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Prior colonisation with *Candida* species fails to guide empirical therapy for candidaemia in critically ill adults

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Summary *Objectives:* Pre-emptive fluconazole (fcz) anti-fungal therapy is often based upon *Candida* colonisation of at least 2 non-contiguous non-sterile sites. The aim of this study was to evaluate the relationship between candidaemia and prior colonisation of non-sterile sites.
Methods: A retrospective observational study was performed in the intensive care unit/high dependency unit (ICU/HDU) of a University hospital on alternate years from 1999–2007, where a pre-emptive anti-fungal therapy policy was introduced in 2005.
Results: A higher proportion of blood isolates were *Candida glabrata* compared with non-sterile isolates (16/46 vs 106/1062; $p < 0.001$), similarly a greater proportion of blood isolates were fcz-resistant compared with non-sterile isolates (15/46 vs 101/1062; $p < 0.001$). No trend over time was detected in the proportion of *C. glabrata* and *Candida albicans* isolates from blood and non-sterile sites, or in the fcz-sensitivity of isolates from these sites. *C. glabrata* candidaemia was more likely to occur in the absence of non-sterile site colonisation compared with non-glabrata candidaemia (12/16 vs 8/30; $p = 0.005$). Of candidaemic patients, 43% had no preceding colonisation by any *Candida* spp.; in 67% of these patients, candidaemia was due to *C. glabrata*.
Conclusions: Pre-emptive therapy based upon colonisation of at least two sites may be inadequate as 43% of candidaemic patients had no evidence of prior colonisation, 67% of whom had

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candidaemia due to *C. glabrata*. Furthermore if pre-emptive anti-fungal therapy is instituted in non-colonised patients there is a risk of selecting an inappropriate anti-fungal for *C. glabrata*. Despite the introduction of pre-emptive fcz therapy, no time trend was detected in the proportion of fcz-sensitive isolates from blood and non-sterile sites.

Introduction

Candida spp. causes around 9% of health-care associated bloodstream infections in ICU patients[1] and have an attributable mortality of approximately 30%[2] with delayed anti-fungal therapy an independent predictor of mortality.[3] Preceding *Candida* colonisation has been reported to be the strongest predictor of subsequent invasive disease,[4] and is therefore accepted as an indication for early anti-fungal treatment in at-risk patients.[4–8]

Pre-emptive prescribing of anti-fungal drugs is based upon the species and drug susceptibilities of *Candida* spp. isolated from at least 2 non-contiguous skin and mucosal sites of high risk patients; fcz is typically the drug of choice.[9] However the relationship between colonising *Candida* spp. and those causing invasive disease has not been definitively established.

The aim of this study was to evaluate the relationship between candidaemia and prior colonisation of non-sterile sites in a mixed adult ICU/HDU, where a pre-emptive anti-fungal therapy policy was introduced in 2005. Accordingly, the following measures were examined: firstly whether the distribution of *Candida* spp. is similar between blood and non-sterile isolates; secondly, whether the proportion of fcz-sensitive *Candida* isolates is similar between blood and non-sterile sites; and finally to establish the proportion of patients with candidaemia who had prior *Candida* colonisation of at least 2 non-contiguous anatomic sites and to describe the species involved.

Methods

Study design, setting and definitions

The Royal Victoria Hospital is a 1000-bed University hospital with a 28-bed ICU/HDU. A retrospective observational study of patients in ICU/HDU with *Candida* spp. colonisation or candidaemia, as identified from screening swabs or blood culture, was carried out using the prospectively collected laboratory records. The difference in *Candida* spp. distribution and fcz-sensitivity of isolates from blood and non-sterile sites were compared per isolate, using data from the selected cohort. Colonisation of non-sterile sites preceding candidaemia was investigated in the subgroup of patients with confirmed candidaemia.

Screening swabs were sent from each patient on admission to the unit and then weekly thereafter until discharge from ICU/HDU as part of standard patient care. They were typically sampled from the following: nose, groin, axilla, perineum, sputum and urine, with the aim to detect MRSA colonisation; however if *Candida* spp. were identified these were routinely reported. Additional superficial swabs and blood cultures were sent at the clinicians' discretion. *Candida* colonisation was defined as the growth of at least 5 cfu *Candida* spp. from a non-sterile site, that is, any sample type other than blood. Central line tips were excluded from the analysis as these could not consistently be categorised as either sterile or non-sterile specimens. Candidaemia was defined as at least one blood culture taken during ICU/HDU admission, either peripherally or from a central line, positive for *Candida* spp.

Participant selection and exclusion criteria

Patients admitted to ICU/HDU had undergone severe trauma, neurosurgery or vascular or abdominal surgery, although demographic data or antimicrobial therapy received during ICU/HDU admission was not available. All patients admitted to ICU/HDU on alternate years from 1999—2007 who isolated *Candida* spp. from either a non-sterile site or from blood, were eligible for inclusion in the analysis. Patients with candidaemia for whom data on surface colonisation was unavailable were excluded from the analysis. Pre-emptive anti-fungal therapy was introduced in 2005 for patients with colonisation at 2 or more non-sterile sites and signs of unexplained sepsis. Additionally, patients with perforation of the oesophagus, small intestine or large bowel following either (i) a course of broad-spectrum antibiotics, or (ii) recent bowel surgery, received pre-emptive therapy. In this category of patient the addition of an anti-fungal was not dependent upon colonisation or clinical status.

Data was not available for which patients received pre-emptive anti-fungal therapy.

Isolate identification

The blood culture detection method used was the BacT/Alert automated system (BioMerieux, Basingstoke, UK).

Non-sterile specimens were cultured on blood agar; a positive culture was defined as the detection of at least 5-cfu of any *Candida* spp. within 48 h incubation at 37 °C.

Candida colonies from blood or non-sterile specimens were subcultured onto CAN2 chromogenic agar (BioMerieux, Basingstoke, UK) for purity. *Candida albicans* was identified by germ-tube formation in horse serum and confirmed using CANDIFAST ES Twin (ELITECH, France); the identity of non-albicans species was confirmed using the API 32C (BioMerieux, Basingstoke, UK) identification system. Fcz-sensitivity was determined using SENSITITRE YeastOne Y08 (Trek Diagnostics Systems Ltd, East Grinstead, UK).

Only the first isolate of each species from each non-sterile site tested and from blood, for each patient, was included in this study; isolates were categorised as "albicans", "glabrata" and "non-albicans/non-glabrata".

Statistics

Comparisons of proportions of categorical variables were investigated using Pearson chi-square and Yates' corrected test. Age, APACHEII score and length of stay were compared between the candidaemic group and total cohort using the independent samples *t*-test. Trends in fcz-sensitivity and the number of isolates of albicans, glabrata and non-albicans/non-glabrata over time were calculated using the chi-square test for trend. The number of samples sent from candidaemic patients, and length of ICU stay, was compared using the Mann–Whitney *U* test. The 5% significance level was used for all tests. Analyses were performed using SPSS 15.0 for Windows software (SPSS inc., Chicago, Illinois, U.S.A.).

Results

A total of 3500 patients were admitted to ICU/HDU during the study. The study cohort comprised 974 patients; 46 patients with candidaemia and 928 with colonisation. From these 974 patients there were 46 positive blood cultures and 1062 positive non-sterile specimens. Table 1 shows the source of non-sterile isolates. Complete records of non-sterile site samples were not available for 4 of the 46 patients with confirmed candidaemia and these were excluded from this analysis. As 4 patients had mixed candidaemias, 46 blood isolates from 42 patients comprised the candidaemia subgroup. Therefore the final cohort comprised 970 patients; 42 with candidaemia and 928 with colonisation. Table 2 shows the demographic data for the total ICU/HDU cohort and the candidaemic group. There was no significant difference in either age or APACHEII score between the candidaemic patients and the total ICU/HDU cohort. However, candidaemic patients had a significantly longer duration of stay; mean (standard deviation (SD)) total cohort 7.4 (11.7); candidaemic group 24.5 (21.5); $p \leq 0.001$.

In candidaemic patients, the median number of blood cultures taken per patient, prior to the candidaemia, was 2 including the positive culture. Data is not available for the number of non-sterile samples taken from the 928 colonised patients. In the 42 candidaemic patients, a median of 12 sterile and non-sterile specimens (interquartile (IQ) range 22.5) were taken from each patient; for non-sterile samples only, the median value was 9.5 (IQ range 21.25). The median number of non-sterile specimens sent before detection of candidaemia was 3 (IQ range 9) from candidaemic patients with no prior colonisation and 20 (IQ range 21) from colonised candidaemic patients. The median time between the last positive non-sterile culture and first positive blood culture was 4 days (range 1–13) for isolates of the same *Candida* spp. and 14.5 days (range 12–17) where isolates were of different species.

Table 1 Source of non-sterile isolates of *Candida* spp.

Specimen type	No of samples	% of 1062 samples
Sputum/tracheal secretions	359	33.8
Throat/oral swab	290	27.3
Urine	158	14.9
Nasal swab	69	6.5
Rectal swab	53	5.0
Skin swab	51	4.8
Penile/Vaginal/perineal swab	24	2.3
Pleural or abdominal drain fluid	20	1.9
Wound swab	16	1.5
Bronchoalveolar lavage fluid	11	1.0
Tracheostomy-site swab	7	0.7
Line swab	4	0.4

Distribution of *Candida* spp. from blood and non-sterile isolates

The distribution of species from blood and non-sterile sites are shown in Table 3.

A significantly higher proportion of *Candida* spp. from non-sterile sites were *C. albicans* compared with blood ($\chi^2 = 15.67$, df = 1, $p < 0.001$); however a significantly higher proportion of *Candida* spp. from blood were *Candida glabrata* compared with non-sterile sites ($\chi^2 = 25.21$, df = 1, $p < 0.001$). There was no significant difference, between blood and non-sterile sites, in the proportion of non-albicans/non-glabrata species isolated ($p = 0.88$).

Trends over time in species distribution

Although there was a significant difference in the proportion of glabrata isolates detected from non-sterile sites between the years investigated ($\chi^2 = 12.10$, df = 4, $p = 0.017$), there was no linear trend to the difference ($\chi^2 = 0.53$, df = 1, $p = 0.47$) indicating overall no progressive change in the proportion of isolates with time.

No difference was found in the proportion of *C. glabrata* isolates detected from blood over time ($\chi^2 = 1.28$, df = 4, $p = 0.87$). Likewise, there was no significant difference over time in the proportion of either *C. albicans* or non-albicans/non-glabrata isolates from blood or non-sterile sites.

Fluconazole sensitivity

Of the 46 blood isolates, 15 were resistant or susceptible dose-dependent (SDD) to fcz. All but one of these was *C. glabrata*.

A significantly greater proportion of *Candida* isolates from non-sterile sites were fcz-sensitive compared with blood ($\chi^2 = 25.10$, df = 1, $p < 0.001$) (Table 3). There was no significant difference between blood and non-sterile sites in the proportion of fcz-sensitive isolates for each *Candida* spp. (albicans, glabrata and non-albicans/non-glabrata). There was no trend in fcz-sensitivity over time in either blood ($\chi^2 = 0.14$, df = 1, $p = 0.71$) or non-sterile ($\chi^2 = 2.12$, df = 1, $p = 0.15$) isolates.

Candida colonisation preceding candidaemia

In only 13 (31%) patients with candidaemia, was there prior isolation by the same *Candida* spp. of 2 or more non-sterile

Table 2 Admissions to ICU/HDU.

Year of admission	No of admissions	Mean age in years (SD)	% Male; female	Mean APACHEII (SD)	Mean length of stay in days (SD)
1999	712	52.6 (19.2)	58.3; 41.7	21.9 (7.9)	6.2 (10.5)
2001	644	52.4 (19.6)	62.4; 37.6	18.0 (6.9)	6.9 (9.9)
2003	559	52.8 (19.2)	62.6; 37.4	17.5 (7.5)	9.5 (15.3)
2005	807	53.4 (20.0)	62.2; 37.8	17.9 (7.2)	7.3 (12.1)
2007	778	54.7 (19.8)	61.3; 38.7	17.9 (7.1)	7.5 (10.4)
Total cohort	3500	53.2 (19.7)	61.3; 38.7	18.7 (7.5)	7.4 (11.7)
Candidaemic patients	42	55.9 (18.9)	61.9; 38.1	20.5 (6.4)	24.5 (21.5)

non-contiguous sites; a further 7 patients were colonised at one site.

Eighteen (43%) patients developed candidaemia without evidence of preceding colonisation of non-sterile sites by any *Candida* spp.. Four patients with candidaemia had colonisation of 2 or more non-contiguous sites with a different *Candida* spp. (discordant colonisation). Of the patients with discordant colonisation, 2 were colonised with fcz-sensitive *C. albicans* but developed candidaemia with a fcz-resistant species. Fig. 1 illustrates the distribution of *Candida* colonisation in patients with subsequent candidaemia at the time of the first episode of candidaemia.

C. glabrata candidaemia

Sixteen patients had *C. glabrata* candidaemia of which only 2 were fcz-sensitive.

Table 4 shows non-sterile colonisation preceding *C. glabrata* and non-glabrata candidaemia.

A significant difference was detected in the total number of samples sent from patients with *Candida glabrata* candidaemia (median 5.5 (IQ range 9.25)) compared with patients with non-glabrata candidaemia (median 19 (IQ range 22), $p = 0.002$). Likewise a significant difference was detected in the number of non-sterile samples sent from these patient groups (*C. glabrata* candidaemia: median 3.5 (IQ range 9.25); non-glabrata candidaemia: median 14 (IQ range 22); $p = 0.003$). However, patients with non-glabrata candidaemia had a significantly longer duration of stay than patients with *C. glabrata* candidaemia ($p = 0.02$).

C. glabrata candidaemia was significantly less likely than non-glabrata candidaemia to be preceded by colonisation of 2 or more non-sterile sites with the same species (2 sites: 1/16 vs 12/30; $\chi^2 = 4.32$, df = 1:$P = 0.04$).

Table 3 Distribution and fcz-sensitivity of *Candida* spp. from blood and non-sterile sites.

	Blood	Non-sterile
No of *C. albicans* isolates (%)	25 (54.3)	848 (79.8)
No of *C. glabrata* isolates (%)	16 (34.8)	106 (10.0)
No of non-albicans/non-glabrata isolates (%)	5 (10.9)	108 (10.2)
No fcz-sensitive (%)	31 (67.4)	961 (90.5)
No fcz SDD/resistant (%)	15 (32.6)	101 (9.5)

Of *C. glabrata* candidaemias, 75% occurred with no prior evidence of colonisation; in 67% of patients with candidaemia in the absence of colonisation, the species detected was *C. glabrata*.

Discussion

In this retrospective study 65.7% of candidaemias were caused by non-albicans species, a figure in keeping with the SENTRY surveillance programme.[10] *C. glabrata* was the most frequent isolate from both blood and non-sterile sites after *Candida albicans*, in agreement with a previous large study,[11] although the authors did not investigate the relationship between colonising species and those causing subsequent invasive infection. In this study the proportion of *Candida* spp. isolated from blood and non-sterile sites was significantly different, with a higher proportion of *C. glabrata* isolates detected from blood compared with non-sterile specimens whereas a higher proportion of *Candida* isolates from non-sterile sites were *C. albicans*; a finding reflected in the proportion of fcz-sensitive isolates from these sites, with a higher proportion of isolates from blood being fcz-resistant compared with non-sterile isolates. This cannot be explained by the hypothesis that *C. albicans* colonisation leads to pre-emptive fcz therapy and the selection of non-albicans/fcz-resistant isolates, increasing the likelihood that subsequent candidaemias are due to a fcz-resistant species[12–14]; in this study only 2-patients (4.8%) who were colonised at 2 or more non-sterile sites with a fcz-sensitive species developed candidaemia with a fcz-

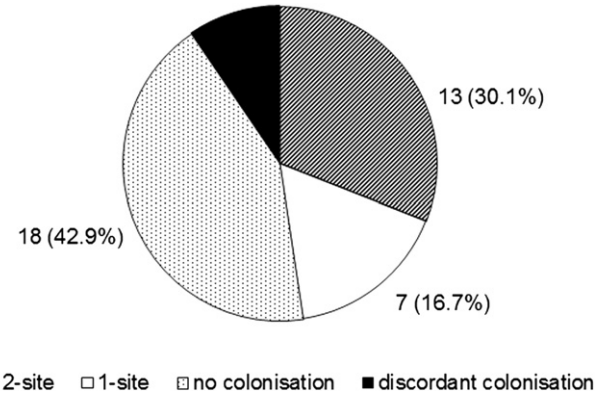

Figure 1 Number of candidaemic patients (%) with prior non-sterile site colonisation at first candidaemic episode.

Table 4 Non-sterile colonisation preceding each *C. glabrata* and non-glabrata candidaemia.

No of sites colonised prior to blood culture isolate	No of *C. glabrata* candidaemias ($n = 16$)	No non-glabrata candidaemias ($n = 30$)
≥2 sites same *Candida* spp.	1	12
≥1sites other *Candida* spp.	1	3
1 sites same *Candida* spp.	2	7
0 sites	12	8

resistant species; furthermore *C. glabrata* candidaemia was significantly more likely to occur in the absence of non-sterile site colonisation by any species. However, as the aim of the screening policy was to detect MRSA, blood agar rather than CAN2 chromogenic agar was used as the primary isolation medium. It is therefore likely that this reduced overall the detection of *Candida* from non-sterile sites, and potentially leads to a detection bias towards *C. albicans* isolation from non-sterile sites, due to the characteristic colonial morphology.

No trend was detected over time in either the proportion of *C. glabrata* or *C. albicans* isolated from blood or non-sterile sites, or in fcz-sensitivity of these isolates, despite the formal introduction of a pre-emptive anti-fungal therapy policy at our institution in 2005. This is unexpected; it was hypothesised that there would be a species shift towards fcz-resistant species. This could be explained if *C. albicans* candidaemia had occurred in the absence of colonisation and thus in patients not exposed to pre-emptive fcz therapy; however this seems improbable as in this study non-glabrata candidaemia was significantly more likely to be preceded by 2-site colonisation than *C. glabrata* candidaemia, which was more likely to occur in the absence of non-sterile site colonisation by any species. Previous studies have demonstrated a decrease in invasive fungal infections with pre-emptive or prophylactic fcz therapy; however they failed to provide convincing evidence of an increase in the incidence of invasive disease or colonisation by fcz-resistant *Candida* spp..[7,15] Nevertheless the possibility of such a relationship cannot be ruled out[15]; data from the US indicated a decrease in the incidence of candidaemia during the last decade, mainly due to a reduction in *C. albicans* candidaemia but with an increase in the proportion of candidaemias caused by non-albicans species.[16] In addition, recent studies have raised the possibility that preceding antimicrobial therapy have a role in the development of candidaemia.[17,18] Hence further work is required to elucidate the relationship between prophylaxis and subsequent *Candida* colonisation and invasive disease.

Of the 16 *C. glabrata* candidaemias, 4 occurred in 2001; 3 in 2003; 1 in 2005; and 8 in 2007. Isolates in 2001 and 2003 occurred in different calendar months, separated by a minimum of 19 days. This is unlikely to be caused by cross-infection by health-care workers due to the transient carriage of yeasts on the hands of healthy workers, and the time period over which the isolates were detected. In 2007, 3 blood isolates were detected over an 11-day period in May—June, and in October, 2 *C. glabrata* candidaemias occurred on the same day. In these instances, health-care associated spread cannot be excluded. Exogenous transmission of *Candida* spp. has been reported,[19–21] although endogenous colonisation is thought to cause most candidiasis.[19,22,23]

Candida colonisation of multiple sites is an independent risk factor for invasive disease and it has been suggested that colonisation of 2 or more non-contiguous sites in a high risk patient is sufficient to initiate treatment.[8,24–26] In this retrospective study, only 31% of patients who developed candidaemia would have received appropriate pre-emptive treatment based on colonisation of 2 non-contiguous sites, whereas 20 patients (48%) would have received either inappropriate or anti-fungal therapy as they had either no colonisation or candidaemia with a fcz-resistant species despite colonisation with fcz-sensitive species. This finding is at odds with the results of a previous prospective study by Pittet et al.,[4] which found that all patients who developed invasive infections were colonised previously with genetically identical species. This differing result may be due to the systematic prospective sampling and daily calculation of colonisation index by Pittet et al.,[4] a method which could not be employed in this study, where colonisation in the candidaemic subgroup was investigated retrospectively. In this current study a further 7 patients (16.7%) had colonisation of only one non-sterile site, and therefore would have been unlikely to receive pre-emptive therapy if based on colonisation of 2 or more non-contiguous sites alone. Therefore not only does pre-emptive therapy based upon colonisation of at least two sites inadequately select patients at high risk of candidaemia but, based on the results of this study, it appears that colonisation is not a necessary pre-requisite for invasive infection, as was previously thought.[4]

More recently colonisation index (ratio of the number of different body sites colonised by *Candida* spp. to the total number of body sites cultured)[4] has been proposed as a method of detecting which at-risk, colonised patients would benefit from pre-emptive anti-fungal therapy.[4,5,7,27] It has been shown to have a higher positive predictive value for subsequent invasive *Candida* disease than 2-site colonisation,[4] although should be incorporated into overall clinical evaluation of an at-risk patient, rather than used as a definitive test of need for pre-emptive therapy.[28] However, in this study even if colonisation index was used to determine pre-emptive therapy, at least 43% of patients who developed candidaemia would not have received pre-emptive therapy. It is possible that colonisation may be a more robust indicator of subsequent candidaemia for non-glabrata species; in this study, in 67% of patients with de novo candidaemias the species detected was *C. glabrata*. This implies that even if pre-emptive anti-fungal therapy was initiated in patients with no evidence of colonisation, but who had other risk factors for invasive disease,[29] if fcz was prescribed the therapy would

be inappropriate as non-colonisation appears to be a feature of *C. glabrata* invasive disease. In this study, patients with *C. glabrata* candidaemia had significantly fewer non-sterile specimens sent than patients with non-glabrata candidaemia. However, non-sterile sampling is confounded by duration of ICU stay, with *C. glabrata* candidaemias occurring earlier in the patients' admission compared with non-glabrata candidaemias, therefore it is not unexpected that these patients had fewer non-sterile samples sent. *C. glabrata* may be inherently more pathogenic than other *Candida* spp. and so more likely to cause invasive disease than colonisation in critically ill patients, although this hypothesis is not borne out by mortality rates from candidaemia due to *C. glabrata* compared with *C. albicans*.[30] Alternatively the patients who develop *C. glabrata* candidaemia without prior colonisation may represent a more severely debilitated subgroup of the ICU population. In this study, candidaemic patients had a significantly longer ICU/HDU admission compared with the total cohort, which could denote a more debilitated group.

Previous studies investigating colonisation index have not explored its predictive value according to species[4,5,7,27]; further work is needed to explore the relationship between non-sterile site colonisation, patient risk factors and *C. glabrata* candidaemia.

In conclusion, this study suggests that despite the introduction of pre-emptive anti-fungal treatment, there has been no increase in the proportion of either non-albicans or fcz-resistant species isolated from blood or non-sterile sites.

However pre-emptive therapy based upon 2-site colonisation or colonisation index may be inadequate as almost half of candidaemic patients in this study had no prior colonisation; two thirds of these candidaemias were due to *C. glabrata*, which is more likely to present as candidaemia without evidence of colonisation. Non-glabrata candidaemias are more likely to be preceded by 2-site colonisation; therefore colonisation index may be a more robust predictor of invasive disease by non-glabrata *Candida* spp.. Further work is needed to elucidate the relationship between non-sterile site colonisation and *C. glabrata* candidaemia.

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