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Update from a twelve-year nationwide fungaemia surveillance: increasing intrinsic and acquired resistance causes concern

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ABSTRACT

New data from the Danish National Fungaemia Surveillance 2012-15 is reported and epidemiological trends are investigated in a 12-year perspective (2004-15). During 2012-15, 1900 out of 1939 (98%) fungal bloodstream isolates were included. The average incidence was 8.4/100,000 inhabitants and this appears to be stabilizing after the increase to 10.1/100,000 in 2011. The incidence was higher in males than females (10.0 vs 6.8) and in patients above 50 years, mainly driven by an increasing incidence among 80-89 years old males (65.3/100,000 in 2014-15). The proportion of *Candida albicans* decreased 2004-15 (64.4% to 42.4%) in parallel with a doubling of *Candida glabrata* (16.5% to 34.6%, p<0.0001). *C. glabrata* was more common among females (34.0% vs. 30.4% in males). Following an increase in 2004-11 the annual drug use stabilised during the last 2-3 years but remained higher than in other Nordic countries. This was particularly true for the fluconazole and itraconazole use in the primary healthcare sector which exceeded the combined national use of these compounds in each of the other Nordic countries. Fluconazole susceptibility decreased (68.5%, 65.2% and 60.6% in 2004-7, 2008-11 and 2012-15, respectively, p<0.0001) and echinocandin resistance in *Candida* emerged (0%, 0.6% and 1.7%, respectively, p<0.001). Amphotericin B susceptibility remained high (98.7%). Among 16 (2.7%) echinocandin-resistant *C. glabrata* isolates (2012-15), 13 harboured FKS mutations and five (31%) were multidrug resistant.

The epidemiological changes and the increased incidence of intrinsic and acquired resistance emphasise the importance of continued surveillance and of strengthened focus on antifungal stewardship.

INTRODUCTION

Candidaemia remains a threat to susceptible patients and continues to carry a high crude 30-days mortality of 30-40% (1–3). The mortality is linked to infecting species, co-morbidity, underlying disease, age, and time to initiation of appropriate treatment (3, 4). However, as diagnosis of invasive candidiasis is often delayed, diligent attention to intravascular devices are important and empiric treatment recommendations must be founded on reliable contemporary epidemiology (5). Institutional studies are affected by referral practices and departments served. In general, population-based studies are less prone to bias, especially when the study base becomes large and representative for entire countries. Nationwide surveillance data is available from Australia, Scotland, Finland, Iceland, Norway, Sweden, and Denmark (6–18). In Denmark, an increasing candidaemia incidence was demonstrated during 8 years reaching 10.1/100,000 inhabitants in 2011 (18). Although increasing incidence rates have also been found elsewhere, the Danish incidence is notably high compared to other reported contemporary nationwide incidence rates of 2.4-5.7/100.000 inhabitants (12–16).

Besides increasing incidence rates another common observation is the changing species distribution to non-*albicans* species and particularly *C. glabrata* in the northern hemisphere, Australia and Taiwan and *C. parapsilosis* in southern Europe and South America (3, 12, 13, 19, 20)) at the expense of *C. albicans*. The increase in *C. glabrata* has been coupled with an increased use of azoles for which *C. glabrata* is intrinsically less susceptible (18, 21, 22).

From 2001 the echinocandins were introduced in Europe and the USA and in 2009-12 incorporated into candidaemia management guidelines as the first-line treatment (23–27). Following the increased use, reports of acquired resistance has emerged among several different

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83 species but especially *C. glabrata* (28–31), thereby warranting continued surveillance and
84 monitoring. The aim of this population based nationwide study was to update and assess
85 incidence and susceptibility patterns of fungaemia in Denmark during a 12-year period.

MATERIAL AND METHODS

Population and surveillance 2012-15

Incidences per 100,000 inhabitants were calculated using the population by January 1st each year (www.statistikbanken.dk). The Danish population increased 1.4% from 5,580,516 to 5,659,715 inhabitants from 2012-15. The mean was used for incidence rates calculated for periods of >1 year. National annual number of admissions and bed days were available from The Danish Health Data Authority at www.eSundhed.dk. The local study representative reported population, numbers of admissions and bed days for hospitals within geographical capture area serviced by each centre of clinical microbiology. All Danish residents have access to universal tax-supported free-of-charge healthcare. All centres of clinical microbiology have specific geographic capture areas as specified previously (18), however, due to public health reforms the number was reduced from 13 to 11 (as centres 3 and 4 were administratively merged in 2012 (geographically separated blood culturing sites remained) and centres 12 and 13 fully merged in 2013). Original numbering of centres was retained to allow direct comparison with previous reports (17, 18).

Isolates were referred to the National Reference Mycology Laboratory for species verification and susceptibility testing (see below). Completeness was ensured through comparison with local electronic laboratory records. A total of 39 out of 1939 (2.0%) isolates were not referred and therefore excluded from the susceptibility paragraphs (14 *C. albicans,* 14 *C. glabrata*, 2 *C. tropicalis,* 2 *C. parapsilosis*, 1 each of *C. krusei, C. lusitaniae* and *C pellliculosa,* and 2 unidentified *Candida* species).

Two blood culture (BC) systems were used: BACT/ALERT (bioMérieux, Marcy l'Etoile, France) and BACTEC (Becton Dickinson, Franklin Lakes, NJ, USA), accounting for the detection of 77.9% and

22.1% of cases, respectively. For fungaemia patients with successive fungal bloodstream infections over time, subsequent episodes were included if they occurred at least 21 days apart or were caused by a different species consistent with previous reports (17, 18, 32).

Species identification

Identification was performed as previously described including matrix- assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker, Bremen, Germany) (18) with the addition of DNA sequencing as described below.

Susceptibility testing

Susceptibility testing was done contemporaneously for the referred isolates and included amphotericin B, voriconazole and isavuconazole (98.0% of the isolates), anidulafungin and micafungin (97.9% of the isolates), and fluconazole (97.6% of isolates) according to the EUCAST definitive document E.Def 7.3 (33). Exceptions were amphotericin B (prior to January 2015) for which E-test (bioMérieux, Herlev, Denmark) and RPMI-1640 2% glucose agar buffered with MOPS (SSI Diagnostika, Hillerød, Denmark) were used. Manufacturers and stock solutions (5000 mg/L in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Brøndby, Denmark)) were the following: fluconazole and amphotericin B (Sigma-Aldrich), anidulafungin and voriconazole (Pfizer A/S, Ballerup, Denmark), micafungin (Astellas Pharma Inc., Tokyo, Japan) and isavuconazole (Basilea, Pharmaceutica Ltd., Basel, Switzerland). *C. parapsilosis* ATCC22019 and/or *C. krusei* ATCC6258 were included as quality controls in each run (34). Susceptibility classification was performed according to established or proposed EUCAST breakpoints and ECOFFs (Supplementary table 1) (35–38). Finally, amphotericin B: ≤1 mg/L: S and fluconazole: ≤2 mg/L: S was used for remaining

129 species to illustrate the overall susceptibility of those species or groups of fungi but should not be
130 interpreted as an exact figure of clinical susceptibility or resistance.

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

132 Sequencing of internal transcribed spacer regions ITS1 and ITS2 (ITS) (18) and translation
133 elongation factor (TEF, for *Fusarium*) was performed as previously described (39). Echinocandin
134 target hot-spots in *FKS1*, and for *C. glabrata* also *FKS2*, DNA sequence analysis was performed for
135 resistant isolates and sequences compared to relevant reference sequences including Genbank
136 accession no. JX899422 for *C. kefyr* (40).

137 **Consumption of antifungal compounds**

138 Information concerning overall use of antifungal agents in Denmark 2000–15 was retrieved from
139 the Danish Medicines Agency (at www.medstat.dk). Posaconazole tablet and iv formulation were
140 marketed in Denmark in 2014. The licensed maintenance dose of these formulations is 300
141 mg/day compared to 800 mg/day for the oral suspension. To reflect the actual number of
142 individual dosages given in 2014-15, a corrected use was calculated (0.3 g enterotablet or iv
143 infusion and 0.8 g oral solution translated to 1 DDD). The antifungal use (DDD/1000
144 inhabitants/year) from Norway, Sweden and Finland was retrieved from
145 www.legemiddelforbruk.no, www.socialstyrelsen.se, and www.fimea.fi. Posaconazole formulation
146 information was not available and unadjusted posaconazole DDDs were used for comparison
147 between the Nordic countries and Denmark.

148 **Statistics**

149 Chi-square test or Fisher's exact test was used for comparison of proportions and the Chi-square
150 test for trend used for evaluation of changes in species distribution over the 12-year surveillance
151 period. Calculations were performed using GraphPad Prism Version 6.04 (GraphPad Software Inc.,
152 La Jolla, CA, USA). For twelve episodes (10 in 2004 and one each in 2005 and 2007) the gender was
153 unknown. When possible, episodes were allotted evenly to genders in specific 10-year age groups,
154 but in four instances single cases within age groups were excluded from analysis of gender- and
155 age-specific incidence rates, conducted with linear Poisson regression/incidence ratio rate
156 calculation (package: epitools). P-values <0.05 (two-tailed) were considered statistically significant.
157 Binomial univariate logistic regression was used to investigate associations between species
158 distribution and year, age, gender and BC system using R (R Foundation for Statistical Computing,
159 Vienna, Austria. URL https://www.R-project.org/). For this analysis and to assure independence of
160 observations only incident cases were included. Covariates with a p<0.1 were investigated further
161 in a binomial multivariate analysis. Year, age, gender, and BC system were all retained in the
162 multivariate analysis and independent significant findings displayed. The p-value also calculated
163 when excluding the main tertiary referral hospital serviced by centre 1-RH.

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RESULTS

Current epidemiology (2012-2015) in a 12-year perspective

A total of 1939 isolates from 1883 unique episodes and 1813 patients were included in the period 2012-15. The mean and median age of patients were 65 years (range: 0-98 years) and 69 years (interquartile range 58-77 years), respectively. Overall, 59.7% of patients were males and the proportion increased (p=0.002) (Table 1). In a 12-year perspective, both the age of candidaemia patients and the male proportion increased (Table 2).

The average episode rate was 8.38/100,000 inhabitants (range 7.6-9.1) in 2012-15 and overall stable over twelve year although a significant increase in incidence was evident from 2004-11 (p=0.001). The population grew with 4.9% from 2004-15, the number of discharges increased by 20.2%, but the number of bed days decreased by 18.5% (2005-15). Consequently, the incidence rate/1,000 admissions declined whereas the incidence rate/10,000 bed days increased (Table 2). The incidence varied across centres in 2012-15, from 3.1-13.1/100,000 inhabitants, 0.2-0.7/1000 discharges and 0.6-1.8/10,000 somatic bed days (Supplementary table 2).

The highest incidence was seen at the extremes of age, i.e. 9.5/100,000 in the <1-year old and 17.2, 31.4, 39.9 and 21.2 per 100,000 inhabitants in the 60-69, 70-79, 80-89, and 90+ years old age groups, respectively, in 2012-15. Moreover, the incidence was significantly higher in males compared to females (10.0 vs 6.8, IRR 1.5; 95% CI (1.3-1.6)) (Figure 1). In the 12-y perspective, decreasing age-specific incidence rates were observed in all age groups ≥50 years of age except ≥80 years (Figure 1). Whereas the overall female incidence rate numerically decreased over the three 4-year periods (p=0.05), the male incidence remained stable and with a significant increase between 80-89 years (Figure 1).

186 *C. albicans* accounted for the majority of isolates (47.9%) in 2012-15, followed by *C. glabrata*
187 (31.8%). *C. tropicalis, C. krusei*, *C. dubliniensis* and *C. parapsilosis* each accounted for ≤4.3%. Sixty-
188 nine (3.6%) of the isolates belonged to other *Candida* species, thirty-two (1.7%) to other fungal
189 genera whereof eleven were mould and another eleven *Saccharomyces cerevisiae* isolates (Table
190 1). Poly-fungal episodes (n=53) involved 5.6% of isolates. Over the twelve years, the proportion of
191 *C. albicans* decreased (p<0.0001) whereas the proportion of *C. glabrata* increased (p<0.0001)
192 (Figure 3). This development was still observed despite conservatively assigning non-*C. albicans* as
193 *C. glabrata* among isolates from 2004-09. The proportion of *C. dubliniensis* increased significantly
194 from 2.3% to 3.6% (p=0.01) whereas no change was detected for other species (Figure 3). The
195 increase in *C. glabrata* was significant for age groups 1-9, 30-39, 50-59, and 70-79 again despite
196 assigning early isolates identified as only non-albicans to be *C. glabrata* (p<0.03 for all groups;
197 Figure 2).

198 The species distribution in 2012-15 varied by age, gender, and by centre (Supplementary table 2
199 and 3). Correlation between species and age, gender, BC system, and calendar year was
200 investigated for all incident cases (only first episode included in the full 12-year period) in a
201 univariate and multivariate logistic regression analysis (Table 3). A decrease in *C. albicans* and an
202 increase in *C. glabrata* and *C. dubliniensis* episodes over time was found. *C. glabrata* was positively
203 associated with female sex whereas the opposite was the case for *C. tropicalis*. *C. albicans*, *C.*
204 *parapsilosis, C. dubliniensis,* and "other fungi" were associated with younger patients whereas the
205 odds of being infected with *C. glabrata* increased with age. Candidaemia involving *C. glabrata* was
206 positively associated with BACT/ALERT whereas the opposite was true for *C. parapsilosis, C. krusei,*
207 and "other fungi" (multivariate analysis). For "other fungi" this association disappeared when
208 excluding centre 1-RH.

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Susceptibility

For the six most common *Candida* species susceptibility patterns were largely as predicted by the species identification (Table 4). However, acquired resistance in *Candida* was occasionally detected and is detailed below per drug class.

Azoles: Overall, significantly fewer 1147/1892 (60.6%) of the isolates were fluconazole susceptible in 2012-15 compared to 1137/1745 (65.2%) in 2008-11 and 972/1420 (68.5%) in 2004-7, respectively (trend test p<0.0001). Among *C. albicans*, *C. dubliniensis*, *C. parapsilosis* and *C. tropicalis*, 2.1% (24/1128) were non-wild-type (wt) for fluconazole and 1.4% (16/1128) resistant (0.4% *C. albicans*, 4.2% *C. dubliniensis*, 6.5% *C. parapsilosis*, and 6.2% *C. tropicalis*) (Table 4). Two of four fluconazole-resistant *C. parapsilosis* isolates were voriconazole resistant. Six of seven fluconazole non-susceptible *C. tropicalis* isolates had a trailing phenotype making MIC determination difficult due to a 50% growth inhibition over a broad range of MIC values (which was also the case for voriconazole and isavuconazole). Altogether fourteen isolates (1.2%) of the four species were voriconazole resistant/non-wt and ten (0.9%) were isavuconazole non-wt, none of which were fluconazole susceptible.

For *C. glabrata* a bimodal fluconazole MIC distribution was observed, with the peaks at MIC values of 4 mg/L and 64 mg/L; 9.1% were resistant (MIC>32 mg/L). This proportion declined from 2012-13 to 2014-15 (11.4 % vs 6.6%, p=0.04). Finally, 8.1% of *C. glabrata* were also non-wt for voriconazole and 1.4% of *C. krusei* were non-wt for voriconazole.

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Echinocandins: Acquired echinocandin resistance increased compared to the previous years: 29/1754 (1.7%) compared to 10/1581 (0.6%) and 0/1294 (0%), in 2008-11 and 2004-7, respectively ($p<0.001$).

The twenty-nine *Candida* isolates displaying acquired anidulafungin resistance in 2012-15 consisted of 4.2% of *C. dubliniensis,* 2.7% *C. glabrata*, 6.8% *C. krusei*, 2.5% *C. tropicalis*, and 23% (3/13 isolates) *C. kefyr* isolates (Table 4). *FKS* sequencing detected hot spot alterations in 13/16 *C. glabrata*, 1/5 *C. krusei* (with the remaining four having L701M outside the hotspot which has not been found uniformly associated with echinocandin resistance in our laboratory), 0/3 *C. dubliniensis*, 2/2 *C. tropicalis*, and 1/3 *C. kefyr*. Five of the 16 (31%) *C. glabrata* isolates were fluconazole cross-resistant and thus multidrug resistant. For less common species see supplementary data 4.

Amphotericin B: Acquired amphotericin B resistance was found in 1.3% of fungaemia isolates and 1.0% (18/1851) of *Candida* isolates in 2012-15 (Table 4) including 1.5% *C. glabrata* isolates, 8.2% *C. krusei* isolates, two *C. nivariensis,* and one *C. norvegensis* isolates. In all instances, the MIC was 2 mg/L and thus one dilution step above the breakpoint.

Antifungal consumption

The antifungal consumption in Denmark has increased over the first 10 years of observation but stabilised or decreased during the last 2-3 years except for posaconazole (17, 18) (Figure 4). Most

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fluconazole, ketoconazole, itraconazole, and terbinafine was used in the primary health care sector 2004-15 (69.9%, 87.9%, 94.7% and 99.8%, respectively).

Denmark had a higher consumption of systemic antifungal drugs per 1,000 inhabitants compared to the other Nordic countries (2015: total 790 DDDs (DK) vs 512, 321, and 762 DDDs (Norway, Sweden and Finland)) and especially of the azoles (+44-174%; 237 DDDs (DK) vs 87, 111, and 164 DDDs (Norway, Sweden, and Finland, respectively)) despite a continued annual increase in fluconazole consumption until 2012 (Finland) or 2015 (Norway and Sweden). Caspofungin was the main echinocandin used. Anidulafungin was introduced in 2009 and accounted for 6-13% of the echinocandin use until 2013 and 25% in 2015.

DISCUSSION

The Danish fungaemia incidence rate declined slightly in 2012-15 after an increase in the preceding 8 years. Whether this is just annual variations or an actual declining incidence is not yet known. In the other Nordic countries and Scotland incidence rates have been increasing during the 1990s/the early 2000s, but for most parts appear now to have stabilized around a lower level compared to Denmark (7–11, 13–16, 41) (Supplementary Figure 1). Outliers are Australia with a low incidence but modest increase, and metropolitan Spain with a notable increase to 8.1/100,000 partly driven by a doubling of the incidence among children <1 year old (3, 12). A study of US community hospital discharge records of invasive candidiasis of >1 month olds also demonstrated a minor decrease from 2005-12 for both genders (42). This finding was corroborated by a population based study from two metropolitan areas in the USA where their unprecedented high incidence rates declined from 2008-13 (Atlanta 14.1 to 9.5 and Baltimore 30.9 to 14.4). This change was found in almost all age groups but limited to patients with central venous catheters (85%) and was hypothesized to be related to the introduction of an infection control bundle focusing on i.v. catheter management (43).

A recent study has examined the observed differences in incidence rates from 2010-11 between the Nordic countries. Denmark had a higher prevalence of malignant haematological disease compared to the other Nordic countries, but no demographic differences could justify the higher rate. Despite a similar overall antibacterial consumption (DDDs/1000 inhabitants/day), the use of penicillin, piperacillin-tazobactam, metronidazole, carbapenem, and colistin was significantly higher in Denmark (16). Metronidazole and broad-spectrum beta-lactams are associated with profound impact on the GI flora thereby potentially selecting for yeast (44, 45). Moreover, a

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281 higher use of broad-spectrum antibiotics and the increasing utilisation of BC reported in Denmark
282 (particularly in the ≥ 65 years old population) may be markers of Danish patients being more
283 severely ill or the introduction of sepsis packages (including timely diagnostics) (46).

284 Despite the high overall incidence, a more diverse picture was observed among children and the
285 elderly. The incidence rate has remained constant in the <1-year old (10.8/100.000 population) in
286 the 12-year perspective and was comparable to rates reported from Norway, Finland, and England
287 and Wales (6.6-11/100.000) (8, 15, 47) but low in a global perspective (20 to >90/100,000) (3, 6,
288 14, 48, 49). Such differences suggest that socioeconomic factors and infection control practises
289 may play an important role as also suggested in the study from Atlanta and Baltimore (43). In
290 contrast, the incidence rate in the elderly population was higher than in any population based
291 study in Caucasians ((3, 6, 8, 14, 15, 48, 49) and only exceeded by the rate from the mixed
292 population in Atlanta and Baltimore (43). The high Danish rates in the elderly were mainly driven
293 by a significantly higher and increasing rate in males aged 80-89 years. The reason for this is
294 unclear. Colon cancer and haematological malignancies are recognised risk factor for candidaemia.
295 Both malignancies are more common in males increasing with advancing age and the total
296 prevalence of gastro-intestinal tract (incl. pancreas and liver) cancers as well as large groups of
297 haematological disease (leukaemia and non-Hodgkin lymphoma) have increased more in males
298 during the last decade 2004-14 (http://www.ancr.nu).

299 The previously observed species-shift towards non-albicans species and particularly *C. glabrata*
300 continued 2012-15. This trend has been observed in several population-based candidaemia
301 surveys (3, 12, 13, 20, 50). The increase in the proportion of *C. glabrata* has happened
302 concomitantly with an increase in azole use in Denmark and with the population getting older.

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303 Sweden, Norway, Finland, and Iceland have not witnessed the same increase in the proportion of
304 *C. glabrata* during the last decade of surveillance although sharing demographic characteristics.
305 Despite an increase in systemic azole use in all four countries, the overall use was substantially
306 lower in the other Nordic countries (Figure 4, (14)). In 2015, the overall systemic azole
307 consumption in Norway was comparable to the fluconazole use alone in Denmark in 2004. In
308 contrast, the use of topical azoles for vaginitis was twice as high in Norway compared to Denmark
309 (an average of 375 vs 213 DDD/1000 inhab./year). We speculate whether this increasing selection
310 pressure mediated by systemic azoles over the twelve-year period has facilitated the increasing
311 proportion of *C. glabrata* in the Danish setting.
312 The age-dependent species distribution of *C. parapsilosis* and *C. glabrata* and the influence of
313 blood culture system on the detection of *C. glabrata* were confirmed in a multivariate analysis (15,
314 17, 18, 51). Although the gradual change from BACTEC to BACT/ALERT may have contributed to
315 the increase in *C. glabrata* no centres have changed to BACT/ALERT since January 2011. Less
316 importance has been placed on the impact of gender in relation to *C. glabrata*. *C. glabrata* was
317 correlated to the female sex and was especially common above the age of 40 years, an
318 observation also made in our previous study but to our knowledge not reported elsewhere (18).
319 One reason for this could be the gender-inequality in the antifungal consumption in the primary
320 health care sector. Prior fluconazole use has been shown to be associated with emergence of *C.*
321 *glabrata* (18, 22, 52, 53). Fluconazole is the main azole used in Denmark, and the majority is
322 administered in the primary sector. In this setting (2012-15), 2/3 of the sale of fluconazole was
323 prescribed for the age groups 20-65 years and with a 4.8 female/male ratio of DDDs/1000
324 inhabitants/day. Genotyping studies have confirmed that the infecting organism derives from the
325 colonizing flora (54). We therefore hypothesize that the considerable use of fluconazole in adult

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females in the primary health care sector may play a role in the over-representation of *C. glabrata* in adult females. Consequently, the recommended use of topical azoles rather than systemic treatment whenever possible has been reinforced in the 2012 national guidelines (55). We did not see an increase in *C. krusei.* Although inherently resistant to fluconazole and potentially selected for by azole treatment, this species is also less pathogenic (56). No nationwide study has, to our knowledge, reported an increase in *C. krusei* proportions reflecting that infections occur primarily in a well-defined subset of patients most of whom are already recipients of prophylaxis.

Fluconazole non-susceptibility was detected in more than one third of isolates, mainly driven by an increase in *C. glabrata*. Of note, 9.1% of *C. glabrata* isolates were fluconazole and voriconazole cross-resistant and unlikely to respond to even high dosages of azoles. Non-susceptibility to fluconazole in *C. tropicalis* (8.6%) was primarily caused by heavy trailing growth and affected all azoles equally, impeding precise and reproducible MIC determination. Less than 50% trailing is commonly observed in *C. tropicalis* and was not associated with differential clinical efficacy of fluconazole in 21 *C. tropicalis* cases included in multivariate analysis. The overall mortality for these patients was less than 10% suggesting the majority were not severely ill (57, 58). Therefore, it still remains to be elucidated if isolates displaying heavy trailing are indeed good targets for fluconazole treatment particularly in the setting of severe disease.

Acquired echinocandin resistance remained low but increased. All isolates with an MIC elevated ≥2 dilution step above the breakpoint had an FKS hot spot mutation whereas this was only the case in 4/16 isolates with an MIC one dilution step above the breakpoint. The majority of isolates with hot spot mutations were *C. glabrata* and 31% were fluconazole cross-resistant as reported elsewhere (31, 59, 60). Studies from the USA has demonstrated increasing echinocandin resistance rates in *C. glabrata* (60, 61). In this context, it is worrying that we now see emerging resistance in

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2.7% of our *C. glabrata,* particularly when no isolates were found in 2004-7 and only 1.4% during 2008-12. Furthermore, we are now seeing 2.5% confirmed resistance in *C. tropicalis* and *FKS* mutations in *C. krusei* and *C. kefyr*. Echinocandin resistance has been associated with prior therapy and in *C. glabrata* particularly with the presence of mutations in the DNA mismatch repair gene MSH2 (62). The emergence of echinocandin resistance in Denmark follows a significant increase in echinocandin consumption from 2004-15. These observations suggest that longer term echinocandin should be minimized if possible including when used empirically (63, 64).

In contrast to the increasing resistance observed for azoles and echinocandins, 98% of all isolates were amphotericin B susceptible which is in agreement with previous reports showing broad activity and no indication of acquired resistance (18).

In conclusion, Denmark remains a high incidence country for fungaemia where less than two thirds of isolates are now fully fluconazole susceptible and where acquired echinocandin resistance is on the increase. Continued epidemiological surveillance is important and efforts should be directed towards improved diagnostics and lowering the antifungal selection pressure including regulation of the fluconazole use in the primary health care sector.

Preliminary data has been presented as a short oral poster at the 27th ECCMID in Vienna 2017.

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381 F2G, Gilead, MSD, Novartis, Pfizer and T2Biosystems. She is the current chairman for the EUCAST-
382 AFST has before this served on advisory boards for MSD (until 2014), and Pfizer (until 2012).

383 **REFERENCES**

384 1. Lausch KR, Søgard M, Rosenvinge FS, Johansen HK, Boysen T, Røder BL, Mortensen KL,
385 Nielsen L, Lemming LE, Olesen B, Leitz C, Kristensen L, Dzajic E, Østergård L, Schønheyder
386 HC, Arendrup MC. 2017. Behind candidaemia: risk factors and outcome in a high-incidence
387 nationwide setting.Abstract #b. 27th ECCMID, 22-25 April.

388 2. Lortholary O, Renaudat C, Sitbon K, Madec Y, Denoeud-Ndam L, Wolff M, Fontanet A,
389 Bretagne S, Dromer F. 2014. Worrisome trends in incidence and mortality of candidemia in
390 intensive care units (Paris area, 2002–2010). Intensive Care Med 40:1303–1312.

391 3. Puig-Asensio M, Padilla B, Garnacho-Montero J, Zaragoza O, Aguado JM, Zaragoza R,
392 Montejo M, Muñoz P, Ruiz-Camps I, Cuenca-Estrella M, Almirante B. 2014. Epidemiology
393 and predictive factors for early and late mortality in Candida bloodstream infections: a
394 population-based surveillance in Spain. Clin Microbiol Infect 20:O245–O254.

395 4. Morrell M, Fraser VJ, Kollef MH. 2005. Delaying the Empiric Treatment of Candida
396 Bloodstream Infection until Positive Blood Culture Results Are Obtained: a Potential Risk
397 Factor for Hospital Mortality. Antimicrob Agents Chemother 49:3640–3645.

398 5. Liu CY, Huang LJ, Wang WS, Chen TL, Yen CC, Yang MH, Hsiao LT, Liu CY, Chen PM, Chiou TJ.
399 2009. Candidemia in cancer patients: Impact of early removal of non-tunneled central
400 venous catheters on outcome. J Infect 58:154–160.

401 6. Chen S, Slavin M, Nguyen Q, Marriott D, Playford EG, Ellis D, Sorrell T. 2006. Active
402 Surveillance of Candidemia, Australia. Emerg Infect Dis 12:1508–1516.

403 7. Odds FC, Hanson MF, Davidson AD, Jacobsen MD, Wright P, Whyte JA, Gow NAR, Jones BL.

Journal of Clinical Microbiology

2007. One year prospective survey of Candida bloodstream infections in Scotland. J Med Microbiol 56:1066–1075.

8. Poikonen E, Lyytikäinen O, Anttila V-J, Koivula I, Lumio J, Kotilainen P, Syrjälä H, Ruutu P. 2010. Secular trend in candidemia and the use of fluconazole in Finland, 2004-2007. BMC Infect Dis 10:312.

9. Asmundsdottir LR, Erlendsdottir H, Gottfredsson M. 2002. Increasing Incidence of Candidemia: Results from a 20-Year Nationwide Study in Iceland. J Clin Microbiol 40:3489–3492.

10. Sandven P, Bevanger L, Digranes A, Haukland HH, Mannsaker T, Gaustad P. 2006. Candidemia in Norway (1991 to 2003): Results from a Nationwide Study. J Clin Microbiol 44:1977–1981.

11. Ericsson J, Chryssanthou E, Klingspor L, Johansson AG, Ljungman P, Svensson E, Sjölin J. 2013. Candidaemia in Sweden: a nationwide prospective observational survey. Clin Microbiol Infect 19:E218–E221.

12. Chapman B, Slavin M, Marriott D, Halliday C, Kidd S, Arthur I, Bak N, Heath CH, Kennedy K, Morrissey CO, Sorrell TC, van Hal S, Keighley C, Goeman E, Underwood N, Hajkowicz K, Hofmeyr A, Leung M, Macesic N, Botes J, Blyth C, Cooley L, George CR, Kalukottege P, Kesson A, McMullan B, Baird R, Robson J, Korman TM, Pendle S, Weeks K, Liu E, Cheong E, Chen S. 2017. Changing epidemiology of candidaemia in Australia. J Antimicrob Chemother 72:1270–1270.

13. Rajendran R, Sherry L, Nile CJ, Sherriff A, Johnson EM, Hanson MF, Williams C, Munro CA,

425 Jones BJ, Ramage G. 2016. Biofilm formation is a risk factor for mortality in patients with
426 Candida albicans bloodstream infection—Scotland, 2012–2013. Clin Microbiol Infect 22:87–
427 93.

428 14. Asmundsdottir LR, Erlendsdottir H, Gottfredsson M. 2013. Nationwide Study of Candidemia,
429 Antifungal Use, and Antifungal Drug Resistance in Iceland, 2000 to 2011. J Clin Microbiol
430 51:841–848.

431 15. Hesstvedt L, Gaustad P, Andersen CT, Haarr E, Hannula R, Haukland HH, Hermansen N-O,
432 Larssen KW, Mylvaganam H, Ranheim TE, Sandven P, Nordøy I, Kanestrøm A, Grub C, Onken
433 A, Thielsen C, Skaare D, Tofteland S, Sønsteby L-J, Hjetland R, Hide R, Vik E, Kümmel A,
434 Åsheim S. 2015. Twenty-two years of candidaemia surveillance: results from a Norwegian
435 national study. Clin Microbiol Infect 21:938–945.

436 16. Hesstvedt L, Arendrup MC, Poikonen E, Klingpor L, Friman V, Nordøy I. 2017. Differences in
437 epidemiology of candidaemia in the Nordic countries - what is to blame? Mycoses 60:11–
438 19.

439 17. Arendrup MC, Bruun B, Christensen JJ, Fuursted K, Johansen HK, Kjaeldgaard P, Knudsen JD,
440 Kristensen L, Moller J, Nielsen L, Rosenvinge FS, Roder B, Schonheyder HC, Thomsen MK,
441 Truberg K. 2011. National Surveillance of Fungemia in Denmark (2004 to 2009). J Clin
442 Microbiol 49:325–334.

443 18. Arendrup MC, Dzajic E, Jensen RH, Johansen HK, Kjaldgaard P, Knudsen JD, Kristensen L,
444 Leitz C, Lemming LE, Nielsen L, Olesen B, Rosenvinge FS, Røder BL, Schønheyder HC. 2013.
445 Epidemiological changes with potential implication for antifungal prescription
446 recommendations for fungaemia: data from a nationwide fungaemia surveillance

447 programme. Clin Microbiol Infect 19:e343–e353.

448 19. Guinea J. 2014. Global trends in the distribution of Candida species causing candidemia. Clin
449 Microbiol Infect 20:5–10.

450 20. Zhou Z-L, Lin C-C, Chu W-L, Yang Y-L, Lo H-J. 2016. The distribution and drug susceptibilities
451 of clinical Candida species in TSARY 2014. Diagn Microbiol Infect Dis 86:399–404.

452 21. Diekema D, Arbefeville S, Boyken L, Kroeger J, Pfaller M. 2012. The changing epidemiology
453 of healthcare-associated candidemia over three decades. Diagn Microbiol Infect Dis 73:45–
454 48.

455 22. Lortholary O, Desnos-Ollivier M, Sitbon K, Fontanet A, Bretagne S, Dromer F. 2011. Recent
456 Exposure to Caspofungin or Fluconazole Influences the Epidemiology of Candidemia: a
457 Prospective Multicenter Study Involving 2,441 Patients. Antimicrob Agents Chemother
458 55:532–538.

459 23. Hope WW, Castagnola E, Groll AH, Roilides E, Akova M, Arendrup MC, Arikan-Akdagli S,
460 Bassetti M, Bille J, Cornely OA, Cuenca-Estrella M, Donnelly JP, Garbino J, Herbrecht R,
461 Jensen HE, Kullberg BJ, Lass-Flörl C, Lortholary O, Meersseman W, Petrikkos G, Richardson
462 MD, Verweij PE, Viscoli C, Ullmann AJ. 2012. ESCMID* guideline for the diagnosis and
463 management of Candida diseases 2012: prevention and management of invasive infections
464 in neonates and children caused by Candida spp. Clin Microbiol Infect 18:38–52.

465 24. Cornely OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortholary O, Meersseman W,
466 Akova M, Arendrup MC, Arikan-Akdagli S, Bille J, Castagnola E, Cuenca-Estrella M, Donnelly
467 JP, Groll AH, Herbrecht R, Hope WW, Jensen HE, Lass-Florl C, Petrikkos G, Richardson MD,

468 Roilides E, Verweij PE, Viscoli C, Ullmann AJ. 2012. ESCMID* guideline for the diagnosis and
469 management of Candida diseases 2012: non-neutropenic adult patients. Clin Microbiol
470 Infect 18:19–37.

471 25. Ullmann AJ, Akova M, Herbrecht R, Viscoli C, Arendrup MC, Arikan-Akdagli S, Bassetti M,
472 Bille J, Calandra T, Castagnola E, Cornely OA, Donnelly JP, Garbino J, Groll AH, Hope WW,
473 Jensen HE, Kullberg BJ, Lass-Flörl C, Lortholary O, Meersseman W, Petrikkos G, Richardson
474 MD, Roilides E, Verweij PE, Cuenca-Estrella M. 2012. ESCMID* guideline for the diagnosis
475 and management of Candida diseases 2012: adults with haematological malignancies and
476 after haematopoietic stem cell transplantation (HCT). Clin Microbiol Infect 18:53–67.

477 26. Lortholary O, Petrikkos G, Akova M, Arendrup MC, Arikan-Akdagli S, Bassetti M, Bille J,
478 Calandra T, Castagnola E, Cornely OA, Cuenca-Estrella M, Donnelly JP, Garbino J, Groll AH,
479 Herbrecht R, Hope WW, Jensen HE, Kullberg BJ, Lass-Flörl C, Meersseman W, Richardson
480 MD, Roilides E, Verweij PE, Viscoli C, Ullmann AJ. 2012. ESCMID* guideline for the diagnosis
481 and management of Candida diseases 2012: patients with HIV infection or AIDS. Clin
482 Microbiol Infect 18:68–77.

483 27. Pappas PG, Kauffman CA, Andes D, Benjamin DK, Calandra TF, Edwards JE, Filler SG, Fisher
484 JF, Kullberg B-J, Ostrosky-Zeichner L, Reboli AC, Rex JH, Walsh TJ, Sobel JD. 2009. Clinical
485 practice guidelines for the management of candidiasis: 2009 update by the Infectious
486 Diseases Society of America. Clin Infect Dis 48:503–35.

487 28. Dannaoui E, Desnos-Ollivier M, Garcia-Hermoso D, Grenouillet F, Cassaing S, Baixench M-T,
488 Bretagne S, Dromer F, Lortholary O. 2012. Candida spp. with Acquired Echinocandin
489 Resistance, France, 2004–2010. Emerg Infect Dis 18:86–90.

29. Staab JF, Neofytos D, Rhee P, Jimenez-Ortigosa C, Zhang SX, Perlin DS, Marr K a. 2014. Target Enzyme Mutations Confer Differential Echinocandin Susceptibilities in Candida kefyr. Antimicrob Agents Chemother 58:5421–5427.

30. Jensen RH, Johansen HK, Arendrup MC. 2013. Stepwise Development of a Homozygous S80P Substitution in Fks1p, Conferring Echinocandin Resistance in Candida tropicalis. Antimicrob Agents Chemother 57:614–617.

31. Pfaller MA, Castanheira M, Lockhart SR, Ahlquist AM, Messer SA, Jones RN. 2012. Frequency of decreased susceptibility and resistance to echinocandins among fluconazole-resistant bloodstream isolates of Candida glabrata. J Clin Microbiol 50:1199–1203.

32. Arendrup MC, Fuursted K, Gahrn-hansen B, Jensen IM, Knudsen JD, Lundgren B, Schønheyder HC, Tvede M. 2005. Seminational Surveillance of Fungemia in Denmark : Notably High Rates of Fungemia and Numbers of Isolates with Reduced Azole Susceptibility 43:4434–4440.

33. Arendrup MC, Guinea J, Cuenca-EstrellaM, Meletiadis J, Mouton JW, Lagrou K, Howard SJ, and the Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST)*. 2015. EUCAST DEFINITIVE DOCUMENT E.DEF 7.3 Method for the determination of broth dilution minimum Inhibitory concentrations of antifungal agents for yeasts. www.eucast.org.

34. Arendrup MC, Meletiadis J, Mouton JW, Guinea J, Cuenca-Estrella M, Lagrou K, Howard SJ, Arendrup MC, Meletiadis J, Howard SJ, Mouton J, Guinea J, Lagrou K, Arikan-Akdagli S, Barchiesi F, Hamal P, Järv H, Lass-Flörl C, Mares M, Matos T, Muehlethaler K, Rogers TR, Torp Andersen C, Verweij P. 2016. EUCAST technical note on isavuconazole breakpoints for

512 Aspergillus, itraconazole breakpoints for Candida and updates for the antifungal
513 susceptibility testing method documents. Clin Microbiol Infect 22:571.e1-571.e4.

514 35. Lass-Flörl C, Arendrup MC, Rodriguez-Tudela J-L, Cuenca-Estrella M, Donnelly P, Hope W.
515 2011. EUCAST Technical note on Amphotericin B. Clin Microbiol Infect 17:E27–E29.

516 36. Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European
517 Committee for Antimicrobial Susceptibility Testing (EUCAST)*. 2008. EUCAST Technical Note
518 on voriconazole. Clin Microbiol Infect 14:985–987.

519 37. Cavling Arendrup M, Cuenca-Estrella M, Lass-Flörl C, Hope WW. 2014. EUCAST Technical
520 Note on Candida and micafungin, anidulafungin and fluconazole. Mycoses 57:377–379.

521 38. Howard SJ, Lass-Florl C, Cuenca-Estrella M, Gomez-Lopez A, Arendrup MC. 2013.
522 Determination of Isavuconazole Susceptibility of Aspergillus and Candida Species by the
523 EUCAST Method. Antimicrob Agents Chemother 57:5426–5431.

524 39. O'Donnell K, Sutton DA, Rinaldi MG, Sarver BAJ, Balajee SA, Schroers H-J, Summerbell RC,
525 Robert VARG, Crous PW, Zhang N, Aoki T, Jung K, Park J, Lee Y-H, Kang S, Park B, Geiser DM.
526 2010. Internet-Accessible DNA Sequence Database for Identifying Fusaria from Human and
527 Animal Infections. J Clin Microbiol 48:3708–3718.

528 40. Arendrup MC, Garcia-Effron G, Lass-Florl C, Lopez AG, Rodriguez-Tudela J-L, Cuenca-Estrella
529 M, Perlin DS. 2010. Echinocandin Susceptibility Testing of Candida Species: Comparison of
530 EUCAST EDef 7.1, CLSI M27-A3, Etest, Disk Diffusion, and Agar Dilution Methods with RPMI
531 and IsoSensitest Media. Antimicrob Agents Chemother 54:426–439.

532 41. Poikonen E, Lyytikäinen O, Anttila V-J, Ruutu P. 2003. Candidemia in Finland, 1995–1999.

533 Emerg Infect Dis 9:985–990.

534 42. Strollo S, Lionakis MS, Adjemian J, Steiner CA, Prevots DR. 2016. Epidemiology of
535 Hospitalizations Associated with Invasive Candidiasis, United States, 2002–20121. Emerg
536 Infect Dis 23:7–13.

537 43. Cleveland AA, Harrison LH, Farley MM, Hollick R, Stein B, Chiller TM, Lockhart SR, Park BJ.
538 2015. Declining Incidence of Candidemia and the Shifting Epidemiology of Candida
539 Resistance in Two US Metropolitan Areas, 2008–2013: Results from Population-Based
540 Surveillance. PLoS One 10:e0120452.

541 44. Ben-Ami R, Olshtain-Pops K, Krieger M, Oren I, Bishara J, Dan M, Wiener-Well Y, Weinberger
542 M, Zimhony O, Chowers M, Weber G, Potasman I, Chazan B, Kassis I, Shalit I, Block C, Keller
543 N, Kontoyiannis DP, Giladi M. 2012. Antibiotic Exposure as a Risk Factor for Fluconazole-
544 Resistant Candida Bloodstream Infection. Antimicrob Agents Chemother 56:2518–2523.

545 45. Rafii F, Sutherland JB, Cerniglia CE. 2008. Effects of treatment with antimicrobial agents on
546 the human colonic microflora. Ther Clin Risk Manag 4:1343–58.

547 46. Gubbels S, Nielsen J, Voldstedlund M, Kristensen B, Schønheyder HC, Vandenbroucke-Grauls
548 CMJE, Arpi M, Björnsdóttir MK, Knudsen JD, Dessau RB, Jensen TG, Kjældgaard P, Lemming
549 L, Møller JK, Hansen DS, Mølbak K. 2015. Utilization of blood cultures in Danish hospitals: a
550 population-based descriptive analysis. Clin Microbiol Infect 21:344.e13-344.e21.

551 47. Oeser C, Lamagni T, Heath PT, Sharland M, Ladhani S. 2013. The Epidemiology of Neonatal
552 and Pediatric Candidemia in England and Wales, 2000–2009. Pediatr Infect Dis J 32:23–26.

553 48. Laupland KB. 2005. Invasive Candida species infections: a 5 year population-based

554 assessment. J Antimicrob Chemother 56:532–537.

555 49. Hajjeh RA, Sofair AN, Harrison LH, Lyon GM, Arthington-Skaggs BA, Mirza SA, Phelan M,
556 Morgan J, Lee-Yang W, Ciblak MA, Benjamin LE, Thomson Sanza L, Huie S, Yeo SF, Brandt
557 ME, Warnock DW. 2004. Incidence of Bloodstream Infections Due to Candida Species and In
558 Vitro Susceptibilities of Isolates Collected from 1998 to 2000 in a Population-Based Active
559 Surveillance Program. J Clin Microbiol 42:1519–1527.

560 50. Lockhart SR, Iqbal N, Cleveland AA, Farley MM, Harrison LH, Bolden CB, Baughman W, Stein
561 B, Hollick R, Park BJ, Chiller T. 2012. Species Identification and Antifungal Susceptibility
562 Testing of Candida Bloodstream Isolates from Population-Based Surveillance Studies in Two
563 U.S. Cities from 2008 to 2011. J Clin Microbiol 50:3435–3442.

564 51. Horvath LL, George BJ, Murray CK, Harrison LS, Hospenthal DR. 2004. Direct Comparison of
565 the BACTEC 9240 and BacT/ALERT 3D Automated Blood Culture Systems for Candida
566 Growth Detection. J Clin Microbiol 42:115–118.

567 52. Bassetti M, Ansaldi F, Nicolini L, Malfatto E, Molinari MP, Mussap M, Rebesco B, Bobbio
568 Pallavicini F, Icardi G, Viscoli C. 2009. Incidence of candidaemia and relationship with
569 fluconazole use in an intensive care unit. J Antimicrob Chemother 64:625–629.

570 53. Jensen RH, Johansen HK, Søes LM, Lemming LE, Rosenvinge FS, Nielsen L, Olesen B,
571 Kristensen L, Dzajic E, Astvad KMT, Arendrup MC. 2016. Posttreatment Antifungal
572 Resistance among Colonizing Candida Isolates in Candidemia Patients: Results from a
573 Systematic Multicenter Study. Antimicrob Agents Chemother 60:1500–1508.

574 54. Brillowska-Dabrowska A, Bergmann O, Jensen IM, Jarløv JO, Arendrup MC. 2010. Typing of

575 Candida isolates from patients with invasive infection and concomitant colonization. Scand J
576 Infect Dis 42:109–113.

577 55. Saunte DML, Hald M, Lindskov R, Foged EK, Svejgaard EL, Arendrup MC. 2012. Guidelines
578 for superficielle svampeinfektioner.Danish Society of Dermatology.

579 56. Arendrup M, Horn T, Frimodt-Møller N. 2002. In vivo pathogenicity of eight medically
580 relevant Candida species in an animal model. Infection 30:286–91.

581 57. Marcos-Zambrano LJ, Escribano P, Sánchez-Carrillo C, Bouza E, Guinea J. 2016. Scope and
582 frequency of fluconazole trailing assessed using EUCAST in invasive Candida spp. isolates.
583 Med Mycol 54:733–739.

584 58. Rueda C, Puig-Asensio M, Guinea J, Almirante B, Cuenca-Estrella M, Zaragoza O, Padilla B,
585 Muñoz P, Guinea J, Paño Pardo JR, García-Rodríguez J, García Cerrada C, Fortún J, Martín P,
586 Gómez E, Ryan P, Campelo C, de los Santos Gil I, Buendía V, Gorricho BP, Alonso M, Sanz FS,
587 Aguado JM, Merino P, González Romo F, Gorgolas M, Gadea I, Losa JE, Delgado-Iribarren A,
588 Ramos A, Romero Y, Sánchez Romero I, Zaragoza O, Cuenca-Estrella M, Rodriguez-Baño J,
589 Isabel Suarez A, Loza A, Aller García AI, Martín-Mazuelos E, Pérez de Pipaón MR, Garnacho J,
590 Ortiz C, Chávez M, Maroto FL, Salavert M, Pemán J, Blanquer J, Navarro D, Camarena JJ,
591 Zaragoza R, Abril V, Gimeno C, Hernáez S, Ezpeleta G, Bereciartua E, Hernández Almaraz JL,
592 Montejo M, Rivas RA, Ayarza R, Planes AM, Camps IR, Almirante B, Mensa J, Almela M,
593 Gurgui M, Sánchez-Reus F, Martinez-Montauti J, Sierra M, Horcajada JP, Sorli L, Gómez J,
594 Gené A, Urrea M, Valerio M, Díaz-Martín A, Puchades F, Mularoni A. 2017. Evaluation of the
595 possible influence of trailing and paradoxical effects on the clinical outcome of patients with
596 candidemia. Clin Microbiol Infect 23:49.e1-49.e8.

59. Alexander BD, Johnson MD, Pfeiffer CD, Jiménez-Ortigosa C, Catania J, Booker R, Castanheira M, Messer SA, Perlin DS, Pfaller MA. 2013. Increasing Echinocandin Resistance in Candida glabrata: Clinical Failure Correlates With Presence of FKS Mutations and Elevated Minimum Inhibitory Concentrations. Clin Infect Dis 56:1724–1732.

60. Vallabhaneni S, Cleveland AA, Farley MM, Harrison LH, Schaffner W, Beldavs ZG, Derado G, Pham CD, Lockhart SR, Smith RM. 2015. Epidemiology and Risk Factors for Echinocandin Nonsusceptible Candida glabrata Bloodstream Infections: Data From a Large Multisite Population-Based Candidemia Surveillance Program, 2008-2014. Open forum Infect Dis 2:ofv163.

61. Zimbeck AJ, Iqbal N, Ahlquist AM, Farley MM, Harrison LH, Chiller T, Lockhart SR. 2010. FKS Mutations and Elevated Echinocandin MIC Values among Candida glabrata Isolates from U.S. Population-Based Surveillance. Antimicrob Agents Chemother 54:5042–5047.

62. Healey KR, Zhao Y, Perez WB, Lockhart SR, Sobel JD, Farmakiotis D, Kontoyiannis DP, Sanglard D, Taj-Aldeen SJ, Alexander BD, Jimenez-Ortigosa C, Shor E, Perlin DS. 2016. Prevalent mutator genotype identified in fungal pathogen Candida glabrata promotes multi-drug resistance. Nat Commun 7:11128.

63. Pfeiffer CD, Garcia-Effron G, Zaas AK, Perfect JR, Perlin DS, Alexander BD. 2010. Breakthrough Invasive Candidiasis in Patients on Micafungin. J Clin Microbiol 48:2373–2380.

64. Bordallo-Cardona MÁ, Escribano P, Gómez G. de la Pedrosa E, Marcos-Zambrano LJ, Cantón R, Bouza E, Guinea J. 2016. In vitro exposure to increasing micafungin concentrations easily promotes echinocandin resistance in Candida glabrata isolates. Antimicrob Agents Chemother 61:AAC.01542-16.

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621 **FIGURE AND TABLE LEGENDS**

622 **Figure 1:** Age- and gender-specific incidence rates per 100,000 population by three 4-year intervals
623 (2004-2015).

624

625 The overall male incidence rate was stable whereas the female declined numerically. Males had a
626 slight decrease for the age 50-59 (p 0.04) and a significant increase for the age group 80-89 (p
627 <0.0001). Females had significant decreases in age groups 40-49 and 60-69 (and only an increase
628 in the low incidence age group 20-29 years).

629

630 **Figure 2:** Species distribution of blood stream infections on age groups 2012-2015.

631

632 **Figure 3:** Species distribution (isolates in %) over three 4-year periods during 2004-2015.

633

634 The presented percentages are based on isolate numbers during the 4-year periods. Significant p
635 values from a Chi-square test for trend is presented. (for *C. dublinensis* a p value of 0.01 was found
636 due to an increase in isolates 2014-2015).

637

638 **Figure 4:** Annual consumption of systemic antifungal compounds in DDDs/1000 inhabitants in
639 2004-2015. Hospital use is shown in orange and use in the primary healthcare sector in red. The

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640 total annual consumption in Norway, Sweden and Finland is inserted for comparison (dark grey,
641 light grey and white bars, respectively)

642

643 [1]The DDD for caspofungin is 50 mg whereas the DDDs for micafungin and anidulafungin are 0.1g.

644 [2 2] For posaconazole, an enterotablet and an iv formulation was introduced on the Danish market
645 in 2014. The licensed daily dose of these new formulations is 300 mg/day (after a loading dose)
646 whereas the treatment dose of the suspension is 800 mg/day. The official defined DDDs was for all
647 three formulations 0.8 g (recently in 2017 changed to 0.3 g). We have translated DDDs to reflect
648 the actual use (enterotablets and iv formulation: 1 DDD of 0.3 g (red/white stripes) and oral
649 suspension: 1 DDD 0.8 g (solid red)). Total use in the Nordic countries and DK (uncorrected; grey
650 line) is for a DDD of 0.8 g.

651

652 **Table 1:** Characteristics of the national fungaemia surveillance scheme on incidence rates, age,
653 gender, and species distribution 2012-2015

654

655 a) *Candida* spp. includes: *C. lusitaniae* (19*); C. kefyr* (13); *C. fermentati* (8); *C. pelliculosa* (6); *C.*
656 *guilliiermondii* (5); *C. inconspicua* (4); *C. orthopsilosis* (3); *C. magnoliae* and *C. nivariensis* (2 each);
657 *C. fabianii, C. metapsilosis, C. norvegensis, C. palmioleophila* and *C.utilis* (1 each) and finally two
658 *Candida* isolates not referred for species identification.
659 b) Other fungi include: *S. cerevisiae* (11); *Fusarium dimerum* and *Cryptococcus neoformans* (4
660 each); *Fusarium solani* (3); *Fusarium oxysporum, Rhodutorula mucilaginosa* and *Saprochaeta*

661 *clavata* (2 each); *A. fumigatus, Penicillium marneffei, Trichosporon asahii* and *Williopsis saturnus* (1
662 each).
663
664 **Table 2:** Number of isolates, demographics of patients, and blood culture system use during three
665 4-year periods 2012-2015
666
667 For information of isolates included please see table 1 (2012-2015) and refer to references (17, 18)
668 ND: not determined. For the statistical analyses Chi-square trend test was used apart from age and
669 episodes rates where a linear logistic regression analysis was employed.
670 a) Number of bed days 2004 were not available and the figure from 2005 was used for 2004.
671
672 **Table 3:** Binomial logistic regression analysis of variables associated with changing species
673 distribution in Denmark 2004-15. Only incident cases were included, and only significant findings
674 displayed. For the multivariate analysis year, age, gender, and BC system were all kept in the
675 model.
676
677 [a] 95% confidence intervals. Due to an interaction between calendar year and BC system, the year
678 factor is split on [1]BACT/ALERT (top) and [2]BACTEC (bottom).[b] p value when excluding the main
679 tertiary hospital Rigshospitalet (Centre 1-RH).
680

681 **Table 4:** Susceptibility pattern of Danish fungaemia isolates collected in 2012-15 to six antifungal
682 compounds. The EUCAST breakpoints and ECOFFs (Supplementary table 1) are used for the
683 classification as susceptible (S), resistant (R), and non-wildtype (non-wt).

684

685 S: susceptible; R: resistant; non-wt: non-wildtype (as the MIC was above the ECOFF).
686 Empty cells indicate that there were no isolates for which the MIC had the indicated value.
687 - The indicated concentration of that particular drug was not tested
688 Bold numbers indicate isolates for which the MIC were above the ECOFF and with underlined
689 numbers indicating isolates that were resistant.
690 EUCAST breakpoints and ECOFFS were used. For *Candida* species and "other fungi" an
691 amphotericin B breakpoint of 1 mg/L was used. For "other fungi" a fluconazole breakpoint of 2
692 mg/L was used. These last cut-offs are only an indication of the susceptibility profile for the given
693 species/group of isolates (and a conservative estimate of the proportion of cases that are likely
694 good targets for the compound in question).
695 [1] For the echinocandins and fluconazole 29 and 23 isolates were tested, respectively. [2] For the
696 echinocandins and fluconazole, 1898 and 1892 isolates were tested, respectively.

Table 1: Characteristics of the national fungaemia surveillance scheme on incidence rates, age, gender, and species distribution 2012-2015.

	2012	**2013**	**2014**	**2015**	**2012-2015**
Number of Isolates	492	523	441	483	1939
Number of Episodes	479	508	429	467	1883
Number of Patients	461	490	419	443	1813
Mean patient age (95% CI)	66.1 (64.5;67.7)	64.7 (63.2;66.3)	64.8 (63.1; 66.5)	65.7 (63.1; 66.5)	65.3 (64.5; 66.2)
Median patient age (interquartile range)	69 (58; 78)	68 (59; 77)	69 (58; 77)	69 (59; 77)	69 (58; 77)
Gender % male	55.1%	56.8%	63.5%	64.0%	59.7%
Episode rate					
/100,000 inhabitants	8.58	9.07	7.62	8.25	8.38
/1000 discharges	0.35	0.37	0.31	0.34	0.34
/10,000 bed days	1.05	1.14	0.97	1.008	1.06
Isolates on species (%)					
C. albicans	230 (47%)	268 (51%)	225 (51%)	205 (42%)	928 (47.9%)
C. dubliniensis	13 (3%)	12 (2%)	20 (5%)	26 (5%)	71 (3.7%)
C. glabrata	170 (35%)	155 (30%)	125 (28%)	167 (35%)	617 (31.8%)
C. krusei	19 (4%)	19 (4%)	20 (5%)	17 (4%)	75 (3.9%)
C. parapsilosis	16 (3%)	18 (3%)	10 (2%)	20 (4%)	64 (3.3%)
C. tropicalis	18 (4%)	23 (4%)	15 (3%)	27 (6%)	83 (4.3%)
Candida species[a]	18 (4%)	19 (4%)	17 (4%)	15 (3%)	69 (3.6%)
Other fungi[b]	8 (2%)	9 (2%)	9 (2%)	6 (1%)	32 (1.7%)

a) *Candida* spp. includes: *C. lusitaniae* (19*); C. kefyr* (13); *C. fermentati* (8); *C. pelliculosa* (6); *C. guillliermondii* (5); *C. inconspicua* (4); *C. orthopsilosis* (3); *C. magnoliae* and *C. nivariensis* (2 each); *C. fabianii, C. metapsilosis, C. norvegensis, C. palmioleophila* and *C.utilis* (1 each) and finally two *Candida* isolates not referred for species identification.

b) Other fungi include: *S. cerevisiae* (11); *Fusarium dimerum* and *Cryptococcus neoformans* (4 each); *Fusarium solani* (3); *Fusarium oxysporum, Rhodutorula mucilaginosa* and *Saprochaeta clavata* (2 each); *A. fumigatus, Penicillium marneffei, Trichosporon asahii* and *Williopsis saturnus* (1 each).

Table 2: Number of isolates, demographics of patients, and blood culture system use during three 4-year periods 2012-2015.

	2004-7	2008-11	2012-15	Time trend (p value)
Isolates	1932	2049	1939	
Episodes	1874	1994	1883	
Patients	1795	1895	1813	
Mean age (95% CI)	61.9 (60.2; 63.6)	63.0 (61.3; 64.7)	65.3 (64.5; 66.2)	0.0002
Median age (25% quartiles)	65 (54; 75)	66 (56; 74)	69 (58; 77)	ND
Male gender (%)	56.4	59.2	59.7	0.01
Episode rate				
/100,000 inhabitants	8.64	9.03	8.38	0.34
/1000 discharges	0.39	0.38	0.34	<0.0001
/10,000 bed days	0.90[a]	1.03	1.06	<0.0001
Proportion of isolates from BACTEC (%)	45.0	36.2	22.0	<0.0001

For information of isolates included please see table 1 (2012-2015) and refer to references (17, 18).
ND: not determined. For the statistical analyses Chi-square trend test was used apart from age and episodes rates where a linear logistic regression analysis was employed.
a) Number of bed days 2004 were not available and the figure from 2005 was used for 2004.

Table 3: Binomial logistic regression analysis of variables associated with changing species distribution in Denmark 2004-15. Only incident cases were included, and only significant findings displayed. For the multivariate analysis year, age, gender, and BC system were all kept in the model.

Species (no.)	Univariate				Multivariate			
	Odds ratio	95% CI[a]	p	p[b]	Odds ratio	95% CI[a]	p	p[b]
C. albicans (2967)								
Calendar year (Year)	0.94	0.93-0.96	<0.001	<0.001	0.95[1]	0.94-0.97	<0.001	<0.001
					0.92[2]	0.89-0.94	<0.001	<0.001
Age (Year)	0.995	0.992-0.998	<0.001	0.001	0.995	0.992-0.998	0.002	0.002
C. glabrata (1369).								
Calendar year (Year)	1.08	1.06-1.10	<0.001	<0.001	1.06	1.04-1.08	<0.001	<0.001
					1.11	1.07-1.15	<0.001	<0.001
Blood culture system (BACTEC)	0.65	0.57-0.75	<0.001	<0.001	0.78	0.68-0.90	<0.001	0.004
Gender (Female)	1.15	1.01-1.30	0.032	0.023	1.17	1.03-1.33	0.014	0.009
Age (Year)	1.02	1.02-1.03	<0.001	<0.001	1.02	1.02-1.02	<0.001	<0.001
C. tropicalis (237)								
Gender (Female)	0.74	0.56-0.97	0.034	0.018	0.74	0.56-0.97	0.033	0.018
C. parapsilosis (171)								
BC system (BACTEC)	1.87	1.38-2.54	<0.001	0.005	1.70	1.24-2.34	0.001	0.045
Age (Year)	0.98	0.98-0.99	<0.001	<0.001	0.98	0.98-0.99	<0.001	<0.001
C. krusei (219)								
BC system (BACTEC)	2.00	1.52-2.62	<0.001	<0.001	2.07	1.56-2.74	<0.001	<0.001
C. dubliniensis (149)								
Calendar year (Year)	1.06	1.01-1.12	0.014	0.010	1.07	1.02-1.13	0.007	0.001
Age (Year)	0.99	0.982-0.997	0.007	0.009	0.989	0.981-0.997	0.008	0.005
Other fungi (83)								
BC system (BACTEC)	1.87	1.21-2.89	0.005	0.046	1.71	1.09-2.69	0.019	0.10
Age (Year)	0.98	0.97-0.99	<0.001	<0.001	0.98	0.97-0.99	<0.001	0.001

[a] 95% confidence intervals. Due to an interaction between calendar year and BC system, the year factor is split on [1]BACT/ALERT (top) and [2]BACTEC (bottom).[b] p value when excluding the main tertiary hospital Rigshospitalet (Centre 1-RH).

Table 4: Susceptibility pattern of Danish fungaemia isolates collected in 2012-15 to six antifungal compounds. The EUCAST breakpoints and ECOFFs (Supplementary table 1) are used for the classification as susceptible (S), resistant (R), and non-wildtype (non-wt).

	No. of isolates with the given MIC (mg/L)															S		R		non-wt	
	≤0.008	0.015	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	No	%	No	%	No	%
***C. albicans* (914)**																					
Amphotericin B	-		4	67	155	356	331	1		-	-	-	-	-	-	914	100	0	0.0	0	0.0
Anidulafungin	-	874	40							-	-	-	-	-	-	914	100	0	0.0	0	0.0
Micafungin	880	29	**5**							-	-	-	-	-	-	909	99.5	5	0.5	5	0.5
Fluconazole	-	-	-	-	520	349	34	5	**2**		**1**		**3**	-	-	910	99.6	4	0.4	6	0.7
Voriconazole	-	-	909	1	1	**1**					**2**	-	-	-	-	911	99.7	3	0.3	3	0.3
Isavuconazole	-	-	911		1						2	-	-	-	-	ND	ND	ND	ND	3	0.3
***C. dubliniensis* (71)**																					
Amphotericin B	-	16	18	15	20	2				-	-	-	-	-	-	71	100	0	0.0	0	0.0
Anidulafungin	-	43	25	**3**						-	-	-	-	-	-	68	95.8	3	4.2	3	4.2
Micafungin	43	24	**4**							-	-	-	-	-	-	67	94.4	4	5.6	4	5.6
Fluconazole	-	-	-	-	28	19	13	7	**1**			**2**	**1**	-	-	68	95.8	3	4.2	4	5.6
Voriconazole	-	-	68	1		**1**	**1**					-	-	-	-	69	97.2	2	2.8	2	2.8
Isavuconazole	-	-	70		**1**							-	-	-	-	ND	ND	ND	ND	1	1.4
***C. glabrata* (603)**																					
Amphotericin B	-	1	5	13	86	128	218	143	**9**	-	-	-	-	-	-	594	98.5	9	1.5	9	1.5
Anidulafungin	-	129	200	258	**6**	**5**	**1**	**2**	**2**	-	-	-	-	-	-	587	97.3	16	2.7	16	2.7
Micafungin	434	132	25	**3**	**2**	**1**	**3**	**1**	**2**	-	-	-	-	-	-	591	98.0	12	2.0	12	2.0
Fluconazole	-	-	-	-		1	1	22	164	278	53	15	14	**38**	**17**	0	0.0	55	9.1	55	9.1
Voriconazole	-	-	108	248	157	13	10	18	**31**	**17**	**1**	-	-	-	-	IE	IE	IE	IE	49	8.1
Isavuconazole	-	-	426	78	20	6	17	24	17	15	0	-	-	-	-	ND	ND	ND	ND	ND	ND
***C. krusei* (73)**																					
Amphotericin B	-				1	4	30	32	**6**	-	-	-	-	-	-	67	91.8	6	8.2	6	8.2
Anidulafungin	-	11	16	41	**4**				**1**	-	-	-	-	-	-	68	93.2	5	6.8	5	6.8
Micafungin				15	46	11			**1**	-	-	-	-	-	-	IE	IE	IE	E	1	1.4
Fluconazole	-	-	-	-							**1**	**17**	**55**	-	-	0	0.0	73	100	ND	ND
Voriconazole	-	-			16	40	15	1	**1**			-	-	-	-	IE	IE	IE	IE	1	1.4
Isavuconazole	-	-	21	19	24	7	2					-	-	-	-	ND	ND	ND	ND	ND	ND
***C. parapsilosis* (62)**																					
Amphotericin B	-				6	34	20	2		-	-	-	-	-	-	62	100.0	0	0.0	0	0.0
Anidulafungin	-					2	7	37	16	-	-	-	-	-	-	0	0.0	ND	ND	ND	ND
Micafungin						1	13	35	13	-	-	-	-	-	-	0	0.0	ND	ND	ND	ND
Fluconazole	-	-	-	-		5	31	16	3	**3**	**1**	**1**	**2**	-	-	55	88.7	4	6.5	7	11.3

Voriconazole	-	-	53	5	2			**1**	**1**			-	-	-	-	60	96.8	2	3.2	2	3.2
Isavuconazole	-	-	61	**1**								-	-	-	-	ND	ND	ND	ND	1	1.6
***C. tropicalis* (81)**																					
Amphotericin B	-					26	41	14		-	-	-	-	-	-	81	100	0	0.0	0	0.0
Anidulafungin	-	33	33	13	**1**		**1**			-	-	-	-	-	-	79	97.5	2	2.5	2	2.5
Micafungin	23	28	24	5					**1**	-	-	-	-	-	-	80	98.8	1	1.2	1	1.2
Fluconazole	-	-	-	-	10	22	26	15	1	**2**	**1**	**2**	**2**	-	-	74	91.4	5	6.2	7	8.6
Voriconazole	-	-	66	4	4	**3**		**1**		**2**	**1**	-	-	-	-	74	91.4	7	8.6	7	8.6
Isavuconazole	-	-	76		**2**					**2**	**1**	-	-	-	-	ND	ND	ND	ND	5	6.2
***Candida sp.* (65)**																					
Amphotericin B	-			6	12	19	19	6	**3**	-	-	-	-	-	-	62	95.4	3	4.6	ND	ND
Anidulafungin	-	12	9	17	8	2	7	7	3	-	-	-	-	-	-	ND	ND	ND	ND	ND	ND
Micafungin	2	6	12	23	4	3	9	3	3	-	-	-	-	-	-	ND	ND	ND	ND	ND	ND
Fluconazole	-				4	11	16	3	6	8	**3**	**6**	**8**	-	-	40	61.5	17	26.2	ND	ND
Voriconazole	-		35	6	14	3	5	1			1	-	-	-	-	ND	ND	ND	ND	ND	ND
Isavuconazole	-		46	6	6	2	1	3			1	-	-	-	-	ND	ND	ND	ND	ND	ND
Other fungi (31)[1]																					
Amphotericin B	-			2	4	5	7	7	**6**	-	-	-	-	-	-	25	80.6	2	6.5	ND	ND
Anidulafungin	-			4	5	2	0	0	18	-	-	-	-	-	-	ND	ND	ND	ND	ND	ND
Micafungin			1	1	7	2	0	0	18	-	-	-	-	-	-	ND	ND	ND	ND	ND	ND
Fluconazole	-	-	-	-						7	**4**	**5**	**7**	-	-	0	0.0	16	69.6	ND	ND
Voriconazole	-	-		4	8	5	2	1	2	7	2	-	-	-	-	ND	ND	ND	ND	ND	ND
Isavuconazole	-	-	3	7	4	1	3	3	1	1	8	-	-	-	-	ND	ND	ND	ND	ND	ND
Overall (1900)[2]																					
Amphotericin B	-	17	27	103	284	574	666	205	**24**	-	-	-	-	-	-	1876	98.7	24	1.3	ND	ND
Anidulafungin	-	1102	323	336	24	11	16	46	40	-	-	-	-	-	-	ND	ND	ND	ND	ND	ND
Micafungin	1382	219	71	47	59	18	25	39	38	-	-	-	-	-	-	ND	ND	ND	ND	ND	ND
Fluconazole	-	-	-	-	562	407	121	68	177	298	64	48	147	-	-	1147	60.6	177	9.4	ND	ND
Voriconazole	-	-	1239	269	202	66	33	23	35	26	7	-	-	-	-	ND	ND	ND	ND	ND	ND
Isavuconazole	-	-	1614	111	58	16	23	30	18	18	12	-	-	-	-	ND	ND	ND	ND	ND	ND

S: susceptible; R: resistant; non-wt: non-wildtype (as the MIC was above the ECOFF).
Empty cells indicate that there were no isolates for which the MIC had the indicated value.
- The indicated concentration of that particular drug was not tested
Bold numbers indicate isolates for which the MIC were above the ECOFF and with underlined numbers indicating isolates that were resistant.
EUCAST breakpoints and ECOFFS were used. For *Candida* species and "other fungi" an amphotericin B breakpoint of 1 mg/L was used. For "other fungi" a fluconazole breakpoint of 2 mg/L was used. These last cut-offs are only an indication of the susceptibility profile for the given species/group of isolates (and a conservative estimate of the proportion of cases that are likely good targets for the compound in question).

[1] For the echinocandins and fluconazole 29 and 23 isolates were tested, respectively. [2] For the echinocandins and fluconazole, 1898 and 1892 isolates were tested, respectively.