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Trends in fungaemia and antifungal susceptibility in the Netherlands

F. Verduyn Lunel[1,2], J.G.M. Koeleman[3], L. Spanjaard[4], C. Vandenbroucke-Grauls[4,5], C. Schultz[5], H.A. Verbrugh[6], G. Vos[6], A. Troelstra[7], E. Mascini[7], P.E. Verweij[1,2], A. Voss[1,2*]

[1]Department of Medical Microbiology, [2]Nijmegen University Centre for Infectious Diseases, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, [3]Department of Medical Microbiology St Fransiscus Hospital, Rotterdam, the Netherlands, [4]Department of Medical Microbiology, Academic Medical Centre Amsterdam, Amsterdam, the Netherlands, [5]Department of Medical Microbiology and Infection Control, VU University Medical Centre, Amsterdam, the Netherlands, [6]Department of Medical Microbiology and Infectious Diseases, Erasmus Medical Centre, Rotterdam, the Netherlands, [7]Department of Medical Microbiology, University Medical Centre Utrecht, Utrecht, the Netherlands, [*]corresponding author: tel.: +31 (0)24-361 43 56, fax: +31 (0)24-354 02 16

ABSTRACT

We retrospectively evaluated fungaemia over the period 1996 to 2001 in five university hospitals. Over 350,000 blood cultures were collected during more than 7 million days of hospitalisation. The average rate of fungaemia over the six-year period was 0.82 per 10,000 patient days (range 0.65 to 1.21 per 10,000 patient days). The proportion of bloodstream infections caused by *Candida albicans* remained stable throughout the study period with a mean of 53% (range 48 to 62%). This is a change from trends described in previous studies, including a survey performed in the Netherlands. This study shows a new, stable rate of fungaemia and no further signs of increasing rate of infections due to non-*albicans Candida* species. Susceptibility to all tested antifungal agents remained stable throughout the study period.

KEYWORDS

Candida, epidemiology, fungaemia

INTRODUCTION

Candida species are frequent causative agents of fungaemia. During the last decade, there has been a shift in the incidence of causative organisms of fungaemia for these species. Although in some studies *Candida albicans* was still the most frequently isolated species,[1-7] non-*albicans Candida* species have become increasingly prevalent.[8-10] Concomitantly, there may have been a change in the susceptibility for systemic antifungal drugs due to this changing distribution of *Candida* species.[11-15] Moreover, the increasing use of azoles in prophylaxis and treatment may have caused selection of azole-resistant yeasts or induced resistance. In 1996, Voss *et al*. determined the incidence of yeast infections in five Dutch university hospitals over the period 1987 to 1995.[8] That study showed an increase in the rate of fungaemia during this period. *C. albicans* was the most frequently isolated species, but overall non-*albicans Candida* species were increasing significantly. To assess whether this trend has continued in recent years, this study was repeated for the period 1996 to 2001. In addition, the susceptibility to antifungal drugs was determined according to the Clinical Laboratory Standards Institute (CLSI) protocol M27-A2.[16]

METHODS

Microbiology data from computer-generated lists of patients whose blood cultures had yielded yeasts during the period from 1 January 1996 to 31 December 2001 were analysed retrospectively. The data were provided by the five university hospital laboratories and an additional laboratory of a major hospital in Rotterdam. All patients admitted to the aforementioned hospitals were eligible. An episode of fungaemia was defined as at least one positive blood culture yielding yeasts during a single hospitalisation period. The number of blood culture sets were examined and the results were recorded. Automated blood culture systems were used in all participating hospitals. Data to determine patient days were retrieved from the hospital information systems.

Strain identification and susceptibility testing

Yeasts were cultured on Sabouraud-dextrose agar and incubated for four days at 37°C. Identification was performed with standard microbiological techniques. All the isolates were initially kept at -70°C in glycerol broth. The antifungal activity of amphotericin b (AMT, Bristol-Meyers-Squibb, Woerden, the Netherlands), 5-fluorocytosin (5-FC, Valeant, Zoetermeer, the Netherlands), fluconazole (FLU, Pfizer, Capelle aan de IJsel, the Netherlands), itraconazole (ITC, Janssen Pharmaceutica BV, Tilburg, the Netherlands), voriconazole (VOR, Pfizer, Capelle aan de IJsel, the Netherlands), posaconazole (PSZ, Schering-Plough, Maarsen, the Netherlands) and caspofungin (CAS, MSD, Haarlem, the Netherlands) was determined *in vitro* using a broth-microdilution method similar to the CLSI protocol M27-A2.[16] The concentration range for AMT, ITC, VOR, and PSZ was 0.016 to 16 mg/l and for 5-FC, FLU and CAS 0.062-64 mg/l.

For AMT and CAS the minimal inhibitory concentration (MIC) was defined as the lowest concentration that showed no visible growth. For the azoles and 5-FC the MIC was defined as the lowest concentration at which 50% growth inhibition was measured compared with that of the control. MIC was determined after 24 and 48 hours of incubation except for *Cryptocccus neoformans* isolates where the total incubation time was 72 hours. All susceptibility tests were performed in duplicate.

Statistics

ANOVA and Kruskal-Wallis tests were applied for comparing means and the χ^2 test for trends was used for comparing the contingency of causative organisms of fungaemia. MIC dilutions were transformed logarithmically before comparing means. Spearman's rank correlation was used for analysing trends for transformed MIC values over the observed period.

RESULTS

The participating hospitals delivered a total of 7,772,455 hospital days of care (mean 1,295,409 ± 215,042 per year) during the period 1996 to 2001 and the annual frequency did not change significantly during the study period (*table 1*). A total of 837,034 admissions were registered (mean 139,506 ± 21,465 per year) also with no significant changes over the study period. The number of hospital days per admission and the number of blood cultures per 10,000 admission days, 9.3 and 458 respectively, remained stable as well. A total of 355,708 cultures were collected throughout the study period, from which 56,270 (16%) yielded a positive culture. From these positive blood cultures 1688 (3%) yielded yeast divided over 626 episodes of fungaemia. The rate of fungaemia increased from 0.71 in 1996 to 1.21 episodes per 10,000 patient days in 2001; however, this increase did not reach a level of significance. The higher value of 1.21 per 10,000 admission days is an unexplained peak in 2001 whereas the rate over the period 1996 to 2000 only varied by 0.71 to 0.76 per 10,000 admission days (*figure 1*). *C. albicans* was still the most frequently isolated species and its proportion (mean 53%) remained stable during the study period; no significant shift from *C. albicans* to non-*albicans Candida* species was observed (*table 1* and *figure 2*). For susceptibility testing, 357 isolates were available: *C. albicans* (204), *C. glabrata* (70), *C. arapsilosis* (32), *C. tropicalis* (26), *C. krusei* (12), *C. lusitaniae* (6), C. *neoformans* (2), *C. dubliniensis* (1), *C. guilliermondii* (1), *C. inscopicua* (1), *C. kefyr* (1), and one isolate of an unspecified yeast. Of the 357 tested isolates, 53 (15%) were not susceptible to FLU (MIC >8 mg/l). These isolates were *C. krusei* (12), *C. glabrata* (39) and one isolate each of *C. albicans* and *C. tropicalis*. Generally, *C.albicans*

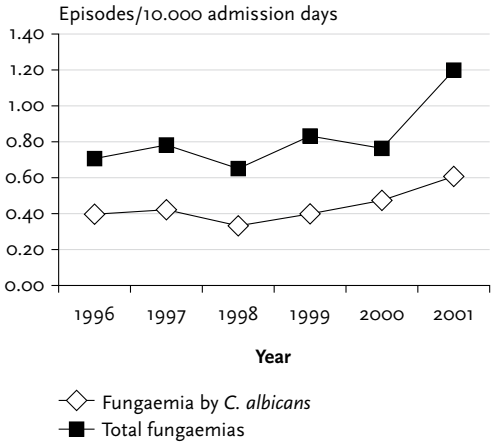
Table 1. *Selected epidemiological and microbiological characteristics over the observed period*

	1996	1997	1998	1999	2000	2001	Total
Admissions*	118,586	158,234	155,068	154,143	143,760	107,243	837,034
Admission days*	1,089,356	1,496,963	1,464,139	1,402,340	1,348,859	970,798	7,772,455
Days per admission	9.2	9.5	9.4	9.1	9.4	9.1	
Blood cultures*	52,282	62,223	61,960	66,012	66,435	46,796	355,708
Blood cultures per 10,000 admission days*	479.93	415.66	423.18	470.73	492.53	482.04	
Positive blood cultures	7324 (14%)	9557 (15%)	10001 (16%)	10660 (16%)	10364 (16%)	8364 (18%)	56270 (16%)
Positive blood cultures per 10,000 admission days*	67.23	63.84	68.31	76.02	76.84	86.16	
Blood cultures containing yeasts	263 (4%)	287 (3%)	332 (3%)	271 (3%)	296 (3%)	239 (3%)	1688 (3%)
Fungaemia*	77	117	95	117	103	117	626
Fungaemia with *C. albicans*	43 (56%)	63 (54%)	49 (52%)	56 (48%)	64 (62%)	59 (50%)	334 (53%)
Fungaemia/10,000 admission days*	0.71	0.78	0.65	0.83	0.76	1.21	
Fungaemia with *C. albicans* per 10,000 admission days*	0.39	0.42	0.33	0.40	0.47	0.61	
Fungaemias per 1000 admissions*	0.65	0.74	0.61	0.76	0.72	1.09	

*No significant changes occurred over the observed period.

isolates tended to be more susceptible to all tested antifungal agents, compared with non-*albicans Candida* isolates. This difference was more pronounced for the azoles. No significant changes in susceptibility of the tested yeast isolates to any of the tested antifungals were observed during the study period (*figures 3A-G*). The new azoles VOR and PSZ as well as the echinocandin CAS showed marked activity against all yeast isolates including the *C. krusei* isolates and non-*C. krusei* isolates with an FLU MIC >8 mg/l.

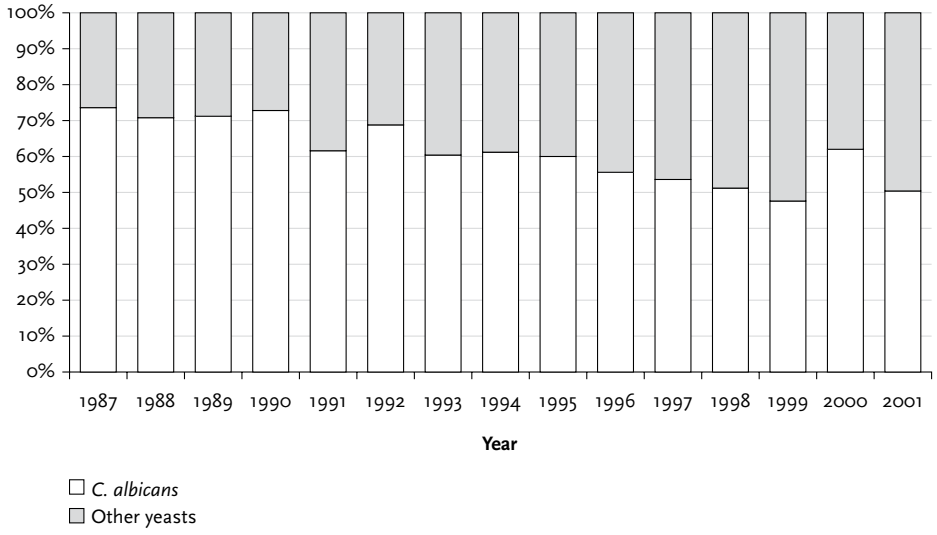
Figure 1. *Number of all episodes of fungaemia per 10,000 admission days and of those caused by C. Albicans per 10,000 admission days*



DISCUSSION

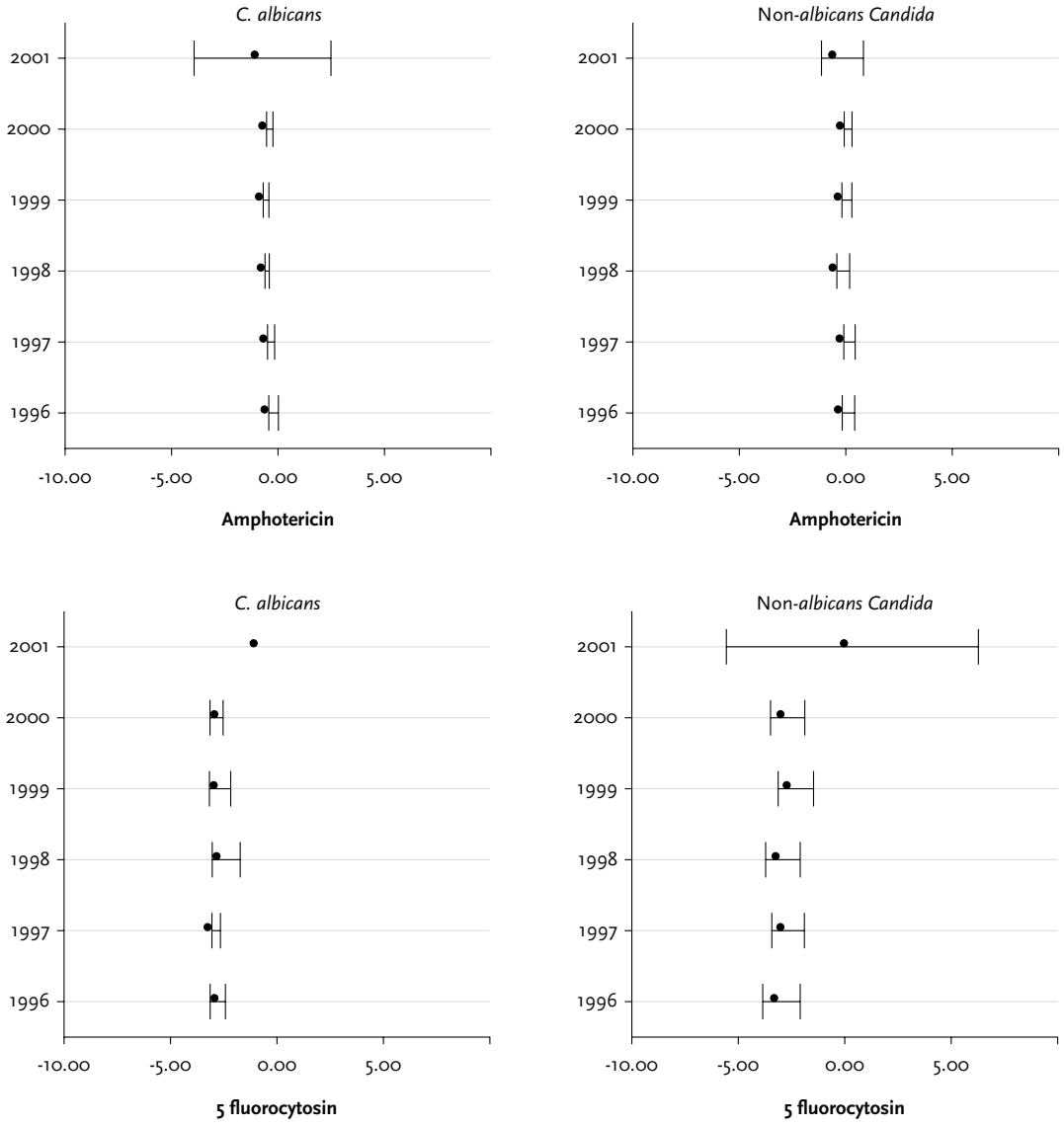
An increase in fungal infections was already reported in the 1980s.[17,18] The main causative agents were *Candida* species of which *C. albicans* was the principal representative. However, in the United States a gradual, relative, increase in non-*albicans Candida* species has been observed in intensive care units.[19] Other studies show similar shifts in fungaemia caused by non-*albicans Candida* species, mainly *C. glabrata*.[5,20] In leukaemia patients the proportion of fungaemia due to *C. krusei* and *C. glabrata* bloodstream infections increased, despite a significant decrease in the overall incidence of fungaemia.[21] This shift in frequency of non-*albicans Candida* infections has been attributed to increased use of FLU,[22] although in one study a causative link between these two parameters was not found.[23] Due to the increase of non-*albicans Candida* species, especially *C. glabrata*, susceptibility to the first-line azole FLU has decreased. More importantly, this decreased susceptibility appears to be accompanied by higher treatment failure rates.[24,25] However, in our study population, both the incidence of fungaemia as well as the proportion of non-*albicans Candida* species remained stable throughout the period 1996 to 2001. There was no significant change in the proportions of fungaemia caused by *C. albicans* and non-*albicans Candida* species. This suggests a break with the trend shown in the period 1987 to 1995, where a significant increase of fungaemia due to non-*albicans Candida* species was observed (*figure 2*).[8] The incidence of fungaemia varied from 0.71/10,000 patient days in 1996 to 0.76/10,000 patient days in 2000. The observed peak of 1.21 episodes

Figure 2. *Contribution of C. albicans candidaemia to total episodes of fungaemia*



Data from the episode 1987 to 1995 were extracted with permission from reference 8 and show a significant increase in the proportion of non-*albicans Candida* species. Over the period 1996 to 2001 this was not significant.

Figures 3A and B. *Mean ^{2}log MIC values after 48 hours of incubation (error bars show 95.0% CI of mean)*

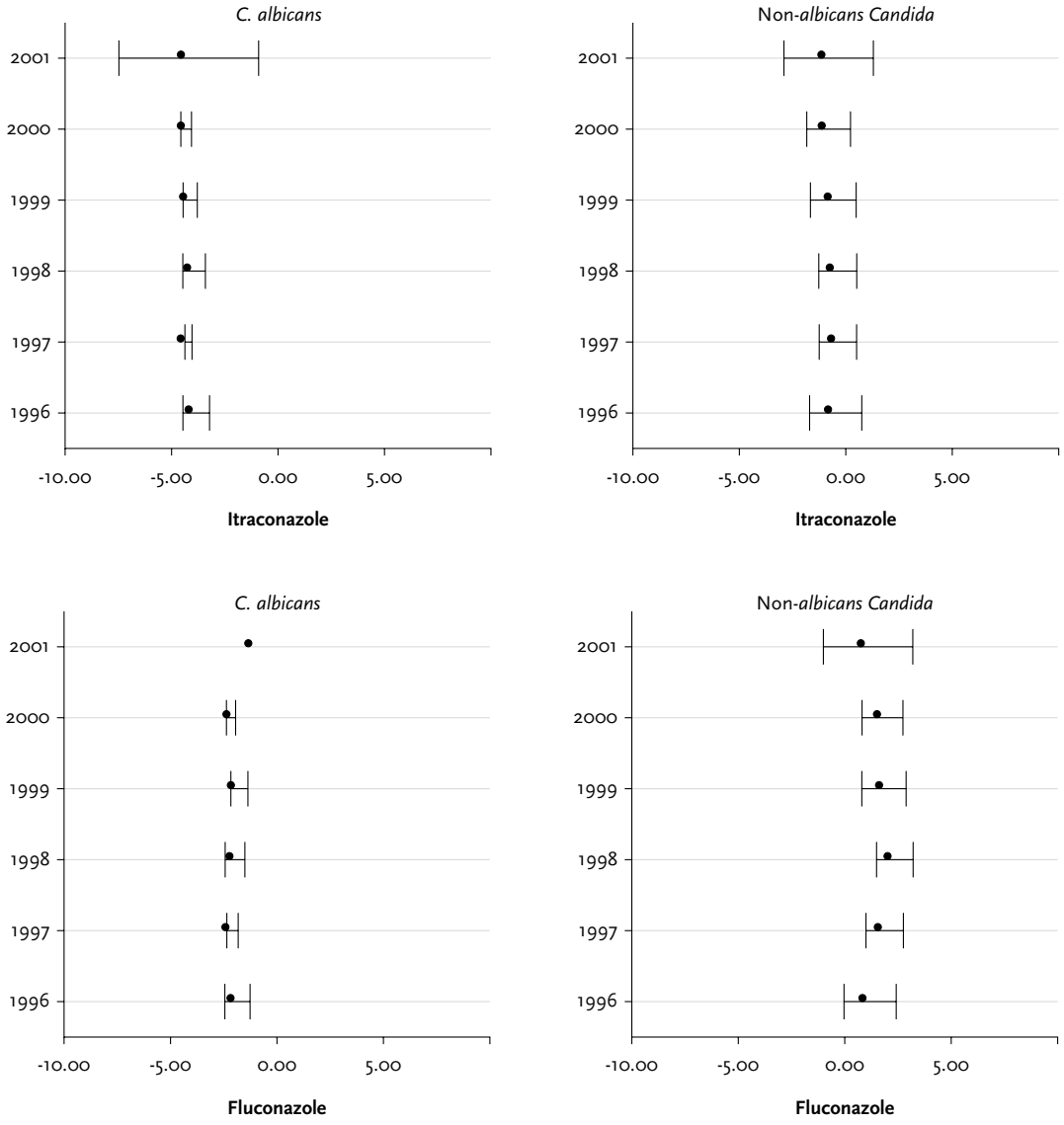


of fungaemia /10,000 patient days in 2001 was not significantly different to the rates in other years. However, additional observational studies are required for the years after 2001. The observed trends in the epidemiology of fungaemia are similar to those found in two Swiss studies, where no shift from *C. albicans* fungaemia to those caused by non-*albicans Candida* species was observed.[26,27] The incidence of fungaemia in Dutch hospitals is lower than the rates reported from hospitals outside Europe, even when the solitary peak of 1.21/10,000 patient days in 2001 is taken into account. In 2004, Hajjeh *et al*. reported incidence rates of 1.5/10,000 hospital days.[28] In a Canadian candidaemia study by Karlowsky *et al*., over the period 1976 to 1996, *Candida* species contributed to approximately 8% of the total bloodstream isolates;[29] this was more than twice the percentage we found (3%). In Iceland, the incidence of fungaemia had increased to 0.55/1000 admissions,[30] but remained lower than the incidence in our study.

C. albicans remains the most frequently isolated yeast in fungaemia; however, other species are on the rise. Over the period 1999 to 2003 Irish investigators observed an average annual incidence of 0.70 episodes /10,000 patient days in a tertiary care hospital. Here, the proportion of *C. albicans* decreased from about 80% in 1996 to 1999 to 58% in 2000 to 2003 in favour of the proportion of *C. glabrata* and *C. parapsilosis*.[20]

It is possible that the stable incidence of fungaemia in the Dutch hospitals in our study is due to FLU use as prophylaxis and vigilant infection control practices.

Figures 3C and D. *Mean 2log MIC values after 48 hours of incubation (error bars show 95.0% CI of mean)*



However, the increased use of FLU might lead to selection of species such as *C. glabrata* and *C. krusei*, which are less susceptible to FLU, as described in a study from the Invasive Fungal Infections Cooperative Group (now Infectious Diseases Group) of the European Organisation for the Research and Treatment of Cancer.[31] We did not observe such a shift. In addition, no decrease in susceptibility was observed for any of the tested antifungal agents. Similar results were observed in a worldwide study on the susceptibility of *Candida* species to FLU over a ten-year period.[32] These observations show that FLU maintains its value for the treatment of systemic fungal infections. The antifungal agents showed good activity against the isolates, including isolates that were less susceptible to FLU.

In conclusion, the incidence of fungaemias in the Netherlands appears to have remained stable between 1996 and 2001, so the increase that we observed in the previous period (1987 to 1995) has levelled off. However, *C. albicans* was still the most frequently isolated species, being recovered from approximately 55% of the patients with fungal bloodstream infections.

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Figures 3E and F. *Mean ^{2}log MIC-values after 48 hours of incubation (error bars show 95.0% CI of mean)*

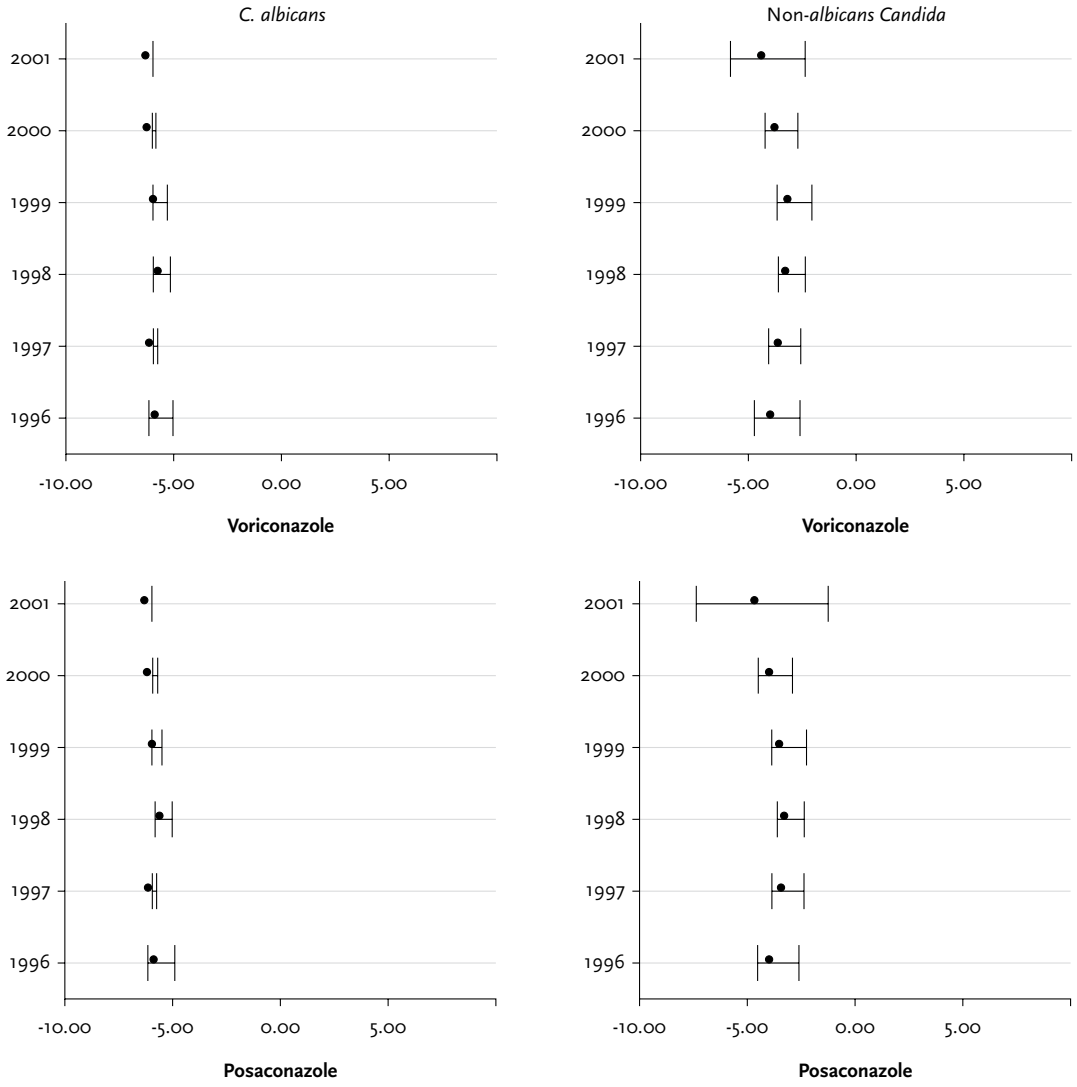
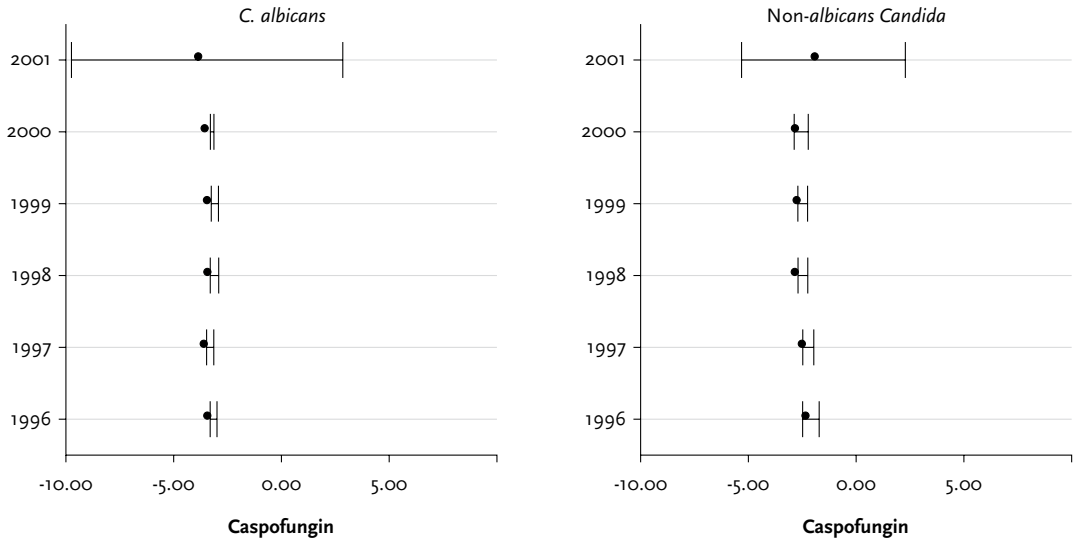


Figure 3G. *Mean ^{2}log MIC values after 48 hours of incubation (error bars show 95.0% CI of mean)*



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