**Candida Colonization as a Risk Marker for Invasive Candidiasis in Mixed Medical-Surgical Intensive Care Units: Development and Evaluation of a Simple, Standard Protocol**

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Colonization with *Candida* species is an independent risk factor for invasive candidiasis (IC), but the minimum and most practicable parameters for prediction of IC have not been optimized. We evaluated *Candida* colonization in a prospective cohort of 6,015 nonneutropenic, critically ill patients. Throat, perineum, and urine were sampled 72 h post-intensive care unit (ICU) admission and twice weekly until discharge or death. Specimens were cultured onto chromogenic agar, and a subset underwent molecular characterization. Sixty-three (86%) patients who developed IC were colonized prior to infection; 61 (97%) tested positive within the first two time points. The median time from colonization to IC was 7 days (range, 0 to 35). Colonization at any site was predictive of IC, with the risk of infection highest for urine colonization (relative risk [RR] = 2.25) but with the sensitivity highest (98%) for throat and/or perineum colonization. Colonization of ≥2 sites and heavy colonization of ≥1 site were significant independent risk factors for IC (RR = 2.25 and RR = 3.7, respectively), increasing specificity to 71% to 74% but decreasing sensitivity to 48% to 58%. Molecular testing would have prompted a resistance-driven decision to switch from fluconazole treatment in only 11% of patients infected with *C. glabrata*, based upon species-level identification alone. Positive predictive values (PPVs) were low (2% to 4%) and negative predictive values (NPVs) high (99% to 100%) regardless of which parameters were applied. In the Australian ICU setting, culture of throat and perineum within the first two time points after ICU admission captures 84% (61/73 patients) of subsequent IC cases. These optimized parameters, in combination with clinical risk factors, should strengthen development of a setting-specific risk-predictive model for IC.

Invasive candidiasis (IC), particularly, candidemia, is a significant cause of mortality in critically ill patients, accounting for almost a third of nosocomial infections in intensive care units (ICUs) (1, 2). Early antifungal therapy reduces IC-related mortality and approximately halves the incidence of IC (3–8). Untargeted prophylactic antifungal use, however, is expensive, has the potential to cause adverse drug reactions, and may select for resistant fungal species (9).

Clinical risk prediction rules that identify the high-risk patients most likely to benefit from prophylaxis have been developed (10–16), but those studies have used different sets of predictors, including (a) clinical risk factors only and (b) clinical risk factors in combination with colonization indices (CIs). Those rules have been applied in various settings and have included assessment at different times postadmission to the ICU and different types of ICU (surgical ICUs only or mixed medical/surgical ICUs—defined as units that house both medical and surgical populations). These differences may explain why predictive models and algorithms have performed poorly outside their derivative populations (17).

Since IC is preceded by colonization of mucosal surfaces with the infecting strain (14, 18–20) and since colonization is an independent risk factor for IC (14, 19, 21, 22), it is logical that it should be incorporated into predictive models (15, 16). Using data from mixed medical/surgical ICUs in Australia, we demonstrated that the post hoc addition of colonization parameters to two published clinical risk factor-only predictive models improved their performance characteristics (17).

Although several studies examining colonization as a risk factor for IC have been published, the methodology has not been standardized and most were confined to surgical ICUs (14, 23–25).
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Study design. MATERIALS AND METHODS

25). Interstudy differences existed with respect to sites of sampling, use of convenience and/or specified sample sites, timing of first samples and frequency of sampling, quantification of colonization density, and culture media and methods used for species identification. Furthermore, nucleic acid-based methods of fungal identification were not included.

A systematic evaluation of Candida colonization in a prospective multicenter cohort study of Australian ICU patients was conducted between June 2007 and January 2012. The overall project aimed to develop and validate a clinical culture and colonization-based risk-predictive model suitable for use in mixed Australian medical and surgical ICUs. In this study, we determined the utility of culture on CHROMagar for semiquantitative estimates of Candida colonization (with parallel molecular testing performed on a subset of patients), the optimal timing of sample collection, and the minimum number of sites to be sampled. Our overall aim was to develop a protocol to optimize detection of colonization status for inclusion into risk-predictive models of IC in critically ill patients.

MATERIALS AND METHODS

Study design. A total of 6,015 nonneutropenic critically ill patients admitted consecutively to mixed medical and surgical ICUs in seven Australian hospitals (Westmead, Sydney [n = 1,163]; Princess Alexandra, Brisbane [n = 1,231]; Royal Brisbane Women’s and Children’s, Brisbane [n = 896]; St Vincent’s Hospital, Sydney [n = 611]; Royal Melbourne Hospital, Melbourne [n = 1,227]; Concord Hospital, Sydney [n = 371]; and Nepean Hospital, Sydney [n = 516]) for at least 72 h were studied prospectively from 15 June 2007 to 1 January 2012. Patients with neutropenia (absolute neutrophil count < 0.5 × 10^9/liter) within the first 72 h of ICU admission were excluded. Approval for the study was obtained from each respective Human Research Ethics Committee.

Surveillance specimen collection. Published literature (23) and a preliminary study at the Westmead Hospital site suggested that the throat, the perineum (rather than the groin or rectum), and urine were the most accessible, feasible, and highest-yield sites for the assessment of Candida colonization. Sampling techniques were standardized across sites. In brief, standard sterile red-top culturette swabs containing liquid Amies media (Copan, Murrieta, CA, USA) were used to sample the oropharynx and perineum (just anterior to the anus), and catheter (or midstream) urine specimens were obtained. Specimens were obtained independently of any clinically indicated samples. Preliminary experiments (performed prior to the commencement of this study) showed that sampling the throat and perineum with dry swabs followed by immersion in 2 ml nutrient broth at 35°C for 48 h was more sensitive and specific than sampling the oral cavity (24). Two sites (throat and perineum) were sampled at the commencement of this study) showed that sampling the throat and perineum provided suitable material for detection of Candida colonization; the sites were selected on the basis of their accessibility in critically ill patients and the most frequent locations of Candida colonization (25). Swabs of the throat and perineum obtained from the first samples were also plated onto blood agar (CHROMagar, Paris, France) using a half-plate quantitative urine streaking method. Plates were incubated at 35°C for 48 h. Candida albicans, Candida tropicalis, and Candida krusei were differentiated by color as described by the manufacturer. All other yeast isolates (nearly all were Candida glabrata or Candida parapsilosis as confirmed by multiplex tandem PCR (MT-PCR)) were collectively designated “other Candida spp.” Growth of each morphologically distinct yeast isolate was enumerated semiquantitatively, and the extent of growth was classified as none, light (<10 colonies), moderate (10 to 100 colonies), or heavy (>100 colonies).

MT-PCR. Swabs of the throat and perineum obtained from the first 205 patients sampled at Westmead Hospital underwent multiplex tandem PCR (MT-PCR) analysis in addition to culture. Following culture, the swabs were plated into 3 ml saline solution containing 10% nutrient broth (Oxoid, Adelaide, South Australia) and stored at 4°C until nucleic acid was extracted. DNA was isolated from 1 ml of the suspension using a nucISENS easyMAG instrument (bioMérieux, Baulkham Hills, New South Wales, Australia) according to the manufacturer’s instructions. The elution volume was 110 μl. As previously described (28–30), the MT-PCR method enabled identification of seven Candida species, including C. albicans, Candida dubliniensis, C. glabrata, Candida guilliermondii, C. krusei, C. parapsilosis complex, and C. tropicalis, as well as Saccharomyces cerevisiae. An internal positive control (artificial DNA) was included with each specimen to test for PCR inhibition, and a negative water control was included in each run to monitor contamination. Urine specimens were not tested by MT-PCR, as the application of this technology to urine samples had not yet been validated during the study period.

Definitions. Colonization was defined as the isolation of a Candida species from at least one surveillance site. Positive samples collected as part of routine clinical management were not included in the analysis. The colonization index (CI) was defined as the ratio of the number of sites colonized by Candida spp. to the number of body sites surveyed. The corrected colonization index (CCI) was defined as the ratio of the number of sites heavily colonized by Candida spp. to the number of body sites surveyed (14). In this present study, the CCI threshold was reduced from ≥0.5 (14) to ≥0.3 to accommodate the reduced number of sites surveyed (three as opposed to five).

Surveillance specimens were collected at 72 h following ICU admission and twice weekly thereafter until ICU discharge or death. Time points 1, 2, and 3 were defined as 3 to 4 days, 6 to 8 days, and 9 to 11 days post-ICU admission, respectively.

IC episodes were defined using modified European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria (12, 17). Patients with candiduria alone were excluded because this was not considered an invasive infection. IC episodes detected at least 72 h following ICU admission and within 72 h after ICU discharge were considered to have been ICU acquired.

Statistical analysis. Surveillance culture results from ICU patients who did develop IC and from those who did not were compared with respect to categorical variables that included the anatomical site of colonization, the colonization indices (CI and CCI), the density of colonization, the number of colonizing species, and the time of surveillance. The relative risk of developing IC based on colonization status was calculated using Fisher’s exact test. A P value of <0.05 was considered significant. The predictive performance characteristics (including sensitivity, specificity, positive predictive value [PPV], and negative predictive value [NPV]) were calculated for each variable studied. MT-PCR and culture results were compared for the detection of colonization status and the time to detection of colonization status using Fisher’s exact test. All statistical calculations were performed using MedCalc (www.medcalc.org).

RESULTS

Patient characteristics. Of the 6,015 patients studied, 73 (1%) developed IC in the ICU. Fifty-eight (79%) of these had proven infection, i.e., candidemia (n = 43) or noncandidemic IC (n = 15); the remaining 15 (21%) patients were classified as probable cases (12, 17). Infections were caused by C. albicans (n = 45, 62%), C. glabrata (n = 8, 11%), C. tropicalis (n = 8, 11%), C. parapsilosis (n = 6, 8%), Candida spp. (n = 5, 7%), and C. krusei (n = 1, 1%). The median time from ICU admission to the development of ICU-acquired IC was 11 days (range, 4 to 41; average, 14 days).

Systemic (oral or parenteral) antifungal drugs had been received by five (7%) patients prior to ICU admission (days −7 to 0) or at time point 1 (days 3 to 4); by an additional seven (10%) between days 5 and 7; and by an additional six (8%) between days 8 and 10. Fluconazole therapy was administered in 16 cases of IC.

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and caspofungin in 2 cases. Twelve (16%) of 73 patients who de-
veloped IC had received empirical antifungal therapy (none had
received antifungal prophylaxis) prior to diagnosis; 9 (7 with in-
fected by C. albicans, 1 with infection due to C. lusitaniae, and
and 1 with infection due to C. tropicalis (75%) of the 12 had been
treated with fluconazole.

Colonization (culture) versus IC: anatomical site of coloni-
zation. Table 1 summarizes the risk of developing IC by site of colo-
12, 84%). Nineteen

<table>
<thead>
<tr>
<th>Variablea</th>
<th>n</th>
<th>RRb</th>
<th>P value</th>
<th>95% confidence interval (low)</th>
<th>95% confidence interval (high)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPVc (%)</th>
<th>NPVd (%)</th>
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<tbody>
<tr>
<td>Time point 1 (n studied = 6,015)</td>
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<tr>
<td>Any site colonized (CI ≥ 0.3)</td>
<td>3,511</td>
<td>2.35</td>
<td>0.002</td>
<td>1.35</td>
<td>4.08</td>
<td>78</td>
<td>40</td>
<td>2</td>
<td>99</td>
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<td>At least throat colonized</td>
<td>2,927</td>
<td>2.04</td>
<td>0.004</td>
<td>1.25</td>
<td>3.34</td>
<td>68</td>
<td>49</td>
<td>2</td>
<td>99</td>
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<tr>
<td>At least perineum colonized</td>
<td>1,967</td>
<td>1.76</td>
<td>0.0155</td>
<td>1.1</td>
<td>2.77</td>
<td>48</td>
<td>66</td>
<td>2</td>
<td>99</td>
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<td>At least urine colonized</td>
<td>630</td>
<td>2.25</td>
<td>0.003</td>
<td>1.32</td>
<td>3.84</td>
<td>23</td>
<td>88</td>
<td>3</td>
<td>99</td>
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<td>2,025</td>
<td>3.69</td>
<td>0.001</td>
<td>1.66</td>
<td>8.17</td>
<td>86</td>
<td>38</td>
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<td>1,555</td>
<td>2.97</td>
<td>0.0007</td>
<td>1.58</td>
<td>5.56</td>
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<td>At least perineum colonized</td>
<td>1,313</td>
<td>1.54</td>
<td>NSf</td>
<td>0.89</td>
<td>2.67</td>
<td>52</td>
<td>59</td>
<td>2</td>
<td>99</td>
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<td>At least urine colonized</td>
<td>450</td>
<td>1.81</td>
<td>NSf</td>
<td>0.95</td>
<td>3.44</td>
<td>24</td>
<td>85</td>
<td>3</td>
<td>99</td>
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<tr>
<td>Any site colonized (CI ≥ 0.3)</td>
<td>1,162</td>
<td>1.41</td>
<td>&lt;0.0001</td>
<td>1.23</td>
<td>1.61</td>
<td>89</td>
<td>37</td>
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<td>100</td>
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<td>837</td>
<td>5.04</td>
<td>0.001</td>
<td>1.93</td>
<td>13.21</td>
<td>82</td>
<td>53</td>
<td>3</td>
<td>99</td>
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<tr>
<td>At least perineum colonized</td>
<td>762</td>
<td>2.04</td>
<td>NSf</td>
<td>0.96</td>
<td>4.34</td>
<td>61</td>
<td>57</td>
<td>2</td>
<td>99</td>
</tr>
<tr>
<td>At least urine colonized</td>
<td>284</td>
<td>NSf</td>
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a Time point 1, days 3 to 4 post-ICU admission; time point 2, days 6 to 8 post-ICU admission; time point 3, days 9 to 11 post-ICU admission.
b RR, relative risk.
c PPV, positive predictive value.
d NPV, negative predictive value.
e CI, colonization index.
f NS, not significant.

and the development of IC. Seven (70%) of the 10 patients had only
one set of surveillance cultures obtained before discharge from the
ICU. The remaining three had two, five, or seven sets of surveillance
cultures obtained; the times from the last surveillance culture to the
development of IC were 2, 15, and 19 days, respectively. All but 1 of
the 10 patients who had negative surveillance cultures prior to devel-
oping IC had undergone surgery just prior to or during ICU admis-
sion. In six instances where the type of surgery was specified, five had
involved penetration of the gastrointestinal tract.

Colonization index (CI), corrected colonization index (CCI), and invasive candidiasis (IC). Of the 58% patients (n = 3,511) in whom Candida colonization was detected at the first time point, 1,671 (48%) had Candida spp. isolated from at least two surveil-
lance sites (CI ≥ 0.5; Table 2). Almost half (n = 35, 48%) of the patients who developed IC had a CI of ≥0.5 (RR = 2.25, P value = 0.0005; Table 2).

The sensitivity, specificity, and risk of developing IC increased slightly when heavy colonization was taken into account (CCI ≥ 0.3; Table 2). At time point 1, 42 (58%) patients who developed IC were heavily colonized in at least one site (RR = 3.7, P value < 0.0001). Specificity increased (74% to 92%) when two sites were
heavily colonized, but this in turn reduced sensitivity from 58% to 21% (Table 2).

Prevalence of Candida colonization and incidence of IC over
time in the ICU. Detection of Candida colonization at any site was
maintained at 60% over the first three time points (Fig. 1). Detec-
tion of perineum colonization increased the most over the first
two screens, while the likelihood of Candida colonization in the
urine increased steadily with prolonged ICU stay. Detection of
Candida colonization in the throat was highest at time point 1 and
decreased with time in the ICU. Of the 73 infected patients, 6, 17,
and 15 patients developed IC within time points 1, 2, and 3, re-

TABLE 1 Risk of invasive candidiasis based on screening time point and anatomical site of colonization (derived from culture results only; entire patient cohort included)

and Caspofungin in 2 Cases. Twelve (16%) of 73 Patients Who De-
veloped IC had Received Empirical Antifungal Therapy (None Had
Received Antifungal Prophylaxis) Prior to Diagnosis; 9 (7 with In-
fected due to C. albicans, 1 with Infection Due to C. lusitaniae, and
and 1 with Infection Due to C. tropicalis (75%) of the 12 Had Been
Treated with Fluconazole.

Colonization (Culture) versus IC: Anatomical Site of Colonization. Table 1 Summarizes the Risk of Developing IC by Site of Colonization Over the First Three Time Points. A Total of 3,511 (60%), 2,025 (63%), and 1,162 (64%) Patients Were Colonized at Time Points 1 (Days 3 to 4 Post-ICU Admission), 2 (Days 6 to 8 Post-ICU Admission), and 3 (Days 9 to 11 Post-ICU Admission), Respectively.

Of the 73 Patients Who Developed IC, Candida Colonization Was Detected in 63 (86%) Prior to Infection; 56 (89%) of These at Time Point 1, an Additional 5 at Time Point 2, and a Further 2 at Time Point 3. Patients With Detected Colonization in the Throat, Perineum, or Urine at Time Point 1 Were All at Increased Risk of Developing IC (Relative Risk [RR] = 2.35, P Value = 0.002, Sensitivity = 78%), With Colonization of the Urine Being Associated With the Highest Relative Risk of IC (RR = 2.25) Compared With Throat and Perineum (RR = 2.04 and 1.76, Respectively; Table 1). At Time Points 2 and 3, Patients Colonized in the Throat or in at Least One of the Three Sites Sampled (CI ≥ 0.3) Were at Increased Risk of Developing IC. NPVs Were Consistently High (99% to 100%; Table 1).

Of the 63 Patients in Whom Candida Colonization Was Detected Prior to Infection, 38 Were Colonized in at Least Two Sites, Most Commonly the Throat and Perineum (n = 32, 84%). Nineteen (30%) Patients With IC Were Colonized in the Throat Alone, Five (8%) in the Perineum Alone, and One (2%) in the Urine Alone.

The Median Time From the First Positive Surveillance Specimen to the First Positive Clinical Culture Was 7 Days (Range, 0 to 35; Average, 9 Days). Ten Patients (14%) Had Negative Surveillance Cultures Prior to
respectively, leading to cumulative incidences of IC of 8%, 32%, and 52% within the first three time points (Fig. 1).

**Distribution of colonizing species.** Most patients were colonized with *C. albicans* (*n* = 2,854, 81%), followed by "other” *Candida* spp. (*n* = 1,059, 30%), *C. tropicalis* (*n* = 343, 10%), and *C. krusei* (*n* = 123, 4%). The majority (80%) of patients were colonized with a single *Candida* species. Mixed cultures generally consisted of two species (*n* = 662), typically *C. albicans* with *C. glabrata* or *C. parapsilosis* (*n* = 467, 67%). All 63 colonized patients who developed IC were colonized with the species that subsequently caused infection. Twenty (32%) patients were colonized with two or more *Candida* species; the dominant colonizing species caused subsequent infection. The remaining 43 patients were colonized with one species (33 with *C. albicans*, 8 with “other” *Candida* spp., and 1 each with *C. krusei* and *C. tropicalis*). The number of colonizing species was not associated with the development of IC (data not shown).

**Comparison of MT-PCR and culture for the detection of Candida colonization in patients with or without IC.** In addition to culture, swabs of the throat and perineum from 205 patients underwent MT-PCR analysis. The median time of IC acquisition in this patient cohort was 5 days (range, 4 to 19 days) post-ICU admission. MT-PCR detected *Candida* DNA in an additional seven patients (*n* = 173, 84%) compared with culture (*n* = 166, 81%). No significant difference was found between MT-PCR and culture for detecting colonization status or between the sampling periods in which colonization was detected (91% and 93% of colonized patients were positive by MT-PCR and culture, respectively, at time point 1).

While only *C. albicans*, *C. tropicalis*, and *C. krusei* were distinguished with certainty on CHROMagar (based on the manufacturer’s protocol), a further five species, including *C. dubliniensis*, *C. glabrata*, *C. guilliermondii*, *C. parapsilosis*, and *S. cerevisiae*, were identified by MT-PCR. In this cohort, *C. albicans* was cultured from 134 (65%) patients whereas *C. albicans* DNA was detected in only 124 (60%) patients; *C. dubliniensis* DNA was detected in these 10 discrepant cases. Non-*Candida albicans* spp. detected by MT-PCR included *C. dubliniensis* (*n* = 27, 13%), *C. glabrata* (*n* = 29, 14%), *C. guilliermondii* (*n* = 3, 1%), *C. krusei* (*n* = 12, 6%), *C. parapsilosis* (*n* = 17, 8%), *C. tropicalis* (*n* = 15, 7%), and *S. cerevisiae* (*n* = 35, 17%). MT-PCR provided species identification in 73 cases where the species of the yeast could not be determined by CHROMagar.

The turnaround time for MT-PCR (3 h [but, in practice, 8 to 24 h, allowing for specimen transport and incorporation into routine laboratory workload]) was shorter than the 48 h required to obtain results from culture-based surveillance.
In this prospective multicenter study, we systematically determined the most effective and practical protocol to measure colonization status and predict IC in nonneutropenic patients in mixed medical/surgical Australian ICUs. As expected, colonization was detected in most patients (86%) prior to development of IC. Standardized cultures of the throat and perineum at time points 1 and 2 captured 97% (61/63) of colonized patients who developed IC, a median of 7 days prior to the diagnosis of IC (designated the diagnostic specimen collection date). Notably, 14% of the patients (n = 10) had negative surveillance cultures prior to the diagnosis of IC, similarly to a Spanish study of mixed medical/surgical ICUs (19). Based on our prior observation (17), we expect that this subgroup will be captured when clinical parameters such as recent gastrointestinal surgery are built into derivation of our model.

Prior Candida colonization is a major risk factor for developing IC; however, the sites surveyed have differed considerably (14, 19, 21, 31–33). Furthermore, proponents of “clinical only” prediction rules have argued for exclusion of colonization data because the process is resource intensive and adds to laboratory costs. Our study determined that collection of samples from three sites alone (throat, perineum, and urine) was practical and feasible but was not necessary for routine surveillance, since 98% (62/63) of colonized patients who subsequently developed IC were captured by throat and perineum surveillance cultures. In contrast to our findings, others have indicated that the mouth is not a good site for routine Candida surveillance due to lower positivity rates (23); the reason for this difference is not clear but may be linked to changes in the oropharyngeal niche and differences in oral hygiene practices among ICUs.

To optimize the timing of surveillance cultures, we compared results of cultures at time points 1, 2, and 3 (Tables 1 and 2 and Fig. 1). In a trend similar to those previously reported by others (21, 34), the majority of colonized patients who subsequently developed IC tested positive on the first screen (n = 56, 89%), indicating that new colonizing strains are acquired in a minority of patients after 72 h in the ICU (21). Detection of throat colonization unexpectedly decreased with time in the ICU (Fig. 1). Selective decontamination of the digestive tract (SDD) was not used in the present cohort, although changes to the oropharyngeal flora upon ICU admission are expected, considering variables such as patient intubation status, nutritional components, and different oral hygiene methods. Since 97% (61/63) of colonized patients who developed IC had positive surveillance in the throat and/or perineum within the first two time points and since the median time from ICU admission to IC was 11 days, we suggest that surveillance cultures taken at 72 h and 7 days post-ICU admission are sufficient for determining Candida colonization status for inclusion into risk-predictive models.

Multifocal colonization (at least two sites, CI > 0.5) and heavy colonization (at least one site, CCI ≥ 0.3) were both significant independent risk factors in our cohort (Table 2), but the decrease (78% to 58%) in sensitivity when CCI was applied indicates its
poor utility for predicting IC in our mixed medical/surgical ICU population. In the original study by Pittet et al. (14), the CCI resulted in a 100% PPV compared with the 2% to 4% in our study (Table 2). This large discrepancy is likely because the CCI (in the Pittet study) was derived from a small cohort of critically ill surgical patients who were selected as “high risk for IC” based on multiple clinical risk factors and because colonization status was determined through a variable number of “convenience” samples received in the laboratory at any time prior to the development of IC (14). This contrasts with populations, such as ours, that were selected on the basis of ICU length of stay, resulting in a low PPV and a high NPV. Defined sampling protocols and the mixed medical/surgical populations in our ICUs may also have contributed to the considerable difference with respect to CCI performance.

In other studies (20, 23), low PPVs were obtained when Candida colonization parameters were applied as the sole criterion for predicting IC—mainly due to its low incidence (1% to 2%, as in our study). With a median time from ICU admission to infection of 11 days (range, 4 to 41), initial screening at 72 h post-ICU admission was necessary to predict the 25% of cases that would have been missed had we used the 7-day entry point proposed by others (16, 22). Excellent NPVs were generated for all Candida colonization parameters investigated, indicating that patients with negative surveillance cultures are highly unlikely to develop disease and would not benefit from early antifungal intervention. An approach to patient care based on these observations would in turn reduce hospital costs, drug-related toxicities, and the likelihood of emerging antifungal resistance.

In a trend similar to those observed in European and North American studies (2, 19, 21, 22, 34), C. albicans was the dominant colonizing (81%) and infecting (77%) species. A rising prevalence of IC due to non-albicans Candida species has been attributed to azole prophylaxis and empirical treatment (6, 35–38). Others, however, have reported negligible effects of systemic antifungal use on colonization status (including that by C. glabrata, specifically) (24, 25). In the present study, only 12 of the 63 colonized IC patients had received antifungal therapy (all empirical; 10 fluconazole); hence, associations could not be tested.

MT-PCR was evaluated on a subset of patients (n = 205). Overall, results of detection of colonization status were similar between MT-PCR and culture (81% and 84%, respectively), although instances of culture misidentification were found (namely, C. dubliniensis was misidentified as C. albicans by CHROMagar). Other than providing species identification in cases where the yeast could not be identified on CHROMagar (14% C. glabrata and 8% C. parapsilosis), MT-PCR did not provide sufficient additional information to justify faster results and a 5-fold cost increase. Furthermore, yeast species determinations can now be performed rapidly and inexpensively from culture using technologies such as matrix-assisted laser desorption ionization—time of flight mass spectrometry (MALDI-TOF MS) (this was not available in clinical laboratories in Australia during the study period).

A potential limitation of this study was its multicenter characteristic, which encompassed possible variations in diagnostic and therapeutic decision making. Australian ICUs, however, are comparable with respect to case mix and management approaches, and a center effect was not demonstrated when data from individual hospitals were analyzed and compared (data not shown). In addition, a large sample size was employed to minimize disparities and improve the generalizability of results. The possibility of systematic bias was eliminated by the inclusion of patients who were admitted consecutively into the ICUs.

In conclusion, after performing a large-scale, multicenter, prospective, systemic evaluation of a variety of Candida colonization parameters, we propose a simplified protocol to optimize detection of colonization status for inclusion into risk-predictive models of IC in critically ill patients. This protocol, consisting of throat and perineum surveillance sampling at 72 h post-ICU admission and 3 to 4 days later, captures most patients likely to develop IC. It should be noted that colonization might not be detected prior to infection in a subset of patients who proceed to develop IC (14% in our cohort), but we expect that these patients will be captured by incorporation of clinical risk factors along with these colonization data into our final risk-predictive model.

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REFERENCES

6. Playford EG, Webster AC, Sorrell TC, Craig JC. 2006. Antifungal agents for preventing fungal infections in nonneutropenic critically ill and surgical patients: systematic review and meta-analysis of randomized clinical


