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# Epidemiological changes with potential implication for antifungal prescription recommendations for fungaemia: data from a nationwide fungaemia surveillance programme

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## Abstract

Significant changes in the management of fungaemia have occurred over the last decade with increased use of fluconazole prophylaxis, of empirical treatment and of echinocandins as first-line agents for documented disease. These changes may impact the epidemiology of fungaemia. We present nationwide data for Denmark from 2010 to 2011. A total of 1081 isolates from 1047 episodes were recorded in 995 patients. The numbers of patients, episodes and recovered isolates increased by 13.1%, 14.5% and 14.1%, respectively, from 2010 to 2011. The incidence rate was significantly higher in 2011 (10.05/100 000) than in 2010 (8.82/100 000), but remained constant in the age groups 0–79 years. The incidence rate was highest at the extremes of age and in males. *Candida albicans* accounted for 52.1% but declined during 2004–11 (p 0.0155). *Candida glabrata* accounted for 28% and increased during 2004–2011 (p <0.0001). *Candida krusei, Candida tropicalis* and *Candida parapsilosis* remained rare (3.3–4.2%). The species distribution changed with increasing age (fewer *C. parapsilosis* and more *C. glabrata*) and by study centre. Overall, the susceptibility rates were: amphotericin B 97.3%, anidulafungin 93.8%, fluconazole 66.7%, itraconazole 69.6%, posaconazole 64.2% and voriconazole 85.0%. Acquired echinocandin resistance was molecularly confirmed in three isolates. The use of systemic antifungals doubled over the last decade (2002–2011) (from 717 000 to 1 450 000 defined daily doses/year) of which the vast majority (96.9%) were azoles. The incidence of fungaemia continues to increase in Denmark and is associated with a decreasing proportion being susceptible to fluconazole. Changes in demography, higher incidence in the elderly and higher antifungal consumption can at least in part explain the changes.

**Keywords:** Amphotericin B, anidulafungin, antifungals, *Candida*, candidaemia, caspofungin, epidemiology, fluconazole, itraconazole, posaconazole, susceptibility, voriconazole
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## Introduction

Significant changes in the management of fungaemia have occurred over the last decade in response to the increasing number of fungaemia (fungal bloodstream infection) cases and a

high mortality in patients in whom antifungal treatment has been delayed [1–8]. In general, antifungal prophylaxis, empirical and pre-emptive treatment approaches, most often with fluconazole, have been extensively explored and the echinocandins have become first-line agents for targeted treatment of documented invasive candidiasis [1,9–16]. *Candida albicans* remains the predominant species. However, there are geographical differences and changes over time in species distributions. Hence, *Candida glabrata* is more common in the northern hemisphere while *Candida parapsilosis* is more common in the southern parts of the world and in Asia [17]. The intrinsic susceptibility patterns of these species are different. Hence, primary antifungal regimens should be adjusted to the local epidemiology. Several papers have reported possible consequences of changes in management of fungaemia with respect to the epidemiology and susceptibility pattern. Previous exposure to fluconazole or caspofungin affects the species distribution for subsequent candidaemia cases, with a higher proportion of *C. glabrata* after one week of fluconazole and of *C. parapsilosis* after echinocandin exposure [18–21]. Surveys conducted in Europe have reported *C. glabrata* proportions from 8 to 22% [22–32]. In this perspective, the surveillance in Denmark revealed an unexpectedly increasing proportion of *C. glabrata* among blood isolates (from 17% in 2004 to 27% in 2009) [1]. Recent reports of echinocandin breakthrough infections and acquired resistance, particularly in *C. glabrata* suggest that acquired resistance may be emerging [33–37]. Nevertheless, it remains uncertain whether these trends reflect chiefly tertiary-centre experiences or represent changes that can be translated into other settings as well.

Under these circumstances close population-based surveillance of epidemiology and susceptibility patterns are important to place these observations in the correct perspective and to allow updated treatment recommendations ensuring appropriate initial treatment, until species identification and susceptibility test results are available. A nationwide fungaemia surveillance programme with prospective collection and susceptibility testing of all blood isolates has been active in Denmark since 2010. It was established on the basis of a previous semi-national programme that combined with retrospective data documented a notably high annual incidence rate of 8.6 episodes per 100 000 inhabitants in 2004–09 compared with most other countries [1,5,8,22,23,25,30,38–42]. The objective of this study was to extend previous observations in a contemporary and nationwide perspective.

## Materials and Methods

### Surveillance and population

Thirteen departments of clinical microbiology together serving the entire country participated in the prospective national surveillance in 2010–11. These centres and their geographic capture areas have been specified previously [1]. 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, USA) blood culture system. In total 70.8% of the cases were detected using BacT/ALERT and 29.2% using BACTEC. For fungaemia patients with successive blood culture isolates, separate episodes were included if they occurred at least 21 days apart or were caused by different species consistent with our previous reports [1,24,43].

The size of the Danish population increased marginally (0.47%, from 5 534 738 in 2010 to 5 560 628 in 2011; www.statistikbanken.dk).

Information on haematological and gastrointestinal cancers was retrieved at the website http://www.ssi.dk/Sundhedsdataogit/Dataformidling/Sundhedsdata/Behandling%20ved%20sygehuse/Sygehusaktivitet%20pa%20diagnoseniveau.aspx.

### 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 and 43°C, rapid tests for the identification of *Candida dubliniensis* and *C. glabrata* (BICHRO-DUBLI and Glabrata RTT, Fumouze Diagnostics, Simoco, Denmark) and assimilation profile by use of a commercial system (ATB ID32C; bioMérieux). Additionally, matrix-assisted laser desorption ionization–time of flight mass spectrometry was gradually implemented during the second year and used as an additional tool for isolates that were difficult to identify by conventional methods. If no reliable species diagnosis was obtained molecular identification was performed as described below.

### Susceptibility testing

Susceptibility testing was carried out for a total of 1060 (98.1%, amphotericin B and anidulafungin), 468 (43.3%, caspofungin) and 1062 (98.2%, fluconazole, itraconazole, posaconazole and voriconazole) isolates, respectively, according to the EUCAST definitive document E.Def 7.2 [44]; exceptions were amphotericin B and caspofungin, for which Etest (AB bioMérieux, Herlev, Denmark) and RPMI 2% glucose agar buffered with MOPS (SSI Diagnostika, Hillerød, Denmark) was used. Manufacturers and stock solutions in DMSO were as follows (dimethyl sulphoxide (DMSO), D8779, Sigma-Aldrich,

Vallensbæk Strand, Denmark): fluconazole (Sigma-Aldrich; 10 000 mg/L), itraconazole (Sigma-Aldrich; 5000 mg/L), posaconazole (Merck, Sharp and Dohme, Glostrup, Denmark; 5000 mg/L), and anidulafungin and voriconazole (Pfizer A/S, Ballerup, Denmark; 5000 mg/L). *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 was included as a quality controls in each run. Accepted EUCAST MIC ranges are previously published [44] whereas the CLSI MIC ranges were used for Etest results [45]. The following breakpoints (mg/L; S ≤ /R>): amphotericin B: 1/1 [45]; anidulafungin: *C. albicans* 0.03/0.03, *C. glabrata*, *C. krusei* and *C. tropicalis* 0.064/0.064 [46]; caspofungin: *C. albicans*, *C. krusei* and *C. tropicalis* 0.25/0.5, *C. glabrata* 0.125/0.25 and *C. parapsilosis* and *Candida guilliermondii*: 2/2 [47]; fluconazole: *Candida* species other than *C. glabrata* and *C. krusei*: 2/4 [48]; itraconazole: 0.125/0.5 [45]; posaconazole: *Candida* species other than *C. glabrata* and *C. krusei*: 0.064/0.064 [49]; and voriconazole: *Candida* species other than *C. glabrata* and *C. krusei*: 0.125/0.125 [50]. For species and antifungal compound combinations for which no breakpoint has yet been proposed, the proportion of isolates with MIC below the breakpoint valid for the other species was reported as the susceptible proportion of the isolates for the given species. This is mainly done to illustrate the overall susceptibility of that species/group of fungi and should not be interpreted as a precise measurement of susceptibility versus resistance.

#### Molecular identification and *FKS* gene sequence analysis (for selected isolates)

DNA was released from fungal colonies as previously described [51]. Species identification was performed using the universal fungal primers (ITS1; CGTAGGTGAACCTG CGG and ITS4; TCCTCCGCTTATTGATATGC). *FKS* gene sequence analysis was performed as previously described [52]. This gene encodes the target enzyme (glucan synthase) for echinocandins.

#### Consumption of antifungal compounds

Information concerning overall use of antifungal agents in Denmark 2000–11 in hospitals and primary health care was available in defined daily doses (DDD) from the Danish Medicines Agency at (www.medstat.dk). Similar information for Norway was available from the Norwegian Institute of Public Health at http://www.legemiddelforbruk.no/english/.

#### Statistics

Incidences per 100 000 inhabitants were calculated using the population sizes for 1 January each year. Numbers of admissions for each geographical region in Denmark were reported by the local study participants.

Chi-square test was used for comparison of changes in incidence rate and species distribution. p-values <0.05 (two-tailed) were considered statistically significant.

## Results

#### Epidemiology

*National data.* During 2010–11 a total of 1081 isolates from 1047 episodes of fungaemia were recorded in 995 patients leading to an annual incidence rate of 9.4/100 000 inhabitants. The incidence rate was significantly higher in 2011 compared with 2010 (10.05 and 8.82/100 000 inhabitants, respectively, p 0.037) (Table 1). The number of patients, episodes and recovered isolates increased by 13.1%, 14.5% and 14.1%, respectively, and by 10.8%, 11.4% and 11.8%, comparing the incidence rate in 2010–11 to that of the preceding 6-year period (2004–09) (Table 1) [1]. The incidence rate is shown in Fig 1 in comparison with the similar figures from Norway and Finland as reported earlier [22,23,39,53].

The median age remained constant over the 2-year period and compared with the previous 6 years (Table 1). *Candida* species accounted for 98.2% of the fungal isolates and *C. albicans* was the predominant species (in total 52.1%) though a continued decline was observed from 2004 to 2011 (p 0.016, Table 1). *Candida glabrata* was the second most frequent species (28%) and increased over the study period as well as compared with the previous years (p <0.0001). *C. krusei*, *C. tropicalis* and *C. parapsilosis* were rare isolates (3.3–4.2%) and their occurrence remained stable. The species distribution did not vary significantly by gender (data not shown).

The age-specific and gender-specific incidence rates are shown in Table 2. The highest incidence rates were seen at the extremes of age (range 0.86–38.17/100 000 inhabitants). Only 1.4% of the patients were below 1 year of age, 3.9% were 1–29 years of age, 67.8% were 60 years or older, and 42.2% were 70 years of age or older. The incidence rate was significantly higher in males than in females (11.7 versus 7.8/100 000, p ≤ 0.0001) with the largest and significant gender differences for inhabitants 30–39 years and inhabitants older than 50 years (Table 2). The age-specific incidence was comparable to those reported in Norway and Finland for children and younger adults; however, it was remarkably higher in the 50 + year age group (Fig 2) [22,23,39,53]. Overall, *C. albicans* and *C. parapsilosis* accounted for 75% of the infections in patients <10 years old. Both *C. glabrata* and *C. krusei* were rare in young patients (two with *C. glabrata* and three with *C. krusei* in patients <20 years old). In contrast, 35.9% of the fungaemia isolates were either *C. glabrata* or

**TABLE 1. Epidemiology and species distribution of fungaemia in Denmark in 2010 and 2011 compared with the previous 6-year period.**

| | 2004–09[a] | 2010 | 2011 | In total 2010–11 |
|---|---|---|---|---|
| Fungal isolates (no.) | 2901 | 505 | 576 | 1081 |
| Episodes (no.) | 2820 | 488 | 559 | 1047 |
| Patients (no.) | 2694 | 467 | 528 | 995 |
| Median age (years (range and interquartile ages)) | 66 (0–98 and 55;74) | 67 (0–96 and 55;75) | 66 (0–105 and 57;75) | 66 (0–105 and 56;75) |
| Gender (% males) | 56.5 | 59.6 | 59.4 | 59.5 |
| Episode rate per 100 000 inhabitants | 8.6 | 8.8 | 10.1 | 9.4 |
| Episode rate per 10 000 discharges | 4.1 | 4.1 | 4.6 | 4.4 |
| Species distribution | | | | |
| *Candida albicans* | 57.1% | 52.9% | 51.4% | 52.1% |
| *Candida dubliniensis* | 2.6% | 1.8% | 1.7% | 1.8% |
| *Candida glabrata* | 21.1% | 26.9% | 29.0% | 28.0% |
| *Candida krusei* | 4.1% | 5.0% | 4.7% | 4.8% |
| *Candida parapsilosis* | 3.7% | 5.1% | 3.3% | 4.2% |
| *Candida tropicalis* | 4.8% | 4.0% | 4.2% | 4.1% |
| *Candida* species[b] | 2.7% | 2.4% | 4.2% | 3.3% |
| Non-*C. albicans* spp. not referred for ID[c] | 2.4% | 0.0% | 0.0% | 0.0% |
| Other fungi[d] | 1.6% | 2.0% | 1.6% | 1.8% |

[a]Compiled from Arendrup *et al.* [1]
[b]*Candida* spp. includes the following species in 2010–11: *C. guilliermondii* 6, *C. inconspicua* 1, *C. kefyr* 6, *C. lambica* 1, *C. lusitaniae* 11, *C. magnolia* 1, *C. norvegensis* 4, *C. orthopsilosis* 2, *C. palmioleophila* 2 and *C. pelliculosa* 2.
[c]Non-*albicans* denotes isolates that were not *C. albicans* but not referred to the mycology reference laboratory for species identification.
[d]Other fungi includes: *Cryptococcus neoformans* 4, *Fusarium oxysporum* 1, *Fusarium proliferatum* 2, *Fusarium solani* 2, *Fusarium* sp. 1, *Geotrichum candidum* 1, *Rhodotorula glutinis* 1, *Saccharomyces boulardii* 1 and *Saccharomyces cerevisiae* 6.

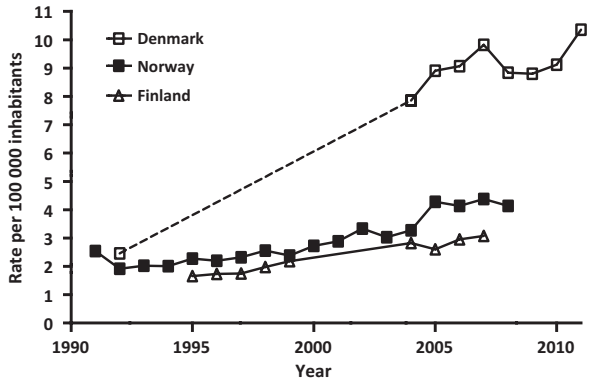


**FIG. 1.** Incidence rate of unique fungal blood stream isolates per 100 000 inhabitants (1992–2011) compared with similar figures from Norway and Finland as reported earlier [22,23,39,53]. The number of isolates in Denmark in 1992 was estimated using the number of cases (57) registered at centres 3, 8, 14 and 15 and the proportion of cases by which these centres contributed during the years 2004–11 (mean 0.45, range 0.42–0.50) leading to an estimate of 127 cases (range 113–136) in a population of 5 162 126 in 1992.

*C. krusei* in patients $\geq$60 years of age (Table 2). *Candida glabrata* was recovered more often at centres using the BacT/ALERT system (207/685 occasions) than at centres using the BACTEC system (96/396 occasions; p 0.035).

Polyfungal infections occurred in 30 patients (2.9%). Twenty-nine of these involved two species whereas one involved three species. In 24 (80.0%) of these patients *C. albicans* or *C. dubliniensis* was isolated in combination with another yeast, among which *C. glabrata* accounted for 12 cases. However, the majority (24/30; 80.0%) of the polyfungal infections included at least one species with intrinsic decreased susceptibility to fluconazole (*C. glabrata* (18), *C. krusei* (7), *C. lambica* (1), *C. glabrata* and *C. krusei* were found together in two cases). Fifty patients (5.0%) had more than one episode (median age 60 years, range 0–83 years), none of which involved selection of isolates with acquired resistance mechanisms. In 24 patients the same species was re-isolated after a median of 53 days (range 22–485 days) whereas at least one new species (with or without the primary species) was isolated in 26 cases after a median of 7 days (range 1–217 days). *Candida albicans* and *C. glabrata* were most commonly involved in a recurrent episode as well as episodes involving new species and there was no difference in the frequency with which *C. glabrata* followed *C. albicans* or vice versa (six each). Nor was there a trend among recurrent episodes towards increasing resistance among the other cases (data not shown).

*Centre-specific data.* The incidence rate varied four-fold among the centres from 3.91 to 16.55/100 000 inhabitants and from 2.38 to 9.84/10 000 hospital discharges (Table 3). The incidence rate was lowest at centres serving district hospitals 2.38–4.82/10 000 and highest at centres serving university hospitals only 4.40–9.84 (Table 3). Also the species distribution varied by centre; for example, *C. albicans* accounted for 45–59% and the proportion of isolates belonging to species with reduced susceptibility to fluconazole (*C. glabrata*, *C. krusei* and other fungi) varied from 23 to 43% (Table 3). Incidence rates were found to be higher and the proportion of *C. glabrata* and *C. krusei* lower or equal to the average ($\leq$32%) at centres 1, 8, 10 and 14. In contrast, the highest

**TABLE 2. Incidence rate (per 100 000 inhabitants) and species distribution by age and gender in the 2-year period 2010–11**

| | Age group (years) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | <1 | 1–9 | 10–19 | 20–29 | 30–39 | 40–49 | 50–59 | 60–69 | 70–79 | 80–89 | 90–99 | 100+ | In total |
| Incidence | 12.65 | 1.09 | 0.86 | 1.31 | 3.14 | 5.10 | 11.51 | 20.29 | 38.17 | 36.82 | 25.36 | 55.56 | 9.4 |
| Female 2010–11 | 12.93 | 0.69 | 0.74 | 0.79 | 1.77 | 4.36 | 9.25 | 15.89 | 30.61 | 24.04 | 16.47 | 66.05 | 7.83 |
| Male 2010–11 | 10.76 | 1.49 | 0.98 | 1.84 | 4.47 | 5.82 | 13.80 | 24.84 | 48.10 | 57.68 | 53.86 | 0.00 | 11.69 |
| p value (Chi square approximation) | NS | NS | NS | NS | 0.0055 | NS | 0.0139 | 0.0003 | <0.0001 | <0.0001 | 0.0136 | NS | <0.0001 |
| No. isolates | 15 | 13 | 12 | 17 | 46 | 83 | 165 | 277 | 295 | 140 | 19 | 1 | 1081 |
| Proportion (%) | 1.4 | 1.2 | 1.1 | 1.6 | 4.3 | 7.7 | 15.3 | 25.6 | 27.3 | 13.0 | 1.8 | 0.1 | 100.0 |
| Species distribution | | | | | | | | | | | | | |
| *Candida albicans* | 60.0% | 61.5% | 50.0% | 47.1% | 45.7% | 45.8% | 57.6% | 52.0% | 53.9% | 47.9% | 42.1% | - | 52.1% |
| *Candida dubliniensis* | 0.0% | 0.0% | 8.3% | 5.9% | 2.2% | 3.6% | 3.0% | 1.4% | 1.0% | 0.7% | 0.0% | - | 1.8% |
| *Candida glabrata* | 6.7% | 0.0% | 8.3% | 23.5% | 17.4% | 19.3% | 25.5% | 29.6% | 28.8% | 37.9% | 52.6% | 1/1 | 28.0% |
| *Candida krusei* | 0.0% | 15.4% | 8.3% | 5.9% | 4.3% | 10.8% | 3.0% | 6.1% | 3.7% | 2.9% | 0.0% | - | 4.8% |
| *Candida parapsilosis* | 13.3% | 15.4% | 8.3% | 5.9% | 2.2% | 4.8% | 1.2% | 4.3% | 6.1% | 2.9% | 0.0% | - | 4.2% |
| *Candida tropicalis* | 6.7% | 0.0% | 0.0% | 0.0% | 13.0% | 9.6% | 4.2% | 2.2% | 3.4% | 3.6% | 5.3% | - | 4.1% |
| *Candida* species[a] | 13.3% | 7.7% | 0.0% | 5.9% | 2.2% | 4.8% | 5.5% | 3.6% | 1.7% | 2.1% | 0.0% | - | 3.3% |
| Other fungi[b] | 0.0% | 0.0% | 16.7% | 5.9% | 13.0% | 1.2% | 0.0% | 0.7% | 1.4% | 2.1% | 0.0% | - | 1.8% |

[a]*Candida* spp. includes the following species in 2010–11: *C. guilliermondii* 6, *C. inconspicua* 1, *C. kefyr* 6, *C. lambica* 1, *C. lusitaniae* 11, *C. magnolia* 1, *C. norvegensis* 4, *C. orthopsilosis* 2, *C. palmioleophila* 2 and *C. pelliculosa* 2.
[b]Other fungi includes: *Cryptococcus neoformans* 4, *Fusarium oxysporum* 1, *Fusarium proliferatum* 2, *Fusarium solani* 2, *Fusarium* sp. 1, *Geotrichum candidum* 1, *Rhodotorula glutinis* 1, *Saccharomyces boulardii* 1 and *Saccharomyces cerevisiae* 6.

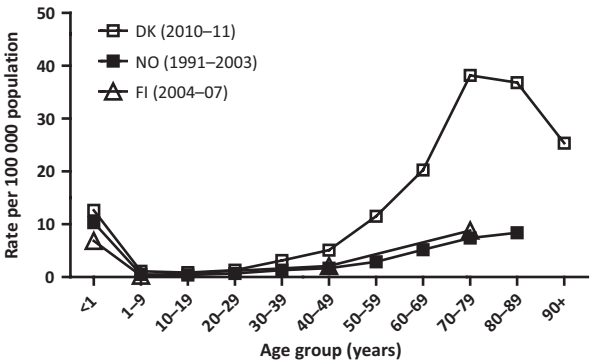


**FIG. 2.** Age-specific incidence rate of unique fungal bloodstream isolates per 100 000 inhabitants compared with the similar figures from Norway and Finland as reported earlier [22,23,39,53].

proportion (>40%) of *C. glabrata* and *C. krusei* were found at centres 2, 3, 4, 5–7 and 12, for which the incidence rates were lower than the average (Table 3).

### Antifungal susceptibility

If adopting the breakpoints for *Candida* spp. and other fungi for which no species-specific breakpoints have been established, the proportion of isolates that were susceptible was 97.3% for amphotericin B, 93.8% for anidulafungin, 84.4% for caspofungin, 66.7% for fluconazole, 69.6% for itraconazole, 64.2% for posaconazole and 85.0% for voriconazole (Table 4).

Most species and isolates were susceptible to amphotericin B (Table 4). Exceptions were *C. krusei* and the group of other fungi for which 73.1% and 68.4% were susceptible, respectively. However, for the majority of the non-susceptible *C. krusei* isolates the MIC was 2 mg/L (11/14 isolates, 78.6%) and no isolates were found with amphotericin MICs above 4 mg/L. Hence, these isolates did not truly separate from the wild-type population (Table 4). On the contrary, the MICs for other fungi formed a tri-modal distribution with six isolates separating from the rest of the isolates, including five isolates of *Fusarium* and one of *Geotrichum*.

Overall, the susceptibility to echinocandins was high. Caspofungin microdilution testing has been associated with an unacceptable lot to lot variation prohibiting the selection of meaningful breakpoints. Therefore, caspofungin susceptibility testing with Etest was used in 2010 before the EUCAST anidulafungin breakpoint was established and recommended as a marker of echinocandin susceptibility in 2011 [46]. Comparing the proportion of isolates classified as anidulafungin versus caspofungin susceptible by species two discrepancies were observed. First, fewer *C. glabrata* and *C. krusei* isolates were classified as susceptible to caspofungin (65% and 28%, respectively) than to anidulafungin (99.3% and 100%, respectively) and second, all *C. parapsilosis* and *C. guilliermondii* were classified as susceptible to caspofungin but not to anidulafungin because of the different recommendations and breakpoints for CLSI and EUCAST (Table 4). The risk of misclassifying susceptible wild-type isolates of *C. glabrata* and *C. krusei* as non-susceptible using caspofungin Etest and CLSI breakpoints has recently been addressed, and the apparent discrepancy between the proportion of isolates that are classified as susceptible to caspofungin and anidulafungin among these two species is likely to be a laboratory issue rather than a true difference in antifungal activity [54]. Two *C. glabrata* isolates were classified as echinocandin resistant because of anidulafungin MICs of 0.125 mg/L. For one of these, *FKS1* and *FKS2* sequencing revealed a S663P alteration in hot spot 1 of the FKS2p protein. Additionally, one *C. tropicalis* isolate was anidulafungin and caspofungin resistant (MIC 0.25 mg/L and >32 mg/L, respectively) and harboured a heterozygous S80S/P

**TABLE 3.** Centre-specific incidence rates (number of bloodstream fungal isolates per 100 000 inhabitants and per 10 000 discharges) and species distribution

| | 1-RH[a] | 2-Cph City Hospitals | 3-Cph County Herlev[a] | | 4-Frederiksborg | 5-7-Central & SW-Sealand | 8-Funen[b] | 9-S-Jutland | 10-Esbjerg | 11-Vejle | 12-Herning | 13-Viborg | 14-N-Jutland | 15-Aarhus | In total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Centre type U/D[c] | U | UD | U | UD | | D | UD | D | D | D | D | D | UD | UD | UD |
| No. of isolates | | | | | | | | | | | | | | | |
| 2010 | 71 | 48 | 45 | 24 | | 50 | 68 | 8 | 18 | 19 | 13 | 18 | 64 | 59 | 505 |
| 2011 | 90 | 80 | 42 | 16 | | 53 | 80 | 10 | 21 | 19 | 13 | 15 | 55 | 82 | 576 |
| Incidence (2010–11) | | | | | | | | | | | | | | | |
| /100 000 inhabitants | NA | 7.91 | 8.34 | 6.45 | | 6.28 | 16.55 | 3.91 | 8.21 | 6.71 | 4.55 | 7.17 | 10.26 | 9.51 | 9.74 |
| /10 000 discharges | 9.84 | 3.89 | 4.40 | 2.54 | | 3.16 | 7.65 | 2.38 | 4.82 | 3.30 | 2.66 | 3.17 | 5.07 | 4.42 | 4.52 |
| Species (2010–11) | | | | | | | | | | | | | | | |
| *Candida albicans* (%) | 48 | 50 | 45 | 49 | | 49 | 58 | 56 | 59 | 45 | 46 | 55 | 54 | 58 | 52 |
| *Candida dubliniensis* (%) | 2 | 2 | 3 | 0 | | 0 | 4 | 0 | 0 | 3 | 0 | 0 | 2 | 1 | 2 |
| *Candida glabrata* (%) | 24 | 34 | 31 | 38 | | 38 | 21 | 33 | 26 | 29 | 42 | 24 | 25 | 26 | 28 |
| *Candida krusei* (%) | 8 | 5 | 10 | 5 | | 5 | 2 | 0 | 3 | 0 | 0 | 0 | 7 | 4 | 5 |
| *Candida parapsilosis* (%) | 7 | 0 | 3 | 3 | | 3 | 5 | 11 | 8 | 5 | 0 | 6 | 3 | 3 | 4 |
| *Candida tropicalis* (%) | 2 | 2 | 5 | 6 | | 6 | 3 | 0 | 0 | 8 | 8 | 6 | 3 | 6 | 4 |
| *Candida* spp.[d] (%) | 4 | 5 | 1 | 0 | | 0 | 5 | 0 | 5 | 11 | 4 | 0 | 2 | 3 | 3 |
| Other fungi[e] (%) | 4 | 2 | 1 | 0 | | 0 | 1 | 0 | 0 | 0 | 0 | 9 | 4 | 0 | 2 |

[a]Centre using the BACTEC blood culture system (the remaining using BacT/ALERT).
[b]Centre using BACTEC blood culture system in 2010 but BacT/ALERT in 2011.
[c]Characteristics of the hospitals served by the centre: U, University hospitals; D, district hospitals. NA, not applicable. RH is a tertiary hospital without a unique geographic uptake area.
[d]*Candida* spp. includes the following species in 2010–11: *C. guilliermondii* 6, *C. inconspicua* 1, *C. kefyr* 6, *C. lambica* 1, *C. lusitaniae* 11, *C. magnolia* 1, *C. norvegensis* 4, *C. orthopsilosis* 2, *C. palmioleophila* 2 and *C. pelliculosa* 2.
[e]Other fungi includes: *Cryptococcus neoformans* 4, *Fusarium oxysporum* 1, *Fusarium proliferatum* 2, *Fusarium solani* 2, *Fusarium* sp. 1, *Geotrichum candidum* 1, *Rhodotorula glutinis* 1, *Fusarium boulardii* 1 and *Saccharomyces cerevisiae* 6.

hot spot alteration [55]. Finally, the proportion of other fungi that were classified as echinocandin susceptible was low and limited to *Saccharomyces* isolates.

For the azoles all *C. albicans* and *C. dubliniensis* isolates were fluconazole susceptible except three *C. albicans* isolates with fluconazole MICs of $\geq 32$ mg/L. Two of these were also highly resistant to the other three azoles (MICs of 2 mg/L for itraconazole, posaconazole and voriconazole) whereas the MIC for one isolate was borderline (itraconazole, posaconazole and voriconazole MICs of 0.25, 0.25 and 0.125 mg/L, respectively). Similarly, one *C. tropicalis* was fluconazole resistant and posaconazole resistant (MICs 8 mg/L and 0.25 mg/L, respectively), intermediate to itraconazole (MIC 0.25 mg/L) and had the voriconazole MIC in the upper susceptibility range of 0.125 mg/L. The azole MICs for *C. glabrata*, *C. krusei* and other fungi were in general elevated compared with those for *C. albicans* (Table 4). For *C. glabrata* in particular, the MIC distribution was somewhat asymmetric with a tail of isolates spanning a wide concentration range to the right of the peak and 13.8% (41/298) $\geq 32$ mg/L, suggesting that a proportion of these isolates may harbour acquired resistance mechanisms (Table 4). Among *Candida* spp., fluconazole MICs for *C. guilliermondii*, *C. lambica*, *C. palmioleophila*, *C. pelliculosa*, *C. norvegensis*, *C. inconspicua* and *C. magnolia* were consistently >2 mg/L and for the majority of these isolates MICs were also elevated for itraconazole and posaconazole, as reflected by the similar proportion of isolates being classified as susceptible if adopting the breakpoints for these species (57.1%, 62.9% and 65.7% for fluconazole, itraconazole and posaconazole, respectively, whereas this was somewhat higher for voriconazole (88.6%) (Table 4). Similarly, more isolates in the group of other fungi were classified as voriconazole susceptible than susceptible to the other three azoles if applying the *Candida* breakpoints. However, this difference reflected that more *Saccharomyces* isolates were classified as susceptible to voriconazole. For the six *Fusarium* isolates the MICs were as follows: fluconazole >16 mg/L, itraconazole $\geq 4$ mg/L, posaconazole 0.25 to $\geq 4$ mg/L and voriconazole 1–4 mg/L.

*Consumption of antifungals.* The national use of systemic antifungal compounds was investigated as the use in the primary as well as the hospital setting may impose a selection pressure on the colonizing fungal flora and species distribution of subsequent fungaemia isolates. During the 2-year study period a total of 10 423 000 DDD was prescribed (939 DDD/1000 inhabitants/year) (Fig 3). Excluding terbinafine, a total of 2 841 000 DDD was used (256 DDD/1000 inhabitants/year) of which 72% was prescribed in the primary healthcare setting. For comparison, in Norway the total consumption of systemic antifungal compounds excluding terbinafine was 70.6 DDD/

**TABLE 4.** **Susceptibility of the fungaemia isolates to seven systemic antifungal compounds by species. The MIC was determined by EUCAST reference methodology (anidulafungin, fluconazole, itraconazole, posaconazole and voriconazole) or by Etest (amphotericin and caspofungin). Grey boxes indicate concentrations not tested. The official EUCAST breakpoints were adopted for interpretation except for caspofungin and itraconazole for which the revised CLSI breakpoints were applied. These are indicated by solid lines. For species and compounds without breakpoints the proportion below the breakpoint for the other species (dotted lines) is indicated in parenthesis as an indication of the susceptibility profile for the given species/group of isolates (and a rough estimate of the proportion of cases that are likely good targets for the compound in question).**

| | No. | ≤0.032 | 0.064 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | ≥32 | S | S (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amphotericin B | | | | | | | | | | | | | | |
| *Candida albicans* | 551 | 7 | 28 | 72 | 278 | 164 | 2 | 0 | 0 | 0 | 0 | 0 | 551 | 100.0 |
| *Candida dubliniensis* | 18 | 8 | 3 | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 100.0 |
| *Candida glabrata* | 297 | 0 | 4 | 17 | 43 | 155 | 72 | 5 | 1 | 0 | 0 | 0 | 291 | 98.0 |
| *Candida krusei* | 52 | 0 | 0 | 0 | 2 | 13 | 23 | 11 | 3 | 0 | 0 | 0 | 38 | 73.1 |
| *Candida parapsilosis* | 44 | 0 | 0 | 6 | 14 | 19 | 5 | 0 | 0 | 0 | 0 | 0 | 44 | 100.0 |
| *Candida tropicalis* | 44 | 0 | 2 | 1 | 6 | 23 | 11 | 1 | 0 | 0 | 0 | 0 | 43 | 97.7 |
| *Candida* spp.[a] | 35 | 3 | 3 | 8 | 9 | 9 | 1 | 2 | 0 | 0 | 0 | 0 | 33 | 94.3 |
| Other fungi[b] | 19 | 0 | 0 | 3 | 3 | 6 | 1 | 0 | 4 | 0 | 0 | 2 | 13 | 68.4 |
| In total | 1060 | 18 | 40 | 113 | 356 | 389 | 115 | 19 | 8 | 0 | 0 | 2 | 1031 | 97.3 |
| Anidulafungin | | | | | | | | | | | | | | |
| *Candida albicans* | 551 | 551 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | 551 | 100.0 |
| *Candida dubliniensis* | 18 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | 18 | 100.0 |
| *Candida glabrata* | 298 | 293 | 3 | **2** | 0 | 0 | 0 | 0 | 0 | 0 | | | 296 | 99.3 |
| *Candida krusei* | 52 | 52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | 52 | 100.0 |
| *Candida parapsilosis* | 45 | 0 | 0 | 0 | 2 | 8 | 18 | 16 | 1 | 0 | | | 0 | (0.0) |
| *Candida tropicalis* | 44 | 43 | 0 | 0 | **1** | 0 | 0 | 0 | 0 | 0 | | | 43 | 97.7 |
| *Candida* spp.[a] | 35 | 27 | 0 | 0 | 3 | 2 | 2 | 1 | 0 | 0 | | | 27 | (77.1) |
| Other fungi[b] | 17 | 3 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | | | 7 | (41.2) |
| In total | 1060 | 987 | 7 | 2 | 6 | 10 | 20 | 17 | 1 | 10 | | | 994 | (93.8) |
| Caspofungin | | | | | | | | | | | | | | |
| *Candida albicans* | 250 | 73 | 124 | 49 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 249 | 99.6 |
| *Candida dubliniensis* | 9 | 1 | 4 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 100.0 |
| *Candida glabrata* | 120 | 1 | 5 | 72 | 41 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 78 | 65.0 |
| *Candida krusei* | 25 | 0 | 0 | 0 | 7 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 28.0 |
| *Candida parapsilosis* | 24 | 0 | 1 | 0 | 4 | 9 | 7 | 3 | 0 | 0 | 0 | 0 | 24 | 100.0 |
| *Candida tropicalis* | 19 | 0 | 9 | 6 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | **1** | 18 | 100.0 |
| *Candida* spp.[a] | 12 | 0 | 1 | 4 | 2 | 4 | 0 | 0 | 0 | 0 | 1 | 0 | 8 | (66.7) |
| Other fungi[b] | 10 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 7 | 2 | (20.0) |
| In total | 468 | 75 | 144 | 134 | 63 | 34 | 7 | 3 | 0 | 0 | 1 | 7 | 395 | (84.4) |
| Fluconazole | | | | | | | | | | | | | | |
| *Candida albicans* | 551 | | | 534 | 11 | 2 | 1 | 0 | 0 | 0 | 0 | 3 | 548 | 99.5 |
| *Candida dubliniensis* | 18 | | | 17 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 100.0 |
| *Candida glabrata* | 298 | | | 0 | 0 | 0 | 1 | 33 | 113 | 88 | 22 | 41 | 34 | (11.4) |
| *Candida krusei* | 52 | | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 12 | 39 | 0 | (0.0) |
| *Candida parapsilosis* | 45 | | | 5 | 5 | 13 | 14 | 5 | 2 | 1 | 0 | 0 | 42 | 93.3 |
| *Candida tropicalis* | 44 | | | 30 | 5 | 5 | 2 | 1 | 0 | **1** | 0 | 0 | 43 | 97.7 |
| *Candida* spp.[a] | 35 | | | 8 | 7 | 3 | 1 | 1 | 3 | 1 | 5 | 6 | 20 | 57.1 |
| Other fungi[b] | 19 | | | 1 | 0 | 0 | 1 | 1 | 5 | 1 | 2 | 8 | 3 | (15.8) |
| In total | 1062 | | | 595 | 29 | 23 | 20 | 41 | 123 | 93 | 41 | 97 | 708 | (66.7) |
| Itraconazole | | | | | | | | | | | | | | |
| *Candida albicans* | 551 | 538 | 10 | 0 | **1** | 0 | 0 | 0 | 0 | **2** | | | 548 | 99.5 |
| *Candida dubliniensis* | 18 | 17 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | 18 | 100.0 |
| *Candida glabrata* | 298 | 0 | 5 | 41 | 68 | 77 | 43 | 24 | 22 | 18 | | | 46 | 15.4 |
| *Candida krusei* | 52 | 0 | 0 | 15 | 25 | 10 | 2 | 0 | 0 | 0 | | | 15 | 28.8 |
| *Candida parapsilosis* | 45 | 16 | 17 | 11 | 0 | 0 | 1 | 0 | 0 | 0 | | | 44 | 97.8 |
| *Candida tropicalis* | 44 | 32 | 8 | 3 | **1** | 0 | 0 | 0 | 0 | 0 | | | 43 | 97.7 |
| *Candida* spp.[a] | 35 | 12 | 6 | 4 | 5 | 5 | 3 | 0 | 0 | 0 | | | 22 | 62.9 |
| Other fungi[b] | 19 | 1 | 0 | 2 | 2 | 2 | 5 | 1 | 6 | 0 | | | 3 | (15.8) |
| In total | 1062 | 616 | 47 | 76 | 102 | 94 | 54 | 25 | 28 | 20 | | | 739 | (69.6) |
| Posaconazole | | | | | | | | | | | | | | |
| *Candida albicans* | 551 | 548 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | | | 548 | 99.5 |
| *Candida dubliniensis* | 18 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | 18 | 100.0 |
| *Candida* glabrata | 298 | 1 | 5 | 33 | 76 | 99 | 39 | 12 | 18 | 15 | | | 6 | (2.0) |
| *Candida krusei* | 52 | 1 | 1 | 31 | 14 | 5 | 0 | 0 | 0 | 0 | | | 2 | (3.8) |
| *Candida parapsilosis* | 45 | 29 | 12 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | | | 41 | 91.1 |
| *Candida tropicalis* | 44 | 36 | 7 | 0 | **1** | 0 | 0 | 0 | 0 | 0 | | | 43 | 97.7 |
| *Candida* spp.[a] | 35 | 14 | 9 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | | | 23 | (65.7) |
| Other fungi[b] | 19 | 1 | 0 | 1 | 4 | 6 | 2 | 1 | 0 | 4 | | | 1 | (5.3) |
| In total | 1062 | 648 | 34 | 73 | 100 | 114 | 41 | 13 | 18 | 21 | | | 682 | (64.2) |
| Voriconazole | | | | | | | | | | | | | | |
| *Candida albicans* | 551 | 548 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | | | 549 | 99.6 |
| *Candida dubliniensis* | 18 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | 18 | 100.0 |
| *Candida glabrata* | 298 | 27 | 71 | 102 | 46 | 11 | 5 | 12 | 17 | 7 | | | 200 | (67.1) |
| *Candida krusei* | 52 | 0 | 0 | 6 | 33 | 10 | 2 | 0 | 1 | 0 | | | 6 | (11.5) |
| *Candida parapsilosis* | 45 | 41 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | | | 45 | 100.0 |
| *Candida tropicalis* | 44 | 42 | 1 | **1** | 0 | 0 | 0 | 0 | 0 | 0 | | | 44 | 100.0 |
| *Candida* spp.[a] | 35 | 20 | 3 | 8 | 0 | 3 | 1 | 0 | 0 | 0 | | | 31 | (88.6) |
| Other fungi[b] | 19 | 2 | 2 | 6 | 1 | 1 | 3 | 2 | 2 | 0 | | | 10 | (52.6) |
| In total | 1062 | 698 | 80 | 125 | 80 | 25 | 11 | 14 | 20 | 9 | | | 903 | (85.0) |

[a]*Candida* spp. (no. 35) included the following isolates (with the number tested for caspofungin in parenthesis (no. 12)): *C. guilliermondii* 5 (1), *C. kefyr* 6 (3), *C. inconspicua* 1 (1), *C. lambica* 1 (0), *C. lusitaniae* 11 (4), *C. magnolia* 1 (1), *C. norvegensis* 4 (1), *C. orthopsilosis* 2 (0), *C. palmioleophila* 2 (1) and *C. pelliculosa* 2 (0).
[b]Other fungi (no. 19) included the following isolates (with the number tested for caspofungin in parenthesis (no. 10)): *Cryptococcus neoformans* 4 (2), *F. oxysporum* 1 (0), *F. proliferatum* 2 (0), *F. solani* 2 (2), *Fusarium* sp. 1 (1), *Geotrichum candidum* 1 (1), *Rhodutorula glutinis* 1 (1), *S. boulardii* 1 (0) and *S. cerevisiae* 6 (3). (however the *Fusarium* sp. and the *Geotrichum candidum* were not tested for anidulafungin susceptibility leading to a total of 17 tested for this compound.

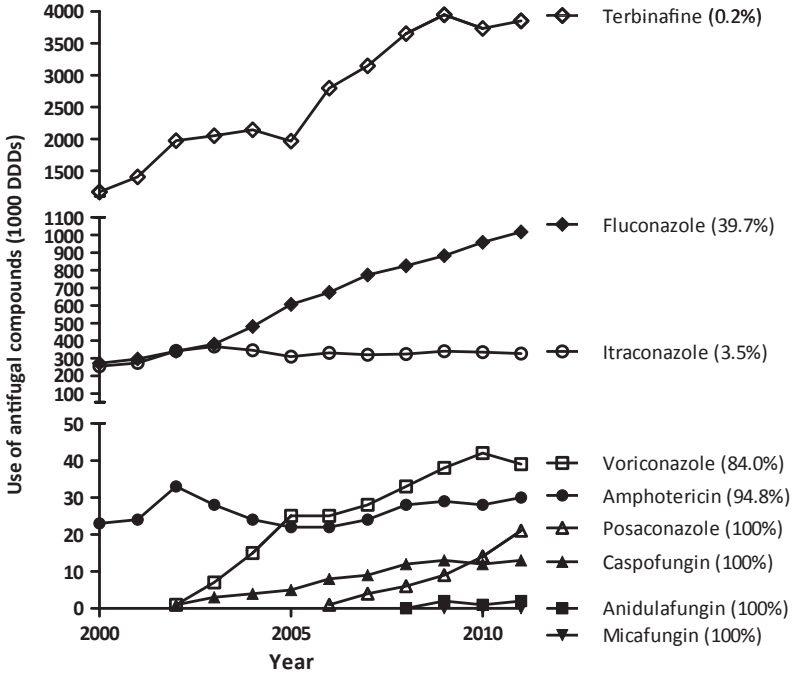


**FIG. 3.** National antifungal consumption (1000 DDD) in the years 2000–11 in Denmark. The hospital use in percentage of the total use in the study period 2010–11 is indicated in parenthesis for each compound

1000 inhabitants/year in 2010 and 2011; the majority thereof was fluconazole (63.7 DDD/1000 inhabitants/year) (http://www.legemiddelforbruk.no/english/).

The main drug class used in the hospital setting in Denmark was the azoles (713 000 DDD, 88.2% of the hospital antifungal use) with fluconazole being the most frequently prescribed agent (587 000 DDD, 72.6% of the hospital use) followed by voriconazole (68 000 DDD, or 8.4% of the hospital use) (Fig 3). Amphotericin B formulations constituted 6.8% of the hospital use (55 000/808 000 DDD) followed by the echinocandins 3.5% (28 000/808 000 DDD) among which the vast majority was caspofungin. Of note, the azole use in the hospital setting constituted only 25.9% of the national use, because of the extensive use in the primary healthcare sector of fluconazole and itraconazole. Consumption of the other agents, again excluding terbinafine, was mainly in the hospital setting (Fig 3).

## Discussion

The most important findings in this nationwide fungaemia surveillance programme are a continuously increasing incidence rate reaching 10/100 000 inhabitants in 2011, a continuously changing species distribution with a shift towards species, particularly *C. glabrata*, with intrinsic reduced susceptibility to azoles and a low prevalence of acquired resistance.

In the early 1990s the incidence rate in Denmark was around 2/100 000 and comparable with the other Nordic countries [39,53]. It has increased since then and more conspicuously than in the neighbouring countries [1,23,24,39,43]. In a recent nationwide study covering 2004–09 the mean annual incidence rate was 8.6/100 000 with a peak incidence rate in 2007 and a modest decline thereafter [1]. However, the data for the last 2 years show a further increase in the incidence rate that appears to be a continuation of the trend observed since the early 1990s. The age-specific and gender-specific incidence rates in this study are comparable with those reported in our previous studies with the notable exception of the population above 80 years of age and particularly among males in this age group [1]. Hence, the main driver of the increasing incidence rate over the last years in Denmark appears to be a changing demography with a growing proportion of the elderly. Likewise, it is noticeable that the age-specific incidence rate was comparable across the Nordic countries and among males and females in the young population whereas the elderly population in Denmark and especially men distinguished themselves from their Nordic counterparts [23,39]. Important underlying diseases like gastrointestinal cancer and leukaemia are more common in the elderly population and also 34% more common in men compared with women (according to number of hospital admissions), which may at least in part explain the gender

specific differences and changing epidemiology (http://www.ssi.dk/Sundhedsdataogit/Dataformidling/Sundhedsdata.aspx). Together, these observations suggest that underlying host factors rather than genetic differences in susceptibility to fungal bloodstream infection explain the differences in the incidence rate among the countries today and are compatible with differences in co-morbidity and frailty. This is also consistent with differences in longevity between populations in the Nordic countries (http://www.norden.org/en/publications/publikationer/2011-001).

*Candida albicans* and *C. parapsilosis* were the predominant species in children, whereas *C. glabrata* became increasingly frequent by age in agreement with previous observations [1,39,42]. Nevertheless, we report for the first time that more than half of the isolates are non-*albicans* species in several of the age-groups and that *C. glabrata* alone accounted for as many as a third to one-half of the isolates from patients more than 80 years old. *Candida glabrata* was found more frequently at centres using the BacT/ALERT system in agreement with previous observations suggesting the BACTEC may be less sensitive for the detection of this species [1,24,56]. Therefore the fact that the number of centres that use the BACTEC system has decreased from six to two from 2004 to 2011 may have contributed to the increased detection of this species. From this perspective it is important that a previous study demonstrated a significantly better outcome for patients with *C. glabrata* when the initial treatment was caspofungin rather than fluconazole, an observation that is in agreement with findings by others [18,57]. Hopefully the publication of the recent European and American candidiasis guidelines will lead to better outcome for this significant patient population [16,58–63].

The incidence rate and species distribution varied between the participating centres. For the first time a *C. albicans* proportion <50% and a combined proportion of *C. glabrata* and *C. krusei* exceeding 40% were found at many centres. An inverse relationship between the incidence rate and the proportion of cases involving *C. glabrata* or *C. krusei* was observed, suggesting that the use of azole prophylaxis may be a significant driver of the centre variation in incidence rate and species distribution. In this context it is important to note that the azole use in Denmark is notably higher than in Norway on the national level and that the vast majority is prescribed in the primary healthcare sector and for rather benign conditions. Examples are itraconazole for skin and nail infection where either topical agents or terbinafine could be used, and systemic fluconazole or itraconazole for vaginitis where topical azoles are valid alternatives. Changes to prescription practices are warranted to reduce the selection pressure on the normal colonizing flora from which most invasive fungal infections originate [64].

Acquired resistance was a rare event and limited to a few *C. albicans* isolates with azole resistance and a few *C. glabrata* and *C. tropicalis* isolates with echinocandin resistance, as documented by detection of underlying *FKS* hot spot mutations [55]. In recent years echinocandin resistance in particular has attracted attention because of its emergence in some settings and because echinocandins are now the first-line agent for invasive candidiasis [33–35,65–67]. In this perspective the finding of only a few isolates with acquired resistance among a thousand cases is reassuring and suggests that species identification in this setting is more important than susceptibility testing, in contrast with recent findings [68]. However, the design of the surveillance programme is not sensitive with respect to detection of emerging resistance as only the initial isolate from each episode is included. This is typically obtained at a time-point with the lowest antifungal exposure and particularly so for the echinocandins, which are mainly used for documented invasive infections. From an epidemiological point of view, this is a sound strategy because inclusion of multiple isolates per patient would lead to bias and skew the epidemiological data set. Only a prospective cohort study of fungaemia patients with inclusion of subsequent isolates can determine whether we have just detected the tip of the resistance iceberg. That this may be a relevant concern is suggested by discrepancies found between the resistance rates among deep-seated isolates and mucosal isolates in a recent study [69].

The major limitations related to this study are the lack of data on comorbidities, previous antifungal drug exposure, and whether fungaemia was nosocomial or of community-onset. In spite of this the study has highlighted several important findings and will form the basis for further studies with the goal of addressing these issues in a nationwide perspective.

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## Transparency Declaration

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