to the previous surveillance results and compared them to the current results.

Multi-species episodes. A multispecies episode was defined as when a *Candida* species other than the incident isolate species was recovered from a patient within the 30 days encompassing the incident episodic period or when two species were recovered simultaneously as part of the incident episode.

Defining multidrug resistance. To screen for multidrug-resistant (MDR) isolates, the most frequently used antifungal agents in our surveillance areas were selected; fluconazole and voriconazole representing the azoles, caspofungin, micafungin and anidulafungin representing the echinocandins and amphotericin B as the polyene. Isolates were considered multidrug resistant if they were resistant to one or more representative antifungal(s) of two of the above classes. Since there are no resistance breakpoints for amphotericin B, the arbitrary but accepted breakpoint of 2.0 µg/ml for resistance was used.

RESULTS

Cases. A total of 2,227 incident episodes had one or more isolates available, from which 2,329 eligible isolates were collected (Table 1). Incident isolates were available for 2,136 (80%) patients. There were 94 episodes with two species, 42 in Atlanta and 52 in Baltimore, one episode with three species, one episode with four species, and one episode with five species, all in Baltimore. *Candida albicans* was the most frequently isolated species, comprising 34% of the isolates from Baltimore and 41% of the isolates from Atlanta (Figure 1). *Candida glabrata* was the second most prevalent species from both sites (27% in Atlanta and 31% in Baltimore) followed by *C. parapsilosis* and *C. tropicalis*. In Baltimore, *C. dubliniensis* was the fifth most

prevalent species but in Atlanta, *C. lusitaniae* was the fifth most prevalent species. Overall, *C. albicans*, *C, glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* comprised 95% of the isolates; with the addition of *C. dubliniensis* and *C. lusitaniae*, they comprised 98% of the isolates. Two hundred seventeen (8%) case-patients had >1 30 day episode of candidemia. Of those, 69 (32%) were *C. glabrata*, 45 (21%) were *C. albicans*, 43 (20%) were *C. parapsilosis*, and 29 (13%) were *C. tropicalis*."

Susceptibility. Among the 2329 isolates tested (Tables 2,3), overall resistance to fluconazole was 7.3%, primarily due to *C. glabrata* and *C. krusei,* which together comprised 31% of the total isolates for which breakpoints were available but 68% of the fluconazole resistant isolates. Resistance to fluconazole varied by species with *C. glabrata* having the highest rate (11.9%) followed by *C. tropicalis* (6.2%), *C. dubliniensis* (5.6%), *C. parapsilosis* (4.1%), and *C. albicans* (2.3%). Resistance to itraconazole was high (21.1%), again primarily due to *C. glabrata* (59.6% of which were resistant) and *C. krusei* (21.9% resistant). Overall voriconazole resistance was low (1.0%), ranging from 0% among *C. krusei* to 2.5% among *C. tropicalis* isolates. There are no currently approved breakpoints for posaconazole but the MIC_{90} value for most species was ≤0.5 µg/ml (Table 2).

Resistance to echinocandins was low overall, ranging from 1.0% to caspofungin or anidulafungin to 1.1% for micafungin. Again, resistance was primarily found in *C. glabrata* which accounted for 65% (20/31) of the isolates resistant to any echinocandin, followed by *C. albicans* (19% of the resistant isolates).

There are no established breakpoints for amphotericin B but an MIC breakpoint of 2 µg/ml is widely considered to be elevated. Only four isolates had amphotericin B MIC values of 2 µg/ml: two *C. glabrata* isolates and one isolate each of *C. tropicalis* and *C. parapsilosis*.

Susceptibility by surveillance site. The overall proportion of isolates that were resistant to fluconazole was slightly higher in Atlanta than it was in Baltimore. When analyzed by species, fluconazole resistance was nearly identical for *C. albicans* in Baltimore and Atlanta (Tables 4A and 4B). Fluconazole resistance was higher in Atlanta among *C. glabrata, C. tropicalis* and *C. parapsilosis*, and nearly twice as high in Atlanta as in Baltimore for the latter two species. Overall resistance to voriconazole was again almost identical in Atlanta and Baltimore and only *C. tropicalis* isolates in Atlanta (3.8%) had resistance higher than 2%. Overall resistance to echinocandins was the same at the two sites (Tables 4A and 4B).

Temporal comparison of susceptibility results. We compared the previously published population-based surveillance results (10,12) to the current results (Table 5). Resistance to fluconazole in *C. albicans* remained relatively unchanged in both Atlanta and Baltimore. Fluconazole resistance among *C. glabrata* isolates in Atlanta was 20% in 1993 and 13.2% in 2011, however there were only 35 isolates available for testing in 1993, just over 10% of the number of isolates available for testing in 2011. Fluconazole resistance among *C. glabrata* isolates in Baltimore was 8.0% in 2008 and 10.8% in 2011. Resistance of *C. parapsilosis* to fluconazole was only slightly different over time in both Atlanta (from 1.9% to 5.6%) and Baltimore (from 0% to 2.3%) and the same was true for *C. tropicalis* in Atlanta (from 3.3% to 8.6%) and in Baltimore (from 8.8% to 4.3%).

Multidrug-resistant isolates. Among isolates with acquired resistance, there were three MDR isolates from Atlanta, all *C. glabrata* resistant to fluconazole and one or more echinocandin. There were six MDR isolates From Baltimore, five *C. glabrata* and one *C. albicans* all resistant to fluconazole and one or more echinocandin.

Multi-species incident episodes. There were 42 multi-species episodes in Atlanta and 55 in Baltimore. In Atlanta, there were 23 episodes where two species were isolated simultaneously, and 19 episodes where two species were isolated on different days during the 30 day episodic period (range 1-27 days; average of 9 days apart). In Baltimore, there were 36 episodes with two species isolated simultaneously (including one patient with three species simultaneously) and 21 episodes where two species were isolated on different days (including one patient with four species isolated on four different days and one patient with two simultaneous species, then a different species three days later and then two different species again simultaneously) during the 30 day episodic period (range 1-26 days; average of 7 days apart). *Candida albicans* was the most common species occurring in multispecies episodes, in Baltimore most often with *C. glabrata* (27 times) followed by *C. parapsilosis* (six times) and in Atlanta most often with *C. glabrata* (12 times) or *C. parapsilosis* (seven times). *Candida glabrata* occurred with *C. parapsilosis* six times in Atlanta but only once in Baltimore.

DISCUSSION

There were a number of important results from this population-based surveillance. First, the distribution of species at both of the surveillance sites has changed, with the proportion of *C. albicans* infections decreasing and the proportion of *C. glabrata* and *C. parapsilosis* infections

248 increasing. Second, multispecies episodes were frequently encountered. Third, when *C.*
249 *glabrata* and *C. krusei* were not considered, overall resistance to fluconazole was low. Finally,
250 although echinocandins are relatively new to the antifungal armamentarium, resistance was
251 detected, especially among *C. glabrata* isolates.

252 *Candida albicans* has traditionally been the leading cause of candidemia worldwide, but
253 temporal data show that the proportion caused by non-*C. albicans* species, especially *C. glabrata*
254 and *C. parapsilosis,* has been increasing (26,30,36). That trend has also been demonstrated in
255 this surveillance: the proportion of *C. albicans* candidemia cases dropped from 52% to 41% in
256 Atlanta and from 43% to 34% in Baltimore (Figure 1). At the same time, the proportion of *C.*
257 *glabrata* candidemia cases rose from 12% to 27% in Atlanta and the proportion of *C.*
258 *parapsilosis* candidemia cases rose from 11% to 16% in Baltimore. Among global population-
259 based surveillance studies, these are both the lowest reported percentages of *C. albicans* and the
260 highest reported percentages of *C. glabrata* (3,5,6,9,13,19,33,34). Only recent surveillance
261 studies in Scotland, Canada, Denmark, and the US have reported proportions of *C. glabrata*
262 candidemia at or above 20% (2,9,19,28,29,33). This rise in the proportion of *C. glabrata*
263 candidemia has been reported elsewhere in the US. In surveillance from intensive care units, *C.*
264 *glabrata* increased from the fourth most common *Candida* BSI in 1989 to second most common
265 in 1999 (36). While the relative proportions of each species have changed over the last 20 years,
266 *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis* still comprise 93.5% of all isolates, a
267 number that has not substantially changed in 20 years in the US (12,18).

268 In recent global surveillance the percentage of multispecies candidemia has ranged from
269 1.2% to 3.4% of all episodes with an average of 2.1% in any given surveillance (2,5,6,15,32-35).
270 Our percentages were somewhat higher, 3.6% in Atlanta and 5.1% in Baltimore. However, these

numbers are probably an underestimate as not all of the laboratories participating in the surveillance used chromogenic agar for initial plating of blood cultures, and even when chromogenic agar is used, only *C. albicans/C. dubliniensis*, *C. tropicalis* and *C. krusei* can be reliably distinguished. Thirteen of the 23 simultaneous multispecies episodes in Atlanta and all but one of the 36 simultaneous multispecies episodes in Baltimore would have been readily distinguished on chromogenic agar, indicating the value of this clinical tool in species distinction. Timely and correct identification of isolates in multispecies episodes is essential for correct clinical management of the patient.

The overall proportion of resistance of *Candida* isolates to fluconazole did not change in Atlanta in the 16 years between the two surveillance reports or in Baltimore in the 10 years between surveillance reports. Applying the new breakpoints to the archived susceptibility data from 1992-1993 in Atlanta, the overall fluconazole resistance rate among isolates was 7.0%. In 2008-2011 the overall fluconazole resistance rate was slightly higher at 8.2% and an increase in the proportion of resistant isolates was noted for *C. albicans*, *C. parapsilosis* and *C. tropicalis.* The proportion of fluconazole resistant isolates that were *C. krusei* and *C. glabrata* also only increased slightly from 64% to 65% of all resistant isolates. The largest increases in fluconazole resistance were found in Atlanta for *C. parapsilosis* which increased from 1.9% to 5.6% and in *C. tropicalis* which increased from 3.3% to 8.6%. In Baltimore, in 1998-2000, the overall fluconazole resistance among tested isolates was 7.3% which dropped to 6.5% in 2008-2010. The proportion of overall resistant isolates that were *C. krusei* and *C. glabrata* again increased slightly from 60% to 72% of all resistant isolates. Resistance rates were slightly lower for *C. albicans* and *C. tropicalis*, and slightly higher for *C. glabrata* and *C. parapsilosis*. Because this was the first non-global surveillance report employing the new species-specific *Candida*

susceptibility breakpoints, it was not possible to directly compare our resistance rate results to older surveillance studies performed outside of our institution.

Although the echinocandins are a relatively new class of antifungal agents, they were used during treatment in 61% of the patients in this surveillance (data not shown). Despite this high level of usage, there were very few resistant isolates (approximately 1% of total isolates). However, the one species with a notable level of resistance to echinocandins was *C. glabrata* (38). This is concerning given that this low level of resistance has developed in a species already less susceptible to the triazole antifungals. The Infectious Disease Society of America guidelines recommend echinocandins as primary therapy for *C. glabrata* candidiasis in both neutropenic and non-neutropenic patients (20), so resistance should be monitored closely as usage increases.

In summary, candidemia remains an important cause of healthcare-associated BSI. Laboratories should be aware that mixed-species infections are not rare and that the two most prevalent species in those mixed infections (*C. albicans* and *C. glabrata*) may have very different antifungal susceptibility patterns. Although the overall rates of resistance to antifungal agents remain low, species-specific resistance raise some concerns, especially among isolates of MDR *C. glabrata* with resistance to both fluconazole and echinocandins. Currently, echinocandins are highly effective in vitro against all *Candida* species and fluconazole remains highly effective in vitro against *C. albicans*. As the use of echinocandins becomes more commonplace in both empiric and primary therapy, it is imperative that we continue to monitor candidemia isolates for changing levels of resistance and use these data to update future treatment guidelines.

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485 **FIGURE LEGENDS**

486 Figure 1. Differences in species distribution between past (dark gray) and present (light gray)

487 surveillance in Atlanta (A) and Baltimore (B).

488

489

Table 1. Species distribution of 2008-2011 surveillance.

	Number of isolates (percentage of total)		
Species	Combined	Atlanta	Baltimore
C. albicans	877 (38)	489 (41)	388 (34)
C. glabrata	671 (29)	318 (27)	353 (31)
C. parapsilosis	390 (17)	213 (18)	177 (16)
C. tropicalis	242 (10)	104 (9)	138 (12)
C. dubliniensis	36 (2)	5	31 (3)
C. lusitaniae	34 (1)	22 (2)	12 (1)
C. krusei	32 (1)	19 (2)	13 (1)
C. orthopsilosis	13	6	7
C. metapsilosis	10	8	2
C. guilliermondii	8	5	3
C. nivariensis	4	3	1
C. fermentati	4	2	2
C. bracarensis	2	1	1
C. catenulata	2	0	2
C. pararugosa	2	1	1
C. famata	1	1	0
C. kefyr	1	1	0
C. pelliculosa	1	0	1
C. norvegensis	1	1	0
C. rugosa	1	1	0
Total	2332	1200	1132

Table 2. In vitro susceptibilities of all 2008-2011 surveillance *Candida* isolates.

Species (# of isolates)	Antifungal agent	MIC (µg/ml)			% Resistant
		Range	MIC_{50}	MIC_{90}	(#)
All isolates (2332)	Fluconazole	0.125-256	1	16	7.3 (165)
	Voriconazole	0.008-16	0.06	0.25	1.0 (16)
	Itraconazole	0.015-16	0.25	1	21.1 (493)
	Posaconazole	0.008-16	0.125	1	
	Caspofungin	0.008-16	0.06	0.25	1.0 (22)
	Anidulafungin	0.008-4	0.03	1	1.0 (23)
	Micafungin	0.008-4	0.03	1	1.1 (25)
	Flucytosine	0.125-256	0.125	1	0.8 (19)
	Amphotericin B	0.002-2	0.125	0.38	
C. albicans (877)	Fluconazole	0.125-256	0.5	1	2.3
	Voriconazole	0.008-16	0.03	0.125	0.9
	Itraconazole	0.03-16	0.125	0.25	1.6
	Posaconazole	0.008-16	0.125	0.25	
	Caspofungin	0.008-2	0.03	0.06	0.5
	Anidulafungin	0.008-2	0.015	0.06	0.3
	Micafungin	0.008-2	0.03	0.03	0.3
	Flucytosine	0.125-256	0.25	2	0.9
	Amphotericin B	0.002-0.5	0.094	0.19	
C. glabrata (671)	Fluconazole	0.5-256	8	64	11.9
	Voriconazole[a]	0.008-16	0.25	2	
	Itraconazole	0.06-16	1	4	59.6
	Posaconazole	0.008-16	0.5	2	
	Caspofungin	0.015-16	0.06	0.125	2.4
	Anidulafungin	0.008-4	0.06	0.125	2.7
	Micafungin	0.008-4	0.015	0.03	2.7
	Flucytosine	0.125-256	0.125	0.125	0.6
	Amphotericin B	0.002-2	0.25	0.5	
C. parapsilosis (390)	Fluconazole	0.125-64	1	2	4.1
	Voriconazole	0.008-2	0.03	0.06	0.8
	Itraconazole	0.015-1	0.25	0.5	1.5
	Posaconazole	0.008-1	0.125	0.25	
	Caspofungin	0.008-2	0.25	0.5	0
	Anidulafungin	0.008-8	1	2	0.3
	Micafungin	0.008-4	1	2	0
	Flucytosine	0.125-8	0.125	0.25	0
	Amphotericin B	0.002-2	0.064	0.19	
C. tropicalis (242)	Fluconazole	0.125-128	0.5	2	6.2
	Voriconazole	0.008-16	0.06	0.125	2.5
	Itraconazole	0.015-16	0.5	1	22.4
	Posaconazole	0.008-1	0.125	0.5	

	Caspofungin	0.008-2	0.03	0.125	0.8
	Anidulafungin	0.008-0.25	0.015	0.06	0
	Micafungin	0.008-1	0.03	0.125	1.2
	Flucytosine	0.125-128	0.125	0.25	1.7
	Amphotericin B	0.008-1	0.25	0.5	
C. dubliniensis (36)[a]	Fluconazole	0.125-16	0.25	0.5	5.6
	Voriconazole	0.008-0.125	0.015	0.03	0
	Itraconazole	0.03-0.5	0.125	0.25	0
	Posaconazole	0.03-0.25	0.06	0.125	
	Caspofungin	0.03-0.25	0.06	0.125	0
	Anidulafungin	0.015-1	0.03	0.06	2.8
	Micafungin	0.008-1	0.06	0.06	2.8
	Flucytosine	0.125-0.25	0.125	0.125	0
	Amphotericin B	0.006-0.125	0.023	0.064	
C. lusitaniae (34)	Fluconazole	0.125-8	0.5	1	
	Voriconazole	0.008-0.015	0.008	0.015	
	Itraconazole	0.03-1	0.25	0.5	
	Posaconazole	0.03-0.5	0.125	0.25	
	Caspofungin	0.03-1	0.25	0.5	
	Anidulafungin	0.008-1	0.25	0.5	
	Micafungin	0.015-2	0.25	0.5	
	Flucytosine	0.125-128	0.125	2	
	Amphotericin B	0.016-0.38	0.064	0.125	
C. krusei (32)	Fluconazole[b]	4-64	16	64	100
	Voriconazole	0.015-0.5	0.25	0.25	0
	Itraconazole	0.125-2	0.5	1	21.9
	Posaconazole	0.125-1	0.25	0.5	
	Caspofungin	0.06-0.5	0.125	0.25	0
	Anidulafungin	0.015-0.25	0.03	0.06	0
	Micafungin	0.06-0.125	0.125	0.125	0
	Flucytosine	4-64	8	16	6.3
	Amphotericin B	0.023-1	0.5	0.75	
Other species (50)	Fluconazole[a]	0.25-16	2	8	
	Voriconazole[a]	0.008-0.25	0.06	0.25	
	Itraconazole[a]	0.06-2	0.25	1	18.0
	Posaconazole	0.03-2	0.25	0.5	
	Caspofungin	0.03-0.5	0.125	0.5	
	Anidulafungin	0.015-2	0.25	1	
	Micafungin	0.008-2	0.25	1	
	Flucytosine	0.125-8	0.125	0.25	0
	Amphotericin B	0.002-1	0.064	0.5	

[a]*C. albicans* breakpoints were applied
[b]Percent resistant was based on intrinsic resistance of *C. krusei* and did not follow actual MIC values

Table 3. MIC values for less common *Candida* species

Species	# of isolates	Range of MIC values in µg/ml							
		fluconazole	voriconazole	itraconazole	posaconazole	caspofungin	micafungin	anidulafungin	Amphotericin B
C. orthopsilosis	13	0.25-8	0.008-0.06	0.06-0.5	0.06-0.25	0.06-0.5	0.25-1	0.25-2	0.023-0.094
C. metapsilosis	10	1-4	0.015-0.06	0.125-0.5	0.03-0.25	0.06-0.5	0.125-0.5	0.125-0.5	0.023-0.19
C. guilliermondii	8	0.5-8	0.015-0.25	0.25-1	0.125-0.5	0.06-0.5	0.25-2	0.25-2	0.032-0.125
C. nivariensis	4	8-16	0.125-0.25	1-2	1-2	0.03-0.125	0.015-0.03	0.015-0.06	0.19-1
C. fermentati	4	1-16	0.03-0.125	0.5-1	0.25	0.25-0.5	0.25-1	0.25-1	0.032-0.5

Table 4A. In vitro susceptibilities of 2008-2011 *Candida* surveillance isolates to selected antifungal agents in Atlanta.

Species (#)	Antifungal agent	µg/ml MIC_{50}	µg/ml MIC_{90}	% Resistant
All species (1148[a])	Fluconazole	1	16	8.2
	Voriconazole	0.06	0.5	1.1
	Caspofungin	0.06	0.25	1.0
	Anidulafungin	0.03	1	1.0
	Micafungin	0.03	1	1.0
C. albicans (489)	Fluconazole	0.5	2	2.2
	Voriconazole	0.03	0.125	0.6
	Caspofungin	0.03	0.06	0.6
	Anidulafungin	0.015	0.06	0.4
	Micafungin	0.03	0.03	0.4
C. glabrata (318)	Fluconazole	8	64	13.2
	Voriconazole	0.25	2	
	Caspofungin	0.06	0.125	2.5
	Anidulafungin	0.06	0.125	3.1
	Micafungin	0.015	0.03	2.8
C. parapsilosis (213)	Fluconazole	1	4	5.6
	Voriconazole	0.03	0.06	0.9
	Caspofungin	0.25	0.5	0
	Anidulafungin	1	2	0
	Micafungin	1	2	0
C. tropicalis (104)	Fluconazole	0.5	2	8.6
	Voriconazole	0.03	0.25	3.8
	Caspofungin	0.03	0.125	0
	Anidulafungin	0.015	0.06	0
	Micafungin	0.03	0.06	0
C. krusei (19)	Fluconazole	16	64	100
	Voriconazole	0.25	0.5	0
	Caspofungin	0.125	0.25	0
	Anidulafungin	0.03	0.06	0
	Micafungin	0.125	0.125	0

[a]Number of isolates for which interpretive criteria are available

Table 4B. In vitro susceptibilities of 2008-2011 *Candida* surveillance isolates to selected antifungal agents in Baltimore.

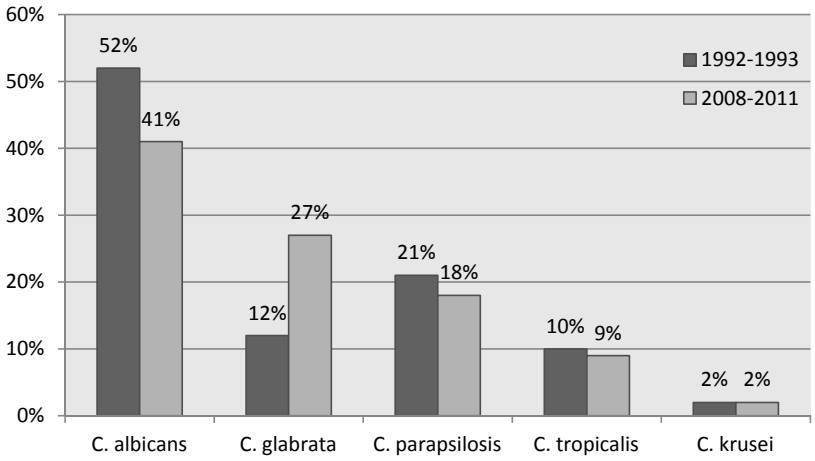
Species (#)	Antifungal agent	µg/ml MIC_{50}	µg/ml MIC_{90}	% Resistant
All species (1100[a])	Fluconazole	1	16	6.5
	Voriconazole	0.06	0.5	0.9
	Caspofungin	0.06	0.25	1.0
	Anidulafungin	0.03	1	1.0
	Micafungin	0.03	1	1.0
C. albicans (388)	Fluconazole	0.5	1	2.3
	Voriconazole	0.03	0.125	1.3
	Caspofungin	0.03	0.06	0.3
	Anidulafungin	0.03	0.06	0.3
	Micafungin	0.015	0.03	0.3
C. glabrata (353)	Fluconazole	8	64	10.8
	Voriconazole	0.25	1	
	Caspofungin	0.06	0.125	2.3
	Anidulafungin	0.06	0.125	2.3
	Micafungin	0.015	0.03	2.5
C. parapsilosis (177)	Fluconazole	1	2	2.3
	Voriconazole	0.03	0.06	0.6
	Caspofungin	0.25	0.5	0
	Anidulafungin	1	2	0.6
	Micafungin	1	2	0
C. tropicalis (138)	Fluconazole	0.5	2	4.3
	Voriconazole	0.06	0.125	1.4
	Caspofungin	0.03	0.06	1.4
	Anidulafungin	0.015	0.06	0
	Micafungin	0.03	0.125	1.4
C. krusei (13)	Fluconazole	16	32	100
	Voriconazole	0.125	0.25	0
	Caspofungin	0.125	0.25	0
	Anidulafungin	0.03	0.06	0
	Micafungin	0.06	0.125	0

[a]Number of isolates for which interpretive criteria are available

Table 5. Comparison of fluconazole susceptibilities from past and present surveillance in Atlanta (A) and Baltimore (B).

	Species	Year	Number tested	MIC_{50}	MIC_{90}	% Resistant
A.	*C. albicans*	1993	103	0.25	0.5	1.0%
		2011	489	0.5	2	2.2%
	C. glabrata	1993	35	16	128	20%
		2011	318	8	64	13.2%
	C. parapsilosis	1993	52	1	2	1.9%
		2011	213	1	4	5.6%
	C. tropicalis	1993	30	1	2	3.3%
		2011	104	0.5	2	8.6%
B.	*C. albicans*	2000	232	0.125	1	3.4%
		2011	388	0.5	1	2.3%
	C. glabrata	2000	150	4	32	8.0%
		2011	353	8	64	10.8%
	C. parapsilosis	2000	58	0.5	1	0%
		2011	177	1	2	2.3%
	C. tropicalis	2000	80	0.5	4	8.8%
		2011	138	0.5	2	4.3%

A. n = 1198



B. n = 1131