

National Surveillance of Fungemia in Denmark (2004 to 2009)[∇]

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A 6-year nationwide study of fungemia in Denmark was performed using data from an active fungemia surveillance program and from laboratory information systems in nonparticipating regions. A total of 2,820 episodes of fungemia were recorded. The incidence increased from 2004 to 2007 (7.7 to 9.6/100,000) and decreased slightly from 2008 to 2009 (8.7 to 8.6/100,000). The highest incidences were seen at the extremes of age (i.e., 11.3 and 37.1/100,000 for those <1 and 70 to 79 years old, respectively). The rate was higher for males than for females (10.1 versus 7.6/100,000, $P = 0.003$), with the largest difference observed for patients >50 years of age. The species distribution varied significantly by both age and gender. *Candida* species accounted for 98% of the pathogens, and *C. albicans* was predominant, although the proportion decreased (64.4% to 53.2%, $P < 0.0001$). *C. glabrata* ranked second, and the proportion increased (16.5% to 25.9%, $P = 0.003$). *C. glabrata* was more common in adults and females than in children and males, whereas *C. tropicalis* was more common in males ($P = 0.020$). *C. krusei* was a rare isolate (4.1%) except at one university hospital. Acquired resistance to amphotericin and echinocandins was rare. However, resistance to fluconazole (MIC of >4 µg/ml) occurred in *C. albicans* (7/1,183 [0.6%]), *C. dubliniensis* (2/65 [3.1%]), *C. parapsilosis* (5/83 [6.0%]), and *C. tropicalis* (7/104 [6.7%]). Overall, 70.8% of fungemia isolates were fully fluconazole susceptible, but the proportion decreased (79.7% to 68.9%, $P = 0.02$). The study confirmed an incidence rate of fungemia in Denmark three times higher than those in other Nordic countries and identified marked differences related to age and gender. Decreased susceptibility to fluconazole was frequent and increasing.

A seminal active fungemia surveillance program was initiated in Denmark in 2003 (5) and until 2009 included two-thirds of the Danish population (5, 6). The data from 2004 to 2006 has previously been reported and documented a notably high annual incidence rate of 10 to 11 episodes per 100,000 inhabitants (6). In comparison, in three other Nordic countries the rate of candidemia has been in the range of 2.2 to 3.5/100,000, with 65 to 70% of the cases being caused by *Candida albicans* (24, 29, 36, 37, 44). In European surveys, rates of 2.5 to 5.0/100,000 have been reported (3, 9, 30, 33, 49), with 56% (43 to 67%) of the cases being caused by *C. albicans* in a recent European study (49). Finally, in the United States, rates of 6.0 to 8.7/100,000 have been reported in most population-based studies, with 45 to 57% of the cases being caused by *C. albicans* (18, 20, 22). One major exception is a study reporting a rate as high as 24/100,000 in Baltimore compared to 7.1/100,000 in Connecticut despite the fact that rates calculated per number of admissions were comparable (2).

C. albicans remains the predominant species on both sides of the Atlantic Ocean. However, geographical differences in *Candida* species distributions exist. Thus, *C. glabrata* is more common in the northern hemisphere, while *C. parapsilosis* and *C. tropicalis* are more common in the southern parts of the world (4). This has significant importance due to the difference in susceptibility patterns, with *C. glabrata* having reduced susceptibility to fluconazole and *C. parapsilosis* to the echinocandins; primary antifungal regimens should therefore be adjusted to the local epidemiology. Surveys conducted in Europe have reported *C. glabrata* proportions from 8 to 22% (3, 6, 23, 24, 29, 30, 37, 38, 45, 47, 48). In this perspective, the first years of surveillance in Denmark revealed an unexpectedly high proportion of blood isolates to be *C. glabrata* (23%) (6).

However, species distribution and incidence data may have been skewed due to underrepresentation of regions without university hospitals, and therefore, we found it of interest to extend the previous 3-year seminal study to a nationwide study that includes data from all of Denmark during the 6-year period from 2004 to 2009. The objective was to determine population-, gender-, and age-specific incidence rates and species distributions and thereby extend previous seminal observations of trends in a high-incidence country.

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TABLE 1. Characteristics of the 14 participating centers^a

Center no. and name	No. of hospitals (university/district)	Population (mean 2004–2009)	Blood culture system	No. of bottles/BC (ae/an/myc)	Incubation time (days)	No. of isolates/yr (mean 2004–2009)	Incidence/100,000 population ^d (mean 2004–2009)	Incidence/1,000 admissions ^e (mean 2004–2009)	Proportion of <i>C. albicans</i> / <i>C. glabrata</i> (%)
1 Rigshospitalet	1 (1/0)	NA	Bactec	2 (1/1/0) [*]	7	79	NA	1.22	51/20
2 Copenhagen city hospitals	6 (1/5)	634,750	BacT/Alert	4 (2/2/0)	5	47	7.33	0.39	51/30
3 Copenhagen county	4 (3/1)	619,088	Bactec	4 (2/2/0) [*]	5	48	7.75	0.35	60/19
8 Funen	9 (1/8)	479,564	Bactec	2 (1/1/0)	6	55	11.54	0.53	60/17
15 Aarhus	8 (3/5)	664,413	BacT/Alert	2 (1/1/0)	6	67	10.01	0.49	61/20
14 N-Jutland	7 (1/6)	503,174	BacT/Alert	3 (2/1/0)	7	45	8.94	0.42	62/21
4 Frederiksberg	4 (0/4)	364,333	BacT/Alert	4 (2/2/0)	5–6	26	7.23	0.36	54/25
5 Roskilde	2 (1/1)	243,451	Bactec	3 (2/1/0)	5	19	7.80	0.37	55/18
6 & 7 SW-Sealand*	9 (0/9)	576,756	BacT/Alert	3 (2/1/0)	6	24	4.16	0.21	54/15
11 Vejle*	5 (0/5)	345,000	BacT/Alert	2 (1/1/0)	5,6	25	7.39	0.37	58/23
12 Herning*	4 (0/4)	276,842	BacT/Alert	2 (1/1/0)	6	17	6.14	0.37	52/37
13 Viborg*	3 (0/3)	224,789	Bactec → BacT/Alert**	2 (1/1/0) or 4 (2/2/0)	6	10	4.45	0.20	73/13
10 Esbjerg*	2 (0/2)	235,863	Bactec → BacT/Alert**	3 (2/1/0)	5	13	5.44	0.30	68/16
9 S-Jutland*	4 (0/4)	285,390	BacT/Alert	2 (1/1/0)	7	10	4.06	0.25	51/22

^a Incidence calculation based upon number of isolates.

^b N/A, not applicable; center 1 is a tertiary hospital receiving patients referred from other hospitals from the entire country. ^c, center 1 (since 1 January 2007) and center 3 (since 1 November 2006) recommend inclusion of a mycosis bottle in blood cultures for risk patients. ^{*}, centers participating in the retrospective part of the study. ^{**}, center 13 changed the blood culture system from Bactec to BacT/Alert in 2009, and center 10 changed the blood culture system from Bactec to BacT/Alert in 2007. BC, blood culture; ae/an/myc, aerobic/anaerobic/mycosis.



FIG. 1. Geographical uptake area of the 14 participating centers designated by the following numbers: 1, Rigshospitalet; 2, Copenhagen city hospitals; 3, Copenhagen county; 4, Frederiksberg; 5, Roskilde; 6 and 7, SW-Sealand; 8, Funen; 9, S-Jutland; 10, Esbjerg; 11, Vejle; 12, Herning; 13, Viborg; 14, N-Jutland; and 15, Aarhus. The island Bornholm, 16, was served by Roskilde, 5, in 2004 but in the rest of the period by Copenhagen city hospitals, 2. No other laboratories process blood cultures in Denmark. (Adapted from a map available on Wikipedia under a Creative Commons license.)

MATERIALS AND METHODS

Surveillance and population. (i) Retrospective part. Six departments of clinical microbiology serving one-third of the Danish population participated in the retrospective part of the study; these departments were situated at Slagelse Hospital (center no. 6 and 7), Vejle Hospital (center no. 11), Herning Hospital (center no. 12), Viborg Hospital (center no. 13), Esbjerg Hospital (center no. 10), and Sønderborg Hospital (center no. 9). Characteristics of uptake area, number of admissions, and population sizes are summarized in Table 1, and geographical locations are shown in Fig. 1. Information on total numbers of bloodstream isolates and species diagnosis was retrieved from the departments' laboratory information systems.

(ii) Prospective part. Eight Danish departments of clinical microbiology at Rigshospitalet (center no. 1), Hvidovre Hospital (center no. 2), Herlev Hospital (center no. 3), Odense University Hospital (center no. 8), Skejby Hospital (center no. 15), Aalborg Hospital (center no. 14), Hillerød Hospital (center no. 4), and Statens Serum Institut (center no. 5) have participated in the prospective semination surveillance since 2004, covering two-thirds of the Danish population. Isolates were referred to the National Mycology Reference Laboratory for verification of species identification and susceptibility testing (see below). Completeness was ensured through comparison with local laboratory records.

Two blood culture systems were used: the BacT/Alert (bioMérieux, Marcy l'Etoile, France) and the Bactec (Becton Dickinson, Franklin Lakes, NJ) blood culture system (Table 1). In total, 55.5% of the cases were detected using BacT/Alert, and 44.5% using Bactec. For fungemia patients with successive blood culture isolates, separate episodes were included if they occurred at least 21 days apart or were caused by different species.

The size of the Danish population increased marginally during the study period (2.1%, 5,397,640 to 5,511,451; www.statistikbanken.dk). Information on the national number of nonpsychiatric admissions, trauma, violent injuries, and poisoning (classified together) and of hematological and gastrointestinal cancers was retrieved at the website www.sundhedsdata.sst.dk.

Species identification. Species identification at the reference laboratory was based on colony morphology on chromogenic agar (CHROMagar Co., Paris, France), microscopic morphology on corn meal agar and rice plus Tween agar (SSI Diagnostika, Hillerød, Denmark), growth at 35°C and 43°C, and assimilation profile by use of a commercial system (ATB ID32C; bioMérieux). Rapid tests for the identification of *C. dubliniensis* and *C. glabrata* (Bichro-Dubli and Glabrata RTT; Fumouze Diagnostics, Simoco, Denmark) were used increasingly over the study period. If no reliable species diagnosis was obtained, molecular identification was performed as described below.

TABLE 2. Number of cases, episodes, and isolates each year and distribution of fungal isolates according to species and year^a

Parameter ^b	2004		2005		2006		2007		2008		2009		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Patients	403	NA	436	+8.2	455	+4.4	501	+10.1	455	-9.2	444	-2.4	2694	NA
Cases	414	NA	462	+11.6	474	+2.6	524	+10.5	474	-9.5	472	-0.4	2820	NA
Isolates	424	NA	482	+13.7	492	+2.1	534	+8.7	484	-9.5	485	+0.2	2901	NA
<i>C. albicans</i>	273	64.4	299	62.0	276	56.1	279	52.2	272	56.2	258	53.2	1657	57.1
<i>C. dubliniensis</i>	5	1.2	11	2.3	14	2.8	15	2.8	15	3.1	14	2.9	74	2.6
<i>C. glabrata</i>	70	16.5	96	19.9	104	21.1	119	22.3	98	20.2	125	25.8	612	21.1
<i>C. krusei</i>	12	2.8	10	2.1	28	5.7	22	4.1	28	5.8	19	3.9	119	4.1
<i>C. parapsilosis</i>	17	4.0	11	2.3	22	4.5	21	3.9	18	3.7	19	3.9	108	3.7
<i>C. tropicalis</i>	17	4.0	24	5.0	20	4.1	29	5.4	27	5.6	21	4.3	138	4.8
<i>Candida</i> species*	12	2.8	7	1.5	11	2.2	22	4.1	11	2.3	16	3.3	79	2.7
Non- <i>Candida albicans</i> **	13	3.1	16	3.3	10	2.0	13	2.4	6	1.2	11	2.3	69	2.4
Other fungi***	5	1.2	8	1.7	7	1.4	14	2.6	9	1.9	2	0.4	45	1.6

^a The percentages for the numbers of patients and cases and total number of isolates indicate change in number compared to the previous year, whereas for each individual species, percentages indicate the species distribution that particular year. NA, not applicable.

^b *, *Candida* spp. include indicated number of the following isolates: *C. ciferrii*, 1; *C. colliculosa*, 1; *C. fermentati*, 3; *C. guilliermondii*, 14; *C. holmii*, 1; *C. inconspicua*, 1; *C. inconspicua/norvegensis*, 1; *C. intermedia*, 2; *C. kefir*, 11; *C. lipolytica*, 2; *C. lusitanae*, 22; *C. nivariensis*, 1; *C. norvegensis*, 1; *C. palmiophila*, 9; *C. pelliculosa*, 5; *C. pulcherrima*, 1; *C. rugosa*, 1; *C. utilis*, 1; and *C. valida*, 1. **, non-*C. albicans* denotes isolates that were not *C. albicans* but not referred to the mycology reference laboratory for species identification. ***, other fungi include indicated number of the following isolates: *Cryptococcus neoformans*, 9; *Cryptococcus* sp., 1; *F. dimorum*, 2; *F. solani*, 5; *Fusarium* sp., 4; *Geotrichum capitatum*, 1; *Pichia fabii*, 1; *Pichia caribica*, 1; *Rhodotorula glutinis/Rhodotorula mucilaginosa*, 1; *Rhodotorula mucilaginosa*, 1; *Rhodotorula* sp., 1; *S. cerevisiae*, 15; *Trichosporon inkin*, 1; *Trichosporon mucoides*, 1; and *Williopsis* sp., 1.

Susceptibility testing. Susceptibility testing for amphotericin B, anidulafungin, caspofungin, fluconazole, itraconazole, voriconazole, and posaconazole was done for a total of 2,091 (72.1%), 514 (17.7%), 2,086 (72.0%), 2,103 (72.5%), 2,079 (71.7%), 2,010 (69.3%), and 1,055 (36.4%) isolates, respectively, according to the EUCAST definitive document E7.1 (40); exceptions were amphotericin B (2006 to 2009) and caspofungin (2008 to 2009), for which Etest (AB bioMérieux, Herlev, Denmark) and RPMI 2% glucose agar (SSI Diagnostika, Hillerød, Denmark) were used. Manufacturers and stock solutions were as follows: dimethyl sulfoxide (DMSO), D8779, Sigma-Aldrich, Vallensbæk Strand, Denmark; fluconazole, Pfizer A/S, Ballerup, Denmark, or Sigma-Aldrich (10,000 µg/ml); amphotericin B, A2411, Sigma-Aldrich (5000 µg/ml in DMSO); caspofungin, Merck, Sharp and Dohme, Glostrup, Denmark (5,000 µg/ml in DMSO); itraconazole, Janssen-Cilag, Birkerød, Denmark, or Sigma-Aldrich (5,000 µg/ml in DMSO); and voriconazole, Pfizer A/S, Ballerup, Denmark (5,000 µg/ml in DMSO). Two-fold dilutions in RPMI supplemented to a final concentration of 2% glucose were prepared in microtiter plates and stored at -20°C until use. Microtiter plates were read spectrophotometrically at 490 nm. The MIC was defined as the lowest drug dilution giving 100% growth inhibition for amphotericin B and 50% growth inhibition for the other compounds. *C. krusei* ATCC 6258 was included as a quality control in each run. For this strain the accepted ranges for amphotericin B, fluconazole, itraconazole, and voriconazole were as published in reference 15 and 0.25 to 2 µg/ml for caspofungin. Susceptibility classification was done according to the following breakpoints (susceptible [S≤]/resistant [R>]) (µg/ml): for amphotericin B, 1/1 (14); for anidulafungin, 0.015/0.015 for *C. albicans*, 0.064/0.064 for *C. glabrata* and *C. tropicalis*, and 0.125/0.125 for *C. krusei* (8); for caspofungin, 2/2 for *Candida* species other than *C. parapsilosis* (8, 14); for fluconazole, 2/4 for *Candida* species other than *C. glabrata* and *C. krusei* (42); for voriconazole, 0.125/0.125 for *Candida* species other than *C. glabrata* and *C. krusei* (41); and for itraconazole, 0.125/0.5 (14). For posaconazole, no breakpoints have yet been proposed, but 0.064/0.064 was applied for *Candida* species other than *C. glabrata* and *C. krusei*, based upon wild-type distributions (data not shown).

Molecular identification and FKS gene sequence analysis. DNA was released from fungal colonies as previously described (12). Species identification was performed using universal fungal primers (internal transcribed spacer 1 [ITS1] [CGTAGGTGAACCTGCGG] and ITS4 [TCCTCCGCTTATTGATATGC]) (51). These primers amplify the intervening 5.8S ribosomal DNA (rDNA) and the adjacent ITS1 and ITS2 (51). Sequences were subjected to BLAST analyses with the online sequence databases available through NCBI and aligned to reference strains for species identification. Sequence analysis, alignments, and phylogenetics were performed with the bioinformatics software CLC DNA Workbench (CLC bio, Denmark). *FKS* gene sequence analysis was performed as previously described (8). This gene encodes the target enzyme (glucan synthase) for echinocandins.

Consumption of antifungal compounds. Information concerning overall use of antifungal agents in defined daily doses (DDD) in Denmark over a 10-year period from 2000 to 2009 both in hospitals and primary health care settings were available from the Danish Medicines Agency at <http://dkma.medstat.dk/MedStatDataViewer.php>. Similar information for Norway was available from the Norwegian Institute of Public Health at <http://www.legemiddelforbruk.no/english/>.

Data regarding fluconazole use by gender in the primary health care setting were available for the most recent 5-year period from 2005 to 2009 at <http://dkma.medstat.dk/MedStatDataViewer.php>. However, gender-specific data are not available for use at hospitals.

Statistics. Incidences per 100,000 inhabitants are calculated using the population sizes per January 1 each year. The mean is used when incidences for the entire 6-year period are calculated. Numbers on admissions for each geographical region in Denmark were reported by the local study participant.

The chi-square test was used for comparison of changes in species distribution. *P* values of <0.05 (two-tailed) were considered statistically significant.

RESULTS

Epidemiology. During the 6-year period from 2004 to 2009, a total of 2,820 episodes of fungemia comprising 2,901 isolates were recorded in 2,694 patients, leading to a mean annual incidence of 8.6/100,000 inhabitants. The number of patients, episodes, and recovered isolates increased by 24.3%, 26.6%, and 26.2%, respectively, over the first 4-year period (2004 to 2007) and then decreased to an intermediate level in 2008 to 2009 (Table 2). Similarly, the incidence rate increased over the first 4 years from 7.7 to 9.6/100,000 and then stabilized at 8.7 and 8.6/100,000 per year in 2008 and 2009, respectively. The rate of fungemia per 10,000 somatic admissions was 4.1 for the entire study period (rate by year from 2004 to 2009: 3.7, 4.1, 4.1, 4.5, 4.1, and 3.9, respectively). The highest rates were seen at the centers serving university hospitals (Table 1).

The median age (66 years, range of 0 to 98 years, interquartile range of 55 to 74 years) and the proportion of males (56.5%) remained constant. The highest incidence rates were seen at the extremes of age (Table 3). Only 1.5% of the patients were below the age of 1 year (rate, 11.3/100,000), 2.9%

TABLE 3. Age-specific incidence of fungemia by year

Yr studied	No. of isolates per 100,000 population for indicated age (yr) group											Total
	0	1-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90+	
2004	12.3	0.8	1.3	1.1	2.7	4.0	9.1	20.7	33.7	19.5	29.5	7.9
2005	9.3	0.8	0.9	0.9	2.3	5.3	11.1	20.9	36.0	38.7	5.8	8.9
2006	12.4	0.8	0.8	1.4	1.5	5.4	8.9	23.1	44.3	25.0	17.2	9.1
2007	10.7	1.7	1.5	1.1	4.0	5.9	12.1	21.7	36.0	34.4	22.6	9.8
2008	9.3	1.2	1.2	1.4	2.4	5.7	9.3	20.6	36.2	27.0	25.1	8.8
2009	13.8	1.2	1.0	1.3	1.5	5.3	9.1	21.3	36.6	28.4	16.5	8.8
Total	11.3	1.1	1.1	1.2	2.4	5.3	10.0	21.4	37.1	28.8	19.4	

were 1 to 20 years of age (rate, 1.1/100,000), 65.1% were older than 60 years (rate, 27.0/100,000), and 27.4% were older than 70 years of age (rate, 33.2/100,000). The rate was significantly higher for males than for females (10.1 versus 7.6/100,000, $P = 0.003$), with the largest gender difference for patients 20 to 29 years and older than 50 years (Fig. 2).

The overall species distribution is shown in Table 2, and the ratio of the two most common species by center in Table 1. *Candida* species accounted for 98.4% of the fungal isolates, and *C. albicans* was the predominant species (in total, 57.1%). However, the proportion of *C. albicans* isolates varied considerably among the participating hospitals (range of 51 to 73%) (Table 1), and there was an overall decline throughout the study period (64% to 53%, $P < 0.0001$) (Table 2). *C. glabrata* was the second most frequent species (21%), again with considerable variation in frequency according to center (13% to 37%) and with an increase over the study period (17% to 26%, $P = 0.003$). The variation in distribution of *C. glabrata* between centers using Bactec (17.9%, 231/1,289) and those using BacT/Alert (23.6%, 381/1,612) was statistically significant ($P = 0.0002$). This was also the case if the analysis was performed separately for the group of 60 to 79 years of age (Bactec, 19.2% [131/684]; BacT/Alert, 24.9% [219/880], $P = 0.0071$). *C. krusei*, *C. tropicalis*, and *C. parapsilosis* were rare isolates (3.7% to 4.8%), and their occurrence remained stable. Thirty-seven percent (44/119) of the *C. krusei* isolates were from center 1 (Rigshospitalet, the major tertiary center in Denmark), where this species accounted for 9.3% of the fungal isolates.

The distribution of species varied by gender and by age

group. Thus, *C. glabrata* was significantly more common in females (23.1% versus 19.7%, $P = 0.03$), whereas *C. tropicalis* was more common in males (5.6% versus 3.7%, $P = 0.02$) (Table 4). Overall, *C. albicans* and *C. parapsilosis* accounted for 90% of the infections in patients less than 10 years old, and notably, *C. glabrata* and *C. krusei* were not recovered in any patients younger than 20 years of age, with the exception of five *C. krusei* isolates (all from center 1) and four *C. glabrata* isolates (two from center 1) (Table 5). On the contrary, 28% of the fungemia isolates were either *C. glabrata* or *C. krusei* in patients older than 60 years of age.

Polyfungal infections occurred in 82 cases (2.9%, range of 10 to 20 per year). Seventy-two of these involved two species, whereas 10 involved three species. In 57 (69.5%) of these cases, *C. albicans* was isolated in combination with another yeast, with *C. glabrata* accounting for 48 cases. The majority (70/82, 85.4%) of the polyfungal infections included at least one species with intrinsically decreased susceptibility to fluconazole (*C. glabrata* [in 60 cases], *Saccharomyces cerevisiae* [in 5 cases], *C. krusei* [in 9 cases], *C. guilliermondii* [in 1 case], and *Rhodotorula* sp. [in 1 case]).

Antifungal susceptibility. Overall, MICs below the susceptibility breakpoints were found for 99.0% of the blood isolates for amphotericin B, 98.5% for caspofungin, 93.4% for anidulafungin, 70.8% for fluconazole, 77.4% for itraconazole, 80.4% for voriconazole, and 72.1% for posaconazole. During the study period there was a significant increase in the proportion of isolates with decreased susceptibility to fluconazole (defined as an MIC of ≥ 4 $\mu\text{g/ml}$), with 20.3% (65/320) in 2004, 28.1% (96/342) in 2005, 35.3% (128/363) in 2006, 32.1% (127/396) in

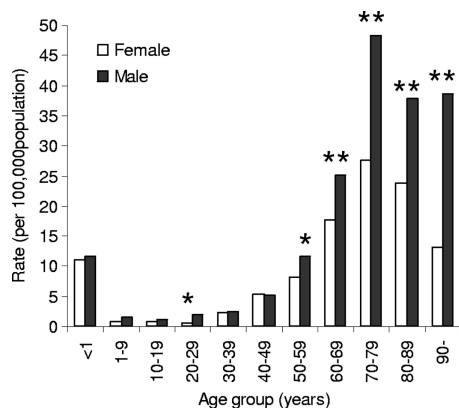


FIG. 2. Annual incidence of fungemia, by age and gender, in Denmark from 2004 to 2009. *, $P = 0.0003$; **, $P < 0.0001$.

TABLE 4. Distribution of fungal isolates according to species and gender^a

	Female		Male		P^*
	No.	%	No.	%	
<i>C. albicans</i>	708	56.3	937	57.4	NS
<i>C. dubliniensis</i>	29	2.3	45	2.8	NS
<i>C. glabrata</i>	290	23.1	322	19.7	0.03
<i>C. krusei</i>	51	4.1	68	4.2	NS
<i>C. parapsilosis</i>	51	4.1	56	3.4	NS
<i>C. tropicalis</i>	46	3.7	91	5.6	0.02
<i>Candida</i> spp.	29	2.3	50	3.1	NS
Non- <i>C. albicans</i>	33	2.6	36	2.2	NS
Other fungi	20	1.6	25	1.5	NS
Total	1,257	43.5	1,630	56.5	<0.0001

^a *, chi-square test, $P < 0.05$ was regarded statistically significant. For 14 patients, the gender was unknown. NS, not significant.

TABLE 5. Distribution of fungal isolates according to species and age group

Species ^a	No. (%) of isolates found for indicated age (yr) group											Total no. (%)
	<1	1-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90+	
<i>C. albicans</i>	32 (73)	32 (73)	29 (74)	24 (52)	65 (58)	143 (57)	252 (58)	417 (54)	474 (60)	167 (51)	22 (54)	1,657 (57)
<i>C. dubliniensis</i>				3 (7)	6 (5)	9 (4)	17 (4)	20 (3)	15 (2)	3 (1)	1 (2)	74 (3)
<i>C. glabrata</i>	1 (2)		3 (8)	9 (20)	15 (13)	49 (20)	79 (18)	177 (23)	174 (22)	93 (29)	12 (29)	612 (21)
<i>C. krusei</i>		3 (7)	2 (5)	2 (4)	1 (1)	7 (3)	23 (5)	55 (7)	17 (2)	8 (2)	1 (2)	119 (4)
<i>C. parapsilosis</i>	8 (18)	7 (16)	2 (5)	2 (4)	6 (5)	7 (3)	16 (4)	27 (4)	23 (3)	10 (3)		108 (4)
<i>C. tropicalis</i>				3 (7)	5 (4)	17 (7)	12 (3)	33 (4)	45 (6)	22 (7)	1 (2)	138 (5)
<i>Candida</i> spp.*	2 (5)		2 (5)	2 (4)	3 (3)	13 (5)	19 (4)	14 (2)	18 (2)	6 (2)		79 (3)
Non- <i>C. albicans</i> **		1 (2)			3 (3)	1 (0)	10 (2)	17 (2)	20 (3)	14 (4)	3 (7)	69 (2)
Other fungi	1 (2)	1 (2)	1 (3)	1 (2)	8 (7)	3 (1)	9 (2)	10 (1)	8 (1)	2 (1)	1 (2)	45 (2)
Total	44	44	39	46	112	249	437	770	794	325	41	2,901

^a *, *Candida* spp. includes indicated number of the following isolates: *C. ciferrii*, 1; *C. colliculosa*, 1; *C. fermentati*, 3; *C. guilliermondii*, 14; *C. holmii*, 1; *C. inconspicua*, 1; *C. inconspicua/norvegensis*, 1; *C. intermedia*, 2; *C. kefyri*, 11; *C. lipolytica*, 2; *C. lusitaniae*, 22; *C. nivariensis*, 1; *C. norvegensis*, 1; *C. palmioleophila*, 9; *C. pelliculosa*, 5; *C. pulcherima*, 1; *C. rugosa*, 1; *C. utilis*, 1; and *C. valida*, 1. **, non-*C. albicans* denotes isolates that were not *C. albicans* but not referred to the mycology reference laboratory for species identification. ***, other fungi include indicated number of the following isolates: *Cryptococcus neoformans*, 9; *Cryptococcus* sp., 1; *F. dimerum*, 2; *F. solani*, 5; *Fusarium* sp., 4; *Geotrichum capitatum*, 1; *Pichia fabii*, 1; *Pichia carribica*, 1; *Rhodotorula glutinis/Rhodotorula mucilaginosa*, 1; *Rhodotorula mucilaginosa*, 1; *Rhodotorula* sp., 1; *S. cerevisiae*, 15; *Trichosporon inkin*, 1; *Trichosporon mucoides*, 1; and *Williopsis* sp., 1.

2007, 27.5% (97/353) in 2008, and 31.1% (103/331) in 2009 (chi-square for trend, $P = 0.02$) (Fig. 3). This was due mainly to a change in species distribution; the proportion of *C. tropicalis* isolates with a fluconazole MIC of >2 µg/ml did, however, increase significantly over the 6-year period ($P = 0.0354$) (Fig. 3).

Nearly all *Candida* isolates were susceptible to amphotericin B. Exceptions were seven *C. glabrata* isolates (1.6%), five *C. krusei* isolates (4.7%), one *C. tropicalis* (1%) isolate, and one *C. norvegensis* isolate (1/1) (Table 6). The echinocandins displayed activity against the majority of *Candida* isolates. One *C. dubliniensis* isolate with a caspofungin MIC of >32 µg/ml and an anidulafungin MIC of 0.5 µg/ml was found to possess an S645P mutation in the *FKSI* gene. Moreover, the anidulafungin MIC was above the applied susceptibility breakpoint for nine additional *Candida* isolates, including one *C. albicans* (0.4%, anidulafungin MIC of 0.06 µg/ml, caspofungin MIC of 0.25), one *C. dubliniensis* isolate (8.7%, anidulafungin MIC of

0.125 µg/ml, caspofungin MIC of 1 µg/ml), two *C. glabrata* isolates (2%, anidulafungin MICs of 0.5 and 1 µg/ml, caspofungin MICs both of 0.5 µg/ml), and one *C. tropicalis* isolate (3.6%, anidulafungin MIC of 1 µg/ml, caspofungin MIC of 0.125 µg/ml). Among other *Candida* species, only *C. guilliermondii* was classified as anidulafungin resistant (MICs of 0.5 to 4 µg/ml), whereas isolates belonging to the closely related species *C. palmioleophila* were susceptible (MICs of <0.03 mg/ml, 8/8 isolates).

The susceptibility pattern for the azoles was more complex. The majority of *Candida* isolates were susceptible to azoles, with the exception of those belonging to the intrinsically less susceptible or resistant species, like *C. glabrata* and *C. krusei* (Table 6). However, 21 *Candida* isolates belonging to species normally susceptible were fluconazole resistant (fluconazole MIC of >4 µg/ml) and included seven *C. albicans* (0.6%), two *C. dubliniensis* (3.1%), five *C. parapsilosis* (6.0%), and seven *C. tropicalis* (6.7%) isolates. Moreover, for 29 additional isolates from other *Candida* species (45.3%), the fluconazole MIC was greater than 4 µg/ml (*C. palmioleophila*, 8/8; *C. inconspicua/C. norvegensis*, 3/3; *C. lusitaniae*, 5/17; *C. guilliermondii*, 5/15; *C. pelliculosa*, 2/5; *C. kefyri*, 1/6; and 1/1 of the following species: *C. ciferrii*, *C. holmii*, *C. rugosa*, *C. valida*, and *C. utilis*).

In comparison, the voriconazole MIC of >0.125 µg/ml was, with few exceptions, seen only for isolates belonging to species with intrinsically reduced azole susceptibility. However, two *C. albicans* (0.3%, MICs of 0.5 and 8 µg/ml), one *C. dubliniensis* (1.5%, MIC of 0.5 µg/ml), one *C. parapsilosis* (1.3%, MIC of 2 µg/ml), and six *C. tropicalis* (6%, MICs of 2, 4, 4, 8, 8, and 8 µg/ml) isolates had voriconazole MICs of >0.25 µg/ml. None of these isolates was susceptible to fluconazole, itraconazole, or posaconazole.

Susceptibility to itraconazole was closely related to fluconazole susceptibility and the species. Thus, 81.1% of the isolates had identical susceptibility classifications for fluconazole and itraconazole. Seventy-one isolates (3.5%) were fluconazole resistant but itraconazole susceptible, and two isolates were categorized as fluconazole susceptible but itraconazole resistant (0.1%). Similarly, for posaconazole, the susceptibility pattern

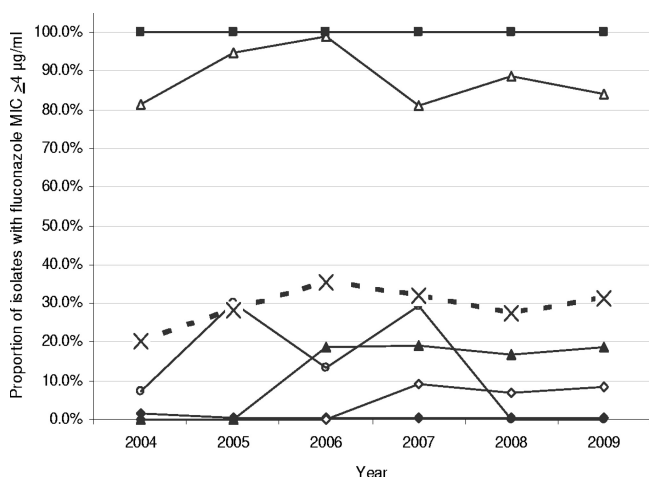


FIG. 3. Proportion of isolates for which fluconazole MIC was >2 µg/ml, by species and year. *C. albicans*, solid line with solid diamonds; *C. dubliniensis*, solid line with open diamonds; *C. glabrata*, solid line with open triangles; *C. krusei*, solid line with solid squares; *C. parapsilosis*, solid line with open circles; *C. tropicalis*, solid line with solid triangles; and all isolates, dotted line with crosses.

TABLE 6. *In vitro* activity of seven antifungal compounds against Danish fungemia isolates^a

Species	Antifungal agent (no. of isolates)	MIC range (μg/ml)	MIC ₅₀ (μg/ml)	MIC ₉₀ (μg/ml)	BP (S≤/R>)	% R
<i>C. albicans</i>	Amphotericin B (1,179)	0.016–1	0.25	0.5	1/1	0
	Anidulafungin (282)	≤0.03–0.06	≤0.03	≤0.03	0.015/0.015	0.4
	Caspofungin (1,174)	0.016–16	0.125	0.5	2/2	0.1
	Fluconazole (1,183)	≤0.125–>16	≤0.125	0.5	2/4	0.6
	Itraconazole (1,167)	≤0.03–>4	0.03	0.03	0.125/0.5	0.1
	Voriconazole (1,121)	≤0.03–>4	≤0.03	0.06	0.125/0.125	0.3
	Posaconazole (563)	≤0.03–>4	≤0.03	0.03	0.064/0.064	0.4
<i>C. dubliniensis</i>	Amphotericin B (65)	0.008–0.5	0.06	0.25	1/1	0
	Anidulafungin (23)	≤0.03–0.5	≤0.03	≤0.03	0.015/0.015	8.7
	Caspofungin (65)	0.03–>32	0.25	1	2/2	3.1
	Fluconazole (65)	≤0.125–>16	0.25	1	2/4	3.1
	Itraconazole (65)	≤0.03–0.125	0.03	0.03	0.125/0.5	0
	Voriconazole (65)	≤0.03–0.5	≤0.03	≤0.03	0.125/0.125	1.5
	Posaconazole (38)	≤0.03–0.06	≤0.03	0.06	0.064/0.064	0
<i>C. glabrata</i>	Amphotericin B (449)	0.06–4	0.5	1	1/1	1.6
	Anidulafungin (104)	≤0.03–1	≤0.03	≤0.06	0.064/0.064	2
	Caspofungin (449)	0.016–2	0.5	1	2/2	0
	Fluconazole (455)	0.5–>16	8	>16	NA	NA
	Itraconazole (448)	≤0.03–>4	0.25	2	0.125/0.5	34.8
	Voriconazole (436)	≤0.03–>4	0.25	2	NA	NA
	Posaconazole (231)	≤0.03–>4	0.25	2	NA	NA
<i>C. krusei</i>	Amphotericin B (106)	0.06–4	1	1	1/1	4.7
	Anidulafungin (28)	≤0.03–0.06	≤0.03	≤0.03	0.125/0.125	0
	Caspofungin (106)	0.06–2	0.5	1	2/2	0
	Fluconazole (106)	8–>16	>16	>16	NA	NA
	Itraconazole (106)	0.03–1	0.25	0.5	0.125/0.5	1.9
	Voriconazole (104)	0.06–2	0.25	0.25	NA	NA
	Posaconazole (62)	0.03–0.5	0.125	0.5	NA	NA
<i>C. parapsilosis</i>	Amphotericin B (82)	0.06–1	0.5	1	1/1	0
	Anidulafungin (22)	0.5–2	1	2	NA	NA
	Caspofungin (83)	0.25–16	2	4	NA	NA
	Fluconazole (83)	≤0.125–>16	1	4	2/4	6.0
	Itraconazole (83)	≤0.03–0.25	0.06	0.125	0.125/0.5	0
	Voriconazole (79)	≤0.03–2	≤0.03	0.06	0.125/0.125	1.3
	Posaconazole (44)	≤0.03–0.25	0.03	0.06	0.064/0.064	2.3
<i>C. tropicalis</i>	Amphotericin B (103)	0.016–2	0.5	1	1/1	1
	Anidulafungin (28)	≤0.03–1	≤0.03	≤0.03	0.064/0.064	3.6
	Caspofungin (104)	0.03–2	0.25	1	2/2	0
	Fluconazole (104)	≤0.125–>16	0.5	4	2/4	6.7
	Itraconazole (104)	≤0.03–>4	0.03	0.25	0.125/0.5	3.8
	Voriconazole (102)	≤0.03–>4	≤0.03	0.25	0.125/0.125	6.0
	Posaconazole (55)	≤0.03–>4	0.03	0.125	0.064/0.064	18.2
<i>Candida</i> spp.	Amphotericin B (64)	0.016–2	0.25	0.5	1/1	1
	Anidulafungin (20)	≤0.03–1	≤0.03	1	NA	NA
	Caspofungin (64)	0.125–8	0.5	2	2/2	3.1
	Fluconazole (64)	≤0.125–>16	4	>16	2/4	45.3
	Itraconazole (63)	≤0.03–>4	0.125	1	0.125/0.5	11.1
	Voriconazole (61)	≤0.03–4	0.125	0.5	0.125/0.125	37.7
	Posaconazole (38)	≤0.03–2	0.06	0.25	0.064/0.064	39.5
Other fungi	Amphotericin B (43)	0.06–>16	0.5	2	1/1	16.3
	Anidulafungin (7)	≤0.03–>4	0.125	>4	NA	NA
	Caspofungin (41)	0.25–>32	2	16	NA	NA
	Fluconazole (43)	≤0.125–>16	8	>16	NA	NA
	Itraconazole (43)	0.03–>4	0.5	>4	0.125/0.5	41.9
	Voriconazole (42)	≤0.03–>4	0.25	2	NA	NA
	Posaconazole (24)	0.03–>4	0.5	>4	NA	NA

^a BP, breakpoint; S, sensitivity; R, resistance.

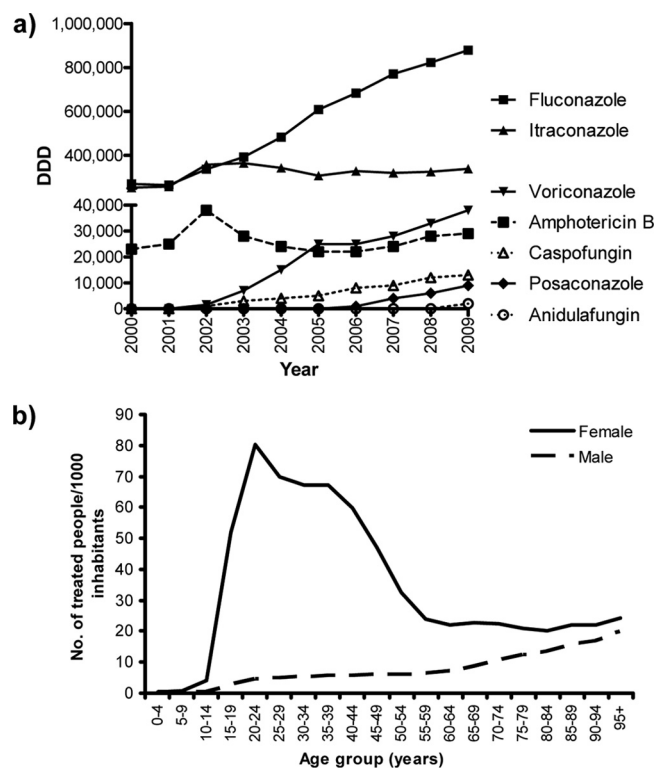


FIG. 4. Consumption of systemic antifungal compounds in Denmark between 2000 and 2009 (in DDD) (a) and for fluconazole by gender and age group (mean no. of treated people per 1,000 inhabitants, 2005 to 2009) (b).

was again closely related to the species (Table 6). Notably, better activity was observed against fluconazole-resistant *Candida* species, though resistance (MIC of >0.125 $\mu\text{g/ml}$) was seen for 1/1 *C. ciferrii*, 4/9 *C. guilliermondii*, 1/2 *C. inconspicua/C. norvegensis*, 5/8 *C. palmiophila*, and 1/2 *C. pelliculosa* isolates.

Isolates belonging to other fungi were less susceptible (Table 6). Amphotericin B displayed the broadest activity against this group and was the only agent with activity against *Rhodotorula* spp. However, for seven other fungal isolates (16.3%), all of which were *Fusarium* species, amphotericin B MICs were 2 to >16 $\mu\text{g/ml}$ (2/2 *F. dimerum*, 3/5 *F. solani*, and 2/4 *Fusarium* species isolates). Caspofungin showed *in vitro* activity against *Saccharomyces*, *Pichia*, and *Williopsis* isolates (MIC₅₀ of 1 $\mu\text{g/ml}$, range of 0.25 to 2 $\mu\text{g/ml}$), but *Fusarium*, *Cryptococcus*, *Geotrichum*, *Trichosporon*, and *Rhodotorula* isolates were resistant (MIC₅₀ of 16 $\mu\text{g/ml}$, range of 4 to >32 $\mu\text{g/ml}$). The majority of other fungi were fluconazole resistant (65.1%), whereas voriconazole MICs against *Fusarium* species were 1 to 4 $\mu\text{g/ml}$ (MIC₅₀ of 1 $\mu\text{g/ml}$), and low MICs (≤ 0.5 $\mu\text{g/ml}$) were found for *Saccharomyces*, *Pichia*, and *Geotrichum* isolates. Posaconazole MICs for *Fusarium* species isolates were 2 to ≥ 8 $\mu\text{g/ml}$.

Consumption of antifungals. The total consumption of antifungals in Denmark is shown in Fig. 4a. The total annual usage of systemic antifungals increased 140% from 546,000 DDD (102.4 DDD/1,000 inhabitants) to 1,309,000 DDD (249.1 DDD/1,000 inhabitants) over a 10-year period from 2000 to

2009. For the systemic azoles, 76% were used in the primary health care sector, and for fluconazole specifically this was true for 67%, a proportion that remained stable over the observation period. Fluconazole was prescribed more often to females than to males in the primary health care sector (Fig. 4b). Thus, the annual number of fluconazole-treated people per 1,000 inhabitants was 36.6 for females versus 5.2 for males, and the dispensed volume of fluconazole per 1,000 inhabitants per day was 0.38 for females versus 0.12 for males in this setting (<http://dkma.medstat.dk/MedStatDataViewer.php>).

DISCUSSION

Almost 3,000 cases were included in this first study of the incidence rate of fungemia in all of Denmark, and to our knowledge it is the largest population-based nationwide fungemia study published so far. A seminal fungemia surveillance program established in 2004 covered two-thirds of the Danish population, with participation of all university hospitals plus all stem cell and organ transplantations centers; it estimated the incidence rate to be 10.4/100,000 inhabitants (6). In Norway the incidence rate of fungemia at county hospitals was half the rate at university hospitals (43), a trend that was also observed in our study. Thus, the observed Danish national incidence rate of 8.6/100,000 is not surprising. The rate increased over the years 2004 to 2007 and then stabilized at an intermediate level in 2008 to 2009. Nationwide studies in the other Nordic countries have reported lower but increasing rates: in Norway, 2.4 to 4 (comparing 1991 to 2003 and 2008) (29, 44); in Iceland, 1.4 to 4.9 (comparing 1980 to 1984 and 1995 to 1999) (9); and in Finland, 1.9 to 3 per 100,000 inhabitants (comparing 1995 to 1999 and 2007) (36, 37). In this context the rate in Denmark appears notably high. The age-specific rate is, however, similar to the rate for the <1 -year age group (Denmark, 11.3; Iceland, around 11; Norway, 10.3; and Finland, 9.4 per 100,000) and lower than those in several other surveys from Europe, Australia, and the United States (24.8 to 38.8 and even >140 /100,000 in black children below the age of 1 year) (3, 13, 20). In contrast, remarkable differences have been seen with the elderly population in Nordic countries (e.g., for 70 to 79 years of age, Denmark, 37.1; Iceland, around 15; Norway, 7.4; and Finland, 8.8 per 100,000), and this Danish rate is also higher than those reported from both Spain and Australia (3, 13). Thus, the high overall rate in Denmark seems to be driven mainly by a high rate in the elderly population.

The incidence of fungemia was higher for males than for females, with the most marked gender difference in the age groups 20 to 29 and >50 years. Furthermore, *C. tropicalis* was significantly more common in males. The reasons for these differences are not clear. Significantly higher rates of hematological (56%) and gastrointestinal (54%) cancers that are risk factors for candidemia are seen with males than with females (the National Board of Health, Denmark; <http://www.sst.dk>). In agreement with this, *C. tropicalis*, which has been associated with hematological malignancy (1, 25, 46, 52), was more commonly isolated from males. It is also likely that the higher rate in males in their twenties is related to a twice-as-high frequency of trauma, violent injury, and poisoning in males compared to females in this age group (the National Board of Health, Denmark <http://www.sst.dk>). However, these gender

differences should be explored further, as they seem not to have been ascertained previously in population-based surveys.

We observed a significant change in species distribution over the 6-year period, with a decline in *C. albicans* and an increase in *C. glabrata*. The same trend is well documented in the other Nordic countries over the last decades (9, 29, 36, 37, 44), and *C. glabrata* has become the second most common pathogen in the northern hemisphere (4). This shift has been associated with increased fluconazole use overall (32) and especially in intensive care and hematological and pediatric settings (19, 27, 28, 50). Invasive disease originates from the colonizing flora (11, 16, 26, 39), and thus, prior antifungal therapy in primary health care or hospital settings may affect species distribution of subsequent invasive infections. Over the last 10 years the consumption of antifungals in Denmark has increased by 140% and reached 249 DDD/1,000 inhabitants in 2009; for comparison, the total annual consumption in Norway was 78.4 DDD/1,000 inhabitants (<http://www.legemiddelforbruk.no/english/>). During the same period, fluconazole use in Denmark increased to a level 2.7 times higher per inhabitant than that in Norway (159.5 versus 58.8 DDD/1,000 inhabitants). In Denmark the majority of fluconazole is prescribed in the primary health care setting and far more often to women than men. These differences may in part explain the prominence of *C. glabrata* in Denmark compared with other Nordic countries, the continuing rise, and the difference among genders. However, as reported previously, *C. glabrata* and *C. krusei* are still remarkably infrequent in the pediatric setting, and hence, fluconazole is still a reasonable option for the treatment of candidemia before species identification in this particular setting, except in children with prior azole exposure.

Recent reports have suggested that the choice of blood culture system may influence the recovery of *C. glabrata* and that the Bactec system may be inferior to the BacT/Alert system, in this respect (6, 44). The present data support this observation, as the recovery rate of *C. glabrata* was significantly lower at centers using the Bactec system than at centers using the BacT/Alert system. A theoretical bias for this observation could be that the age distribution varied between centers using the two blood culture systems, and therefore, the analysis was repeated for the 60- to 79-year-old age group with the same result. It is consequently suggested that the mycosis medium be included in blood cultures at centers using the Bactec system when a patient is at risk for candidemia.

To our knowledge, this is the first surveillance study reporting the emergence of *C. palmiophila* with one isolate in 2006, three isolates in 2007, and five isolates in 2009. This species is phenotypically easily confused with *C. guilliermondii*, but in contrast to *C. guilliermondii*, it is highly susceptible to the echinocandin class of drugs (17, 21). As for many other emerging *Candida* species, susceptibility to fluconazole is low (35), and thus, susceptibility testing and correct species identification is necessary to guide appropriate treatment.

Susceptibility testing confirmed the broad activity of amphotericin B and echinocandins. Thus, 99% were susceptible to amphotericin B, with exceptions being *C. norvegensis*, *C. krusei*, *C. glabrata*, and the majority of *Fusarium* isolates. Amphotericin B MICs were below 1 $\mu\text{g/ml}$ for all 20 *C. lusitanae* isolates. However, this species is known for its reduced clinical susceptibility to amphotericin B and higher mutational rate and thus

should be regarded as having intrinsically reduced amphotericin B susceptibility despite being classified as susceptible based on MIC determinations (10). Also, the echinocandins caspofungin and anidulafungin were active against the vast majority of the isolates. The current CLSI breakpoint of ≤ 2 $\mu\text{g/ml}$ for anidulafungin and caspofungin and *Candida* species is undergoing revision: ≤ 0.25 $\mu\text{g/ml}$ has been proposed for *C. albicans*, *C. glabrata*, *C. krusei*, and *C. tropicalis*, and ≤ 2 $\mu\text{g/ml}$ for *C. parapsilosis* and *C. guilliermondii* (8, 34). Breakpoints for the echinocandins have not yet been established by EUCAST. However, the EUCAST and CLSI methods have been compared using the same set of wild-type and resistant mutants, and the species-specific breakpoints suggested by these data were applied in the present study (8). One limitation is that caspofungin MIC distributions are variable due to unknown factors, and thus, the breakpoint of ≤ 2 $\mu\text{g/ml}$ may be too high (7). We found more isolates classified as susceptible to caspofungin than to anidulafungin and believe this is an artifact due to this issue. One isolate was, however, resistant with an anidulafungin MIC of 0.5 $\mu\text{g/ml}$, a caspofungin MIC of >32 $\mu\text{g/ml}$, and a well-characterized hot spot mutation in the *FKS1* target gene. Furthermore, caspofungin MICs were above the proposed revised breakpoint for three of the other five *Candida* isolates with an increased anidulafungin MIC, suggesting that these unrelated isolates may have acquired resistance mechanisms for echinocandins. Thus, although still rare, acquired echinocandin resistance is detected among Danish blood isolates.

We found that 29% of the blood isolates were not fully fluconazole susceptible, which confirms our previous finding that reduced fluconazole susceptibility is prevalent in Denmark (5, 6). Acquired fluconazole resistance was investigated in two recent surveys, one global and one including patients at two tertiary centers in the United States (31, 35). Among more than 250,000 isolates from 41 countries, fluconazole resistance was found in 2% of *C. albicans*, 3.9% of *C. dubliniensis*, 9% of *C. tropicalis*, and 6.8% of *C. parapsilosis* isolates (35). Notably, at the U.S. tertiary centers, acquired resistance accounted for a third of the resistant isolates (31). In our country, acquired resistance was seen at similar rates among the listed species. But in contrast to the United States, acquired resistance accounted for less than 2.7% of the resistance in candidemia cases, and thus, species identification is still a reliable predictor of susceptibility in Denmark.

In conclusion, this first 6-year nationwide population-based study of fungemia in Denmark has revealed several significant findings. First, the previously reported high rate of fungemia in Denmark was confirmed, and it still stands out in both a Nordic and a global perspective. Also, the high proportion of isolates not fully susceptible to fluconazole remained unchanged after inclusion of fungemias from all district and county hospitals. Second, the species distribution has been changing, with fewer *C. albicans* and more *C. glabrata* isolates, and of note, the newly reported species *C. palmiophila* has emerged as a bloodstream pathogen. Third, acquired resistance continued to be low, particularly for amphotericin B and the echinocandins, but fluconazole resistance in 2009 exceeded 5% for *C. tropicalis* and *C. parapsilosis*. Finally, substantial age- and gender-related differences in incidence rates and species distribution were shown in a population-based setting.

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