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

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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 year olds 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
species but especially *C. glabrata* (28–31), thereby warranting continued surveillance and monitoring. The aim of this population based nationwide study was to update and assess 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 pelliculosa, 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
species to illustrate the overall susceptibility of those species or groups of fungi but should not be interpreted as an exact figure of clinical susceptibility or resistance.

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

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

Consumption of antifungal compounds

Information concerning overall use of antifungal agents in Denmark 2000–15 was retrieved from the Danish Medicines Agency (at [www.medstat.dk](http://www.medstat.dk)). Posaconazole tablet and iv formulation were marketed in Denmark in 2014. The licensed maintenance dose of these formulations is 300 mg/day compared to 800 mg/day for the oral suspension. To reflect the actual number of individual dosages given in 2014-15, a corrected use was calculated (0.3 g enterotablet or iv infusion and 0.8 g oral solution translated to 1 DDD). The antifungal use (DDD/1000 inhabitants/year) from Norway, Sweden and Finland was retrieved from [www.legemiddelforbruk.no](http://www.legemiddelforbruk.no), [www.socialstyrelsen.se](http://www.socialstyrelsen.se), and [www.fimea.fi](http://www.fimea.fi). Posaconazole formulation information was not available and unadjusted posaconazole DDDs were used for comparison between the Nordic countries and Denmark.
Chi-square test or Fisher’s exact test was used for comparison of proportions and the Chi-square test for trend used for evaluation of changes in species distribution over the 12-year surveillance period. Calculations were performed using GraphPad Prism Version 6.04 (GraphPad Software Inc., La Jolla, CA, USA). For twelve episodes (10 in 2004 and one each in 2005 and 2007) the gender was unknown. When possible, episodes were allotted evenly to genders in specific 10-year age groups, but in four instances single cases within age groups were excluded from analysis of gender- and age-specific incidence rates, conducted with linear Poisson regression/incidence ratio rate calculation (package: epitools). P-values <0.05 (two-tailed) were considered statistically significant. Binomial univariate logistic regression was used to investigate associations between species distribution and year, age, gender and BC system using R (R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/). For this analysis and to assure independence of observations only incident cases were included. Covariates with a p<0.1 were investigated further in a binomial multivariate analysis. Year, age, gender, and BC system were all retained in the multivariate analysis and independent significant findings displayed. The p-value also calculated when excluding the main tertiary referral hospital serviced by centre 1-RH.
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).
C. albicans accounted for the majority of isolates (47.9%) in 2012-15, followed by C. glabrata (31.8%). C. tropicalis, C. krusei, C. dubliniensis and C. parapsilosis each accounted for <4.3%. Sixty-nine (3.6%) of the isolates belonged to other Candida species, thirty-two (1.7%) to other fungal genera whereof eleven were mould and another eleven Saccharomyces cerevisiae isolates (Table 1). Poly-fungal episodes (n=53) involved 5.6% of isolates. Over the twelve years, the proportion of C. albicans decreased (p<0.0001) whereas the proportion of C. glabrata increased (p<0.0001) (Figure 3). This development was still observed despite conservatively assigning non-C. albicans as C. glabrata among isolates from 2004-09. The proportion of C. dubliniensis increased significantly from 2.3% to 3.6% (p=0.01) whereas no change was detected for other species (Figure 3). The increase in C. glabrata was significant for age groups 1-9, 30-39, 50-59, and 70-79 again despite assigning early isolates identified as only non-albicans to be C. glabrata (p<0.03 for all groups; Figure 2).

The species distribution in 2012-15 varied by age, gender, and by centre (Supplementary table 2 and 3). Correlation between species and age, gender, BC system, and calendar year was investigated for all incident cases (only first episode included in the full 12-year period) in a univariate and multivariate logistic regression analysis (Table 3). A decrease in C. albicans and an increase in C. glabrata and C. dubliniensis episodes over time was found. C. glabrata was positively associated with female sex whereas the opposite was the case for C. tropicalis. C. albicans, C. parapsilosis, C. dubliniensis, and “other fungi” were associated with younger patients whereas the odds of being infected with C. glabrata increased with age. Candidaemia involving C. glabrata was positively associated with BACT/ALERT whereas the opposite was true for C. parapsilosis, C. krusei, and “other fungi” (multivariate analysis). For “other fungi” this association disappeared when excluding centre 1-RH.
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.
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...
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/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
higher use of broad-spectrum antibiotics and the increasing utilisation of BC reported in Denmark (particularly in the ≥ 65 years old population) may be markers of Danish patients being more severely ill or the introduction of sepsis packages (including timely diagnostics) (46).

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

The previously observed species-shift towards non-albicans species and particularly C. glabrata continued 2012-15. This trend has been observed in several population-based candidaemia surveys (3, 12, 13, 20, 50). The increase in the proportion of C. glabrata has happened concomitantly with an increase in azole use in Denmark and with the population getting older.
Sweden, Norway, Finland, and Iceland have not witnessed the same increase in the proportion of *C. glabrata* during the last decade of surveillance although sharing demographic characteristics. Despite an increase in systemicazole use in all four countries, the overall use was substantially lower in the other Nordic countries (Figure 4, (14)). In 2015, the overall systemic azole consumption in Norway was comparable to the fluconazole use alone in Denmark in 2004. In contrast, the use of topical azoles for vaginitis was twice as high in Norway compared to Denmark (an average of 375 vs 213 DDD/1000 inhab./year). We speculate whether this increasing selection pressure mediated by systemic azoles over the twelve-year period has facilitated the increasing proportion of *C. glabrata* in the Danish setting.

The age-dependent species distribution of *C. parapsilosis* and *C. glabrata* and the influence of blood culture system on the detection of *C. glabrata* were confirmed in a multivariate analysis (15, 17, 18, 51). Although the gradual change from BACTEC to BACT/ALERT may have contributed to the increase in *C. glabrata* no centres have changed to BACT/ALERT since January 2011. Less importance has been placed on the impact of gender in relation to *C. glabrata*. *C. glabrata* was correlated to the female sex and was especially common above the age of 40 years, an observation also made in our previous study but to our knowledge not reported elsewhere (18). One reason for this could be the gender-inequality in the antifungal consumption in the primary health care sector. Prior fluconazole use has been shown to be associated with emergence of *C. glabrata* (18, 22, 52, 53). Fluconazole is the main azole used in Denmark, and the majority is administered in the primary sector. In this setting (2012-15), 2/3 of the sale of fluconazole was prescribed for the age groups 20-65 years and with a 4.8 female/male ratio of DDDs/1000 inhabitants/day. Genotyping studies have confirmed that the infecting organism derives from the colonizing flora (54). We therefore hypothesize that the considerable use of fluconazole in adult
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 byazole 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
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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REFERENCES


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


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


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


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

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

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

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

Figure 4: Annual consumption of systemic antifungal compounds in DDDs/1000 inhabitants in 2004-2015. Hospital use is shown in orange and use in the primary healthcare sector in red. The
total annual consumption in Norway, Sweden and Finland is inserted for comparison (dark grey, light grey and white bars, respectively).

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

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

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

a) Candida spp. includes: C. lusitaniae (19); C. kefyr (13); C. fermentati (8); C. pelliculosa (6); C. guilliermondii (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, Rhodotorula 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 

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. 

a 95% confidence intervals. Due to an interaction between calendar year and BC system, the year factor is split on ¹BACT/ALERT (top) and ²BACTEC (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).

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).

For the echinocandins and fluconazole 29 and 23 isolates were tested, respectively. For the echinocandins and fluconazole, 1898 and 1892 isolates were tested, respectively.

1 For the echinocandins and fluconazole 29 and 23 isolates were tested, respectively. 2 For the...
Table 1: Characteristics of the national fungaemia surveillance scheme on incidence rates, age, gender, and species distribution 2012-2015.

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

- C. albicans includes: C. lusitaniae (19); C. kefyr (13); C. furcata (6); C. pelliculosa (6); C. guilliermondii (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.
- Other fungi include: S. cerevisiae (11); Fusarium dimerum and Cryptococcus neoformans (4 each); Fusarium solani (3); Fusarium oxysporum, Rhodotorula 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.

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

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.

<table>
<thead>
<tr>
<th>Species (no.)</th>
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<th>Multivariate</th>
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<tr>
<td></td>
<td>Odds ratio</td>
<td>95% CI</td>
<td>p</td>
<td>p&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Odds ratio</td>
<td>95% CI</td>
<td>p</td>
<td>p&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>C. albicans (2967)</td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>Calendar year (Year)</td>
<td>0.94</td>
<td>0.93-0.96</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.95&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.94-0.97</td>
<td>&lt;0.001</td>
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<tr>
<td>Age (Year)</td>
<td>0.995&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.992-0.998</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.995</td>
<td>0.992-0.998</td>
<td>0.002</td>
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<tr>
<td>C. glabrata (1369)</td>
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<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Calendar year (Year)</td>
<td>1.08</td>
<td>1.06-1.10</td>
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<td>&lt;0.001</td>
<td>1.06</td>
<td>1.04-1.08</td>
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<td>Blood culture system (BACTEC)</td>
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<td>0.57-0.75</td>
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<td>&lt;0.001</td>
<td>1.11</td>
<td>1.07-1.15</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>Gender (Female)</td>
<td>1.15&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.01-1.30</td>
<td>0.032</td>
<td>0.023</td>
<td>1.17</td>
<td>1.03-1.33</td>
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<td>0.009</td>
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<tr>
<td>Age (Year)</td>
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<td>1.02-1.03</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>1.02</td>
<td>1.02-1.02</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>C. tropicalis (237)</td>
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<tr>
<td>Gender (Female)</td>
<td>0.74</td>
<td>0.56-0.97</td>
<td>0.034</td>
<td>0.018</td>
<td>0.74</td>
<td>0.56-0.97</td>
<td>0.033</td>
<td>0.018</td>
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<td>C. parapsilosis (171)</td>
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<tr>
<td>BC system (BACTEC)</td>
<td>1.87</td>
<td>1.38-2.54</td>
<td>&lt;0.001</td>
<td>0.005</td>
<td>1.70</td>
<td>1.24-2.34</td>
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<td>0.045</td>
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<td>Age (Year)</td>
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<td>0.98-0.99</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.98</td>
<td>0.98-0.99</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<td>C. krusei (219)</td>
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<td>BC system (BACTEC)</td>
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<td>1.52-2.62</td>
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<td>&lt;0.001</td>
<td>2.07</td>
<td>1.56-2.74</td>
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<tr>
<td>Calendar year (Year)</td>
<td>1.06</td>
<td>1.01-1.12</td>
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<td>1.07</td>
<td>1.02-1.13</td>
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<td>Age (Year)</td>
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<td>0.98-0.99</td>
<td>0.007</td>
<td>0.009</td>
<td>0.98</td>
<td>0.98-0.99</td>
<td>0.008</td>
<td>0.005</td>
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<td>Other fungi (83)</td>
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</tr>
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<td>0.98</td>
<td>0.97-0.99</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup> 95% confidence intervals. Due to an interaction between calendar year and BC system, the year factor is split on BACT/ALERT (top) and BACTEC (bottom).<sup>b</sup> p value when excluding the main tertiary hospital Rigshospitalet (Centre 1-RH).
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<table>
<thead>
<tr>
<th>Compound</th>
<th>C. albicans (914)</th>
<th>C. dubliniensis (71)</th>
<th>C. glabrata (603)</th>
<th>C. krusei (73)</th>
<th>C. parapsilosis (62)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of isolates with the given MIC (mg/L)</td>
<td>S</td>
<td>R</td>
<td>non-wt</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.008 0.015 0.032 0.064 0.125 0.25 0.5 1 2 4 8 16 32 64 128</td>
<td>No %</td>
<td>No %</td>
<td>No %</td>
<td></td>
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<tr>
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<td></td>
<td>-</td>
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<td>-</td>
<td>874</td>
<td>40</td>
<td>-</td>
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<td>-</td>
<td>520</td>
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<td>Voriconazole</td>
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<td>-</td>
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<tr>
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<td>-</td>
</tr>
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<td>200</td>
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<tr>
<td>Micafungin</td>
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<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fluconazole</td>
<td></td>
<td>-</td>
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<td>C. krusei (73)</td>
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<tr>
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<td></td>
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<tr>
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<tr>
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<td>---------------</td>
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<td>----------------</td>
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<tr>
<td>EUCAST breakpoint</td>
<td>2 mg/L</td>
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<tr>
<td>Other fungi (31)</td>
<td>-</td>
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<tr>
<td>EUCAST breakpoint</td>
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<td>Overall (1900)</td>
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5: 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).
For the echinocandins and fluconazole 29 and 23 isolates were tested, respectively. For the echinocandins and fluconazole, 1898 and 1892 isolates were tested, respectively.
The diagram illustrates the percentage distribution of various fungal isolates over different time periods:

- **2004-7**
  - p=0.01

- **2008-11**
  - p<0.0001

- **2012-15**
  - p<0.0001

The graph shows a decrease in the percentage of isolates over time, particularly noticeable in the latter period. The labels indicate specific fungal species and groups, such as:

- **C. albicans**
- **C. dubliniensis**
- **C. krusei**
- **C. glabrata**
- **C. parapsilosis**
- **C. tropicalis**
- **Candida spp.**
- **Other fungi**
- **non-C. albicans**

The graph also highlights a significant decrease in the percentage of isolates labeled as **non-C. albicans** and **Other fungi**.