Colonization by *Candida* in children with cancer, children with cystic fibrosis, and healthy controls

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Abstract

A longitudinal, prospective study was conducted intermittently in Norway, from 1999 to 2008, to investigate the *Candida* colonization rates and species distributions in the tonsillopharyngeal and faecal flora in: (i) children with cancer; (ii) children with cystic fibrosis (CF); and (iii) healthy children. The effect of antibiotic treatment on *Candida* colonization was also studied, and we looked for changes in antifungal susceptibility over time within each child and between the different groups of children. In total, 566 tonsillopharyngeal swabs and 545 faecal samples were collected from 45 children with cancer, 37 children with CF, and 71 healthy, age-matched controls. The overall colonization rate with *Candida* was not significantly higher in the two groups of children undergoing extensive treatment with broad-spectrum antibiotics than in healthy controls. Approximately one-third of the cancer patients had a total lack of *Candida* colonization or had only one *Candida*-positive sample, despite multiple samples being taken, treatment with broad-spectrum antibiotics, long hospital stays, and periods with neutropenia. Children with CF had the highest prevalence of *Candida albicans*. Amoxycillin, azithromycin, third-generation cephalosporins and oral vancomycin resulted in a significantly increased *Candida* colonization rate. Phenoxymethylpenicillin, second-generation cephalosporins, metronidazole, trimethoprim–sulphamethoxazole, ciprofloxacin, penicillinase-resistant penicillins and inhaled tobramycin or colistin showed minimal effects on the *Candida* colonization rate. We found no evidence of development of antifungal resistance over time.

Keywords: Cancer, *Candida*, children, colonization, cystic fibrosis

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Background

Children with cancer often undergo chemotherapy, causing severe immunosuppression and neutropenia. Consequently, they are at great risk for serious infections, including invasive fungal infections. If there is any sign of febrile neutropenia, they should be promptly treated with broad-spectrum antibiotics [1]. Diagnosis of a *Candida* infection in these patients is difficult. Blood cultures are often negative, and other diagnostic methods are unreliable. It has been shown that patients with severe *Candida* infections are usually colonized by *Candida* at multiple body sites [2–4]. Also, invasive *Candida* infections regularly occur with the same genotypes as those colonizing the patient [5,6].

Knowledge about the *Candida* flora in children undergoing repeated antibiotic treatment as compared with healthy children is important, e.g. when considering treatment strategies based on the use of surveillance cultures. Children with cancer receive more broad-spectrum antibiotics than most other children. Likewise, children with cystic fibrosis (CF) receive large quantities of antibiotics, usually throughout their lives [7]. Although children with CF have no particular
risk for invasive yeast infections, some studies have shown that airway colonization with Candida may cause symptoms in CF patients [8].

As few studies have focused on the Candida flora in different categories of sick and healthy children, we have examined Candida colonization prospectively and longitudinally in three groups: (i) children with cancer; (ii) children with CF; and (iii) healthy children. The main aim was to investigate and compare the Candida colonization rates and species distributions in the tonsillopharyngeal and faecal flora. Subsequently, we studied the effect of antimicrobial treatment on Candida colonization, and looked for changes in antifungal susceptibility over time.

Materials and Methods

Subjects and clinical information
Serial tonsillopharyngeal swabs (n = 566) and faecal samples (n = 545) were collected from 45 children with cancer at Oslo University Hospital, Norway, during 1999–2000 and 2003–2005, from 37 children with CF at the paediatric outpatient clinic, Oslo University Hospital, during 2004–2008, and from 70 healthy children at day-care centres and schools during 2000–2001 and 2006–2008. Inclusion criteria comprised newly diagnosed cancer that called for at least one course of chemotherapy, a diagnosis of CF, and healthy status with no underlying chronic disease. The number of samples within each group and time interval between first and last sample are shown in Table 1. For each sample, the child’s parents were asked about previous antimicrobial treatment. Hospital medical records for the children with cancer and CF were reviewed for possible invasive fungal disease and information about all antimicrobial treatment in the months before the first sample and during the time of sampling for each individual. Written, informed consent was obtained from the subjects’ parents, and from the subjects themselves when they were 12 years or older. The study was reviewed by the Regional Ethics Committee of South-East Norway.

Sampling, yeast identification, and susceptibility testing
Tonsillopharyngeal swabs (both tonsils and the posterior pharyngeal wall were swabbed) were put directly into transport medium and plated within 5 h after collection. Stool samples, mixed directly into Cary–Blair transport medium, were plated within 5 days. The specimens were inoculated onto Sabouraud dextrose agar containing antibiotics, and incubated at 28°C for a maximum of 5 days. From 2004, CHROMagar Candida (Becton Dickinson, Sparks, MD, USA) was also used. Yeast growth was semiquantitatively noted as none, light (<10 colonies), moderate (10–50 colonies), or heavy (>50 colonies). Morphologically distinct colonies were picked for species identification, performed at the Norwegian Mycological Reference Laboratory, Oslo. Conventional methods were used, e.g. colony colour on chromogenic agar, germ-tube tests, microscopic morphology on corn-meal agar, carbohydrate fermentation and assimilation, urease activity, and morphology on Pal’s agar [9]. Additional identification methods, such as VITEK 2 (bio-Mérieux, Marcy L’Étoile, France), API 32C (bio-Mérieux), an in-house 18S rDNA PCR method, based on Turin et al. [10], and an in-house internal transcribed spacer method based on Ahmad et al. [11], were used when necessary. All non-albicans Candida strains and

### TABLE 1. Background information and rates of Candida colonization in children with cancer, children with cystic fibrosis (CF), and healthy controls, Norway

<table>
<thead>
<tr>
<th>Variable* (n = 45)</th>
<th>Cancer* (A) (n = 45)</th>
<th>CF (B) (n = 37)</th>
<th>Controls (C) (n = 70)</th>
<th>p-values*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>5.3 (0–14)</td>
<td>6.8 (1–16)</td>
<td>6.1 (1–16)</td>
<td>0.30</td>
</tr>
<tr>
<td>Female sex</td>
<td>23 (51)</td>
<td>18 (49)</td>
<td>38 (54)</td>
<td>0.74</td>
</tr>
<tr>
<td>Indwelling central venous catheter</td>
<td>44 (98)</td>
<td>7 (19)</td>
<td>0 (0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Chronic lung infectionb</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>ND</td>
</tr>
<tr>
<td>Invasive fungal disease</td>
<td>3 (6.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>ND</td>
</tr>
<tr>
<td>Time-spanb</td>
<td>10.2 (3–22)</td>
<td>16.4 (7–33)</td>
<td>14.3 (3–26)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tonsillopharyngeal swabs per individual</td>
<td>6.2 (1–13)</td>
<td>3.4 (3–6)</td>
<td>2.4 (1–4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Faecal samples per individual</td>
<td>6.3 (2–15)</td>
<td>2.9 (1–5)</td>
<td>2.5 (1–4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Condido colonization, tonsillipharynx</td>
<td>26 (59)</td>
<td>14 (38)</td>
<td>19 (27)</td>
<td>0.04</td>
</tr>
<tr>
<td>Condido colonization, faeces</td>
<td>32 (71)</td>
<td>24 (73)</td>
<td>35 (52)</td>
<td>0.72</td>
</tr>
<tr>
<td>Condido colonization, either site</td>
<td>35 (78)</td>
<td>25 (68)</td>
<td>38 (54)</td>
<td>0.60</td>
</tr>
</tbody>
</table>

*Continuous data are presented as mean (range) and categorical data as number (%).

*Diagnoses: acute lymphoblastic leukaemia (n = 19), acute myeloblastic leukaemia (n = 6), non-Hodgkin’s lymphoma (n = 6), and various solid tumours (n = 14).

*When numbers were too small, the p-value was not determined (ND).

*Pseudomonas aeruginosa (n = 4), Burkholderia cepacia (n = 2), atypical mycobacterium (n = 1).

*Mean number of months between first and last sample; four controls missing as they provided only one set of samples.

*Number of children with at least one Candida-positive tonsillopharyngeal swab (one child with cancer did not provide samples).

*Number of children with at least one Candida-positive faecal sample (four children with CF and three healthy controls did not provide samples).
some *Candida albicans* strains were re-examined with matrix-assisted laser desorption ionization time-of-flight mass spectrometry [12] in 2010.

The *Candida* isolates were collectively tested, in a blinded fashion, for antifungal susceptibility with the Etest method (bio-Mérieux). Only the first and the last isolates were tested when a child provided more than two positive cultures with the same species.

**Analysis of antibiotic effect**

All samples were examined individually to look for differences in the rates of *Candida* colonization according to the type of antibiotic used: (i) we included samples taken during antibiotic treatment of a minimum of 3 days in duration, and samples were included up to 5 days after antibiotic treatment was stopped; (ii) we excluded samples taken after 1–2 days of antibiotic treatment and samples where antibiotic treatment had been stopped for only 6–20 days, to ensure that samples with unknown antibiotic effects were not used; and (iii) we excluded all samples taken during antifungal treatment, or <21 days after antifungal treatment had been stopped, to avoid antifungal influence.

**Statistics**

SPSS software (version 18.0) was used for all statistical analyses. For comparison of categorical variables in different groups, the Pearson chi-square test was used. For comparison of continuous variables in two different groups, an independent-samples t-test was used. As the number of samples per individual in the cancer group was much greater than in the two other groups, we used linear regression to adjust for number of samples. A 5% significance level was used.

**Results**

Background information and *Candida* colonization rates are presented in Table 1. The rate of *Candida* colonization was not different between the 31 children with haematological cancer and the 14 children with solid tumours (p 0.93), between females and males (p 0.48), or between the two time periods for sampling (p 0.80). The mean age was similar for the colonized (5.9 years) and non-colonized (6.3 years) children (p 0.59). The rate of heavy *Candida* load is shown in Table 2.

Three (6.6%) cancer patients suffered invasive fungal infections: one patient with *C. albicans* candidaemia; one patient with *C. tropicalis* candidaemia and radiological changes compatible with hepatosplenic candidiasis; and one patient who had ultrasonographic and magnetic resonance imaging findings indicative of disseminated fungal infection with lesions in the brain and liver, although blood cultures were negative. No invasive yeast infections occurred in the children with CF or the healthy controls.

**Antibiotic consumption and *Candida* colonization**

All children with cancer and CF received antibiotic treatment during the observation period (Table 4). Only six (8.6%) healthy controls were treated with antibiotics less than 40 days prior to sampling (oral phenoxymethylpenicillin or amoxycillin). Thirty-five (50%) healthy children had never received antibiotics.

**TABLE 3. *Candida* species distribution in the *Candida*-positive children with cancer, children with cystic fibrosis (CF), and healthy controls, Norway**

| Candida species | Cancer (A) (%) | CF (B) (%) | Controls (C) (%) | p-values
|-----------------|---------------|------------|-----------------|--------
| A vs. B | A vs. C | B vs. C |
|----------------|---------------|------------|-----------------|--------|
| *C. albicans* | 22 (63) | 23 (92) | 27 (71) | 0.09 | 0.46 | 0.04 |
| Non-albicans | 20 (57) | 8 (32) | 18 (47) | 0.89 | 0.40 | 0.23 |
| *C. parapsilosis* | 16 (46) | 11 (40) | 11 (29) | 0.04 | 0.14 | 0.01 |
| *C. lusitanei* | 2 (5.7) | 1 (4.0) | 3 (7.9) | ND | ND | ND |
| *C. dublinensis* | 4 (11) | 1 (4.0) | 0 (0) | ND | ND | ND |
| *C. krusei* | 0 (0) | 1 (4.0) | 1 (2.6) | ND | ND | ND |
| *C. tropicalis* | 2 (5.7) | 1 (4.0) | 0 (0) | ND | ND | ND |
| *C. famata* | 1 (2.9) | 0 (0) | 0 (0) | ND | ND | ND |
| *C. guilliermondii* | 2 (5.7) | 0 (0) | 0 (0) | ND | ND | ND |
| *C. magnoliae* | 2 (5.7) | 0 (0) | 0 (0) | ND | ND | ND |
| *C. sphaerica* | 0 (0) | 2 (5.3) | 0 (0) | ND | ND | ND |
| *Pichia guilliermondii* complex | 1 (2.9) | 0 (0) | 1 (2.6) | ND | ND | ND |
| Other *Candida* spp. | 0 (0) | 0 (0) | 2 (5.3) | ND | ND | ND |

| p-values
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A vs. B</td>
<td>A vs. C</td>
<td>B vs. C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>---------------</td>
<td>------------</td>
<td>-----------------</td>
<td>--------</td>
</tr>
</tbody>
</table>

*Number (%) of children with at least one positive culture.

Owing to small numbers, many of the p-values were not determined (ND).

Originally identified as *C. famata* and *C. guilliermondii*. Internal transcribed spacer results listed them as possible *Meyerozyma caribbica/Pichia caribbica/Candida carpophila/Candida fukuyamensis.*

*C. intermedia (n = 1), C. pulcherrima (n = 1).*
We found no or minimal effect on Candida colonization of phenoxy-methylpenicillin, second-generation cephalosporins, metronidazole, trimethoprim–sulphamethoxazole, ciprofloxacin, clonazolin, dicloxacillin, or inhaled tobramycin or colistin. Meropenem was always used in combination with other agents, and was consequently not included in the analyses. Amoxicillin, azithromycin, third-generation cephalosporins and oral vancomycin were predisposing antibiotics (PAs) for Candida colonization. In the tonsillopharynx, the children on a PA had significantly higher Candida colonization rates (11/19) than those without antibiotics (26/129, p <0.01) and those on antibiotics other than a PA (6/29, p 0.01). In faeces, children on a PA had significantly more samples with a heavy Candida load in faeces (11/17) than children without antibiotics (16/46, p 0.03). The difference in heavy load was non-significant in the tonsillopharynx (p 0.98).

The use of antifungals is shown in Table 4. In the cancer group, we observed no significant decrease in Candida colonization rates in the 13 children on oral nystatin prophylaxis as compared with the remaining 32. Twenty tonsillopharyngeal swabs and 26 faecal samples were collected during ongoing systemic antifungal treatment from 11 and nine children, respectively. We found no significant difference in Candida colonization rates between these samples and the samples from cancer patients without antifungal treatment. A change in Candida species after antifungal treatment was observed in three children. Only one acquired a more resistant species (Candida guilliermondii).

### TABLE 4. Antibiotic and antifungal consumption during the study period in the study groups of children with cancer and children with cystic fibrosis (CF), Norway

<table>
<thead>
<tr>
<th>Type of antimicrobial</th>
<th>Cancer (n = 45)</th>
<th>CF (n = 37)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%) of individuals</td>
<td>Mean no. (range) of courses</td>
<td>No. (%) of individuals</td>
</tr>
<tr>
<td>Phenoxymethylpenicillin</td>
<td>6 (13)</td>
<td>1.2 (1–2)</td>
<td>10 (27)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>5 (11)</td>
<td>1.0 (1)</td>
<td>24 (65)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>38 (84)</td>
<td>3.6 (1–9)</td>
<td>1 (2.7)</td>
</tr>
<tr>
<td>Dicloxacillin or clonazolin</td>
<td>12 (27)</td>
<td>1.2 (1–2)</td>
<td>17 (46)</td>
</tr>
<tr>
<td>Second-generation cephalosporins</td>
<td>21 (47)</td>
<td>1.5 (1–3)</td>
<td>23 (62)</td>
</tr>
<tr>
<td>Third-generation cephalosporinsb</td>
<td>29 (64)</td>
<td>2.6 (1–8)</td>
<td>12 (32)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>8 (18)</td>
<td>1.3 (1–3)</td>
<td>5 (14)</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>38 (84)</td>
<td>3.7 (1–10)</td>
<td>15 (41)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>19 (42)</td>
<td>2.1 (1–5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Oral vancomycinc</td>
<td>2 (4.4)</td>
<td>1.5 (1–2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>24 (53)</td>
<td>2.3 (1–6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Trimethoprim–sulphamethoxazolee</td>
<td>38 (84)</td>
<td>ND</td>
<td>25 (68)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>0 (0)</td>
<td>0</td>
<td>15 (41)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>6 (13)</td>
<td>1.0 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>2 (4.4)</td>
<td>2.0 (2)</td>
<td>13 (35)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0 (0)</td>
<td>0</td>
<td>13 (35)</td>
</tr>
<tr>
<td>Inhaled tobramycin or colistin</td>
<td>0 (0)</td>
<td>0</td>
<td>14 (38)</td>
</tr>
<tr>
<td>Other antibiotics</td>
<td>4 (16)</td>
<td>1.0 (1)</td>
<td>6 (16)</td>
</tr>
<tr>
<td>Fluconazolef</td>
<td>22 (49)</td>
<td>2.1 (1–7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>8 (18)</td>
<td>1.5 (1–5)</td>
<td>1 (2.7)</td>
</tr>
<tr>
<td>Voriconazolef</td>
<td>2 (4.4)</td>
<td>1.0 (1)</td>
<td>1 (2.7)</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>0 (0)</td>
<td>0</td>
<td>1 (2.7)</td>
</tr>
<tr>
<td>Mycostatin</td>
<td>20 (44)</td>
<td>ND</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

aNumber (no.) of individuals with at least one course of treatment, and mean no. (range) of courses per individual. Treatment for less than 2 days was excluded. When both short courses and long-term treatment and/or prophylaxis were included, the number of courses per individual was not determined (ND). Owing to small numbers, some of the p-values were not determined (ND).
bCeftazidime, cefotaxime and ceftriaxone in the cancer group; only ceftazidime in the CF group.
cTwo children were treated with oral vancomycin, owing to prolonged hospital-acquired diarrhoea (Clostridium difficile).
dAll three children received more than 2 months of voriconazole treatment.
eFour of the children received several months of fluconazole treatment.

aCandida albicans, Candida glabrata, Candida dubliniensis, Candida glabrata, Candida tropicalis, and Candida krusei. Only one acquired a more resistant species (Candida guilliermondii).
A striking result of this study, comprising 1111 samples, is that the overall Candida colonization rate was not significantly higher in the two groups of children undergoing extensive treatment with broad-spectrum antibiotics than in the healthy controls, who received minimal or no antibiotic treatment. This was unexpected, as treatment with broad-spectrum antibiotics is generally regarded as a predisposing factor for increased colonization by Candida. Many studies have found higher colonization rates in patients than in healthy individuals [13]. However, longitudinal studies are scarce. Agirbasli et al. [14] found that the colonization rate among 80 hospitalized children with repeated sampling was higher than in 61 healthy control children sampled only once. However, the lack of multiple samples in the control group may have led to an underestimation of the rate of colonization in these children. In our study, the only significant differences found were higher Candida colonization rates in the faeces of children with CF and in the tonsillopharynx of children with cancer than in the healthy controls. Interestingly, 13% of children with cancer and repeated episodes of neutropenia, broad-spectrum antibiotic treatment, long hospital stays and multiple samplings for Candida colonization remained negative, and 16% had only one positive sample. This strongly suggests that there are individual mechanisms contributing to Candida colonization resistance.

Several molecular studies have shown that gastrointestinal colonization with Candida precedes invasive disease [5,6,15]. In our study, the prevalence of documented invasive fungal infections was only 6.6%. The two children with candidae-mias had the same species in their blood culture as in their tonsillopharynx and/or faeces, suggesting a common origin. Although controversial, surveillance cultures may be of great value in high-risk patients if used appropriately [2–4,16]. Our study shows that children with cancer are often colonized with Candida. Thus, an isolated Candida-positive oropharyngeal or faecal sample does not call for antifungal treatment. However, one might consider collecting surveillance cultures from certain high-risk children, e.g. prior to certain chemotherapy courses that regularly cause severe mucositis and prolonged neutropenia.

Candida colonization varies with the type of antibiotic used. This has been shown in human and mouse studies [17–20]. Children were investigated in one small study [21]. Antibiotics with a high level of biliary excretion, suppression of anaerobes and a broad spectrum of action tend to promote faecal overgrowth of yeasts. We were able to analyse more than 1000 samples from 172 children with detailed information about antibiotic and antifungal treatment. We identified some antibiotics, including amoxycillin, azithromycin, third-generation cephalosporins, and oral vancomycin, that seemed to have a much greater impact on the Candida colonization rates in the faeces and tonsillopharynx than other agents. Our semiquantitative results suggested that these agents also caused a heavier Candida load than no antibiotic treatment in the faecal flora. This is an important finding, as a heavy Candida load has been associated with risk of invasive disease [3,16].

The use of nystatin prophylaxis and other antifungal agents did not significantly reduce Candida colonization among our patients, as shown previously [22,23]. Some anticancer agents, e.g. cyclophosphamide and dexamethasone, have been shown to increase Candida colonization in mouse models [24,25]. Many of our cancer patients were treated with these

### Table 5. In vitro antifungal susceptibility in Candida isolates from children with cancer, children with cystic fibrosis (CF), and healthy controls, Norway

<table>
<thead>
<tr>
<th>Species (no. of isolates)</th>
<th>Antifungal agent</th>
<th>MIC range (mg/L)</th>
<th>MIC50 (mg/L)</th>
<th>MIC90 (mg/L)</th>
<th>% Resistant</th>
<th>Resistanta</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans (121)</td>
<td>FLC</td>
<td>0.064–0.5</td>
<td>0.25</td>
<td>0.25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C. parapsilosis (40)</td>
<td>FLC</td>
<td>0.125–1</td>
<td>0.25</td>
<td>0.25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C. dubliniensis (8)</td>
<td>FLC</td>
<td>0.125–1</td>
<td>0.25</td>
<td>0.25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C. lusitaniae (7)</td>
<td>FLC</td>
<td>0.25–1</td>
<td>0.5</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C. tropicalis (5)</td>
<td>FLC</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C. krusei (4)</td>
<td>FLC</td>
<td>16–32</td>
<td>16</td>
<td>32</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>C. guilliermondii complex (2)</td>
<td>FLC</td>
<td>0.5–1</td>
<td>0.5</td>
<td>1</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Pichia guilliermondii</td>
<td>FLC</td>
<td>&lt;0.064</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>C. famata (2)</td>
<td>FLC</td>
<td>0.25–4</td>
<td>0.25</td>
<td>0.25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C. magnoliar (2)</td>
<td>FLC</td>
<td>&gt;0.256</td>
<td>&gt;0.256</td>
<td>&gt;0.256</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>C. sphaerica (2)</td>
<td>FLC</td>
<td>0.064–0.125</td>
<td>0.064</td>
<td>0.125</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C. intermedia (1)</td>
<td>FLC</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C. pulcherrima (1)</td>
<td>FLC</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

AMB, amphotericin B; CAS, caspofungin; FLC, fluconazole.

*aClinical breakpoint (CBP) for FLC resistance according to EUCAST [28]: >4 mg/L for C. albicans, C. parapsilosis, and C. tropicalis; C. krusei is considered to be intrinsically resistant. CBP for CAS resistance according to the CLSI [29]: >6.5 mg/L for C. albicans, C. krusei, and C. tropicalis, and >4 mg/L for C. parapsilosis. The AMB breakpoint was set to resistance >1 mg/L according to the EUCAST tradition. For several species, the CBP has not been determined (ND).

All three C. guilliermondii isolates, one isolate from the C. guilliermondii complex and one C. sphaerica isolate showed heteroresistance to FLC. Only the highest MIC value is reported.
and other antineoplastic agents for several months. Thus, antineoplastic drugs may have contributed to an increased Candida colonization in our children with cancer.

C. albicans was the most frequent species in all study groups, and this correlates well with the species distribution in the Norwegian Candidaemia Registry [26,27]. The prevalence of C. albicans was especially high in the CF group. In Norway, fluconazole resistance in C. albicans is rare [26,27]. In our study, we found no evidence of an increase in antifungal drug resistance over time. The MIC values seemed to be stable within each individual, there were no apparent differences in susceptibility between 1999 and 2008, and we found no significant differences between the children with cancer, the children with CF, and healthy controls. Clinical breakpoints for fluconazole are not given for all species [28]. However, we found three C. guilliermondii isolates, one isolate from the C. guilliermondii complex and two C. magnoliae isolates with reduced fluconazole susceptibility, in addition to four C. krusei isolates with intrinsic resistance. Only one child with cancer had a species shift that could have been caused by antifungal treatment: after several courses of fluconazole, a change from a fluconazole-susceptible C. albicans isolate to a fluconazole-resistant C. guilliermondii isolate was observed. In the four children who received months of fluconazole and/or voriconazole treatment, we found no colonization with resistant Candida species. The two children who were colonized with an amphotericin B-resistant C. famata isolate never received antifungal treatment. Caspofungin was used in only one child with CF. The susceptibility data suggest that most of our isolates were susceptible to caspofungin, with MICs within their epidemiological cut-off values [29]. However, all four C. krusei isolates had higher MICs than the epidemiological cut-off value (0.25 mg/L). Whether this has any clinical importance is uncertain.

There are some weaknesses of this study. The relatively low number of children may cause some difficulties in determining significant differences, although multiple samples per individual partially compensates for the relatively low number of subjects. Also, the sampling spanned a 9-year period. There have been some changes in methods, e.g. the inclusion of a chromogenic agar, which makes it easier to detect mixed cultures. Finally, methods other than tonsillopharyngeal swabs may be more sensitive in determining the rate of Candida colonization in the oropharynx [13,30].

In conclusion, in this extensive longitudinal study, we found that both sick children undergoing repeated broad-spectrum antibiotic therapy and healthy children exposed to little or no antibiotic treatment are often colonized with Candida species. Most of the children had stable colonization over time with only one or two species. In addition, some children seemed to be almost resistant to Candida colonization, independently of antibiotic treatment and underlying disease. Amoxycillin, azithromycin, third-generation cephalosporins and oral vancomycin seem to increase both the rate and load of Candida colonization. We found no evidence of antifungal resistance development over time.

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Author Contributions

K. W. Gammelsrud: study design, data and specimen collection, laboratory work, data analysis, and manuscript (conception, design, writing, and revision). P. Sandven: manuscript (conception, design, writing, and revision). E. A. Heiby: study design and manuscript (conception, design, writing, and revision). L. Sandvik: statistical analysis of all the data and manuscript revision. P. Brandtzaeg: study design and manuscript (conception, design, writing, and revision). P. Gaustad: manuscript (conception, design, writing, and revision).

Transparency Declaration

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References